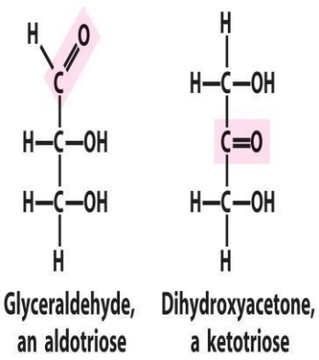
MUHAMMAD IMRAN

Bio 202 – Biochemistry-1

Topic no 1 to 112 in mid term and 113 to 225 final term syllabus

Lesson 1 Carbohydrates

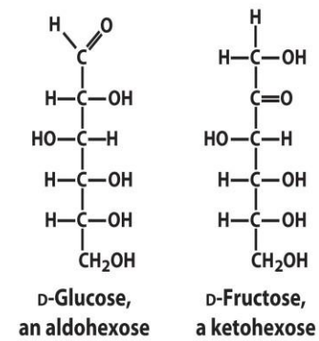
Carbohydrates are polyhydroxy aldehydes or ketones, or substances that yield such compounds on hydrolysis Many, but not all, carbohydrates have the empirical formula ***$(CH_2O)n$***; some also contain nitrogen, phosphorus or sulfur



Classification

- Carbohydrates are classified into **four** major groups:
  1. Monosaccharides
  2. Oligosaccharides
  3. Disaccharides
  4. Polysaccharides

Monosaccharides (simple sugars) are those which cannot be hydrolyzed further into simpler forms The backbones of common monosaccharides are *unbranched* carbon chains in which all the carbon atoms are linked by single bonds In **the open-chain form,** one of the carbon atoms is double-bonded to an oxygen atom to form a ***carbonyl group*** Each of the other carbon atoms has a hydroxyl group



If the carbonyl group is at an end of the carbon chain (that is, in an aldehyde group) the monosaccharide is an ***aldose***

If the carbonyl group is at any other position (in a ketone group) the monosaccharide is a ***ketose*** **The simplest** monosaccharides are the two three-carbon trioses:

- Glyceraldehyde,an aldotriose
- Dihydroxyacetone,a ketotriose

The carbons of a sugar are numbered beginning at the end of the chain nearest the carbonyl group

All common monosaccharides and disaccharides have names ending with the suffix —-ose.‖

Ketoses are designated by inserting "ul" into the name of a corresponding aldose

For example, ribulose is the ketopentose corresponding to the aldopentose ribose • There are a few exceptions however, for example, fructose and dihydroxyacetone

Monosaccharides can be subdivided further depending upon:

Number of carbon atoms Whether aldehyde or ketone groups are present as *aldoses* or *ketoses*

	General formula	Aldosugars	Ketosugars
Trioses	$C_3H_6O_3$	Glyceraldehyde	Dihydroxyacetone
Tetroses	$C_4H_8O_4$	Erythrose	Erythrulose
Pentoses	$C_5H_{10}O_5$	Ribose	Ribulose
Hexoses	$C_6H_{12}O_6$	Glucose	Fructose

The **hexoses** which include the aldohexose D-glucose and the ketohexose D-fructose, are the most common monosaccharides in nature The **aldopentoses** D-ribose and 2-deoxy-D-ribose are components of nucleotides and nucleic acids

Lesson 2

Disaccharides are those sugars which yield two molecules of the same or different monosaccharides on hydrolysis

- Maltose - 2 molecules of glucose on hydrolysis

- Lactose – 1 glucose & 1 galactose on hydrolysis
- Sucrose – 1 glucose & 1 fructose on hydrolysis

Oligosaccharides are short chains of monosaccharide units or residues (3-10) joined by characteristic linkages called glycosidic linkage **Glycosidic bond** of maltose is formed between the OH of carbon 1 and carbon 4 of 2nd glucose monomers. Thereforer, it forms an alpha (1– 4) glycosidic bond.

Lesson 3

Polysaccharides (Glycans) are those which yield more than 10 molecules of monosaccharides on hydrolysis Some have hundreds or thousands of units Some polysaccharides, such as ***cellulose***, are linear chains; others, such as ***glycogen***, are branched

Both glycogen and cellulose consist of recurring units of D-glucose, but they differ in the type of glycosidic linkage and consequently have strikingly different properties and biological roles

Polysaccharides are further divided into two groups:

1. **Homopolysaccharides (homoglycans):** Polymers of same monosaccharide units e.g. starch, glycogen, inulin, cellulose, dextrins, dextrans
2. **Heteropolysaccharides (heteroglycans):** Polymer of different monosaccharide units or their derivatives e.g. Mucopolysaccharides (glycosaminoglycans)

Biomedical Importance

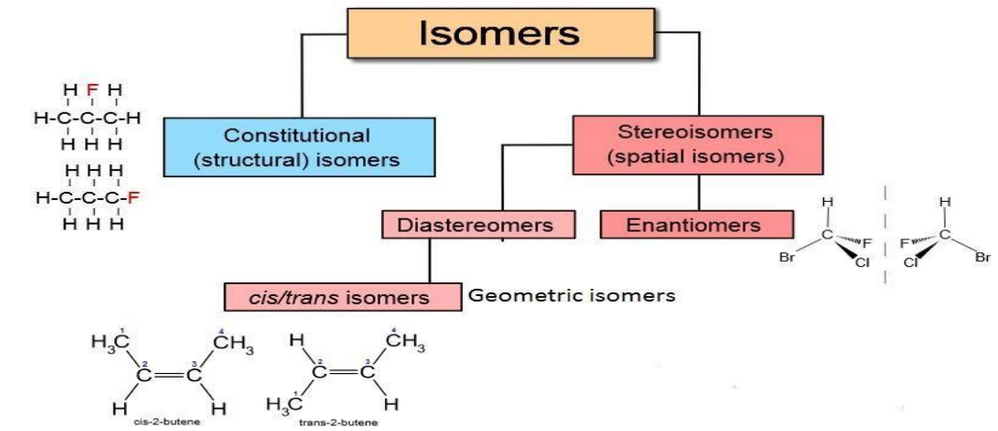
- Chief source of energy Constituents of compound lipids and conjugated proteins Lactose , the principal sugar of milk Degradation products utilized for synthesis of other substances such as fatty acids, amino acids, cholesterol, etc Constituents of mucopolysaccharides which form the ground substance of mesenchymal tissues Certain carbohydrate derivatives are used as drugs like **cardiac glycosides.** Inherited deficiency of certain enzymes in metabolic pathways of different carbohydrates can cause diseases e.g. **galactosemia, glycogen storage diseases, lactose intolerance,** etc

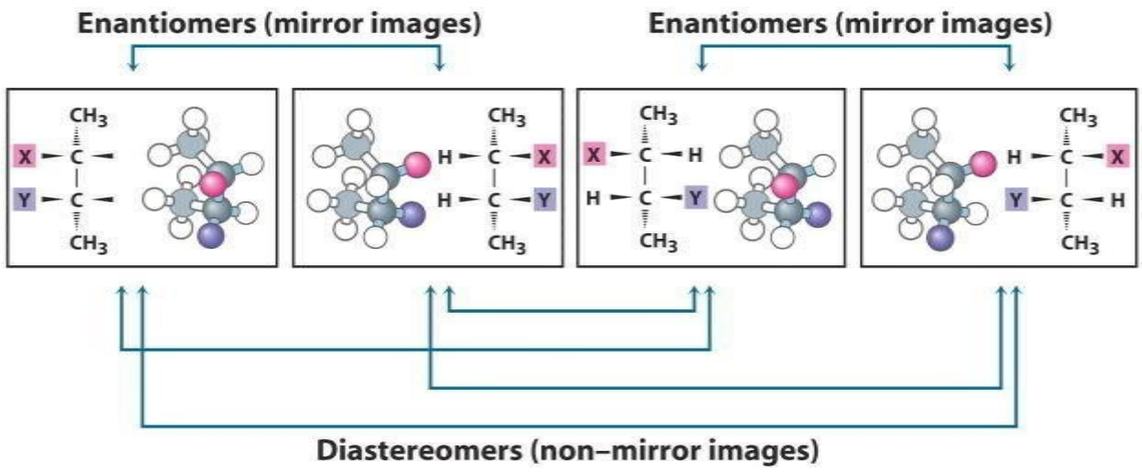
Derangement of glucose metabolism is seen in **Diabetes mellitus**

Lesson 4

Isomerism

- The existence of two or more compounds having the same molecular formula but a different arrangement of atoms within the molecule





Isomerism

Compounds which are identical in composition and differ only in configuration (the fixed spatial arrangement of atoms) are called ***stereoisomers***

Stereoisomers that are mirror images of each other are called ***enantiomers***

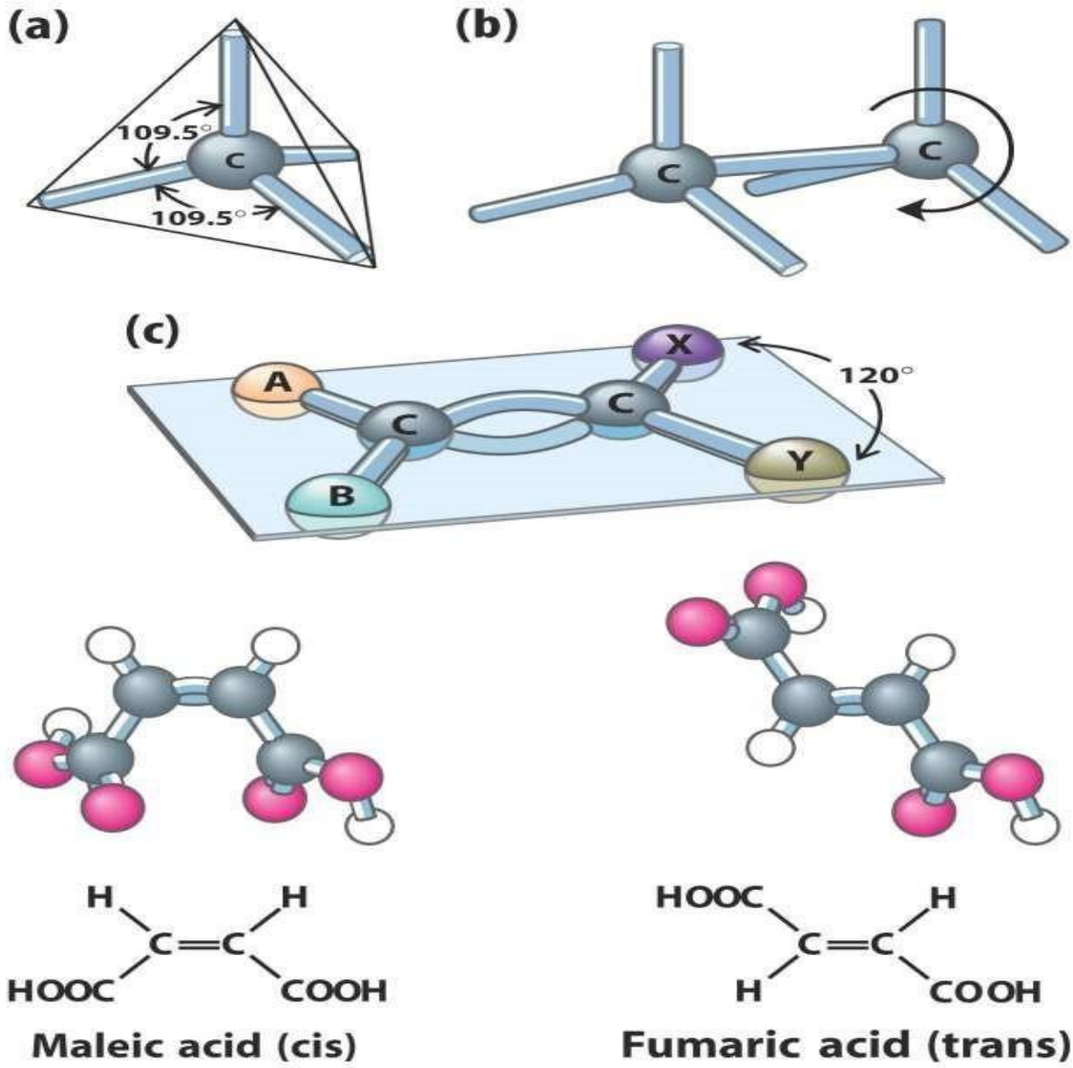
Pairs of stereoisomers that are not mirror images of each other are called ***diastereomers***

The identifying characteristic of stereoisomers is that they cannot be interconverted without temporarily breaking one or more covalent bonds

A carbon atom to which four different atoms or groups of atoms are attached is said to be **asymmetric** or ***chiral***

Lesson 5

Configuration is conferred by the presence of either



– (a) ***chiral centers,*** around which substituent groups are arranged in a specific orientation –
(b) ***double bonds,*** around which there is no freedom of rotation
• **Geometric isomers;** differ in the arrangement of their substituent groups with respect to the nonrotating double bond (each is a well-defined compound that can be separated from the other, and each has *its own unique chemical properties*) • The identifying characteristic of configurational isomers is that they cannot be interconverted without temporarily breaking one or more covalent bonds.
The configurations of maleic acid and its isomer, fumaric acid.
These compounds are **geometric,** or **cis-trans, isomers;**
They differ in the arrangement of their
substituent groups with respect to the nonrotating
double bond

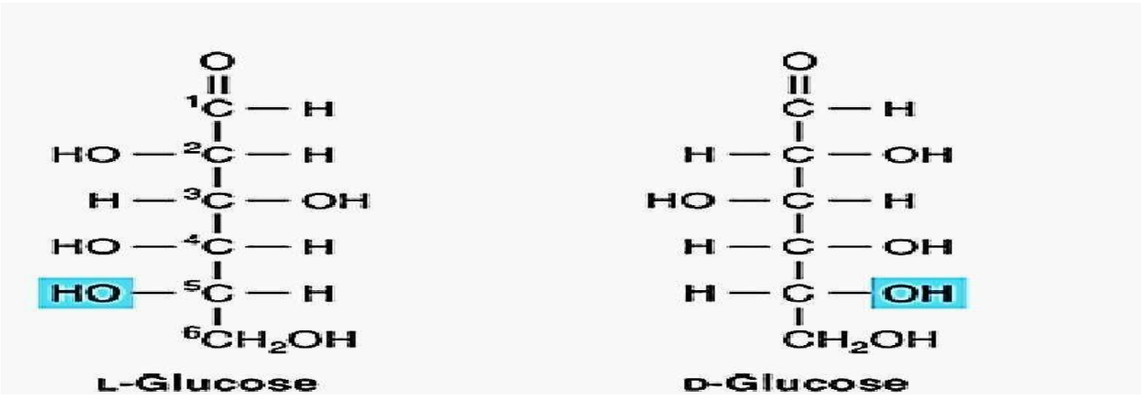
Lesson 6

D and L isomerism:

The designation of a sugar isomer as the D form or as the L form is determined by its spatial relationship to the parent compound of the carbohydrates, the glyceraldehyde
The orientation of the —H and —OH groups around the ***carbon atom adjacent to the terminal primary alcohol or carbon most distant from the carbonyl carbon – the penultimate carbon***
(carbon 5 in glucose) determines whether the sugar belongs to the D or L series.
When the —OH group on this carbon is on the right the sugar is the D isomer;

when it is on the left, it is the L isomer Of the 16 possible aldohexoses eight are D forms and eight are L
Most of the hexoses of living organisms are D isomers and the enzymes responsible for their metabolism are specific for this configuration

Fischer projection formulas



Lesson 7

Four different substituents bonded to a chiral carbon atom may be arranged in two different ways in space, that is, have two stereoisomers (with similar or identical chemical properties ***but differing in certain physical and biological properties)***

A molecule with only one chiral carbon can have two stereoisomers; when two or more (n) chiral carbons are present, there can be 2^n stereoisomers

Enantiomers have nearly identical chemical properties but differ in a characteristic physical property:

- their interaction with plane-polarized light

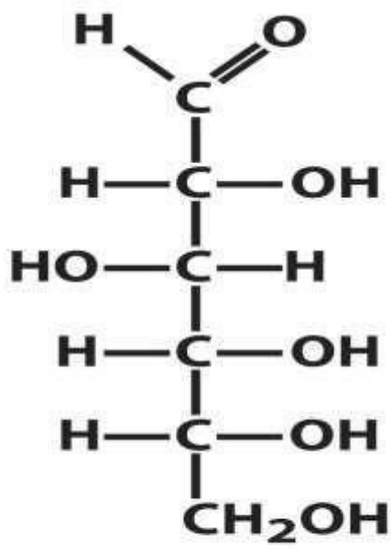
Thus the presence of asymmetric carbon atoms also confers **optical activity** on the compound

When a beam of plane-polarized light is passed through a solution of an **optical isomer,** it rotates either to the right, dextrorotatory (+), or to the left, levorotatory (–)

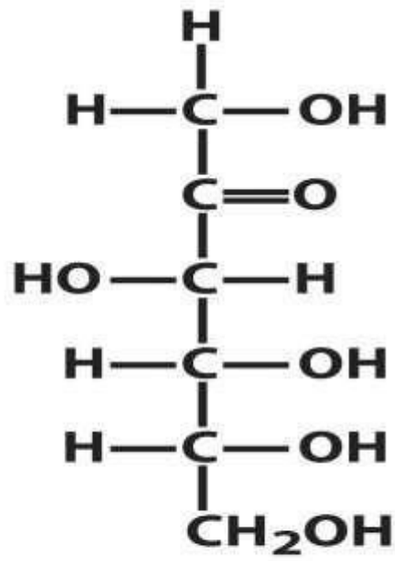
The direction of rotation of plane polarized light is independent of the stereochemistry of the sugar, so it may be designated D(–), D(+), L(–), or L(+)

For example, the naturally occurring form of fructose is the D(–) isomer

In solution, glucose is dextrorotatory, and glucose solutions are sometimes known as **dextrose**



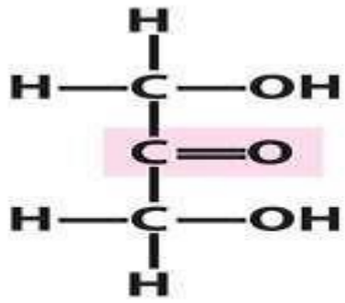
D-Glucose, an aldohexose



D-Fructose, a ketohexose

Lesson 8

When equal amounts of dextrorotatory and levorotatory isomers are present, the resulting mixture has no optical activity
Such a mixture is said to be ***racemic*** and separation of optically active isomers from a racemic mixture is called ***resolution***
All the monosaccharides except dihydroxyacetoe contain one or more asymmetric
(chiral) carbon atoms and thus occur in optically active isomeric forms

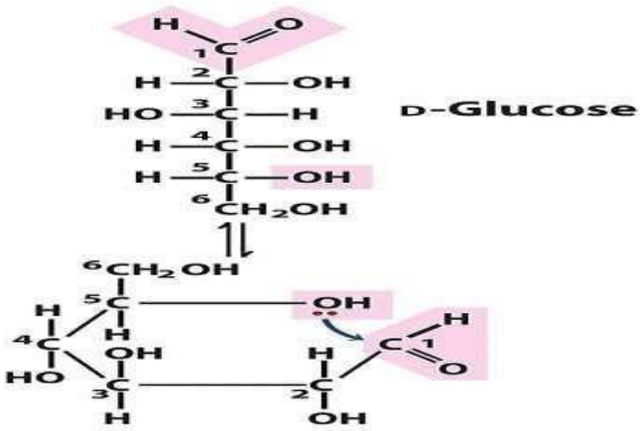


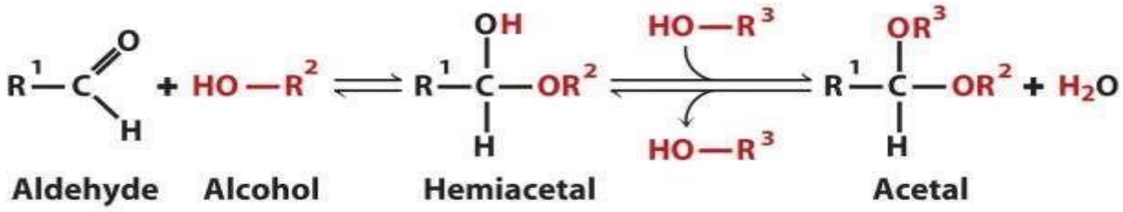
Dihydroxyacetone, a ketotriose

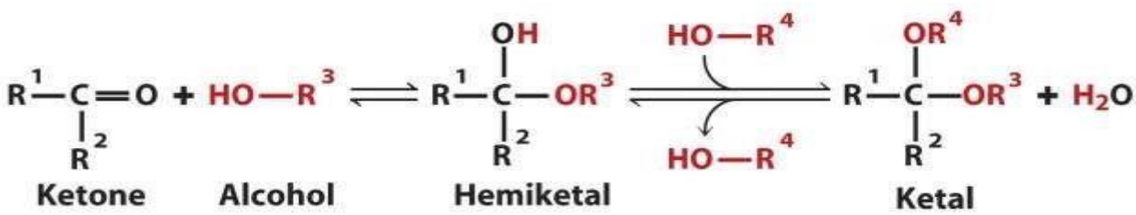
The simplest aldose, glyceraldehyde contains one chiral center (the middle carbon atom) and therefore has two different optical isomers

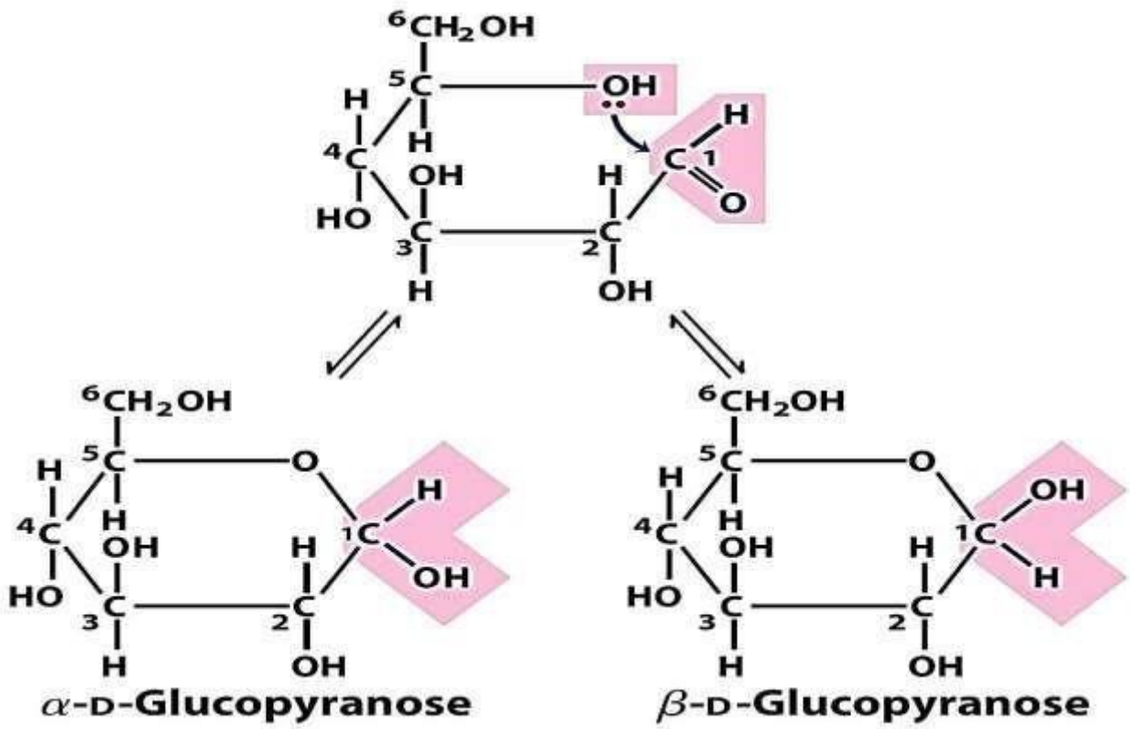
Lesson 9

In aqueous solution, aldotetroses and all monosaccharides with five or more carbon atoms in the backbone occur predominantly as ***cyclic (ring) structures***.
The formation of these ring structures is the result of a general reaction between alcohols and aldehydes or ketones to form derivatives called ***hemiacetals or hemiketals.***
Which contain an additional ***asymmetric carbon atom*** and thus can exist in two stereoisomeric forms
For example,D-glucose exists in solution as an **intramolecular hemiacetal** in which the free hydroxyl group at C-5 has reacted with the aldehydic C-l, •Therefore rendering the latter carbon (aldehydic C-l) asymmetric And producing two stereoisomers, designated ***α and β***
Carbon 1, after cyclization has four different groups attached to it and thus it also becomes ‗asymmetric'
*I*someric forms of monosaccharides that differ only in their configuration about the hemiacetal or hemiketal carbon atom are called **anomers**
The carbonyl carbon atom is called the ***anomeric carbon***









Lesson 10

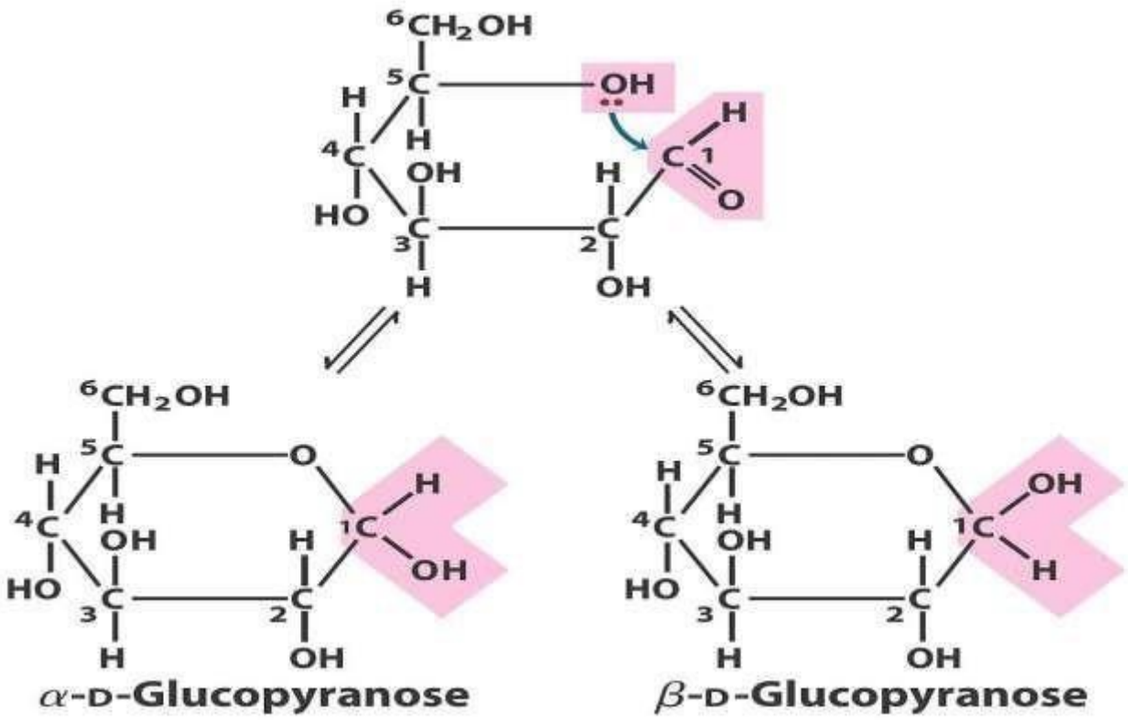
Ring Structure of Carbohydrates

For example,D-glucose exists in solution as an **intramolecular hemiacetal** in which the free hydroxyl group at C-5 has reacted with the aldehydic C-l, • Therefore rendering the latter carbon (aldehydic C-l) asymmetric

And producing two stereoisomers, designated ***α and β***

Carbon 1, after cyclization has four different groups attached to it and thus it also becomes ⎯asymmetric‘

Isomeric forms of monosaccharides that differ only in their configuration about the hemiacetal or hemiketal carbon atom are called **anomers**



Lesson 11

The ring structures of monosaccharides are similar to the ring structures of either pyran (six-membered ring) and are called ***pyranoses*** or furan (a five-membered ring), which are known as ***furanoses***

The six-membered aldopyranose ring is much more stable than the aldofuranose ring Therefore predominates in aldohexose and aldopentose solutions

Similarly Crystalline glucose is **α -D -glucopyranose**

The α and *β* anomers of D-glucose interconvert in aqueous solution by a process called **mutarotation.**

Thus, a solution of *α*-D-glucose and a solution of *β*-D-glucose eventually form identical equilibrium mixtures having identical optical properties.

This mixture consists of about;

one-third *α*-D-glucose, • two-thirds *β*-D-glucose, and very small amounts of the linear and five-membered ring (glucofuranose) forms.

α and β forms are NOT mirror images and are referred to as ***diastereomers***

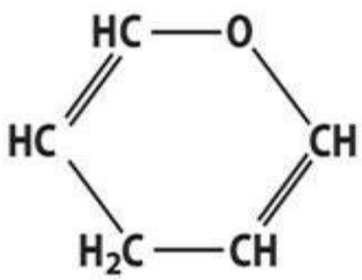
Enzymes are able to distinguish between these two structures and use one or the other preferentially

For example, glycogen is synthesized from α-D-glucopyranose, whereas cellulose is synthesized from β-D-glucopyranose

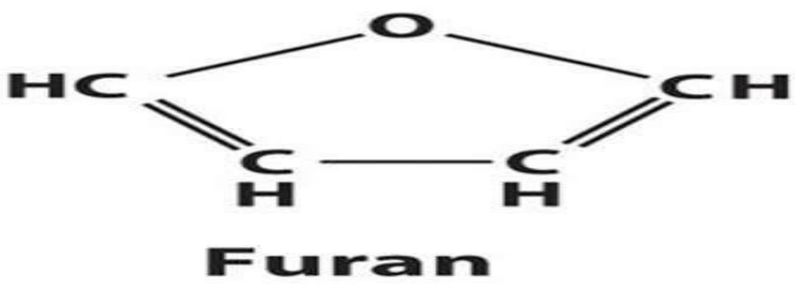
Ketohexoses also occur in α and β anomeric forms

In these compounds the hydroxyl group at C-5 (or C-6) reacts with the keto group at C-2, forming a furanose (or pyranose) ring containing a hemiketal linkage

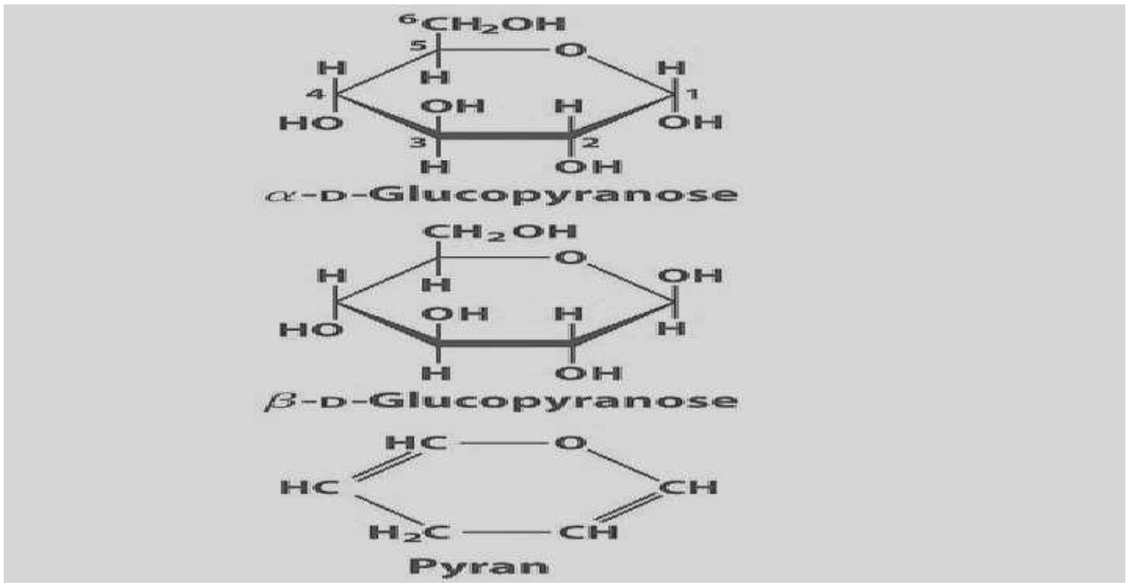
D-Fructose readily forms the furanose ring ; the more common anomer of this sugar in combined forms or in derivatives is *β*-D-fructofuranose



Pyran



Furan



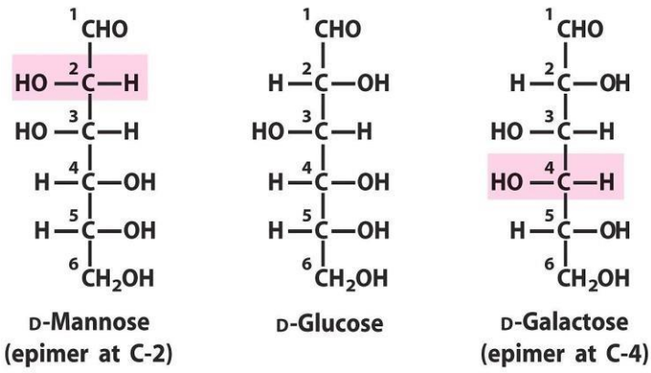
Lesson 12 Epimers

Two sugars which differ from one another only in configuration around a single carbon atom are termed ***epimers***

Glucose and Galactose are an example of an epimeric pair which differ only with respect to C_4

Similarly Glucose and mannose are epimers with respect to C_2

However, Galactose and mannose are NOT epimers as they differ in the position of –OH groups at two carbons (2 and 4), and are therefore defined only as isomers



***Trioses*:**

Both **D-Glyceraldehyde & Dihydroxyacetone** occur as intermediates in glycolysis They are also the precursors of glycerol

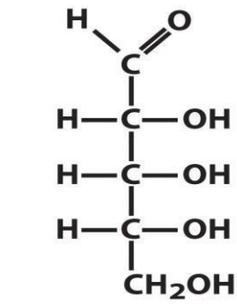
Tetroses:

Erythrose occurs as an intermediate in the Hexose Mono Phosohate shunt which is an alternative pathway for glucose oxidation

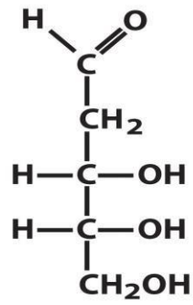
Pentoses*:*

D-Ribose is a constituent of nucleic acid *RNA* and also certain co-enzymes like *FAD, NAD & coenzyme A*

D-2-Deoxyribose is a constituent of *DNA*



D-Ribose, an aldopentose



2-Deoxy-D-ribose, an aldopentose

Hexoses

D-Glucose (Dextrose, Grape sugar):

Most dietary carbohydrate is absorbed into the bloodstream as glucose formed by hydrolysis of dietary starch and disaccharides, and other sugars are converted to glucose in the liver

Sources include fruit juices, hydrolysis of starch, cane or beet sugar, maltose and lactose

Glucose is the major metabolic fuel of mammals

It is the chief physiological sugar present in normal blood at a fairly constant level

All tissues utilize glucose for energy

Erythrocyte utilize glucose solely for energy purposes and Brain cells rely heavily on glucose

It is the precursor for synthesis of all the other carbohydrates in the body, including **glycogen** for storage; **ribose** and **deoxyribose** in nucleic acids; **galactose** in lactose of milk, in glycolipids, And in combination with protein in glycoproteins and proteoglycans

Stored as Glycogen in liver and muscles onlyExcreted in the urine (glucosuria) in poorly controlled diabetes mellitus as a result of hyperglycemia

Lesson 13 D-Galactose:

Occurs as a constituent of milk sugar lactose

It is an epimer of glucose and differs in orientation of H and OH around C-4

Readily metabolized to glucose;

Synthesized in the mammary gland for synthesis of lactose in milk

A constituent of glycolipids and glycoproteins

Hereditary galactosemia as a result of failure to metabolize galactose leads to cataracts D-Fructose:

Commonly known as *fruit sugar* as it occurs free in fruits, honey etc

Also occurs as a constituent of sucrose

It is levorotatory and hence is also called Levulose

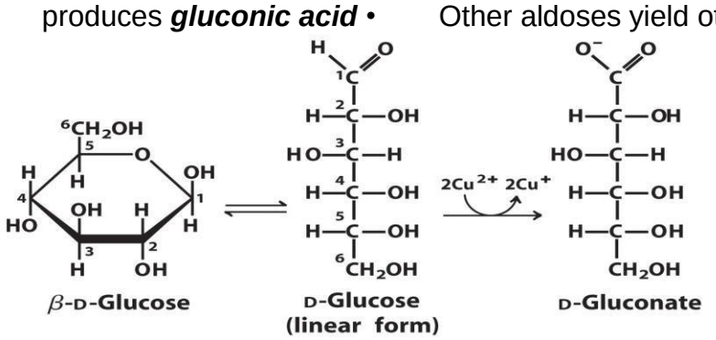
Seminal fluid is rich in fructose and sperm utilize fructose for energy

Readily metabolized either via glucose or directly

Hereditary fructose intolerance leads to fructose accumulation

Properties of Monosaccharides

1. Oxidation of the carbonyl (aldehyde) carbon of glucose to the carboxyl level produces ***gluconic acid*** • Other aldoses yield other ***aldonic acids***



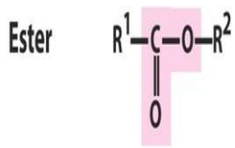
β-D-Glucose D-Glucose (linear form) D-Gluconate

Oxidation of the carbon at the other end of the carbon chain---C-6 of glucose, galactose, or mannoseforms the corresponding ***uronic acid,*** glucuronic, galacturonic, or mannuronic acid

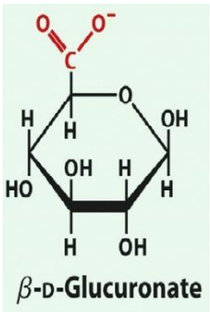
The carboxylic acid groups of the acidic sugar derivatives are ionized at pH 7, and the compounds are therefore correctly named as the carboxylates-glucuronate, galacturonate, and so forth

Both aldonic and uronic acids form stable intramolecular esters called ***lactones***

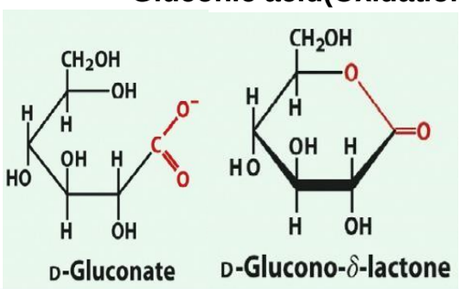
D-Glucono-δ-lactone results from formation of an ester linkage between the C-l carboxylate group and the C-5 (also known as the δ carbon) hydroxyl group of D-gluconate



Glucuronic acid(Oxidation at C-1)



Gluconic acid(Oxidation at C-1)

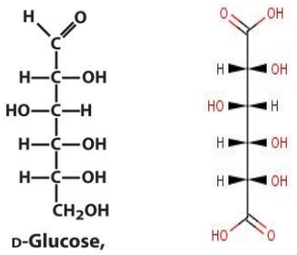


Lesson 14 to 21

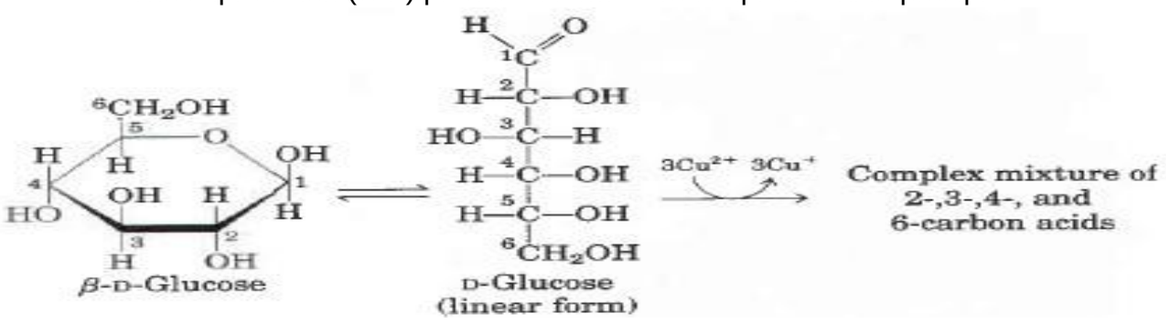
- **Properties of Monosaccharides**
- Glucono delta-lactone (GDL) is commonly found in honey, fruit juices, personal lubricants, and wine . GDL is neutral.
- But hydrolyses in water to gluconic acid which is acidic, adding a tangy taste to foods
- It is metabolized to glucose; one gram of GDL yields roughly the same amount of metabolic energy as one gram of sugar.
- In the body, D-Glucuronic acid is formed from glucose in liver
- It occurs as a constituent of certain polysaccharides
- It is of importance in that it conjugates toxic substances, drugs, hormones and even bilirubin.
- And converts them to a soluble non toxic substance, a glucuronide, which is excreted in urine • Oxidation of the carbon at the both ends of the carbon chain**.**
- **The terminal methyl & the carbonyl carbon** of glucose, galactose, or mannose-forms the corresponding

aldaric acid

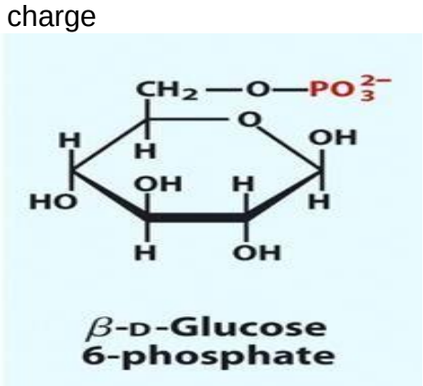
- Glucose forms **Glucaric acid**



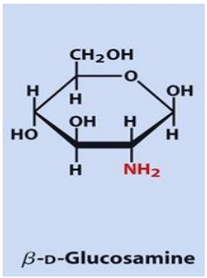
- Monosaccharides can be oxidized by relatively mild oxidizing agents such as cupric (Cu^{2+}) ion
- The carbonyl carbon is oxidized to a carboxyl group
- Glucose and other sugars capable of reducing cupric ion are called ***reducing sugars*** **Sugars as reducing agents**
- Oxidation of the anomeric carbon (and probably the neighboring carbon) of glucose and other sugars under alkaline conditions is the basis for
- Fehling's reaction
- Benedicts reaction
- The cuprous ion (Cu^{+}) produced forms a red cuprous oxide precipitate



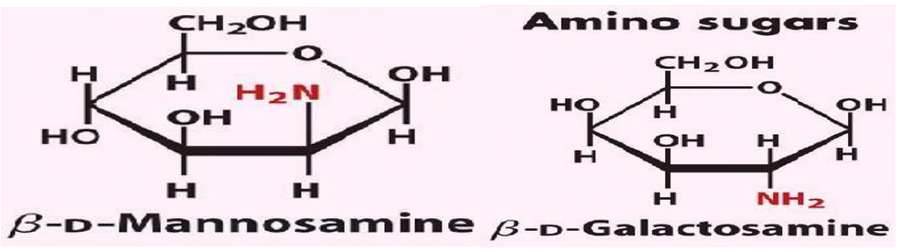
- By measuring the amount of oxidizing agent reduced by a solution of a sugar, it is also possible to estimate the concentration of that sugar
- For many years this test was used to detect and measure elevated glucose levels in blood and urine in the diagnosis of diabetes mellitus
- **Properties of Monosaccharides**
- 2. Condensation of phosphoric acid with one of the hydroxyl groups of a sugar forms a ***phosphate ester***, as in glucose 6-phosphate
- Sugar phosphates are relatively stable at neutral pH and bear a negative charge



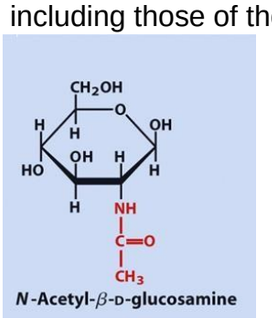
- In fact, one of the strategies of glycolysis is to form three carbon intermediates, that can transfer their phosphate groups to ADP, to achieve a net synthesis of ATP
- In the synthesis and metabolism of carbohydrates, the intermediates are very often not the sugars themselves but their phosphorylated derivatives
- Another function of phosphorylation is the creation of reactive intermediates for the formation of O- and N- glycosidic linkages
- **Properties of Monosaccharides**
- 3.Synthesis of Amino Sugar
- If the hydroxyl at C-2 of the parent monosaccharide is replaced with an amino group, the corresponding ***amino sugar*** is formed



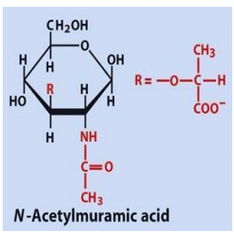
- The amino sugars include:
  - ***D -glucosamine***, a constituent of hyaluronic acid
  - ***D -galactosamine*** (also known as *chondrosamine*), a constituent of chondroitin – ***D -mannosamine***



- The amino group is nearly always condensed with ***acetic acid***, as in N-acetylglucosamine
- Both glucosamine and galactosamine occur as *N*-acetyl derivatives in more complex carbohydrates.
- For example glycoproteins and is part of many structural polymers, including those of the bacterial cell wall



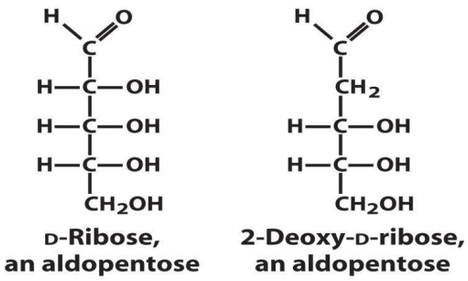
- Bacterial cell walls also contain a derivative of glucosamine, ***N-acetylmuramic acid***, in which lactic acid (a three carbon carboxylic acid) is ether-linked to the oxygen at C-3 of N-acetylglucosamine



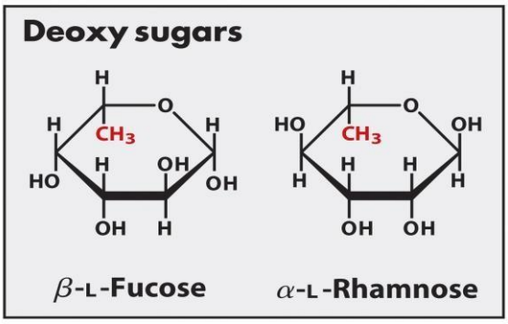
- The Amino Sugars (Hexosamines) are Components of Glycoproteins, Gangliosides, & Glycosaminoglycans
- Several **antibiotics** (e.g, erythromycin) contain amino sugars, which are important for their antibiotic activity
- 4. When oxygen of a

–OH group is removed in a monosaccharide, leaving behind only hydrogen, ***deoxy sugars*** are formed

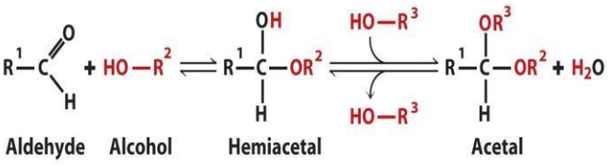
- 2-Deoxy-D-Ribose is derived from D-Ribose and is an important constituent of DNA

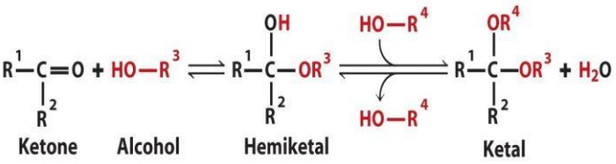


- The substitution of a hydrogen for the hydroxyl group at C-6 of ;
- L-galactose &
- L-mannose produces;
- L-fucose and
- L-rhamnose, respectively.
- ***L-Fucose*** is found in the complex oligosaccharide components of
- glycoproteins and glycolipids;
- **L-Rhamnose** is found in plant polysaccharide

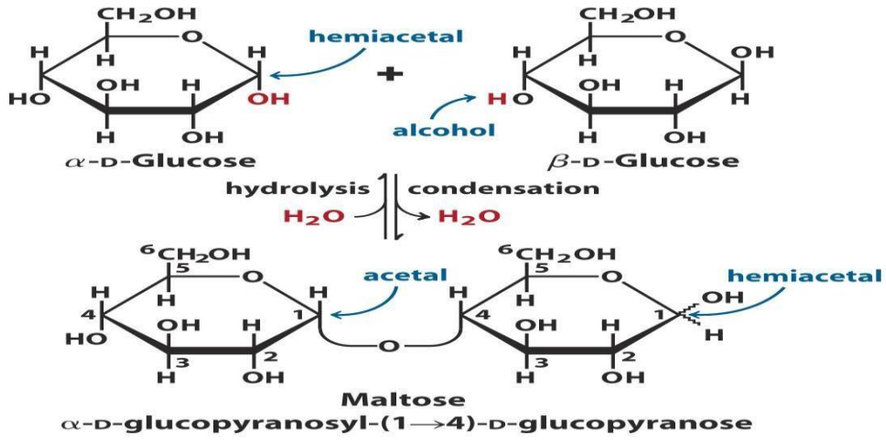


- **5. Glycosides** are formed by condensation between the hydroxyl group of the anomeric carbon of a monosaccharide, and a second compound that may or may not (in the case of an **aglycone**) be another monosaccharide
- If the second group is a ***hydroxyl,*** the ***O-glycosidic*** bond is an **acetal link** because it results from a reaction between a hemiacetal group (formed from an aldehyde and an —OH group) and another —OH group



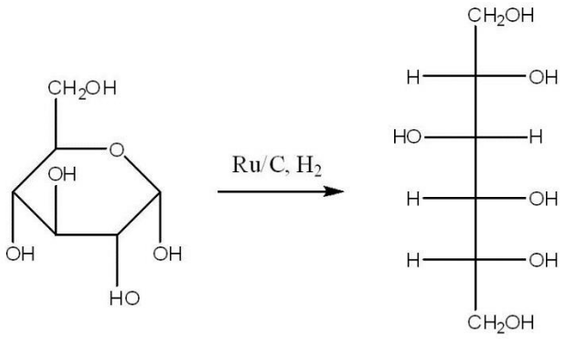


- If the hemiacetal portion is glucose, the resulting compound is a **glucoside**
- If galactose, a **galactoside,** and so on

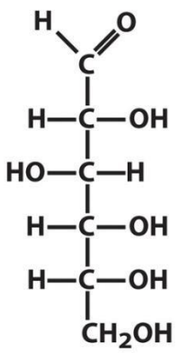


6. The monosaccharides may be reduced to form their ***corresponding alcohols*** by reducing agents;

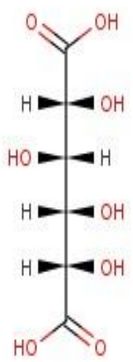
- Examples are
  - D-Glucose yields D-Sorbitol
  - D-Galactose yields D-Dulcitol
  - D-Mannose yields D-Mannitol
  - Ribose to ribitol

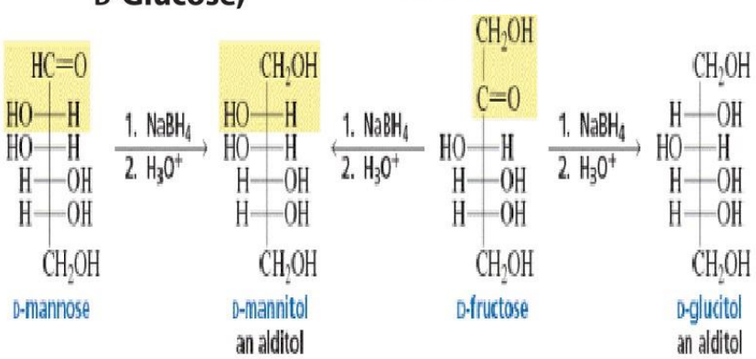


D-Glucose D-Sorbitol



D-Glucose,





Sorbitol:

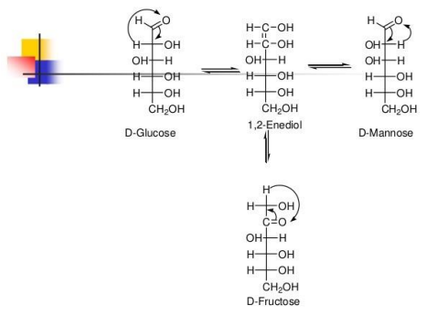
- 35-60% sweetness of glucose
- Used as an artificial sweetner
- Accumulates in tissues such as the eye lens in diabetes mellitus **Mannitol:**
- Administered intravenously as an osmotic diuretic in acute renal failure
- Also used to relieve raised intracranial pressure by forced diuresis

7. Tautomerization

- The process of shifting a hydrogen atom from one carbon atom to another to produce **enediols** is known as tautomerization

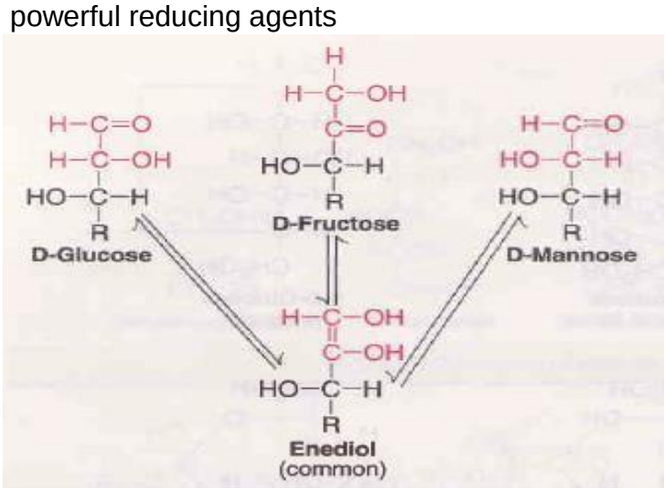
Sugars possessing anomeric carbon atom undergo tautomerization in alkaline solutions

- When glucose is kept in alkaline solution for several hours, it undergoes isomerization to form Dfructose and D-mannose



This reaction results in the formation of a common intermediate-namely enediol--for all the three sugars

- The enediols are highly reactive, hence sugars in alkaline solution are powerful reducing agents



8. ***Osazone formation***:

- It is a useful means of preparing *crystalline derivatives* of sugars called ***osazones***
- They are obtained by adding a mixture of ***phenyl hydrazine hydrochloride*** and ***sodium acetate*** to a sugar solution and heating in water bath for 45 mins
- Crystals are formed after the solution is cooled slowly
- Reaction involves only the carbonyl carbon and the adjacent carbon
- First phenyl hydrazone is formed and then reacts with an additional molecule of phenylhydrazine to form the osazones.
- As is evident from the reaction, the first two carbons (C1 and C2) are involved in osazone formation
- The sugars that differ in their configuration on these two carbons give the same type of osazones, since the difference is masked by binding with phenylhydrazine

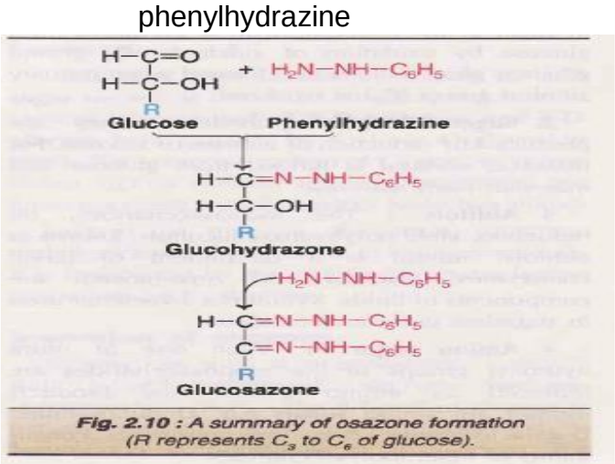
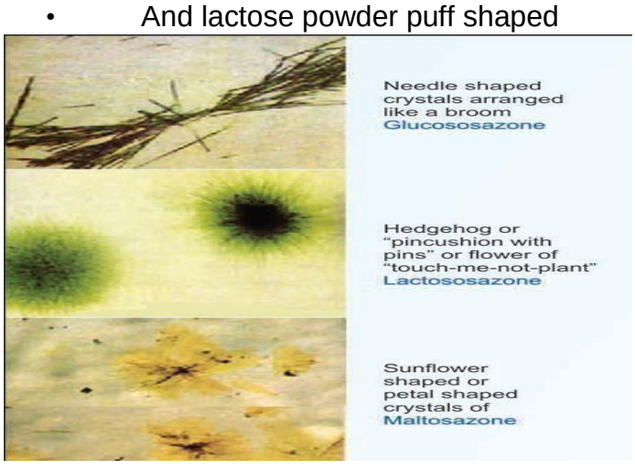


Fig. 2.10 : A summary of osazone formation (R represents C_3 to C_6 of glucose).

- Thus glucose, fructose and mannose give the same type (needle-shaped) osazones
- Reducing disaccharides also give osazones
- Maltose sunflower-shaped

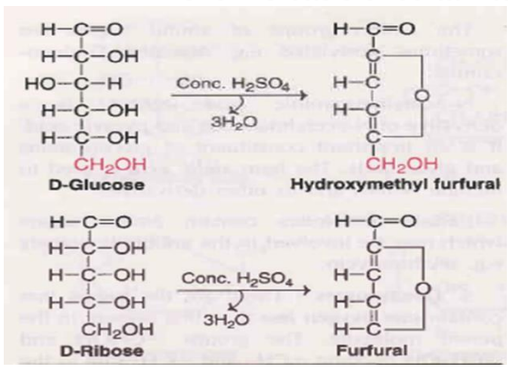
- And lactose powder puff shaped



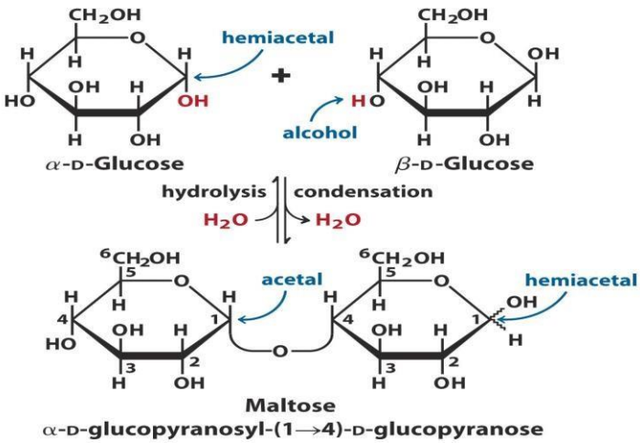
- Osazones have characteristic – Melting points
  - Crystal structures
  - Precipitation time
  - and thus are used in identifying the sugars

9. Action of acids on carbohydrates

- Polysaccharides are hydrolyzed into their constituent monosaccharides by boiling with **dilute** acids
- With **concentrated** acids the monosaccharides are decomposed
- Pentoses yield a cyclic aldehyde *furfural* with 12% HCL
- Hexoses are decomposed by hot strong acids to give *hydroxy methyl furfural*
- The furfural products thus formed can condense with certain organic phenols (α-naphthol) to form compounds having characteristic colours
- This forms basis of certain tests used for detection of sugars



- The furfural products thus formed can condense with certain organic phenols (α-naphthol) to form compounds having characteristic colours
- This forms basis of certain tests used for detection of sugars
- Molisch,s and
- Selivanoff,s are examples of such tests 22 Disaccharides
- **Disaccharides** (such as maltose, lactose, and sucrose) consist of two monosaccharides joined covalently by an **O-glycosidic bond.**
- Which is formed when a hydroxyl group of one sugar reacts with the anomeric carbon of the other
- When the anomeric carbon is involved in **a glycosidic bond**, that sugar residue is not capable of reducing cupric ions and therefore becomes a non-reducing sugar
- In describing disaccharides or polysaccharides, the end of a chain with a free anomeric carbon (one not involved in a glycosidic bond) is commonly called the reducing end
- **Maltose** has two glucose units joined by an **α(1→4)** glycosidic linkage
- Since it has one aldehyde 'free', it has ***reducing properties*** and also exhibits mutarotation



- Maltose comes from the hydrolysis of starch (by **amylase**)
- It is in turn hydrolysed to glucose by **maltase,** which is located on the intestinal brush border
- From nutritional point of view, maltose is easily digestible
- Various food preparations, such as baby and invalid foods, are produced by hydrolysis of grains and contain large amounts of maltose

Lesson 23 •

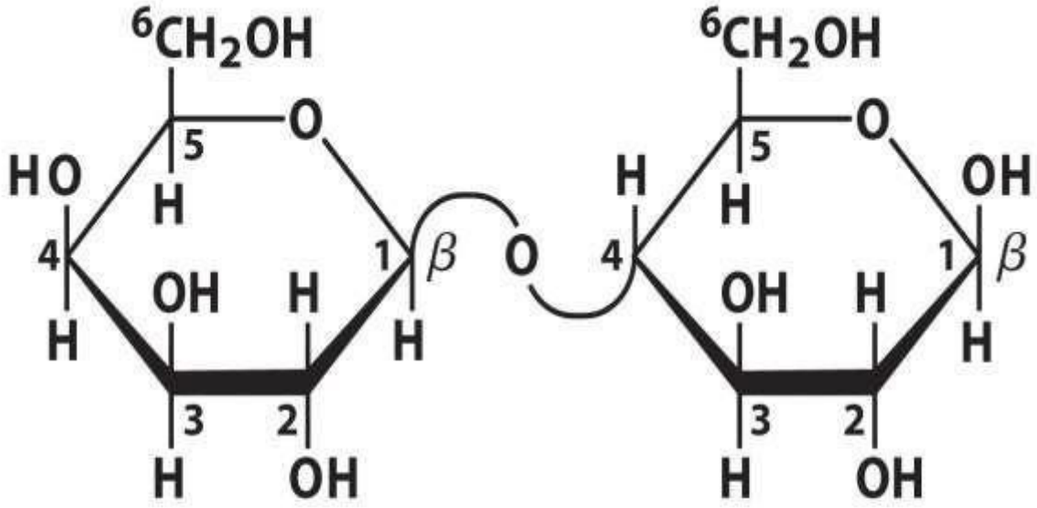
Lactose, the disaccharide of milk consists of galactose joined to glucose by **β (1→4)** glycosidic linkage

- It is dextrorotatory
- As one of the aldehyde group is free, it has reducing properties and exhibits mutarotation

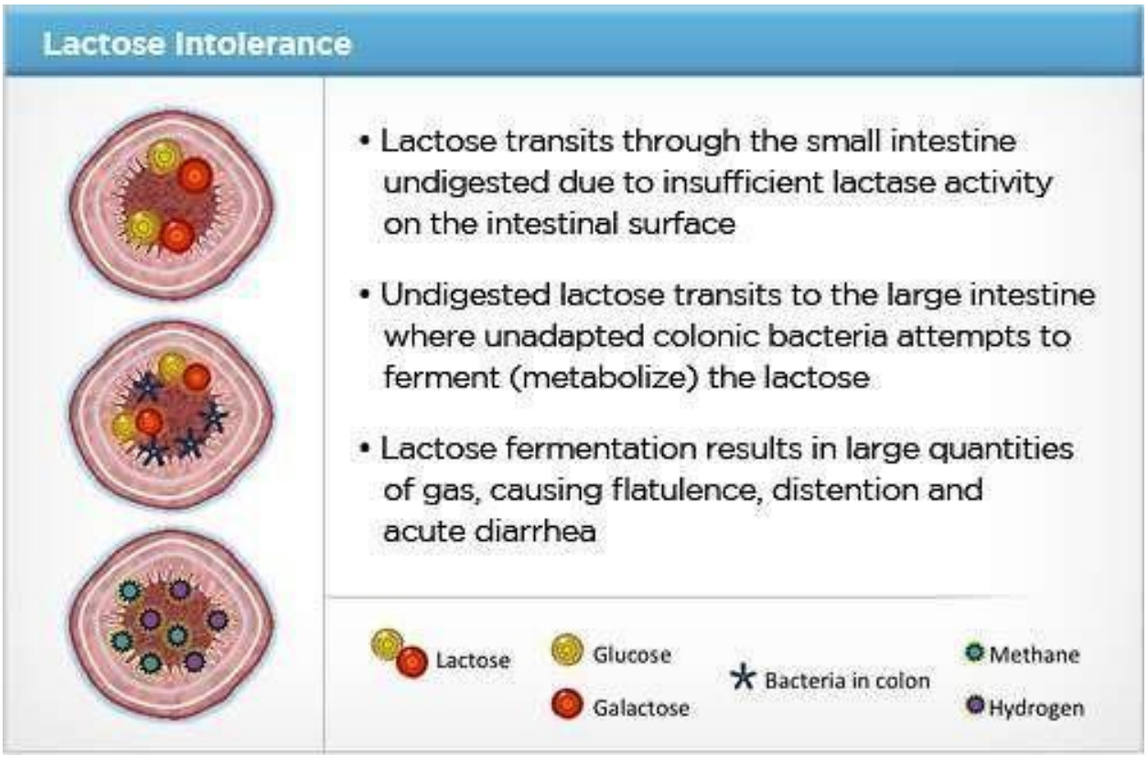
- In lactating mammary gland, the lactose is synthesized from glucose by the duct epithelium
- Many organisms that are found in milk, e.g., *E.Coli* convert lactose of milk to lactic acid
  (by ***β* galactosidase**) thus causing souring of milk

- **Lactase**, the specific enzyme which hydrolyses lactose is present in the intestinal brush border
- **Lactose intolerance** refers to a condition of pain, nausea, and flatulence after the ingestion of foods containing lactose, most notably dairy products
- Although it is often caused by
  - low levels of lactase
  - it also can because by intestinal injury

- In a **lactase deficient** person, lactose accumulates in the lumen of the small intestine after ingestion of milk
- The large osmotic effect of the unabsorbed lactose leads to an influx of fluid into the small intestine
- Hence, the clinical symptoms of **lactose intolerance** are:
  - abdominal distention,
  - nausea,
  - cramping,
  - pain
  - a watery diarrhea



Lactose (β form)
β-D-galactopyranosyl-(1→4)-β-D-glucopyranose
Gal(β1→4)Glc



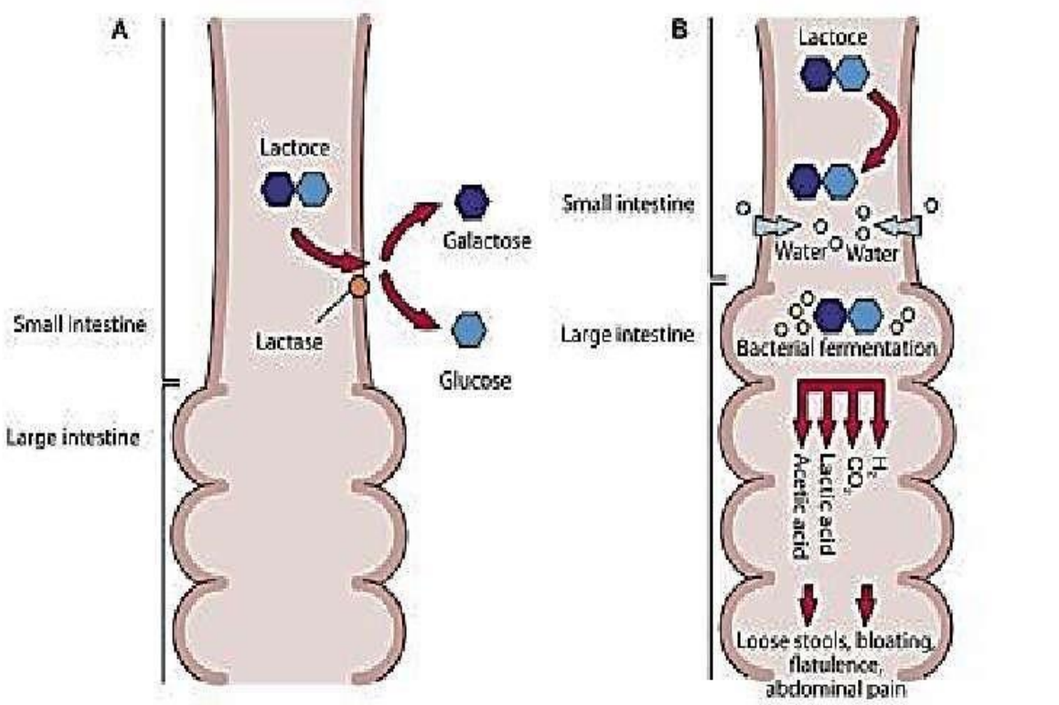
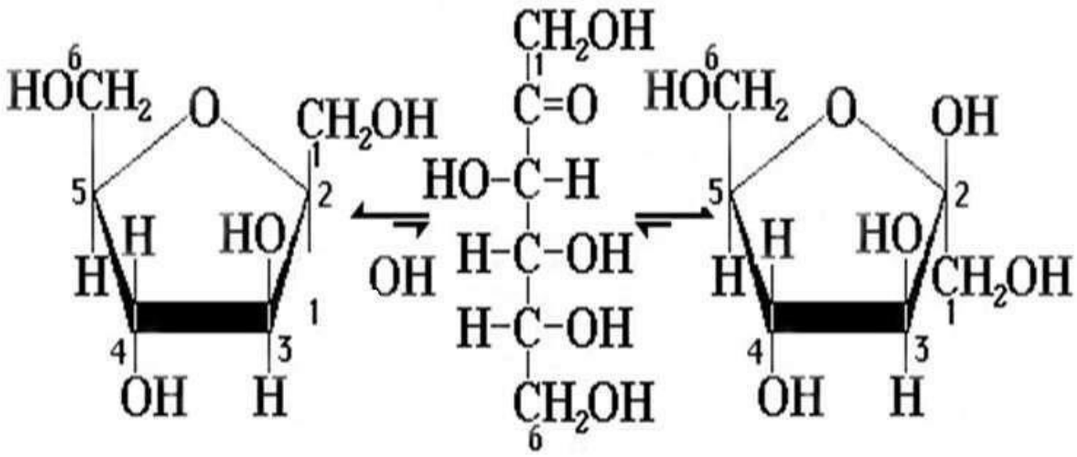


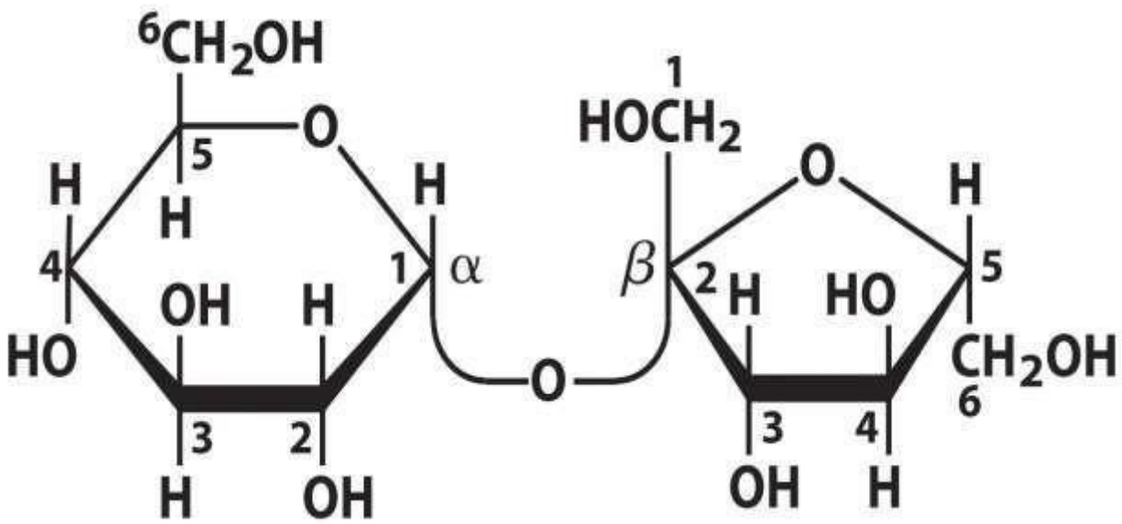
Figure 3. Picture A: Lactase degrades lactose. No symptoms of lactose intolerance. Picture B: Unabsorbed lactose in the large intestine causes lactose intolerance symptoms.

Lesson 24

- **Sucrose** (Ordinary table sugar) is obtained commercially from cane or beet
- The anomeric carbon atoms of a glucose unit and a fructose unit are joined in this disaccharide and the configuration of this glycosidic linkage is α for glucose and β for fructose

- Consequently, sucrose lacks a free reducing group (an aldehyde or ketone end), in contrast with most other sugars
- Sucrose is therefore a non-reducing sugar and does not exhibit mutarotation
- Sucrose is dextrorotatory but its hydrolytic products are levorotatory as fructose has a levorotation greater than the dextrorotation of glucose
- As the hydrolytic products invert the rotation, the resulting mixture is called as **invert sugar** and the process is called as **inversion**
- The hydrolysis of sucrose to glucose and fructose is catalyzed by **sucrase** (also called **invertase**), which is also present in the intestinal brush border like lactase and maltase • Honey is largely invert sugar and the presence of fructose accounts for the greater sweetness of honey





Sucrose

α-D-glucopyranosyl β-D-fructofuranoside

Glc(α1↔2β)Fru

Lesson 25

- **Oligosaccharides** are short polymers of three to ten monosaccharides joined by glycosidic bonds.
- Most are not digested by human enzymes
- At one end of the chain, the reducing end, is a monosaccharide unit with its anomeric carbon not involved in a glycosidic bond

Biomedical Importance

- Integral membrane proteins contain covalently attached carbohydrate units, oligosaccharides, on their extracellular face
- Many secreted proteins such as antibodies and coagulation factors also contain oligosaccharide units

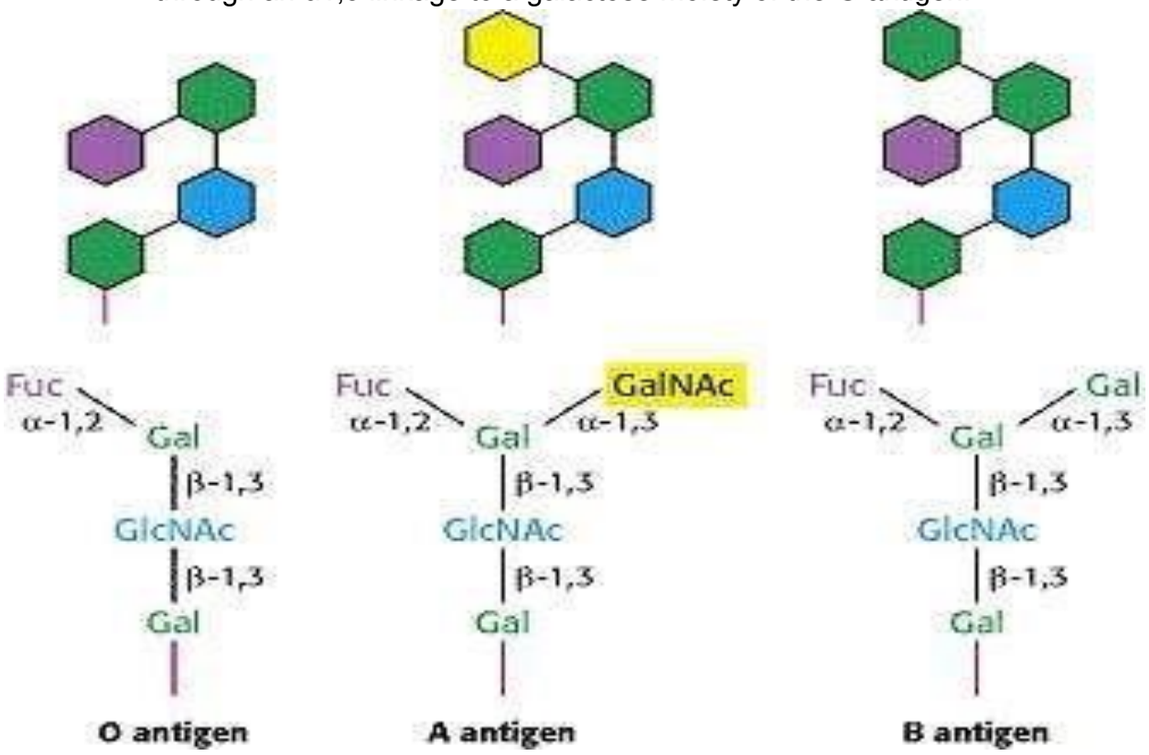
- The oligosaccharides participate in molecular targeting and cell to cell recognition

- They also mark the passage of time and determine when the proteins should be taken out of circulation

- The human ABO blood groups illustrate the effects of glycosyl-transferases

- Carbohydrates are attached to glycoproteins and glycolipids on the surfaces of red blood cells
- For one type of blood group, one of the three different structures, termed A, B, and O, may be present
- These structures have in common an oligosaccharide foundation called the O (or sometimes H) antigen

- The A and B antigens differ from the O antigen by the addition of one extra monosaccharide, either *N*-acetylgalactosamine (for A) or galactose (for B) through an α1,3 linkage to a galactose moiety of the O antigen.



Lesson 26

Polysaccharides

- Most carbohydrates found in nature occur as polysaccharides, polymers of medium to high molecular weight

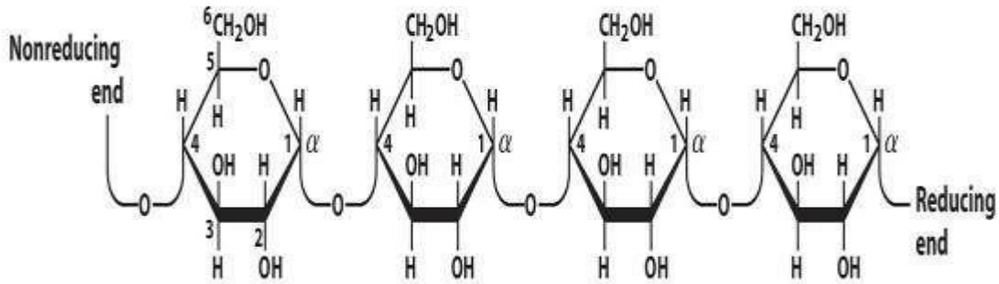
  Polysaccharides also called **glycans**, differ from each other
  – in the identity of their recurring monosaccharide units
  – in the length of their chains
  – in the types of bonds linking the units
  – in the degree of branching
- **Homopolysaccharid*es*** contain only a single monomeric species
- **Heteropolysaccharides** contain two or more different kinds
- Some **homopolysaccharides** serve as storage forms of monosaccharides that are used as fuels
- Other homopolysaccharides (cellulose and chitin) serve as structural elements in plants • The most important storage polysaccharides are **starch in plant cells and glycogen in animal cells**

- Both polysaccharides occur intracellularly as large clusters or granules
- Most plant cells have the ability to form starch and starch storage is especially abundant in tubers (underground stems), such as potatoes and in seeds

- **Heteropolysaccharides** provide extracellular support for organisms of all kingdoms
- For example, the rigid layer of the bacterial cell envelope (the peptidoglycan) is composed in part of a heteropolysaccharide built from two alternating monosaccharide units

- **Starch** contains two types of glucose polymers:
  - Amylose
  - amylopectin
- It is the most important dietary carbohydrate in cereals, potatoes, legumes, and other vegetables

- **Amylose** (13–20%), consists of long, unbranched helical structure of D-glucose residues connected by (α1→4) linkages (as in maltose)



amylose

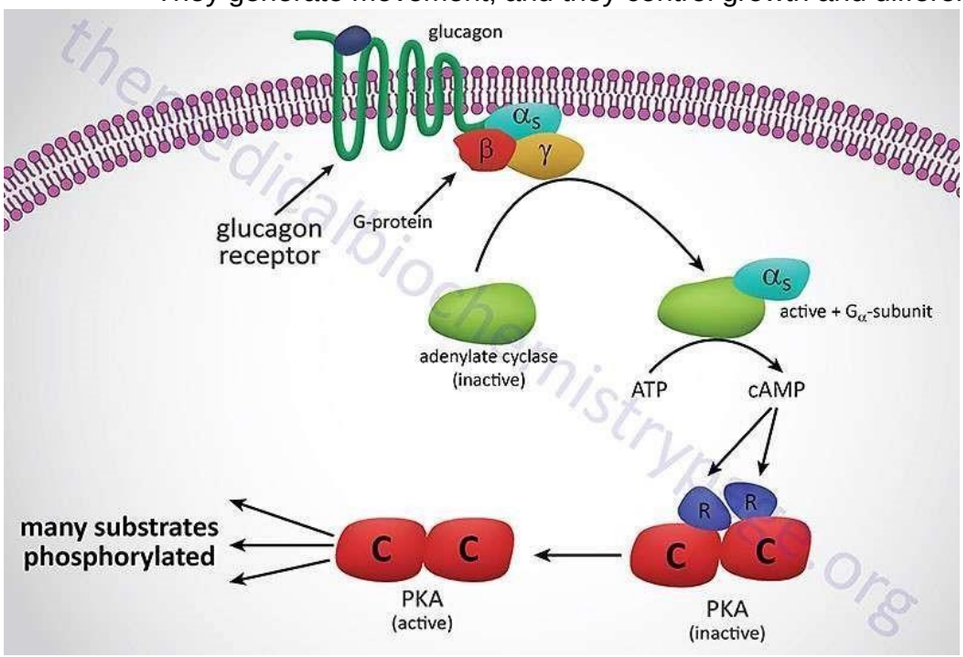
Lesson 27

- Proteins mediate virtually every process that takes place in a cell, exhibiting an almost endless diversity of functions. • To explore the molecular mechanism of a biological process, a biochemist almost inevitably studies one

or more protein

- Proteins are the most abundant biological macromolecules occurring ; in all cells and all parts of cells
- Proteins also occur in great variety; thousands of different kinds may be found in a single cell
- Moreover, proteins exhibit enormous diversity of biological function and are the most important final products of the information pathways
- Example signal transduction
- Biochemical catalysts known as **enzymes** are proteins
- **Immunoglobulins** that serve as the first line of defense against bacteria and viruses and other foreign agents are proteins
- Several **hormones** are proteins
- **Structural proteins** provide **mechanical support** e.g collagen
- **Contractile proteins** help in the **movement** of muscle fiber and microvilli
- Some proteins present in the cell membrane, cytoplasm and nucleus act as **receptors**
- Certain other proteins in the cell membrane act as **channels and transporters**

- **The transport proteins** carry out the function of transporting specific substances across the membrane or in the body fluids
- **Storage proteins** bind with specific substances and store them
- Under certain conditions, proteins can be catabolized to **provide energy**
- Proteins help in the maintenance of water and electrolyte balance in the body by **exerting osmotic pressure**
- Hence proteins are the most versatile macromolecules in living systems and serve crucial functions in essentially all biological processes
- They function as catalysts, they transport and store other molecules such as oxygen,
- They provide mechanical support and immune protection
- They generate movement, and they control growth and differentiation



Lesson 28

- Several key properties enable proteins to participate in such a wide range of functions

1. Proteins are linear polymers built of monomer units called amino acids

- The function of a protein is directly dependent on its three dimensional structure
- Remarkably, proteins spontaneously fold up into three-dimensional structures that are determined by the sequence of amino acids in the protein polymer

2. Proteins contain a wide range of functional groups

These functional groups include

- alcohols,
- thiols,
- thioethers,
- carboxylic acids,
- carboxamides,
- and a variety of basic groups
- When combined in various sequences, this array of functional groups accounts for the broad spectrum of protein function

3. Proteins can interact with one another and with other biological macromolecules to form complex assemblies

- The proteins within these assemblies can act synergistically to generate capabilities not afforded by the individual component proteins
- These assemblies include macromolecular machines that carry out;
- the accurate replication of DNA,
- the transmission of signals within cells, and many other essential processes

4. Some proteins are quite rigid, whereas others display limited flexibility

- Rigid units can function as structural elements in the cytoskeleton (the internal scaffolding within cells) or in connective tissue
- Parts of proteins with limited flexibility may act as
- hinges,
- springs, and levers
- That are crucial to protein function, to the assembly of proteins with one another and with other molecules into complex units
- And to the transmission of information within and between cells

Lesson 29

- All proteins, whether from the most ancient lines of bacteria or from the most complex forms of life, are constructed from the same set of; 20 amino acids, • Which are covalently linked in characteristic linear sequence.
- Each of these amino acids has a side chain with distinctive chemical properties • This group of 20 precursor molecules may be regarded as the alphabet in which the language of protein structure is written
- Proteins are found in a wide range of sizes,
- from relatively small peptides with just a few amino acid residues to huge polymers with molecular weights in the millions
- Cells can produce proteins with strikingly different properties and activities by joining the same 20 amino acids in many different combinations and sequence
- From these building blocks different organisms can make such widely diverse products as
  - enzymes,
  - hormones,
  - antibodies,
  - transporters,
  - muscle fibers,
  - the lens protein of the eye,
  - feathers,
  - spider webs,
  - rhinoceros horn,
  - milk proteins,
  - antibiotics,
  - mushroom poisons etc
- The light produced by fireflies is the result of a reaction involving the protein luciferin and ATP, catalyzed by the enzyme luciferase

RBC contain large amounts of the oxygen-transporting protein hemoglobin. (c) The protein keratin, formed by all vertebrates, is the chief structural component of hair, scales, horn, wool, nails, and feathers

Lesson 30

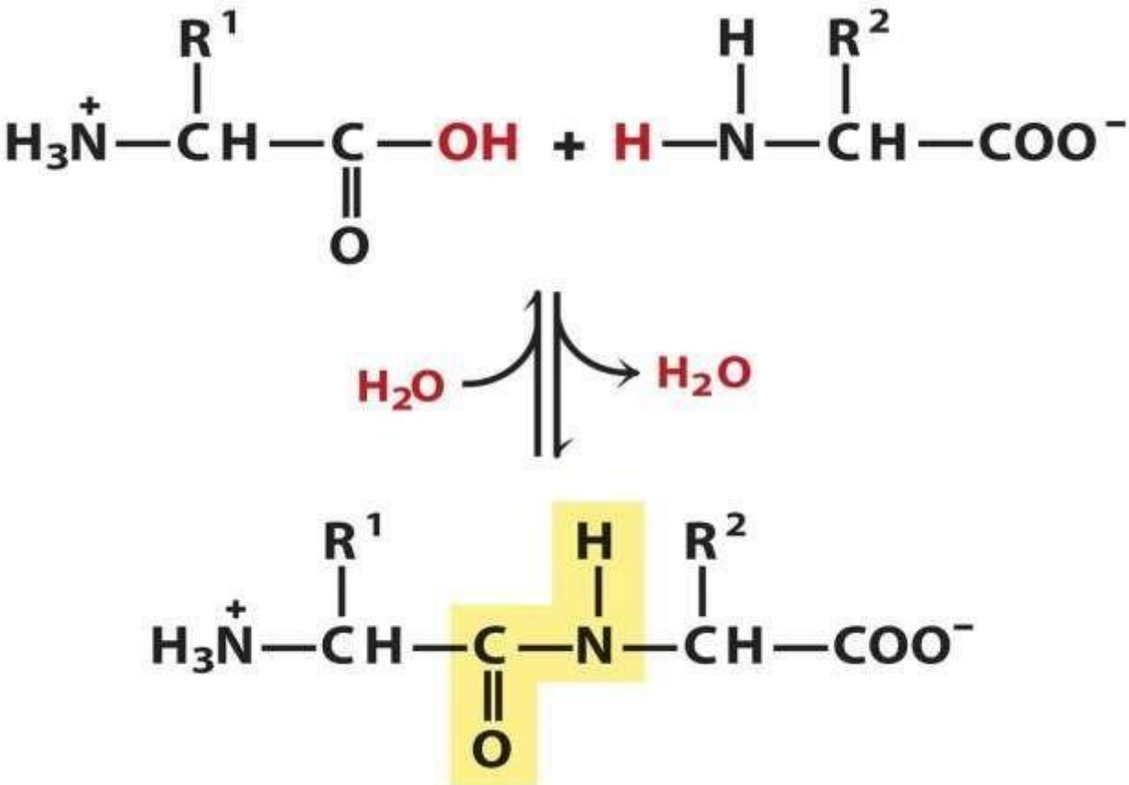
- Proteins are polymers of amino acids, with each amino acid residue joined to its neighbor by a specific type of covalent bond termed the **peptide bond**
- The term "residue" reflects the loss of the elements of water when one amino acid is joined to another
- Twenty different amino acids are commonly found in proteins
- All of the 20 common amino acids are **α amino acids**
- They have a carboxyl group and an amino group bonded to the same carbon atom (the α carbon)
- They differ from each other in their side chains, or R groups, which vary in

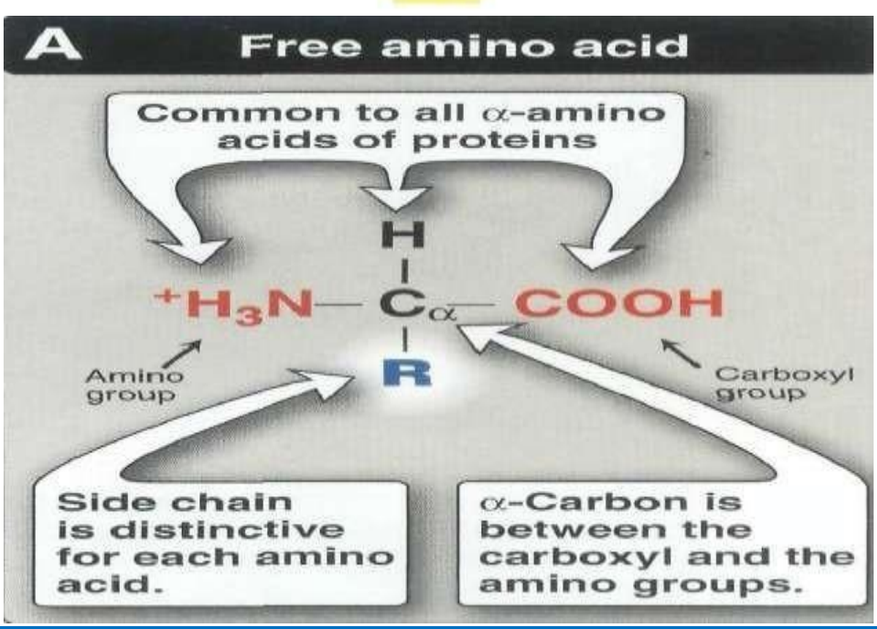
– structure,
– size,
– electric charge,
– and which influence the solubility of the amino acids in water

Standard amino acids

• The 20 amino acids that constitute the monomer units of proteins are **the standard amino acids** as the genetic code specifies only these 20 L -α-amino acids

• In addition to these 20 amino acids there are many less common ones i.e., **the non standard amino acids**





Lesson 31

• Composition of Proteins

• In addition to the 20 common amino acids, proteins may contain residues created by modification of common residues already incorporated in to a polypeptide

Composition of Proteins

- Some proteins contain additional amino acids that arise by modification of an amino acid already present in a peptide i.e., after the protein has been synthesized
- Examples include
  - conversion of peptidyl proline and lysine to
  - 4-hydroxyproline and
  - 5-hydroxylysine
  - the conversion of peptidyl glutamate to carboxyglutamate the methylation,
  - formylation,
  - acetylation,
  - prenylation, and
  - phosphorylation of certain aminoacyl residues

Among these uncommon amino acids
- **4-hydroxyproline**, a derivative of proline
- **5-hydroxylysine**, derived from lysine
- The former is found in plant cell wall proteins, and both are found in collagen

- **6-N-Methyllysine** is a constituent of myosin, a contractile protein

- Another important uncommon amino acid is;
- **γ -carboxyglutamate,** found in the blood clotting protein prothrombin and in certain other proteins that bind Ca^{2+} as part of their biological function

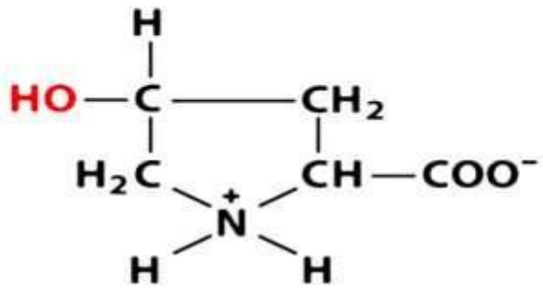
- Some 300 additional amino acids have been found in cells but not as constituents of proteins
- Examples include
  - Ornithine and citrulline (intermediates in urea cycle)

  - Taurine (found in bile acids)
  - δ-Aminolevulinic acid (intermediate in haem synthesis)

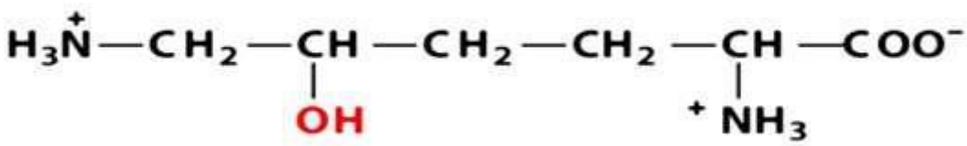
D- AMINO ACIDS

- D-amino acids are also non standard amino acids that occur **naturally** and include – free D- serine, and D-aspartate in brain tissue

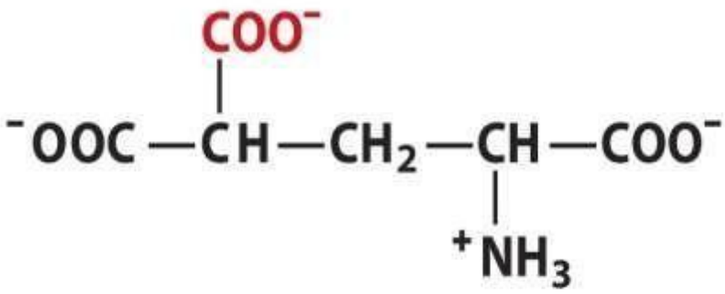
  - D-alanine & D-glutamate in the cell walls of gram positive bacteria
- D-amino acids are also found in some **antibiotics**



4-Hydroxyproline



5-Hydroxylysine



γ-Carboxyglutamate

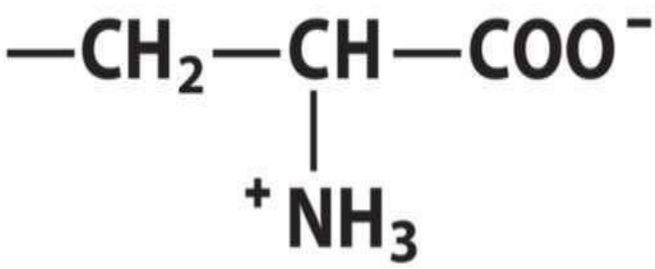
Lesson 32

Selenocysteine, the 21st L-α-Amino Acid?

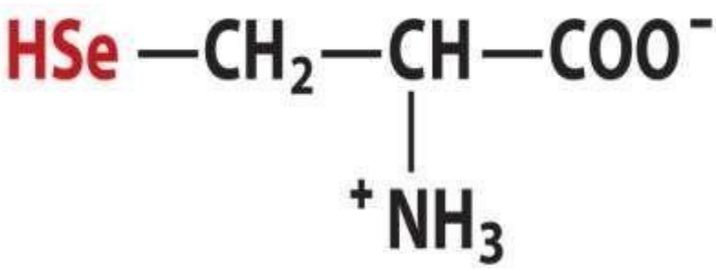
- Selenocysteine is an L-α-amino acid found in a handful of proteins, including certain **peroxidases and reductases** where it participates in the catalysis of electron transfer reactions
- Its synthesis is not a post translational modification, but a modification to a **serine** that occurs while the serine is bound to a unique RNA
- The hydroxyl group of serine is replaced by a selenium atom
- The selenocysteine is then inserted into a protein as it is being synthesized

- Since selenocysteine is inserted into polypeptides during translation, it is commonly referred to as the "21st amino acid"
- However, unlike the other 20 genetically encoded amino acids, selenocysteine is not specified by a simple three-letter codon

- **22nd Amino Acid**
- Pyrrolysine (abbreviated as Pyl or O) is a naturally occurring, genetically coded amino acid used by some methanogenic archaea.



serine



Selenocysteine

Lesson 33

Chiral centers and isomerism

• For all the common amino acids except glycine (the R group is another hydrogen atom), the α carbon is bonded to four different groups:

– a carboxyl group,
– an amino group,
– an R group,
– a hydrogen atom

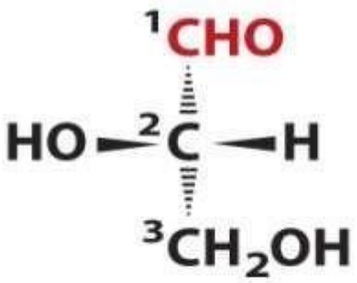
• The α-carbon atom is thus a chiral center and thus amino acids have two possible stereoisomers • All molecules with a chiral center are also optically active-that is, they rotate plane polarized light

• Special nomenclature has been developed to specify the absolute configuration of the four substituents of asymmetric carbon atom

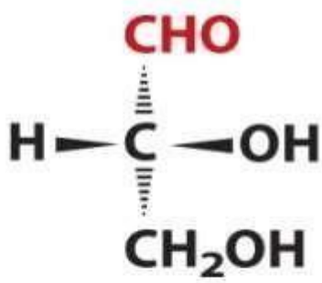
• The absolute configurations of simple sugars and amino acids are specified by the D, L system, based on the absolute configuration of the three-carbon sugar glyceraldehyde

• Thus the amino group of L-alanine occupies the same position about the chiral carbon as does the hydroxyl group of L-glyceraldehyde

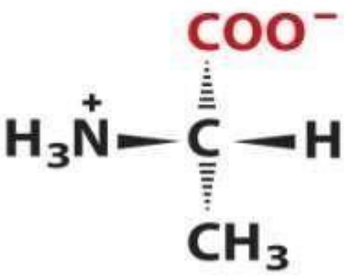
• Since they are nonsuperposable mirror images of each other, the two forms represent a class of stereoisomers called **enantiomers**



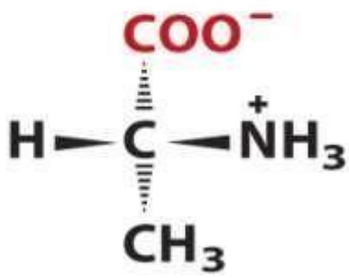
L-Glyceraldehyde



D-Glyceraldehyde



L-Alanine



D-Alanine

Lesson 34

Two conventions are used to identify the carbons in an amino acid

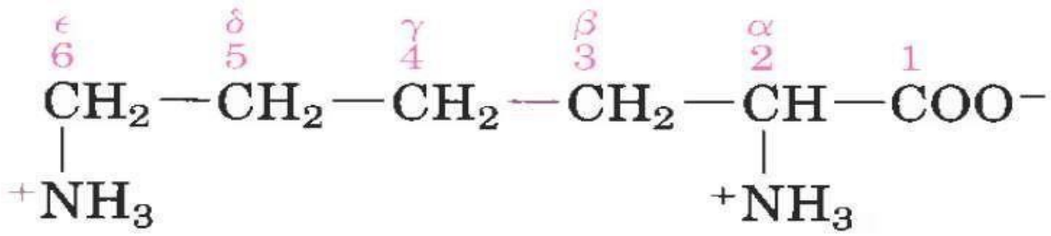
The additional carbons in an R group are commonly designated **β, γ, δ** and so forth, proceeding out from the **α carbon**

- For most other organic molecules, carbon atoms are simply numbered from one end, giving highest priority (C-1) to the carbon with the substituent containing the atom of **highest atomic number**
- Within this latter convention, the carboxyl carbon of an amino acid would be **C-1** and the **α** carbon would be **C-2**
- In some cases, such as amino acids with heterocyclic R groups (such as histidine), the

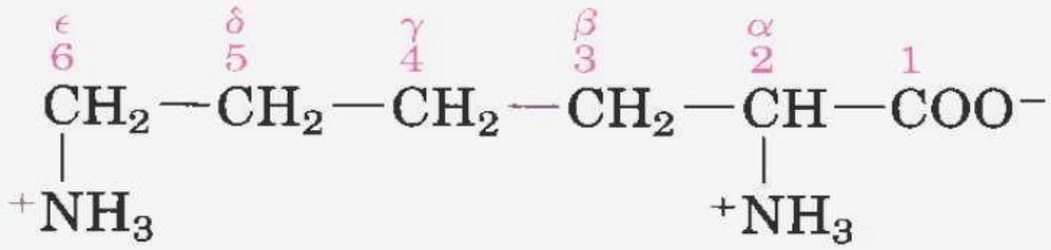
Greek lettering system is ambiguous and the numbering convention is therefore used

- For branched amino acid side chains, equivalent carbons are given numbers after the Greek letters

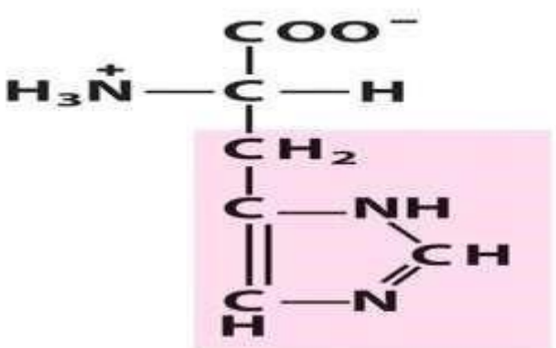
- Leucine thus has δ1 and δ2 carbons



Lysine



Lysine



Histidine

pros (in histidine nomenclature).The nitrogen atoms of the imidazole ring of histidine are denoted by pros (‗near', abbreviated π) and tele (‗far', abbreviated τ) to show their position relative to the side chain. This recommendation arose from the fact that two different systems of numbering the atoms in the imidazole ring of histidine had both been used for a considerable time (biochemists generally numbering as 1 the nitrogen atom adjacent to the side chain, and organic chemists designating it as 3)

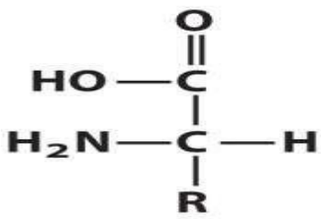
Lesson 35

Acid and base properties of amino acids

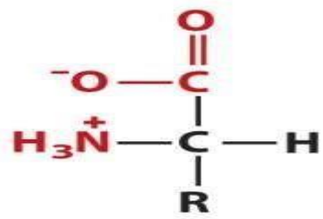
- The form of an amino acid that has both a positive and a negative charge is called a **zwitterion**
- Amino acids in solution at neutral pH exist predominantly as dipolar ions **(zwitterions)**

- In this form, the amino group is protonated ($–NH_3^+$)
- and the carboxyl group is deprotonated ($–COO^-$)
- A zwitterion can act either as an acid (proton donor) or as a base (proton acceptor)
- Substances having this dual nature are **amphoteric** and are often called **ampholytes**

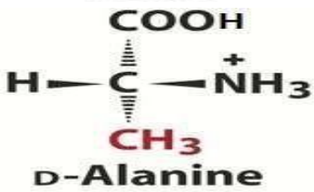
- The ionization state of an amino acid varies with pH
- At physiological pH, the carboxyl group is dissociated , forming the negatively charged carboxylate ion ($-COO^-$) and the amino group is protonated (NH_3^+)
- A simple monoamino monocarboxylic - amino acid, such as alanine, is a diprotic acid when fully protonated—it has two groups, the-COOH group and the - NH_3^+ group, that can yield protons



Nonionic form



Zwitterionic form



D-Alanine

Lesson 36

- Each acid has a characteristic tendency to lose its proton in an aqueous solution
- The stronger the acid, the greater its tendency to lose its proton
- The tendency of any acid (HA) to lose a proton and form its conjugate base (A^-) is defined by the equilibrium constant (K_{eq}) for the reversible reaction
- HA $A^- + H^+$
- Equilibrium constants for ionization reactions are usually called ionization constants or acid dissociation constants, often designated pK_a
- The stronger the tendency to dissociate a proton, **the stronger is the acid and the lower its pK_a**
- The p*K*a expresses, on a logarithmic scale, the relative strength of a weak acid or base:
- $$pH = pK_a + \log \frac{[A^-]}{[HA]}$$
-
- p*K*a = $\log\frac{1}{Ka} = -LogKa$
- The stronger the acid, the lower its p*K*a;
-
- At the midpoint of the titration, the concentrations of the proton donor and proton acceptor are equal, and the pH is numerically equal to the p*K*a.
- The p*K*a can be determined experimentally;
- it is the pH at the midpoint of the titration curve for the acid or base
-
- At that point, [HA] equals [A], and
- pH = p*K*a + log 1
- = p*K*a + 0 = p*K*a
- At that point, [HA] equals [A], and
- pH = p*K*a + log 1
- = p*K*a + 0 = p*K*a

Lesson 37

- pKa is the measure of the tendency of a group to give up (donate) a proton with the tendency decreasing ten-fold as the pKa increases by one unit
- Each titratable group has a pK_a that is numerically equal to the pH at which exactly one half of the protons have been removed from that group
- The pK_a for the most acidic group (COOH) is pK_1, whereas the pK_a for the next most acidic group (NH_3^+) is pK_2
- **In acid solution** (e.g., pH 1), the amino group is protonated ($–NH_3^+$) and the carboxyl group is not dissociated (–COOH)
- As the pH is raised, the carboxylic acid is the first group to give up a proton, as its p*Ka is near 2*
- The dipolar form persists until the pH approaches 9, when the protonated amino group loses a proton

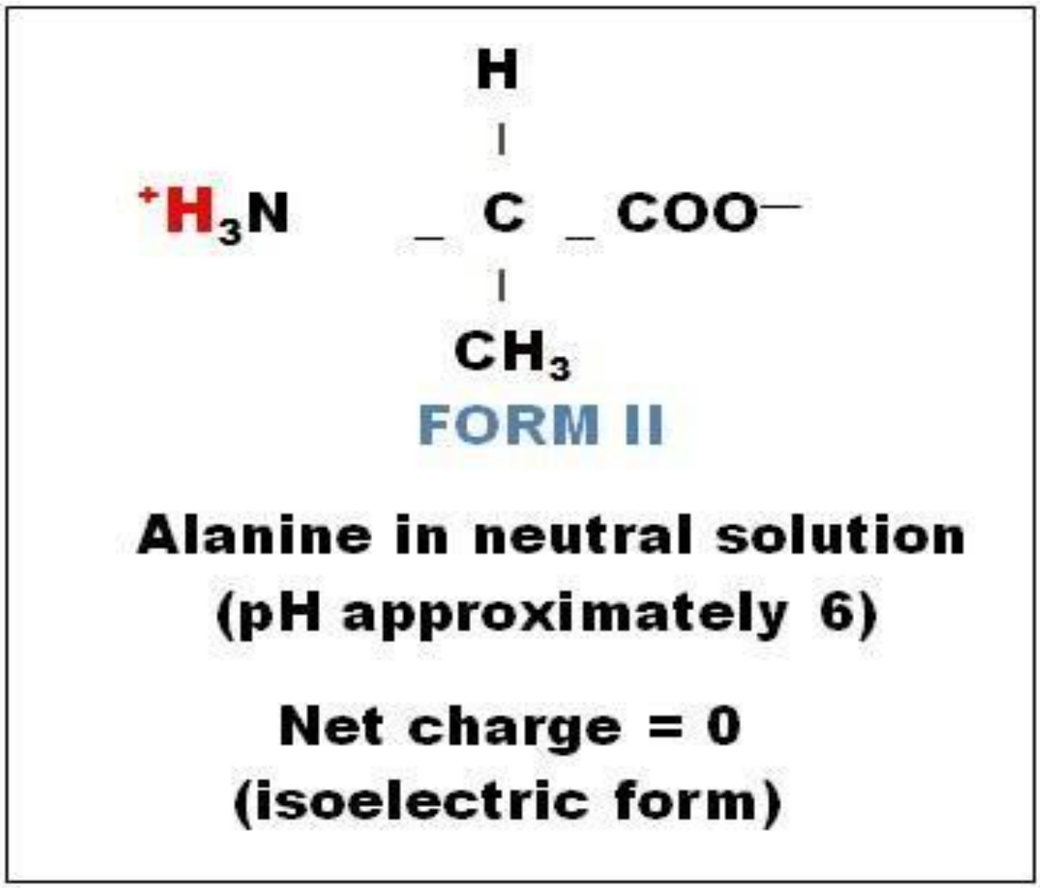
Lesson 38

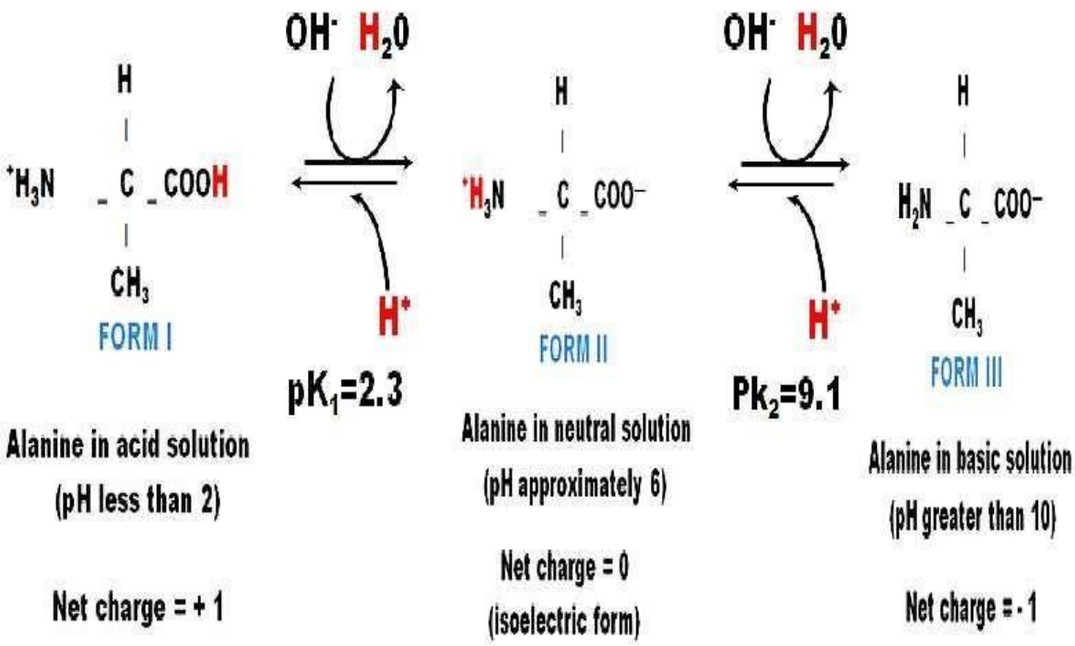
Titration of an amino acid Dissociation of the carboxyl group:

- The titration curve of an amino acid can be analyzed
- Consider alanine, for example, which contains both an α-carboxyl and an α-amino group
- At a low (acidic) pH, both of these groups are protonated
- As the pH of the solution is raised, the -COOH group of form I can dissociate by donating a proton to the medium
- The release of a proton results in the formation of the carboxylate group $–COO^-$
- This structure is shown as form II, which is the **dipolar form of the molecule**
- This form, also called a **zwitterion,** is the **isoelectric form** of alanine
- That is, it has an overall charge of zero
- The dissociation constant of the carboxyl group of an amino acid is called K_1 rather than K_2 because the molecule contains a second titratable group

Dissociation of the amino group:

- The second titratable group of alanine is the amino group
- This is a much weaker acid than the -COOH group and, therefore, has a much smaller dissociation constant, K_2
- Release of a proton from the protonated amino group of form II results in the fully deprotonated form of alanine, form III





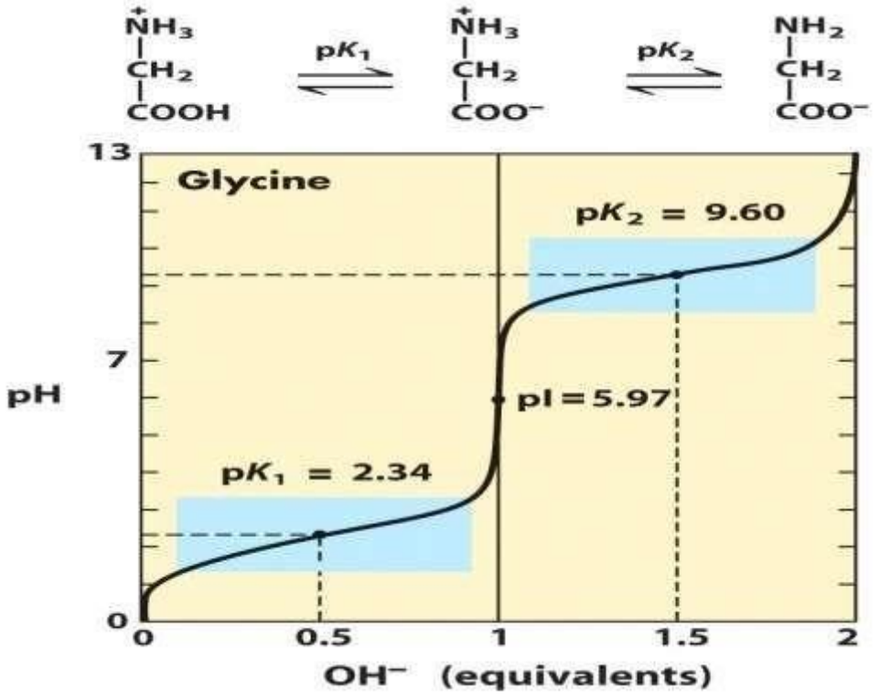
Lesson 39

- A simple amino acid e.g. **glycine** is a diprotic acid when fully protonated
- This means that it has two groups, the COOH and the NH_3^+ group that can yield protons
- Figure shows the titration curve of the diprotic form of glycine
- The two ionizable groups of glycine, the carboxyl group and the amino group, are titrated with a strong base
- The plot has two distinct stages, corresponding to deprotonation of two different groups on glycine
- At very low pH, the predominant ionic species of glycine is **the fully protonated form**,

+H3N-CH2-COOH

- **At the midpoint** of any titration, a point is reached where the **pH is equal to the pK_a** of the protonated group being titrated

- At the midpoint in the first stage of the titration, in which the --COOH group of glycine loses its proton, equimolar concentrations of the protonated ($^{+}H_3N$-CH_2-COOH) and the deprotonated ($^{+}H_3N$-CH_2-COO^{-}) species are present
- As the titration proceeds, another important point is reached at pH 5.97
- This is a point, at which removal of the first proton is essentially complete and removal of the second has just begun
- At this pH glycine is present largely as the dipolar ion (zwitterion) $^{+}H_3N$-CH_2-COO^{-}
- The second stage of the titration corresponds to the removal of a proton from the $–NH_3^{+}$ group of glycine
- The pH at the midpoint of this stage is 9.60, equal to the pK_a for the $–NH_3$ group
- The titration is essentially complete at a pH of about 12, at which point the predominant form of glycine is
- H_2N-CH_2-COO^{-}



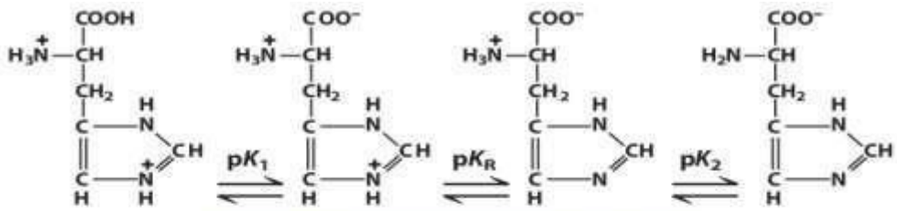
Lesson 40

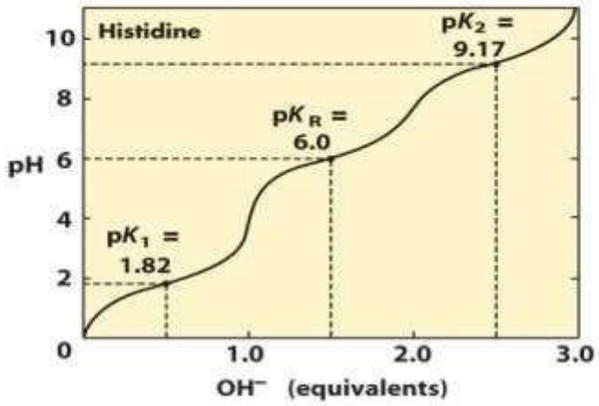
- From the titration curve of glycine we can derive several important pieces of information
- First, it gives a quantitative measure of the pK_a, of each of the two ionizing groups:
- 2.34 for the -COOH group
- 9.60 for the $–NH_3^{+}$ group
- The second piece of information is that this amino acid has **two regions of buffering power**
- One of these is the relatively flat portion of the curve, extending for approximately 1 pH unit on either side of the first pKa, of 2.6, indicating that glycine is a good buffer near this pH
- The other buffering zone is centered around pH 9.60
- Note that glycine is not a good buffer at the pH of intracellular fluid or blood, about 7.4
- Another important piece of information derived from the titration curve of an amino acid is the relationship between its net charge and the pH of the solution
- At pH 5.97, glycine is present predominantly as its dipolar form, fully ionized but with no net electric charge

- The characteristic pH at which the net electric charge is zero is called the **isoeleetric point or isoelectric pH, designated pI**
- For glycine, which has no ionizable group in its side chain;
- the pI is simply the arithmetic mean of the two pKa values
- pI = ½ (pK1+pK2)
- = ½ (2.34+9.60) = 5.97
- The farther the pH of a glycine solution is from its pI, the greater the net electric charge of the population of glycine molecules
- At any pH below its pI, glycine has a **net positive charge** and will move toward the negative electrode (cathode)
- At any pH above its pI, glycine will have a net negative charge and thus will move towards the positive electrode (anode)

Lesson 41

- All amino acids with a
- single α-amino group,
- a single α-carboxyl group, and
- an R group that does not ionize
- have titration curves resembling that of glycine
- These amino acids have very similar, although not identical, pKa values:
- pKa of the –COOH group in the range of 1.8 to 2.4
- pKa of the -NH3+ group in the range of 8.8 to 1 1
- The differences in these pKa values reflect the effects of the R groups
- Alanine has only two dissociable hydrogens (one from α carboxyl & one from α amino group)
- Its pI can be calculated as follows:
- pI = pK1 + pK2 ÷ 2
- pK1 = 2.3 (for carboxyl group)
- pK2 = 9.1 (for amino group)
- pI = 2.3 + 9.1 ÷ 2 = 5.7
- Amino acids with an ionizable R group have more complex titration curves, with three stages corresponding to the three possible ionization steps and thus they have **three pKa values**
- The additional stage for the titration of the ionizable R group merges to some extent with the other two
- In addition, each of the acidic and basic amino acids contains an ionizable group in its side chain
- Thus, both free amino acids and some amino acids combined in peptide linkages can act as **buffers**
- A buffer is a solution that resists change in pH following the addition of an acid or base
- The isoelectric points reflect the nature of the ionizing R groups present
- For example, glutamate has a pI of 3.22, considerably lower than that of glycine
- This is due to the presence of two carboxyl groups, with an average of their pKa values
  of
  3.22
- Histidine has got 3 dissociable hydrogens – one from carboxyl, pK1=1.8,
  - one from imidazole group, pK2=6.0
  - one from amino group, pK3=9.2
- The pI of Histidine, with two groups that are positively charged when protonated, is 7.59 (the average of the pKa values of the amino and imidazole groups)
- Only histidine has an R group (pKa = 6.0) providing significant buffering power near the neutral pH usually found in the intracellular and extracellular fluids of most animals





42 Acidic and Basic properties of amino acids 6 (Conti….)

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- a single α-carboxyl group, and
- an R group that does not ionize
- have titration curves resembling that of glycine
- **These amino acids have very similar, although not identical, pKa values:**
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- **The differences in these pKa values reflect the effects of the R groups**
- **Alanine has only two dissociable hydrogens (one from α carboxyl & one from α amino group)**
- **Its pI can be calculated as follows:**
- **pI = pK1 + pK2 ÷ 2**
- **pK1 = 2.3 (for carboxyl group)**
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- **In addition, each of the acidic and basic amino acids contains an ionizable group in its side chain**
- **Thus, both free amino acids and some amino acids combined in peptide linkages can act as buffers**
- **A buffer is a solution that resists change in pH following the addition of an acid or base**
- **The isoelectric points reflect the nature of the ionizing R groups present**

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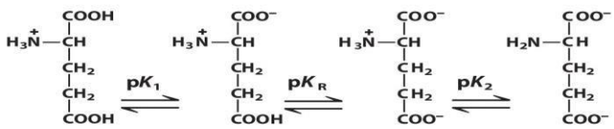
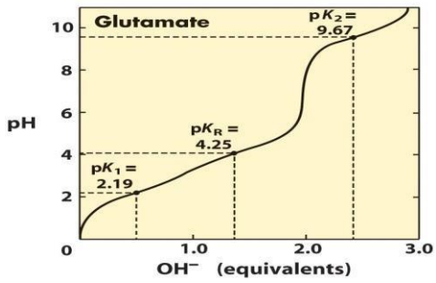
- This is due to the presence of two carboxyl groups, with an average of their pKa values of 3.22





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  - one from amino group, pK3=9.2
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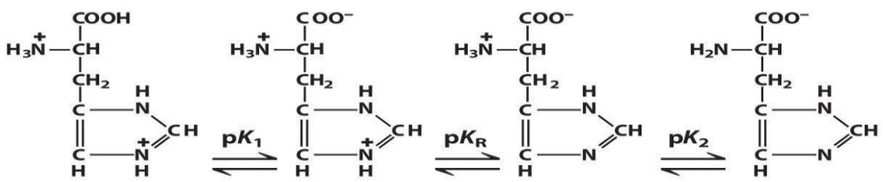
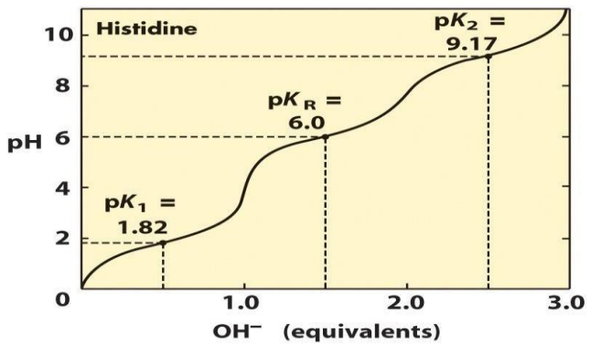




43 Abbreviations and symbols of amino acids

- Abbreviations and symbols for the commonly occurring amino acids
- Each amino acid name has an associated three-letter abbreviation and a one-letter symbol
- The one-letter codes are determined by the following rules:

1. Unique first letter:

- If only one amino acid begins with a particular letter, then that letter is used as its symbol, for example,
- I = isoleucine

1 Unique first letter:

Cysteine	=	Cys	=	C
Histidine	=	His	=	H
Isoleucine	=	Ile	=	I
Methionine	=	Met	=	M
Serine	=	Ser	=	S
Valine	=	Val	=	V

- **2. Most commonly occurring amino acids have priority:**
- **If more than one amino acid begins with a particular letter, the most common of these amino acids receives this letter as its symbol**
- **For example, glycine is more common than glutamate,**
- **So G = glycine**

2 Most commonly occurring amino acids have priority:

Alanine	=	Ala	=	A
Glycine	=	Gly	=	G
Leucine	=	leu	=	L
Proline	=	Pro	=	P
Threonine	=	Thr	=	T

- **Similar sounding names:**
- **Some one-letter symbols sound like the amino acid they represent**
- **For example,**
- **F = phenylalanine, or W= tryptophan (“twyptophan”)**

3 Similar sounding names:

Arginine	=	Arg	=	R (“aRginine”)
Asparagine	=	Asn	=	N (contains N)
Aspartate	=	Asp	=	D (“asparDic”)
Glutamate	=	Glu	=	E (“glutEmate”)
Glutamine	=	Gin	=	Q (“Q-tamine”)
Phenylalanine	=	Phe	=	F (“Fenylalanine”)
Tyrosine	=	Tyr	=	Y (“tYrosine”)
Tryptophan	=	Trp	=	W (dpuble ring in the molecule)

Letter close to initial letter:

- **For the remaining amino acids, a one-letter symbol is assigned that is as close in the alphabet as possible to the initial of the amino acid**

Amino acid	Abbreviation/ symbol	
Nonpolar, aliphatic R groups		
Glycine	Gly	G
Alanine	Ala	A
Proline	Pro	P
Valine	Val	V
Leucine	Leu	L
Isoleucine	Ile	I
Methionine	Met	M
Aromatic R groups		
Phenylalanine	Phe	F
Tyrosine	Tyr	Y
Tryptophan	Trp	W

Amino acid	Abbreviation/ symbol	
Polar, uncharged R groups		
Serine	Ser	S
Threonine	Thr	T
Cysteine	Cys	C
Asparagine	Asn	N
Glutamine	Gln	Q
Positively charged R groups		
Lysine	Lys	K
Histidine	His	H
Arginine	Arg	R
Negatively charged R groups		
Aspartate	Asp	D
Glutamate	Glu	E

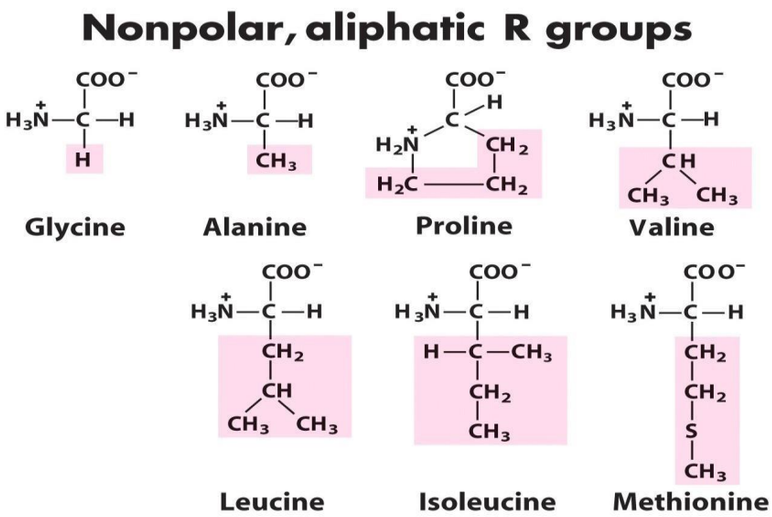
44 Classification of amino acids A. Aliphatic R groups

- **The topic can be simplified by grouping the amino acids into five main classes based on the properties of their R groups, in particular, their polarity, or tendency to interact with water at biological pH (near pH 7.0).**
- **The polarity of the R groups varies widely, from nonpolar and hydrophobic (water-insoluble) to highly polar and hydrophilic (water-soluble)**

A. Nonpolar, Aliphatic R Groups

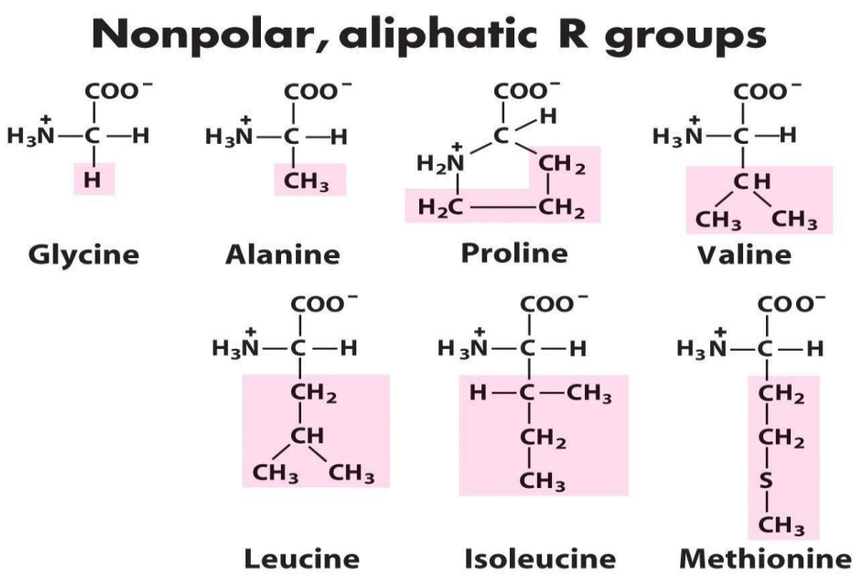
- **These are hydrophobic**
- **The amino acids in this group have non polar side chains that do not bind or give off protons**
- **They also do not participate in hydrogen bonds**
- **They are ‘oily’ or lipid like and promote hydrophobic interactions**
- **These side chains cluster together in the interior of the protein**
- **This class includes**
- **Glycine**
- **Alanine**

- Proline
- Valine
- Leucine
- Isoleucine
- Methionine

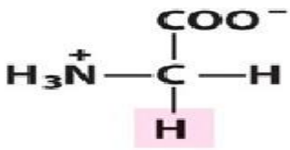


- Electrons are shared equally between the carbon and hydrogen atoms in these side chains, so that they can not hydrogen bond with water
- Within proteins, these amino acid side chains will cluster together to form hydrophobic cores

45 Classification of amino acids A. Aliphatic R groups (Conti...)

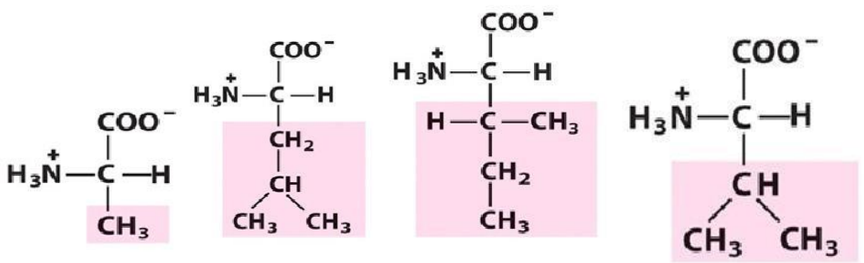


- Glycine has the simplest structure. Although it is formally nonpolar, its very small side chain makes no real
  contribution to hydrophobic interactions
- It is the simplest amino acid, and it really does not fit well into any classification because its side chain is only a hydrogen atom
- Because of this, it causes the least amount of steric hindrance in a protein
- Therefore glycine is often found in bends or in the tightly packed chains of fibrous proteins.



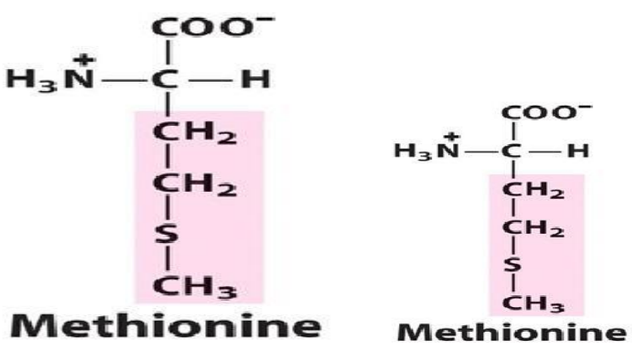
Glycine

- **Alanine and the branched chain amino acids (valine, leucine, and isoleucine) have bulky, nonpolar, *aliphatic(open chain* hydrocarbon) side chains**
- **The side chains of alanine, valine, leucine, and isoleucine tend to cluster together within proteins, stabilizing protein structure by means of hydrophobic interactions.**



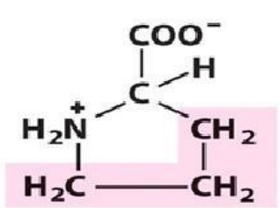
Alanine Leucine Isoleucine Valine

- **Methionine, one of the two sulfur-containing amino acids, has a nonpolar thioether group in its side chain**



Methionine Methionine

- **The role of proline in amino acid structure differ from those of the nonpolar amino acids**
- **The amino acid proline contains a ring involving its α–carbon and its α-amino group, which are part of the peptide backbone**
- **It is an imino acid**
- **This rigid ring causes a kink in the peptide backbone that prevents it from forming its usual configuration**

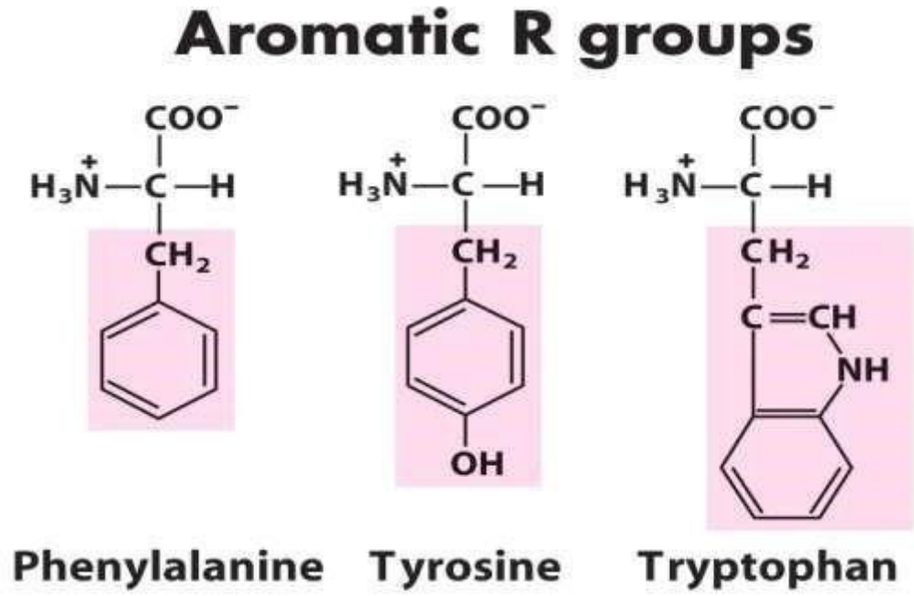


Proline

Lesson 46

Classification of amino acids
B. Aromatic Amino Acids

- The aromatic amino acids have been grouped together because they all contain ring structures with similar properties, **but their polarity differs**
- The aromatic ring is a six-membered carbon–hydrogen ring with three conjugated double bonds (the benzene ring or phenyl group)
- Benzene is an organic chemical compound with the chemical formula C6H6. Its molecule is composed of 6 carbon atoms joined in a ring, with 1 hydrogen atom attached to each carbon atom
- The substituents on this ring determine whether the amino acid side chain engages in polar or hydrophobic interactions
- This group includes
  - Phenylalanine
  - Tyrosine
  - Tryptophan
  - with their aromatic side chains, are relatively nonpolar (hydrophobic). – All can participate in hydrophobic interactions.



- In the amino acid **phenylalanine**, the ring contains no substituents, and the electrons are shared equally between the carbons in the ring, resulting in *a very* **nonpolar hydrophobic** structure in which the rings can stack on each other
- In **tyrosine**, a hydroxyl group on the phenyl ring engages in hydrogen bonds, and the side chain is therefore more polar and **more hydrophilic**
- The more complex ring structure in **tryptophan** is an indole ring with a nitrogen that can engage in hydrogen bonds
- Tryptophan is therefore also more polar than phenylalanine
- Tyrosine and tryptophan are significantly more polar than phenylalanine, because of the tyrosine hydroxyl group and the nitrogen of the **tryptophan** indole ring

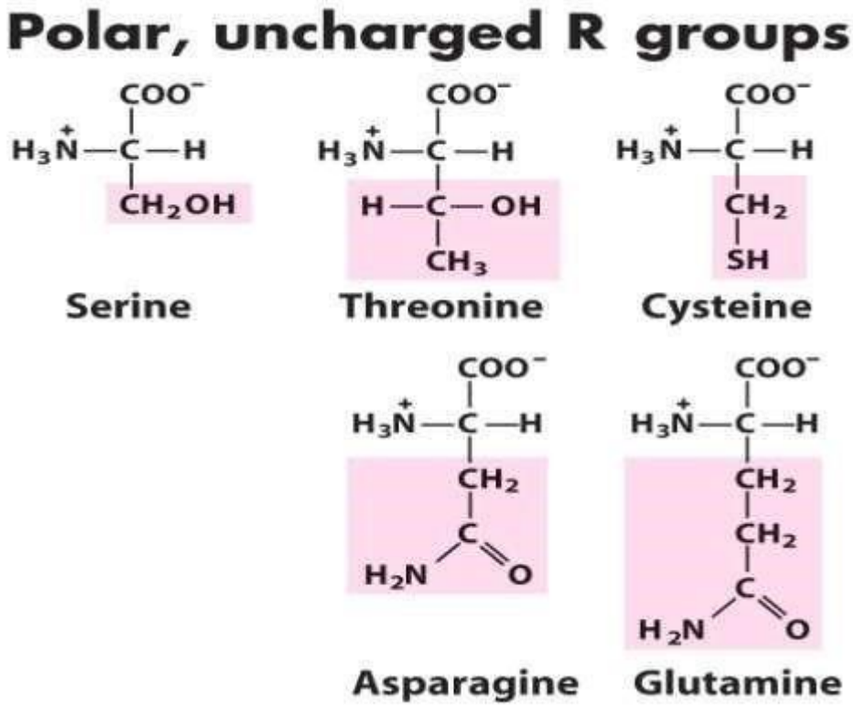
Lesson 47

C. Aliphatic, Polar, Uncharged R Groups

- The R groups of these amino acids are more soluble in water, or more hydrophilic, than those of the nonpolar amino acids.
- Because they contain functional groups that form hydrogen bonds with water.

- This class of amino acids includes
- **serine, threonine,**
- **cysteine,** • **asparagine,** and
- **glutamine.**
- These Amino acids have zero net charge at neutral pH

- **Serine & threonine** of this class contain a polar hydroxyl group
- Similarly side chains of **asparagine & glutamine** contain a carbonyl group & amide group, both of which can participate in hydrogen bonding
  -
- These side chains are sites of attachment for other compounds
- The polar hydroxyl groups of **serine & threonine** serve as a site of attachment for phosphate groups
- The amide group of **asparagine** & the hydroxyl group of **serine & threonine** serve as a site of attachment for oligosaccharide chains in glycoproteins
- Amino acids with side chains that contain an amide group (asparagine and glutamine) or a hydroxyl group (serine and threonine) can be classified as **aliphatic, polar, uncharged amino acids**
- Asparagine and glutamine are amides of the amino acids aspartate and glutamate
- As a consequence of their hydrophilicity, these amino acids are frequently found on the surface of water-soluble globular proteins
- Cysteine, which is sometimes included in this class of amino acids, has been separated into the class of sulfur-containing amino acids

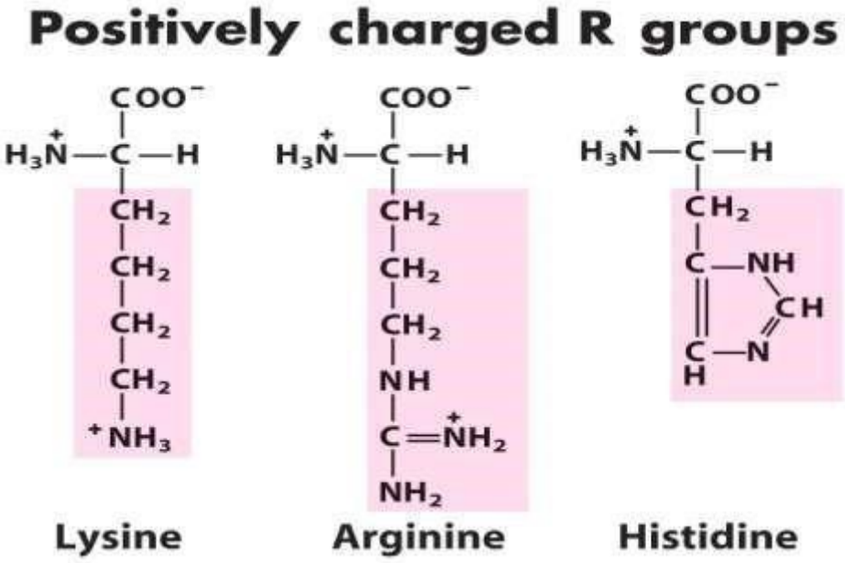


Lesson 48

D. Positively Charged (Basic) R Groups

- The side chains of these amino acids are proton acceptors
- The amino acids in which the R groups have significant **positive charge** at pH 7.0 are:
  - **Lysine**, which has a second primary amino group at the ε position on its aliphatic chain
  - **Arginine,** which has a positively charged guanidinium group
  - **Histidine,** which has an aromatic imidazole group
- The side chains of the two basic amino acids, arginine and lysine, have pK_a values above 10, so that the positively charged form always predominates at physiologic pH
- The side chain of histidine (pK_a ~ 6.0) dissociates near physiologic pH, so only a portion of the histidine side chains carry a positive charge
- **Histidine** is therefore weakly basic & the *free* amino acid is mainly uncharged at physiologic pH

- The amino acid side chains might have very different pK_as than those of the free amino acids if they are involved in hydrogen or ionic bonds with other amino acid side chains
- Therefore, when histidine is incorporated into a protein, its side chain can be either positively charged or neutral depending on the ionic environment provided by the polypeptide chains of the protein

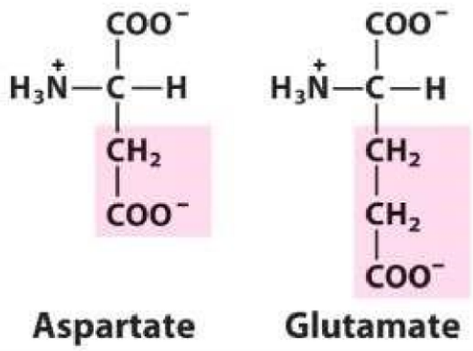


Lesson 49

E. Negatively Charged (Acidic) R Groups

- These amino acids are proton donors
- At neutral pH, the side chains of these amino acids are fully ionized, containing a negatively charged carboxylate group
- (--COO^-)
- Therefore these amino acids are negatively charged at phyisologic pH
- The two amino acids having R groups with a net negative charge at pH 7.0 are **aspartate and glutamate**, each of which has a second carboxyl group
- Humans have no dietary requirement for protein, per se, but, the protein in food does provide essential amino acids.
- Nine of the twenty amino acids needed for the synthesis of body proteins are essentialthat is, they cannot be synthesized in humans at an adequate rate
- **Essential amino acids** can not be made by the body. As a result, they must come from food. The nine **essential amino acids** are: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.
- Of these nine, some amino acids are essential at all times, whereas some are required only during periods of rapid tissue growth characteristic of childhood or recovery from illness

Negatively charged R groups



Aspartate Glutamate

Non essential	Glucogenic	Glucogenic And Ketogenic	Ketogenic
Essential	Alanine Arginine Asparagine Cysteine Glutamate Glutamine Glycine Proline Serine	Tyrosine	
	Histidine Methionine Valine	Threonine Isoleucine Phenylalanine Tryptophan	Leucine Lysine

Essential	Conditionally Non-Essential	Non-Essential
Histidine	Arginine	Alanine
Isoleucine	Asparagine	Asparatate
Leucine	Glutamine	Cysteine
Methionine	Glycine	Glutamate
Phenylalanine	Proline	
Threonine	Serine	
Tryptophan	Tyrosine	
Valine		
Lysine		

Lesson 50

- Amino acids can be classified as **glucogenic or ketogenic** based on what intermediates are produced during their catabolism

A. Glucogenic amino acids

- Amino acids whose catabolism yields **pyruvate are termed glucogenic or glycogenic**
- These intermediates are substrates for **gluconeogenesis** and, therefore, can give rise to the net formation of glucose or glycogen in the liver and glycogen in the muscle

B. Ketogenic amino acids

- Amino acids whose catabolism yields either **acetoacetate or its precursor, (acetyl CoA or acetoacetyl CoA)** are termed **ketogenic**
- Acetoacetate is one of the ketone bodies which also include 3-hydroxybutyrate and acetone
- **Leucine and lysine** are the only exclusively ketogenic amino acids found in proteins
- Their carbon skeletons are not substrates for gluconeogenesis and, therefore, cannot give rise to the net formation of glucose or glycogen in the liver, or glycogen in the muscles

	Glucogenic	Glucogenic And Ketogenic	Ketogenic
Nonessential	Alanine Arginine Asparagine Cysteine Glutamate Glutamine Glycine Proline Serine	Tyrosine	
Essentia	Histidine Methionine Valine	Threonine Isoleucine Phenyl-alanine Tryptophan	Leucine Lysine

TABLE 14–4 Glucogenic Amino Acids, Grouped by Site of Entry

Pyruvate
Alanine
Cysteine
Glycine
Serine
Threonine
Tryptophan*

α-Ketoglutarate
Arginine
Glutamate
Glutamine
Histidine
Proline

Succinyl-CoA
Isoleucine*
Methionine
Threonine
Valine

Fumarate
Phenylalanine*
Tyrosine*

Oxaloacetate
Asparagine
Aspartate

Note: All these amino acids are precursors of blood glucose or liver glycogen, because they can be converted to pyruvate or citric acid cycle intermediates. Of the 20 common amino acids, only leucine and lysine are unable to furnish carbon for net glucose synthesis. *These amino acids are also ketogenic (see Fig. 18–21).

Lesson 51

- Different classifications of proteins are based on their:
  1. Shape and Size
  2. Biological actions/ Functions
  3. Solubility and physical properties

4. Quality **Catalytic Proteins:**

These specialized proteins are called **enzymes** which catalyze the biochemical reactions

2. **Protective Proteins**

a) **Immunoglobins (Igs)**

- These freely circulating proteins protect the body from invading microbes such as bacteria or viruses by inactivating or killing them through various mechanisms. **b) Fibrinogen**
- This forms fibrin clot and stops bleeding from wounds

3. **Regulatory Proteins:**

- Hormones control genetic expression, cellular signalling *and* biochemical reactions catalyzed by enzymes. Enzymes are either activated or inactivated through modification of their structure.
- Examples of protein hormones are
  - growth hormone
  - Insulin
  - Glucagon – Somatostatin

4. **Structural Proteins:**
   - These proteins form various body structures e.g.
     - Collagen
     - Elastin
     - Keratin
5. **Transport Proteins:**
   - These proteins transport various substances from one part of the body to the other e.g.
     - Hemoglobin transport O_2 from lungs to tissues and CO_2 from tissues to lungs – Transferrin transports iron
6. **Contractile Proteins:**
   - These proteins are involved in muscle contraction and relaxation

− Myosin of thick filaments

− Actin of thin filaments of skeletal muscles

52 Functional classification of proteins

7. Respiratory Proteins:

• Heme containing proteins are involved in the function of respiration e.g.

Hemoglobin

Myoglobin

cytochromes

8. Digestive Proteins:

- These proteins are digestive enzymes which digest our food materials such as carbohydrates, proteins, lipids and include

Amylase

Pepsin

Lipases etc.

9. Toxin Proteins:

- These proteins are hydrolytic enzymes found in the venom of poisonous snakes, sting of bees and insects and hydrolyze the compounds forming the structure of the cell membrane

9. Toxin Proteins:

- These proteins are hydrolytic enzymes found in the venom of poisonous snakes, sting of bees and insects and hydrolyze the compounds forming the structure of the cell membrane

- The poisonous mushrooms also have such toxins

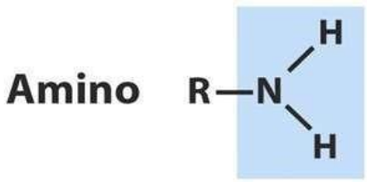
10. Storage Proteins:

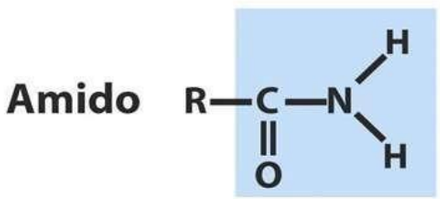
- These proteins store some specific elements or compounds with them
- This is because of the presence of the many binding sites in them for the particular element e.g.
- ferritin stores iron
- ceruloplasmin stores copper

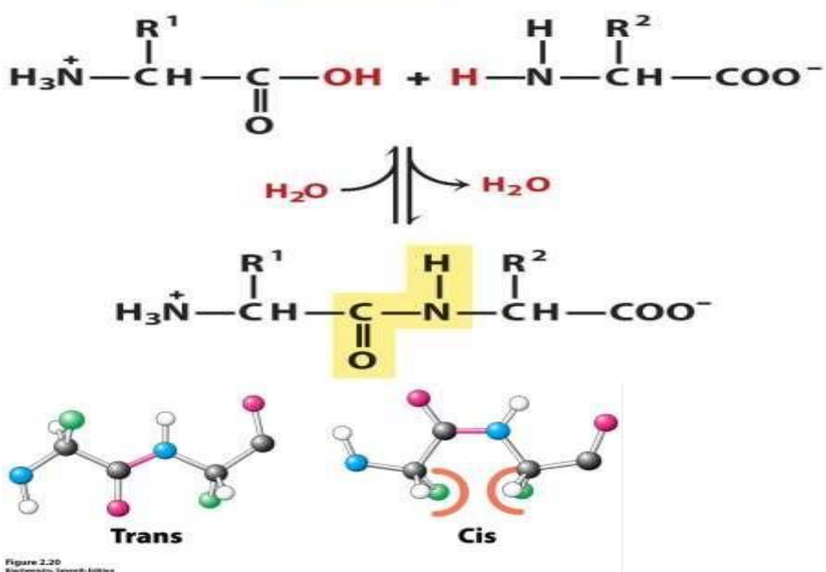
Lesson 53

PEPTIDE BOND

- We now turn to polymers of amino acids, the peptides and proteins
- Biologically occurring polypeptides range in size from small to very large, consisting of two or three to thousands of linked amino acid residues
- Two amino acid molecules can be covalently joined through **an amide linkage,** termed **a peptide bond**
- This linkage is formed by removal of the elements of water from the α-carboxyl group of one amino acid and the α-amino group of another
- Two configurations are possible for a planar peptide bond
- In the trans configuration, the two -carbon atoms are on opposite sides of the peptide bond
- In the cis con-, these groups are on the same side of the peptide bond.
- *Almost all peptide bonds in proteins are trans*
- This preference for trans over cis can be explained by the fact that steric clashes between groups attached to the -carbon atoms hinder formation of the cis form but do not occur in the trans configuration







Lesson 54

The Structure of Proteins

Four levels of protein structure are commonly defined

The Primary Structure

A description of all covalent bonds (mainly **peptide bonds** and **disulfide bonds**) linking amino acid residues in a polypeptide chain is its primary structure

The most important element of primary structure is the **sequence** of amino acid residues **Secondary structure**

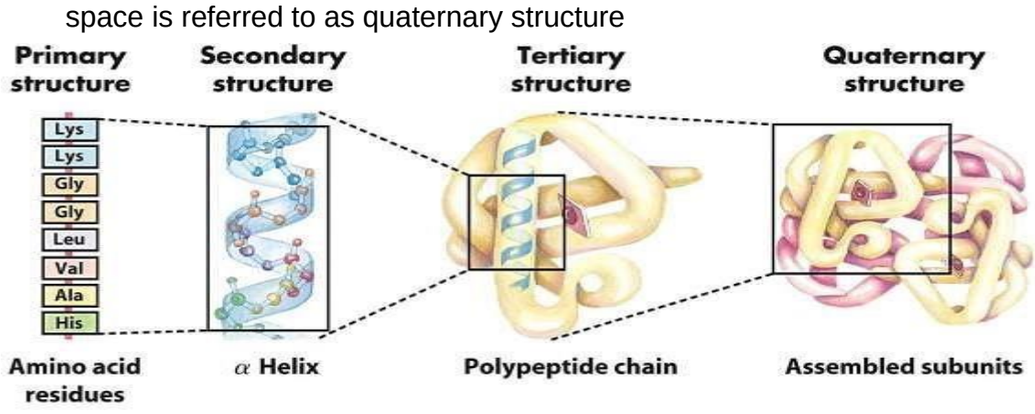
Secondary structure refers to **particularly stable arrangements** of amino acid residues giving rise to recurring structural patterns

Tertiary structure

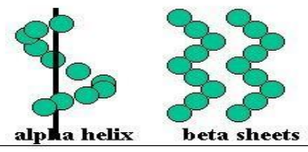
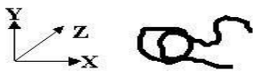
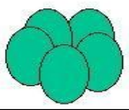
Tertiary structure describes all aspects of the **three-dimensiona**l folding of a polypeptide

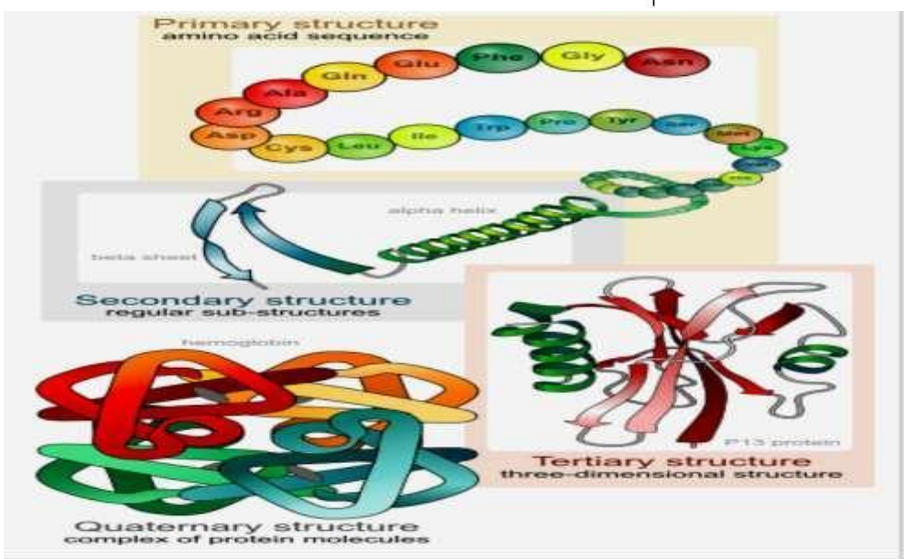
Quaternary structure

When a protein has **two or more polypeptide** subunits, their arrangement in space is referred to as quaternary structure



Protein Structure(Summary)

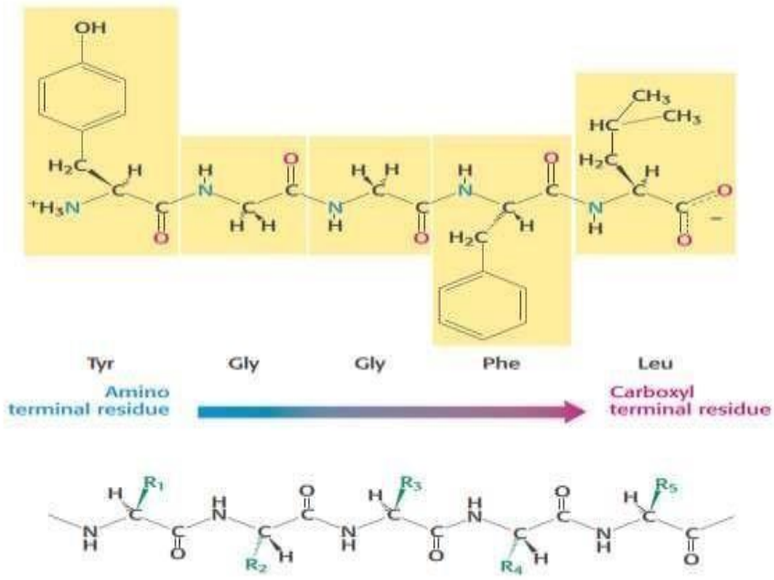
•Primary	The amino acid sequence	Glu-Arg-Phe-Gly
•Secondary	Characteristic structures that occur in many proteins (E.g. alpha helix , beta sheets)	
•Tertiary	Three dimensional structure of proteins	
•Quaternary	Three dimensional structure of proteins composed of multiple subunits	



Lesson 55 Primary structure

- The primary structure of a protein refers to the **linear sequence** of amino acids in the polypeptide chain.
- The primary structure is held together by covalent bonds such as peptide bonds, which are made during the process of protein biosynthesis or translation.
- The two ends of the polypeptide chain are referred to as the **carboxyl terminus** (Cterminus) and the **amino terminus** (N-terminus) based on the nature of the free group on each extremity.
- The primary structure of a protein determines **how it folds up** into its unique threedimensional structure, and this in turn determines the function of the protein
- The function of a protein therefore depends on its amino acid sequence • Proteins have unique amino acid sequences, that are specified by genes**.**
- Each of the 20 amino acids incorporated in the proteins is encoded by one or more *specific* sequences of three nucleotides in DNA or RNA.
- Understanding of the primary structure of proteins is important because many genetic diseases result in proteins with abnormal amino acid sequences which cause loss or impairment of normal function.
- The defect can range from a single change in the amino acid sequence (as in **sickle cell anemia**) to deletion of a larger portion of the polypeptide chain (as in most cases of **Duchenne** muscular dystrophy)
- In Sickle cell anemia a **Glutamate** residue is replaced by **Valine** at position 6 of beta chain of Hemoglobin, resulting in decreased functioning and increased fragility of Hb.

- Duchenne Muscular dystrophy results from a large segment of gene deletion in X chromosome, resulting in small truncated **dystrophin** protein.
- Thus we know that if the primary structure is altered, the function of the protein may also be changed



Lesson 56

Secondary structure**,** the folding of short (3- to 30-residue), contiguous segments of polypeptide into **geometrically ordered units**

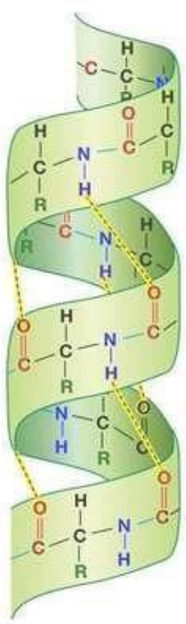
The term secondary structure refers to any chosen segment of a polypeptide chain and describes the local spatial arrangement of its main-chain atoms, without regard to its relationship to other segments

There are a few types of secondary structure that are particularly stable and occur widely in proteins

The most prominent are the **α helix and β conformations The α Helix is a common protein secondary structure**

The simplest arrangement the polypeptide chain can assume, given its rigid peptide bonds (but free rotation around its other, single bonds), is a helical structure, called the α helix

Within the α helix, every peptide bond (except those close to each end of the helix) participates in such hydrogen bonding



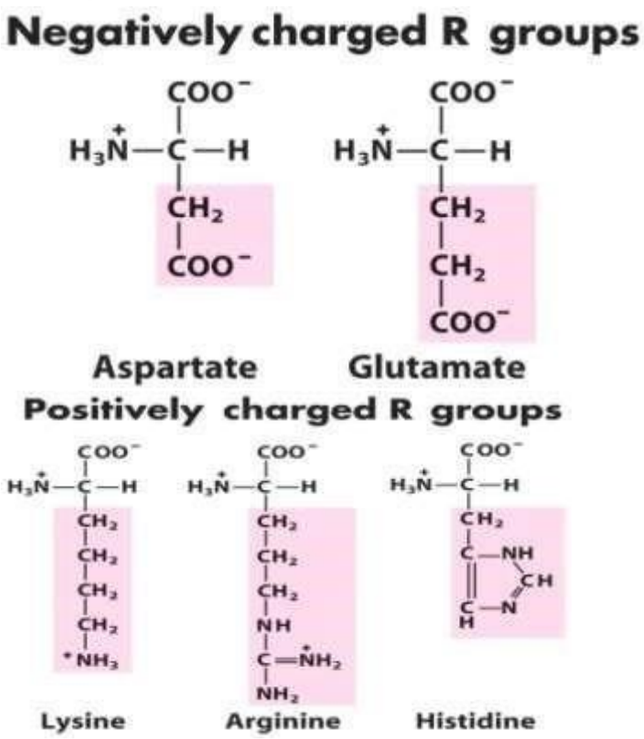
The structure is stabilized by a hydrogen bond between the

hydrogen atom attached to the electronegative nitrogen atom of a peptide linkage and the
electronegative carbonyl oxygen atom of the fourth amino acid on the amino-terminal side of that peptide bond

Lesson 57

- Amino acid residues included in alpha helix have an **intrinsic propensity** to form an alpha helix, reflecting the properties of the R group.
- Amino acids most commonly found in alpha helices are **non-polar** with aliphatic side chains
- **Alanine** shows the greatest tendency to form α helices in most experimental model systems.
- Amino acids most commonly found in alpha helices are **non-polar** with aliphatic side chains
- **Alanine** shows the greatest tendency to form α helices in most experimental model systems.
- The bulk and shape of **Ser, Thr**, and **Cys** residues can destabilize an α helix if they are close together in the chain.
- The bulk and shape of **Ser, Thr**, and **Cys** residues can destabilize an α helix if they are close together in the chain.
- Amino acids with charged R groups such as **Aspartate, Lysine** residues can also disrupt the helix.
- Amino acids with charged R groups such as **Aspartate, Lysine** residues can also disrupt the helix.
- In **proline**, the nitrogen atom is part of a rigid ring, and rotation about the N-C bond is not possible
- Thus, a proline residue introduces a destabilizing kink in an α helix •
  Glycine occurs infrequently in α helices for a different reason.
- It has more conformational flexibility than the other amino acid residues

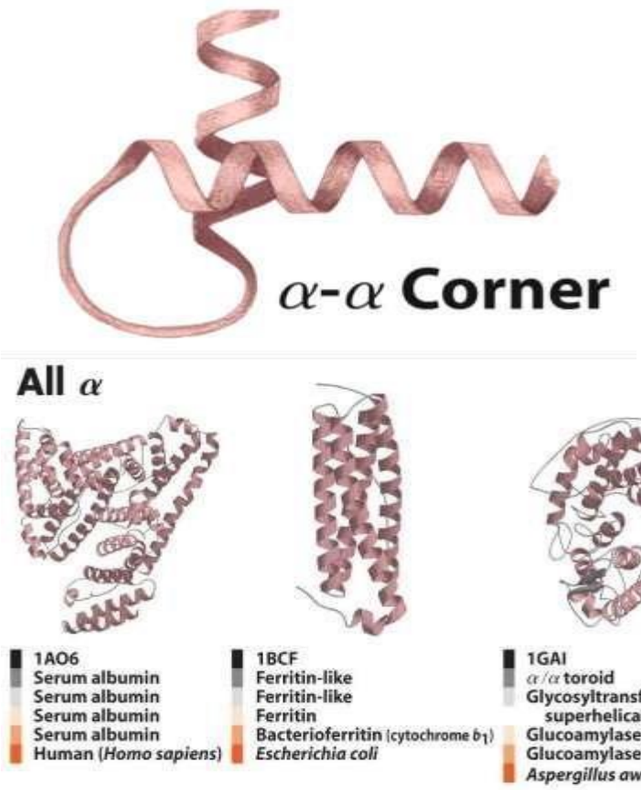
Polymers of glycine tend to take up coiled structures quite different from an α helix



Lesson 58 The Secondary Structure

- **A very diverse group of proteins contains α-helices**
- The alpha-helical content of proteins ranges widely, from nearly none to almost 100%

- For example, the **keratins** are a family of closely related, fibrous proteins whose structure is nearly entirely α-helical.
- They are a major component of tissues such as hair, nails, hoofs and skin.
- In contrast to keratin, myoglobin, whose structure is also highly α-helical, is a globular, flexible molecule.
- Nearly all trans membrane proteins contain alpha helices in their membrane spanning domains.



Lesson 59

The β Conformation organizes polypeptide Chains in β sheets

β Sheets are a second type of regular secondary structure that maximizes hydrogen bonding between the peptide backbones.

The backbone of the polypeptide chain is extended into a zigzag rather than helical structure

The zigzag polypeptide chains can be arranged side by side to form a structure resembling a series of pleats

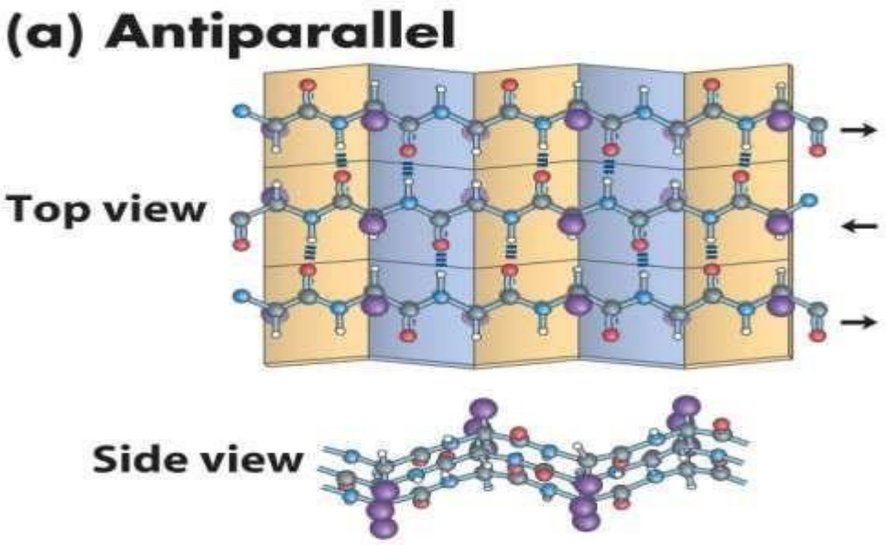
In this arrangement called a β sheet, hydrogen bonds form between adjacent segments of polypeptide chain

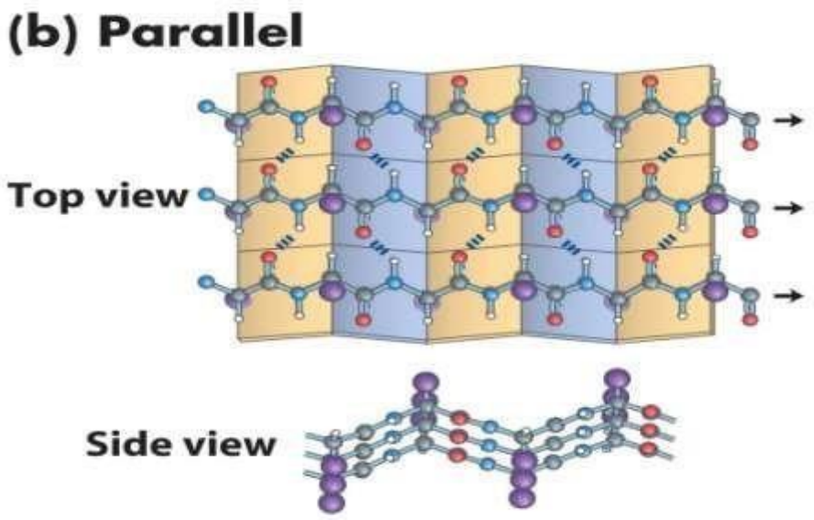
The -pleated sheet is described as parallel if the polypeptide strands run in the same direction (as defined by their amino and carboxy terminals) and anti-parallel if they run in opposite direction

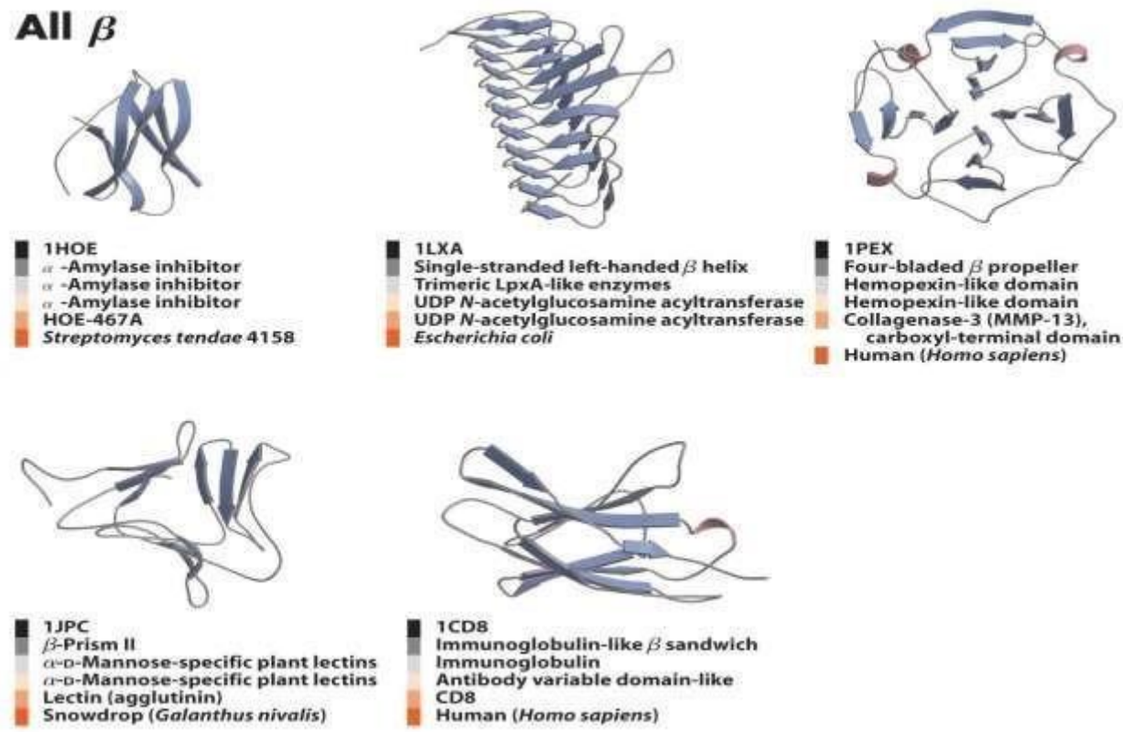
Antiparallel strands are often the same polypeptide chain folded back on itself, with simple hairpin turns or long runs of polypeptide chain connecting the strands The amino acid side chains(R groups) of each polypeptide strand alternate between extending above and below the plane of the β-sheet. Most sheets are not perfectly flat but tend to have a twist.

The individual segments that form a β sheet are usually nearby on the polypeptide chain, but can also be quite distant from each other in the linear sequence of the peptide and may even be in different polypeptide chains.

Clusters of twisted strands of sheet form the core of many globular proteins.







Lesson 60

- In contrast to regular repeating element in the twist of an alpha Helix or a pleat of Beta sheet, **bends, loops,** and **turns are** non- regular secondary structures that do not have a repeating element.

- **Beta-bends** are short regions usually involving four successive amino acid residues.
- They often connect strands of antiparallel beta-sheets.

- β-Bends reverse the direction of a polypeptide chain, helping it form a compact, globular shape.
- The structure is a 180 degree turn involving four amino acid residues

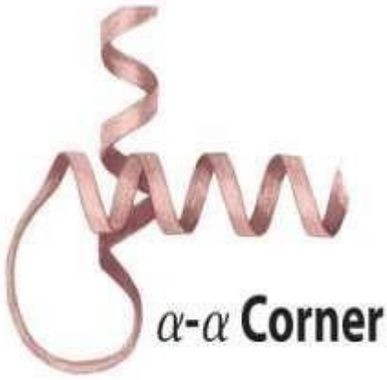
- The carbonyl oxygen of the first residue forms a hydrogen bond with the amino-group hydrogen of the fourth residue.
- β-Bends were given this name because they often connect successive strands of antiparallel β-sheets
- Glycine and Proline residues often occur in β turns
- Glycine because it is small and flexible
- Proline because peptide bonds involving the imino nitrogen readily assume the cis configuration, a form that is particularly amenable to a tight turn

Lesson 61

- A **motif** also called a **super secondary structure** is simply a recognizable folding pattern involving two or more elements of secondary structure and the connection(s) between them.
- Structural motifs are intermediate between secondary and tertiary structures.

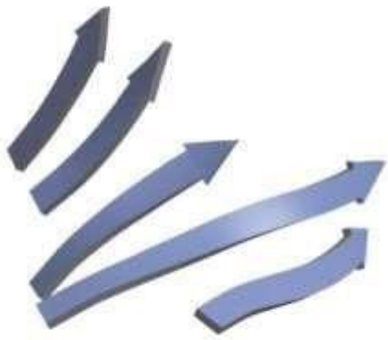
- A motif can be very simple, such as two elements of secondary structure folded against each other, and represent only a small part of a protein
- An example is a **β-α-β loop.**
- A motif can also be a very elaborate structure involving scores of protein segments folded together, such as the **β barrel**

β-α-β Loop



α-α Corner

β Barrel



Twisted β sheet

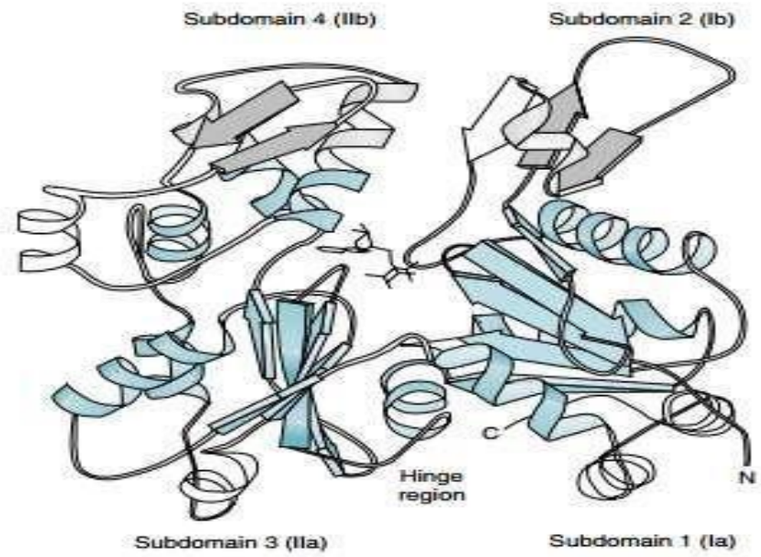
Lesson 62

The tertiary structure

- The tertiary structure of a protein is the folding pattern of the secondary structural elements into a three-dimensional conformation.
- It indicates, in three-dimensional space, how secondary structural features—helices, sheets, bends, turns, and loops—assemble to form domains and how these domains relate spatially to one another.

Domains in the Tertiary Structure

- The tertiary structure of large complex proteins is often described in terms of physically independent regions called structural domains.
- A domain is a section of protein structure sufficient to perform a particular chemical or physical task such as binding of a substrate or other ligand.
- Each domain is formed from a continuous sequence of amino acids in the polypeptide chain that are folded into a three-dimensional structure independently of the rest of the protein.
- Two domains are connected through a simpler structure like a loop.



G-Actin. ATP binds in the center of the cleft. The two domains that form the cleft are further subdivided into subdomains 1–4. The overall structure is found in many ATPbinding proteins and is called the actin fold.

Lesson 63

- The Tertiary Structure
- Molecular forces that stabilise three dimensional tertiary structure include:
- Electrostatic Interactions
- Hydrophobic interactions
- Disulphide bridges
- Hydrogen bonding
- The Tertiary Structure
- The **positive** charges on the basic amino acids enables them to form ionic bonds (electrostatic bonds) with **negatively** charged groups, such as the side chains of acidic amino acids.
- The Tertiary Structure
- **Electrostatic Interactions**
- The **positive** charges on the basic amino acids enables them to form ionic bonds (electrostatic bonds) with **negatively** charged groups, such as the side chains of acidic amino acids.
- The Tertiary Structure
- The Tertiary Structure
- The positive charges on the basic amino acids enables them to form ionic bonds (electrostatic bonds) with negatively charged groups, such as the side chains of acidic amino acids.
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- The Tertiary Structure
- **Hydrophobic Interactions**
- Hydrophobic Interactions occur between non-polar uncharged side chains such as between aromatic groups of phenylalanine side chains.
- The Tertiary Structure
- Hydrophobic interactions occur between non-polar uncharged side chains such as between aromatic groups of phenylalanine side chains.
- The Tertiary Structure **Disulfide Bridges**

- The amino acid cysteine in a protein can form a covalent disulfide bond with another cysteine molecule through spontaneous (nonenzymatic) oxidation of their sulfhydryl groups.
- The Tertiary Structure
- The amino acid cysteine in a protein can form a covalent disulfide bond with another cysteine molecule through spontaneous (nonenzymatic) oxidation of their sulfhydryl groups.
- The Tertiary Structure **Hydrogen bonds**
- Hydrogen bonds in which a hydrogen atom is shared by a nitrogen (or oxygen) in the peptide backbone and an oxygen atom in an amino acid side chain.
- The Tertiary Structure
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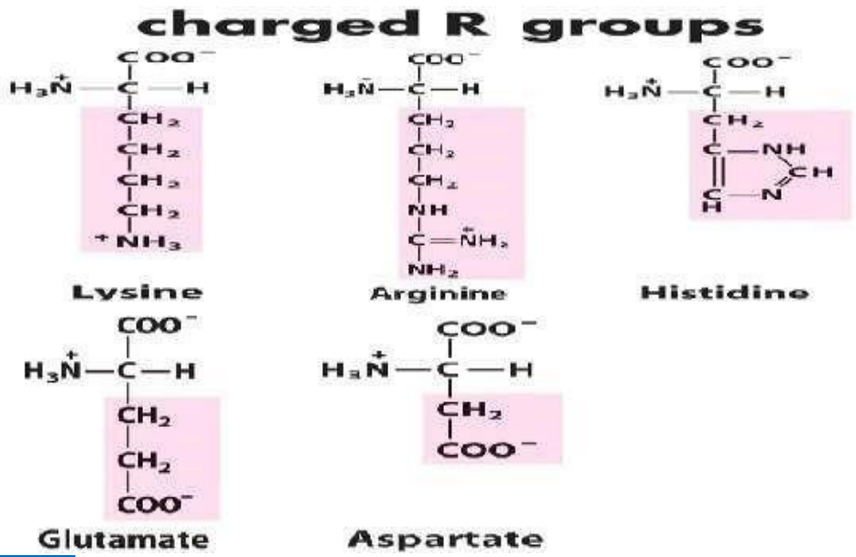
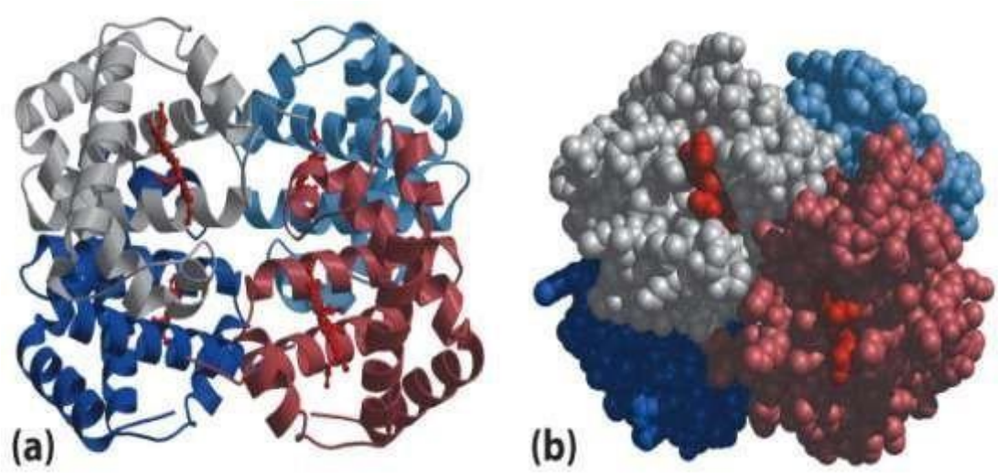


Lesson 64

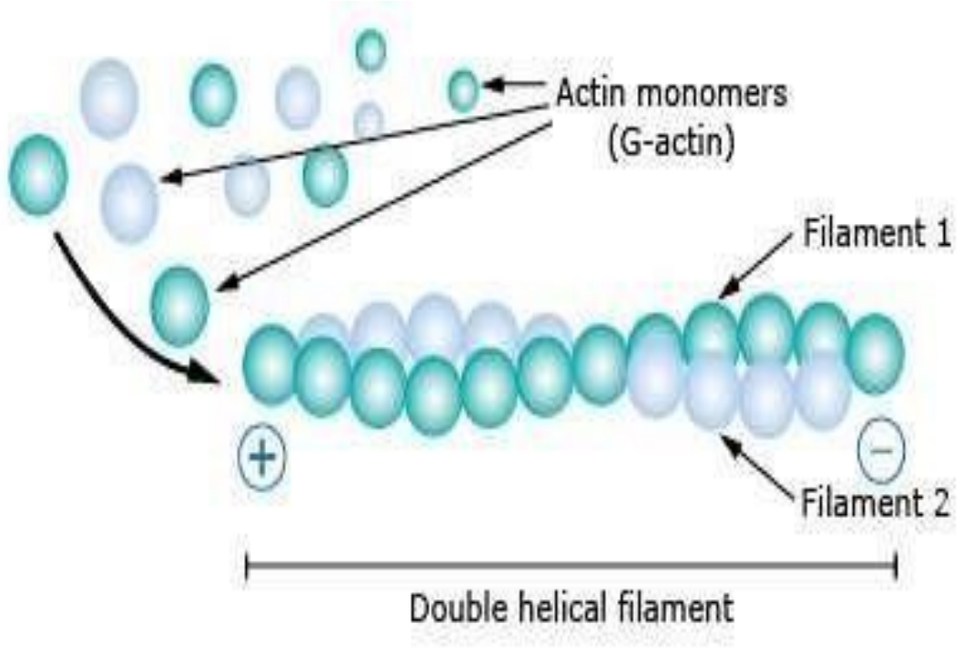
The Quaternary Structure

- Some proteins contain two or more separate polypeptide chains, or subunits, which may be identical or different.
- The arrangement of these protein subunits in three-dimensional complexes constitutes **quaternary structure.**

- Many proteins have multiple polypeptide subunits (from two to hundreds)
- The association of polypeptide chains can serve a variety of functions
- A multisubunit protein is also referred to as a **multimer,** with the prefixes —homoll or —heteroll used to describe identical or different subunits, respectively.
- Most multimers have identical subunits
- or repeating groups of nonidentical subunits, usually in symmetric arrangements.
- The repeating structural unit in such a multimeric protein is **protomer** • Assembly into a multisubunit structure increases the stability of a protein.



X-ray diffraction analysis of deoxyhemoglobin shows how the four polypeptide subunits are packed together. (a) A ribbon representation. (b) A space-filling model. The alpha subunits are shown in gray and light blue; the beta subunits in pink and dark blue. Note that the heme groups (red) are relatively far apart.

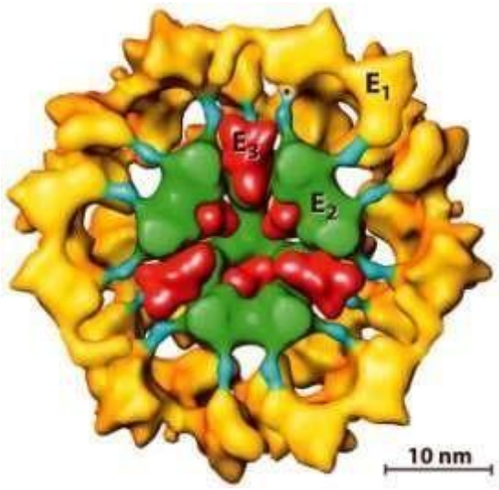


Actin filament is a homo polymer. G-actin, forms the basic unit for actin filaments. Actin filaments together with their associated motor proteins (e.g. myosin superfamily) form an elaborate network known as the actin cytoskeleton involved inn functions including cell motility, muscle contraction, cell division, cytokinesis, vesicle and organelle movement and cell signaling.

Lesson 65

Perks of The Quaternary structure

- The increase in size increases the number of possible interactions between amino acid residues and therefore makes it more difficult for a protein to unfold and refold.
- A multisubunit structure has many advantages besides increased stability.
- It may enable the protein to exhibit cooperativity between subunits in binding ligands (illustrated later with hemoglobin)
- Or it may form binding sites with a high affinity for large molecules (illustrated with antigen binding to the immunoglobulin molecule IgG).
- An additional advantage of a multi-subunit structure is that the different subunits can have different activities and cooperate in a common function.
- For example enzymes that have regulatory subunits or exist as multiprotein complex, such as Pyruvate Dehydrogenase.



- **E1 , pyruvate dehydrogenase;**
- **E2, dihydrolipoyl transacetylase; and**
- **E3,dihydrolipoyl dehydrogenase**

(PDC) is a complex of three enzymes that convert pyruvate into acetyl-CoA by a process called pyruvate decarboxylation

Lesson 66 Protein Folding

Protein folding is the process by which a protein structure assumes its functional shape or conformation.

- Protein Folding

It is the physical process by which a polypeptide folds into its characteristic and functional three-dimensional structure from random coil • Protein Folding

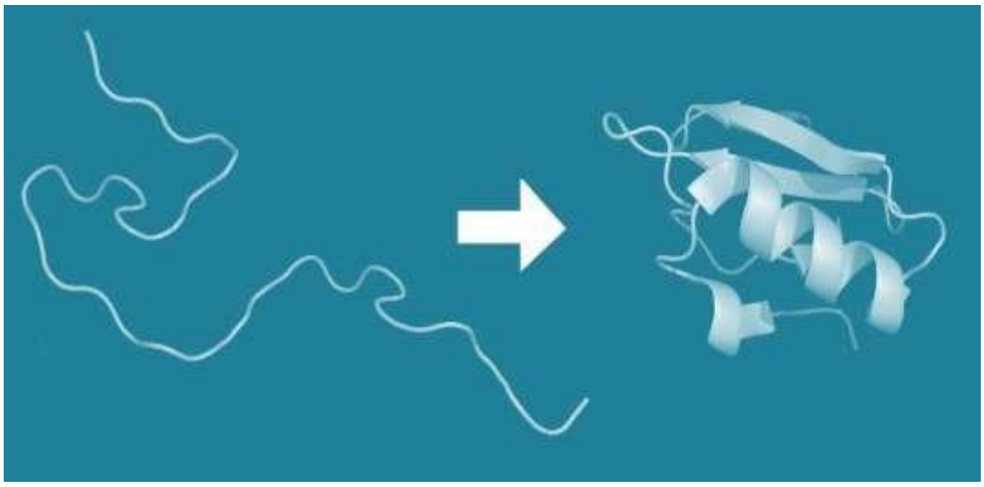
- Protein Folding
- The overall three-dimensional structure of a protein must meet certain requirements to enable the protein to function in the cell or extracellular medium of the body.
- Protein Folding
- The first requirement is the creation of a binding site that is specific for just one molecule, or a group of molecules with similar structural properties.
- The specific binding sites of a protein usually define its functional role.
- Protein Folding
- Moreover, The three dimensional structure must have an external surface appropriate for its environment (e.g., plasma proteins contain polar amino acids on the surface to remain soluble in an aqueous environment.)
- Protein Folding
- Protein Folding
- Proteins are dynamic molecules that can fold into their functionally competent conformation in milliseconds

Protein Folding

- A protein is called a native protein if its amino acid composition and molecular conformation are unchanged from that found in natural states.
- Protein Folding
- Folding into the native state does not involve a haphazard search of all possible structures.
- The native conformation of a Protein is dictated thermodynamically.
- Protein Folding
- The number of potential conformations of even a relatively small—15kDa—polypeptide is unbelievably vast

-
    - Proteins are guided through this vast labyrinth of possibilities by thermodynamics
    - Protein Folding
    - Thermodynamic stability is not evenly distributed over the structure of a protein molecule.
    - It has regions of high and low stability; variations in the stability of regions within a protein are often essential for protein to function.

The regions of low stability allow a protein to alter its conformation between two or more states, such as an inactivated and activated enzyme.



Such as binding site for substrates in enzymes, ligands in receptors, for extracellular matrix in cytoskeletal proteins and for antigens in immunoglobins and so forth.

Lesson 67

- Proteins are dynamic molecules that can fold into their functionally competent conformation in milliseconds
- A protein is called a native protein if its amino acid composition and molecular conformation are unchanged from that found in natural states
- And which is operative and functional
- Folding into the native state does not involve a haphazard search of all possible structures.
- Protein folding in cells takes place in an orderly and guided fashion. Protein folding generally occurs via a stepwise process.
- In the first stage, as the newly synthesized polypeptide emerges from the ribosome, short segments fold into secondary structural units that provide local regions of organized structure
- Then, each element of secondary or super-secondary structure facilitates proper folding by directing the folding process toward the native conformation and away from unproductive alternatives.
- This step-wise folding of the proteins is dictated by thermodynamics resulting in a formation of native form, which is also the most energetically favoured form.

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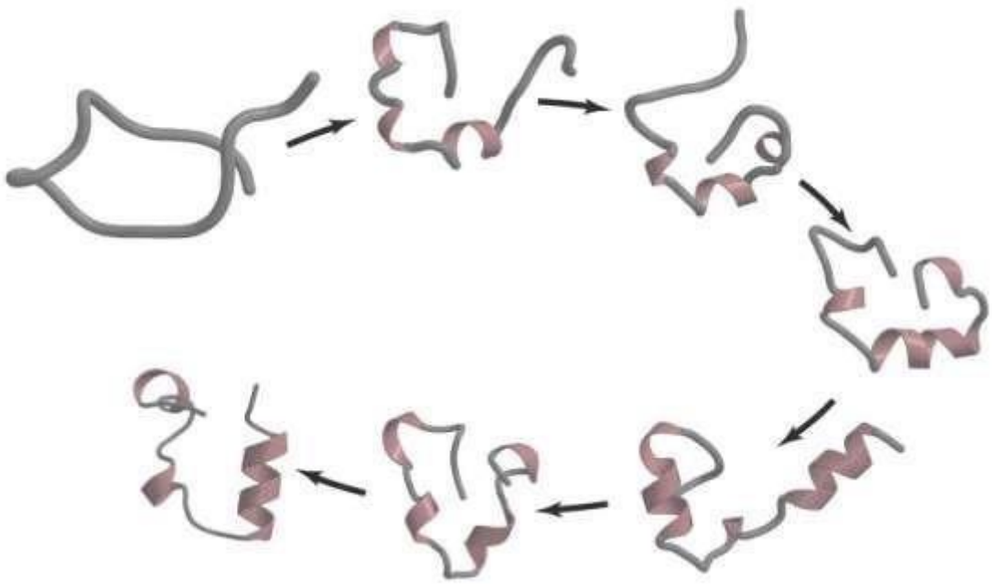


Computer simulated Protein folding showing *local assembly* followed by *global protein folding.*

Lesson 68

- Protein Folding
- The native conformation of a protein is dictated thermodynamically.
- The biologically relevant—or native—conformation of a protein generally is that which is most energetically favored
- The three dimensional structure of a native protein in its normal physiological state is the one in which the Gibbs free energy of the whole system is lowest.
- The free energy change for a process at constant pressure is:
- **ΔG = ΔH – T ΔS**
- **ΔG = Free Energy**
- **ΔH = Enthalpy**
- **ΔS= Entropy**
- Folding of a globular protein is a thermodynamically favored process, i.e. G must be negative.
- **ΔG = ΔH – T ΔS**
  The folding process involves going from a multitude of random-coil conformations to a single folded structure.
- The folding process involves a decrease in randomness and thus a decrease in entropy **- S** and an overall positive contribution to G. This decrease in entropy is termed —conformational entropy‖.
- Difference in energy (free energy) between folded (native) and unfolded (denatured) state is small, 5-15 kcal/mol.
- Protein Folding
- It is very difficult to determine how all factors blend together to give overall $DG_{folding}$
- Use of averages contributions, but
- Each protein is unique
- Large stabilization factors,
- Large destabilization factors,
- But small difference between them
- **ΔG = ΔH – T ΔS**
- Small **ΔG** is necessary because too large a free energy change would mean a very stable protein, one that would never change.
- However, structural flexibility is important to protein function, and proteins need to be degraded.

-
- Enthalpy and entropy differences balance each other, and ΔG is a small positive number.
- Enthalpy is the total energy of the system. **Entropy** is a measure of the randomness or disorder in a system. **Entropy of a system always tend to increase to a maximum** value.

Lesson 69

Protein Folding

- The native state has a smaller Gibbs free energy than the denatured state. The stability of the protein depends on the solvent–solvent, protein–solvent, and protein–protein interactions.
- The free energy of a protein molecule is influenced by
- (1) the hydrophobicity,
- (2) hydrogen bonds,
- (3) electrostatic interactions, and
- (4) the conformational entropy due to the restricted motion
- **Entropy** is a measure of the randomness or disorder in a system.
- According to Second Law of Thermodynamics: **The entropy of a system will tend to increase to a maximum** value.

The folding process involves a decrease in randomness and thus a decrease in entropy **- S** and an overall positive contribution to G. This decrease in entropy is termed —conformational entropy‖.

- the hydrophobic effect and hydrogen bonds are the major stabilizing contributions which counteract entropy.
- Starting from the unfolded protein, the polypeptide chain has to fold partially in order to bring together the residues that need to form the contacts stabilizing the native structure.
- The constrained polypeptide chain has smaller entropy, which means higher Gibbs free energy.
- As native contacts form, the enthalpy term decreases, the protein is stabilized.
- Thermodynamic stability is not evenly distributed over the structure of a protein molecule.
- It has regions of high and low stability; variations in the stability of regions within a protein are often essential for protein to function.
- The rate limiting step in the folding process is the formation of the transition state,
  i.e., the conformation that has the highest Gibbs free energy on the folding pathway

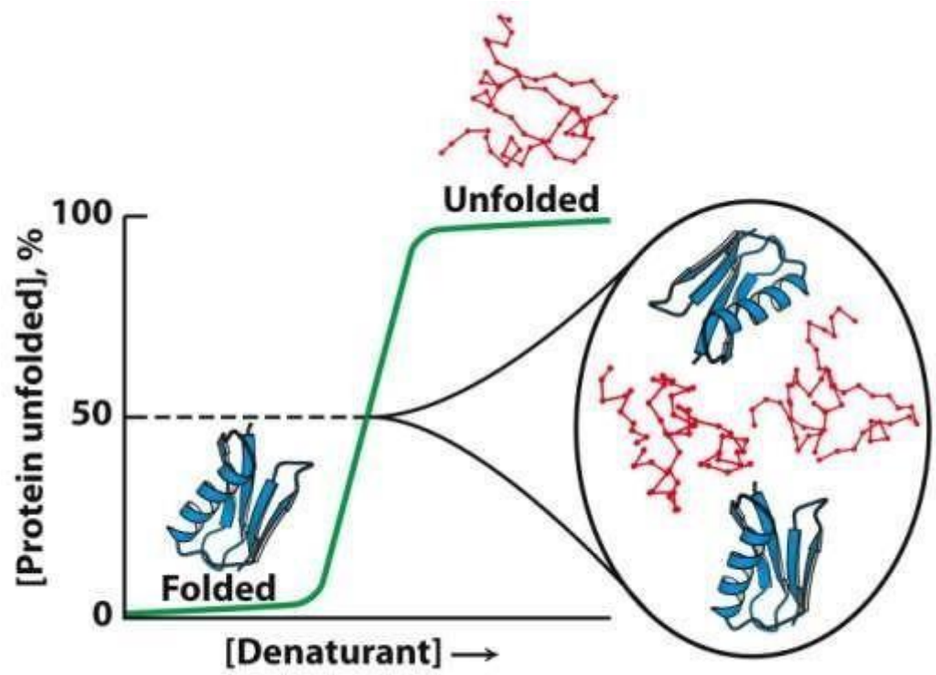


Figure 2.57
Biochemistry, Seventh Edition

Entropy is maximum at unfolded state

The regions of low stability allow a protein to alter its conformation between two or more states, such as an inactivated and activated enzyme.

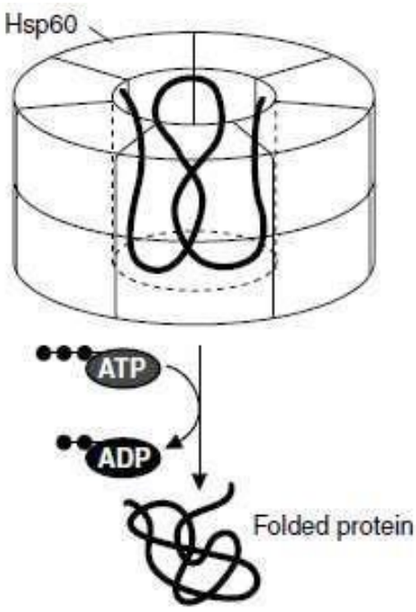
Lesson 70

- Protein Folding
- In the cell, not all proteins fold into their native conformation on their own.
- As the protein folds and refolds while it is searching for its native low energy state, it passes through many high-energy conformations that slow the process
- These high energy conformations are called kinetic barriers.
- Cells employ auxiliary proteins to overcome these kinetic barriers.
- These auxillary proteins are called chaperones.
- Chaperone use energy provided by ATP hydrolysis to assist in the folding process
- **Chaperones** participate in the folding of over half of mammalian proteins.
- They prevent immature folding of the nascent polypeptide and help in the final native protein formation.
- The hsp70 family of chaperones binds short sequences of hydrophobic amino acids in newly synthesized polypeptides.
- Protein Folding
- Chaperones prevent aggregation, thus providing an opportunity for the formation of appropriate secondary structural elements.
- Protein Folding
- Protein Folding
- They also unfold proteins prior to their insertion through the membrane of mitochondria and other organelles.

Lesson 71

Protein Folding

- The hsp60 family of chaperones, sometimes called **chaperonins,** differ in sequence and structure from hsp70 and its homology
- Hsp60 acts later in the folding process, often together with an hsp70 chaperone
- Hsp60 chaperonins form a multi-subunit barrel-shaped structure
- The unfolded protein fits into the barrel cavity that excludes water and serves as a template for the folding process.
- Chaperone proteins can also "rescue" unfolded proteins that have become thermodynamically trapped in a misfolded dead end by unfolding hydrophobic regions and providing a second chance to fold productively
- Finally, the folding pathways of some proteins require two enzymes that catalyze isomerization reactions.
- Protein disulfide isomerase (PDI) is a widely distributed enzyme that catalyzes the interchange, or shuffling, of disulflde bonds until the bonds of the native conformation are formed.



The Hsp60 class of protein has a barrel shape into which the protein fits. It acts as a template, binding and rebinding portions of the unfolded protein until folding is completed. It hydrolyzes many ATP bonds to provide energy for the process.

Lesson 72

Protein Misfolding

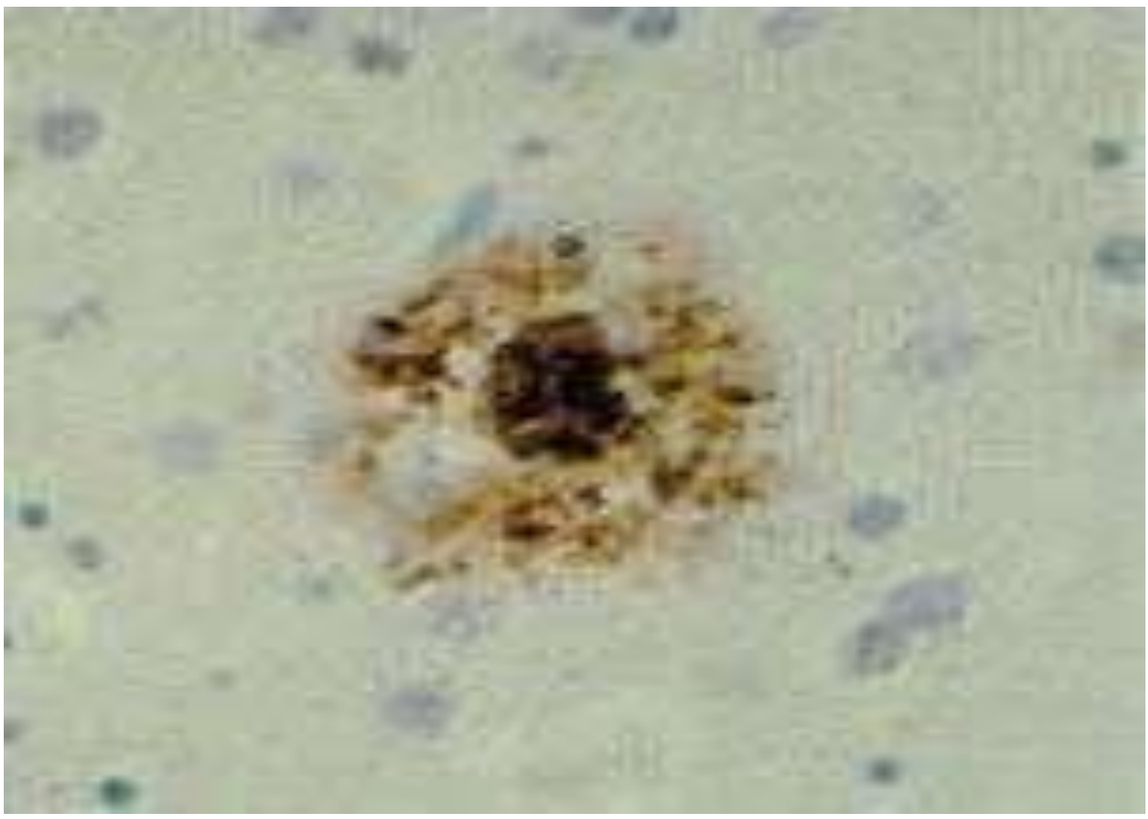
- Despite the many processes that assist in protein folding, misfolding does occur.
- Misfolding of proteins may occur spontaneously

- or caused by a mutation in a particular gene, producing an altered protein.
- In addition some apparently normal proteins can, after abnormal proteolytic cleavage, take on a unique conformational state
- That leads to the formation of long fibrillar protein assemblies consisting of β pleated sheets
- Many conditions, including
- Alzheimer's disease
- Type 2 diabetes,
- Huntington's disease and
- Parkinson's disease, arise from a common misfolding mechanism.
- In most cases, a soluble protein that is normally secreted from the cell is secreted in a misfolded state
- and converted into an insoluble extracellular amyloid fiber.
- The diseases are collectively referred to as amyloidoses.
- Amyloidosis is a generic term that refers to the extracellular tissue deposition of fibrils composed of low molecular weight of a variety of proteins..
- Amyloidoses may be

organ specific or _ generalized

(systemic).

- Primary systemic amyloidosis is caused by deposition of fibrils consisting of misfolded immunoglobulin light chain
- Examples of organ-specific amyloidoses include:
- **Alzheimer's disease** due to deposition of amyloid-b protein cleaved from amyloid precursor protein (APP).
- Islet amyloid polypeptide (IAPP) is commonly seen in diabetes mellitus type 2 and is caused by deposition of amylin in pancreatic islets.
- It has been suggested that the disease-causing mechanism in Huntington's disease (and the other polyglutamine disorders) is the ability of polyglutamine to undergo a conformational change that can lead to the formation of very stable anti-parallel betasheets; more specifically, amyloid structures



Photomicrograph of amyloid plaques in a section of temporal cortex from a patient with

Alzheimer’s disease

Alzheimer's is a type of dementia that causes problems with memory, thinking and behaviour. In August 1994, at the age of 83, Reagan was diagnosed with Alzheimer's disease. Died in 2004.

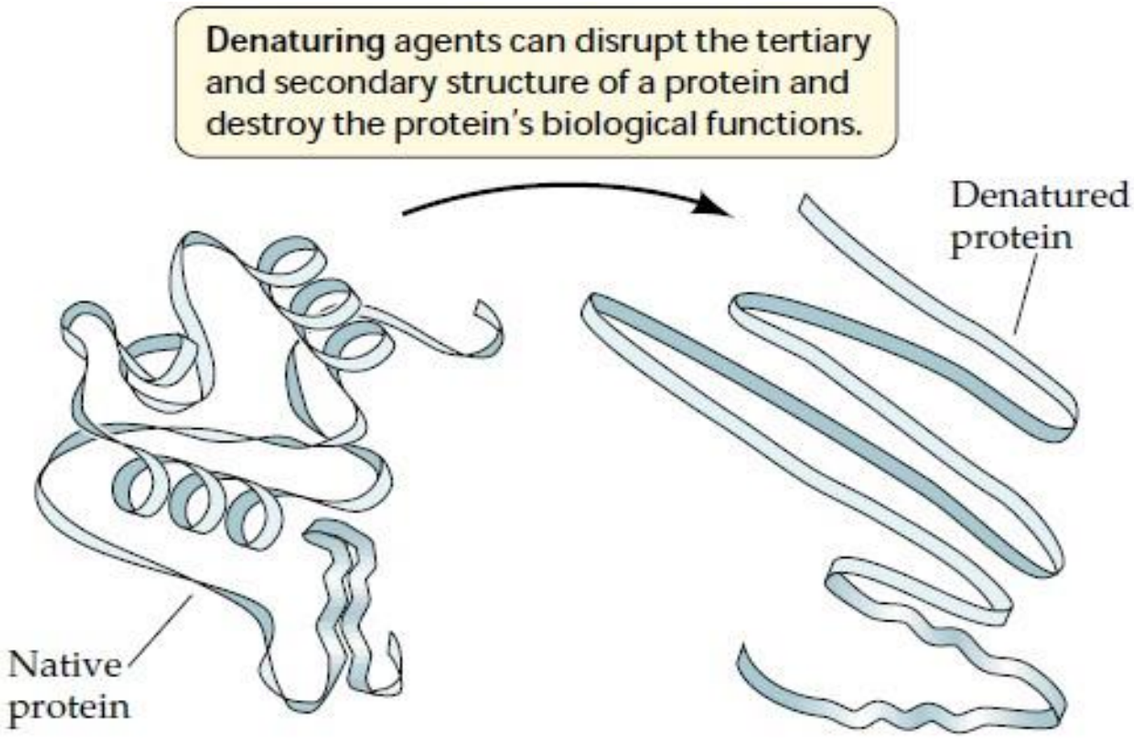
73 Protein denaturation

Protein Denaturation

- **A loss of three-dimensional structure sufficient to cause loss of function is called denaturation**

(Electrostatic bonds,disulfide bonds, Specific hydrogen bonding, Hydrophobic bonds)

- **The denatured state does not necessarily equate with complete unfolding of the protein and randomization of conformation • Under most conditions, denatured proteins exist in a set of partially folded state**

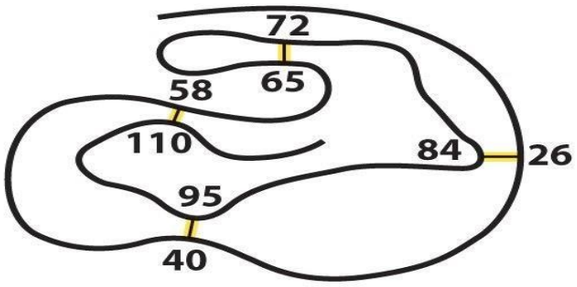


- **Most proteins can be denatured by heat, • which has complex effects on the weak interactions in a protein (primarily hydrogen bonds). • Organic solvents, urea, and detergents act • primarily by disrupting the hydrophobic interactions that make up the stable core of globular proteins; • Extremes of pH alter the net charge on the protein, causing disruption of electrostatic interactions • And the disruption of some hydrogen bonding.**

- **Protein Denaturation**
- **When milk curdles, the acidity increases.**
- **Thermal denaturation by cooking.**
- **Mechanical denaturation when whisking an egg.**
- **Perming hair breaks then reforms the disulphide bonds.**
- **The main purpose of beating an egg is to "denature" the protein within the egg. Proteins are long chains of amino acids and they have lots of internal chemical bonds, which hold them together into tightly contained units. When a protein is denatured, those internal bonds break and the amino acid chains unravel and become elongated. At the same time, atoms that were previously bonded (as part of the internal bonds I mentioned) become available to bond with other molecules**

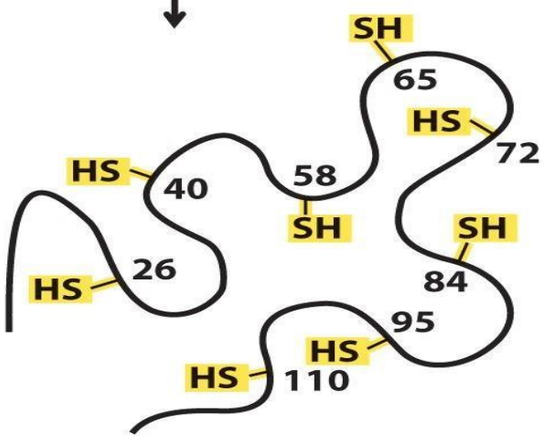
• 74 Protein denaturation (conti...)

- **Certain proteins denatured by heat, extremes of pH, or denaturing reagents • Regain their native structure and their biological activity if returned to conditions in which the native con-formation is stable.**
- **At Very High or Low pH. At Very High Temperatures.By Heavy Metal Ions.By Small Polar Molecules • • This process is called renaturation • Unfolded proteins generally retain a number of contacts and regions of secondary structure that facilitate the refolding process • A classic example is the denaturation and renaturation of ribonuclease A • Purified ribonuclease A denatures completely in a concentrated urea solution in the presence of a reducing agent.**



Native state; catalytically active.

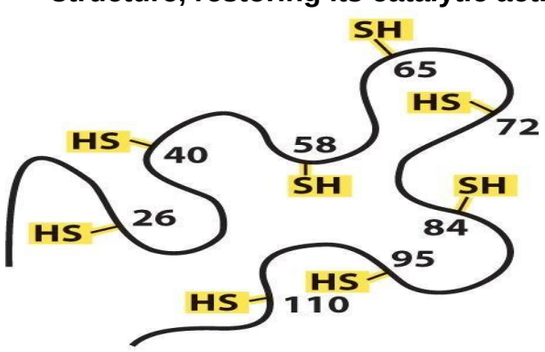
addition of urea and mercapto-ethanol



Unfolded state; inactive. Disulfide cross-links reduced to yield Cys residues.

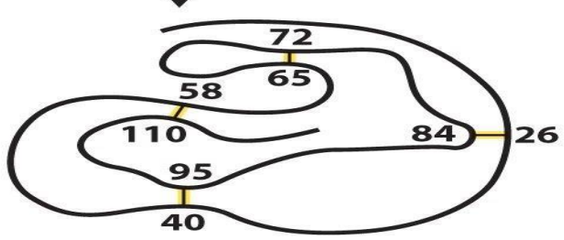
VU Bio Mates https://www.facebook.com/groups/vubiomates/

- When the urea and the reducing agent are removed, the randomly coiled, denatured ribonuclease spontaneously refolds into its correct tertiary structure, restoring its catalytic activity



Unfolded state; inactive. Disulfide cross-links reduced to yield Cys residues.

removal of urea and mercapto-ethanol



Native, catalytically active state. Disulfide cross-links correctly re-formed.

VU Bio Mates
https://www.facebook.com/groups/vubiomates/ denatur
renatur
Incubate 100-fold protein dilution of in protein guanidine

- This proofs that denaturation of some proteins is reversible. • Which tells us that primary structure of a protein determines its three- dimensional conformation.

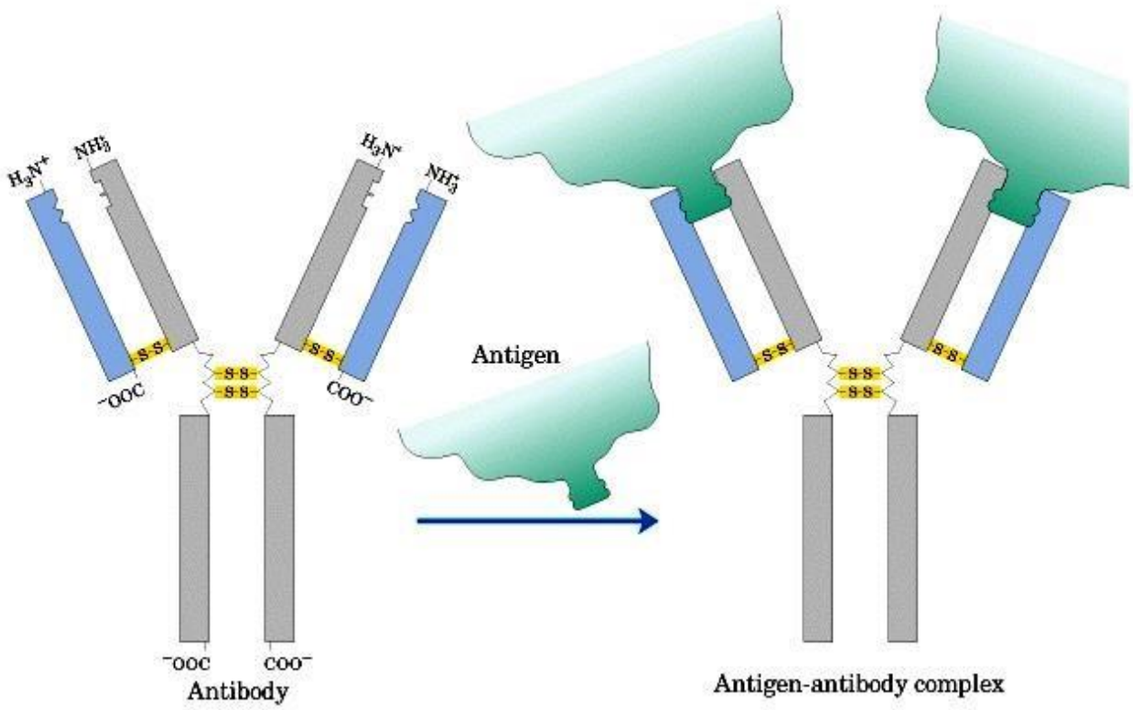
75 Reversible binding of a protein

Reversible binding of a protein to a ligand Oxygen-Binding Proteins • The functions of many proteins involve the reversible binding of other molecules.

• A molecule bound reversibly by a protein is called a ligand. • A ligand may be any kind of molecule, including another protein. • The transient nature of protein ligand interactions is critical to life, allowing an organism to respond rapidly and reversibly to changing environmental and metabolic circumstances.

• A ligand is a small molecule that is able to bind to proteins by weak interactions such as ionic bonds, hydrogen bonds, Van der Waals interactions, and hydrophobic effects. In some cases, a ligand also serves as a signal triggering molecule. A ligand can be a substrate inhibitor, activator or a neurotransmitter.

• A ligand binds at a site on the protein called the binding site, which is complementary to the ligand in – size, – shape, – charge, – hydrophobicity – This complementarity of protein ligand binding gives the interaction a great deal of specificity. • Binding of IgG to an antigen



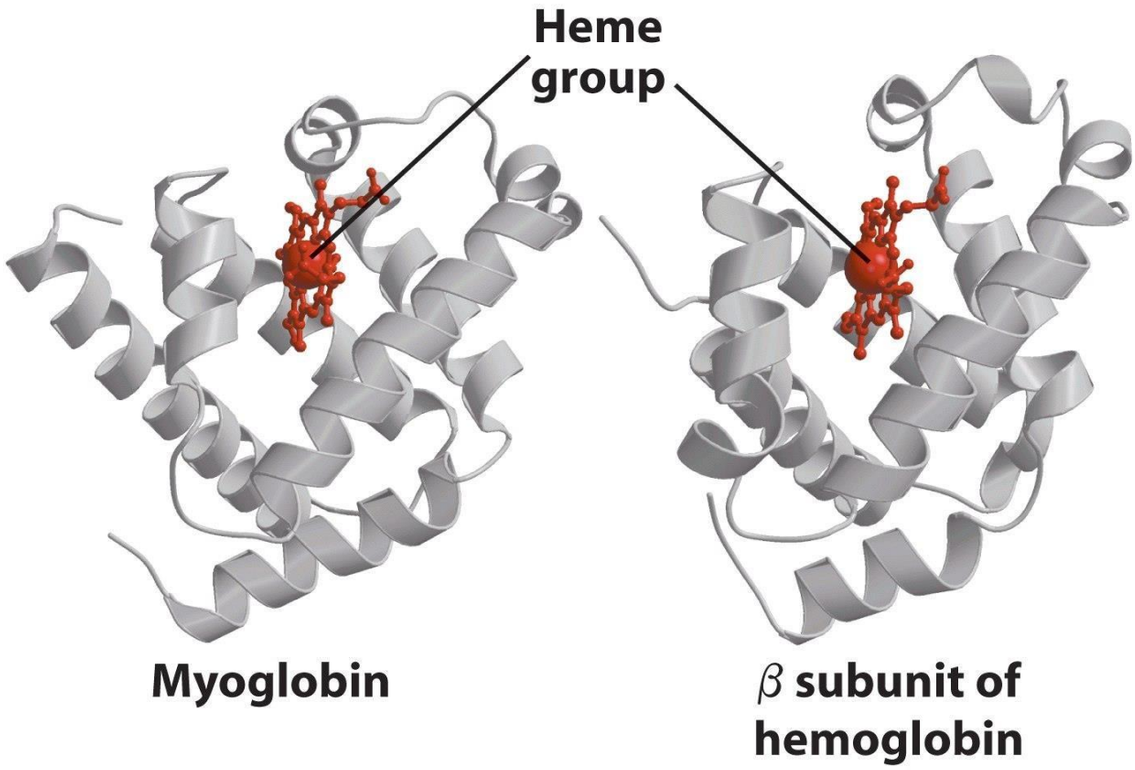
• The protein can discriminate among thousands of different molecules in its environment and selectively bind only one or a few similar compounds. • A given protein may have separate binding sites for several different ligands • The binding of a protein and ligand is often coupled to a conformational change in the protein that makes the binding site more complementary to the ligand, permitting tighter binding • The structural adaptation that occurs between protein and ligand is called induced fit. • Reversible binding of ligands is essential for the function of gloubular proteins – Specificity of ligands and binding sites – Ligand binding is often coupled to conformational changes, sometimes quite dramatic (Induced Fit) • In multisubunit proteins, conformational changes in one subunit can affect the others (Cooperativity)

– Interactions can be regulated • Illustrated by: – Hemoglobin, antibodies, and muscle contraction

76 Reversible binding of a protein (conti...)

Oxygen-Binding Proteins

- Myoglobin and hemoglobin • These two molecules illustrate almost every aspect of that most central of biochemical processes: • The reversible binding of a ligand to a protein • Oxygen is poorly soluble in aqueous solutions and cannot be carried to tissues in sufficient quantity if it is simply dissolved in blood plasma. • Diffusion of oxygen through tissues is also ineffective over distances greater than a few millimeters • The evolution of larger, multicellular animals depended on the evolution of proteins that could transport and store oxygen • Myoglobin is composed of a single polypeptide chain that has one O2 binding site.
- Hemoglobin is a tetramer in which each subunit has a strong sequence homology to myoglobin and contains an O2 binding site. • Myoglobin and hemoglobin are two oxygen-binding proteins with a very similar • primary structure • Hemoglobin is a tetramer composed of two different types of subunits (2α and 2β polypeptide chains, referred to as two αβ protomers
- Each subunit has a strong sequence homology to myoglobin.



- We will compare the; • structure and
- functional relation of each protein in the subsequent discussion to see how both these proteins are suited to perform their required function in their relative sites in the body.

77 Reversible binding of a protein 1 (conti...)

Reversible binding of a protein

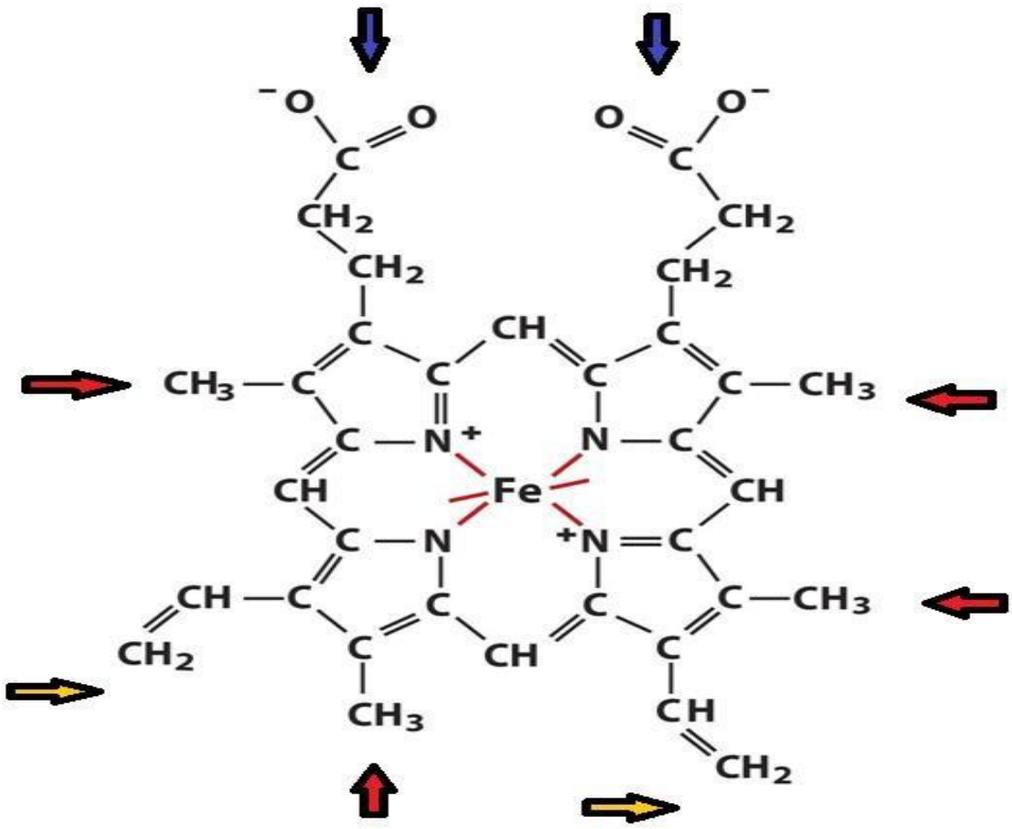
- The tertiary structure of oxygen binding globins consists of; • Eight αhelices. • The helices create a

hydrophobic O2
binding pocket
containing;

- tightly bound heme with an iron atom Ferrous (Fe 2+
) in its center. • 1.Globin. 2.Prosthetic group i.e heme composed of protoporphyri IX ring containing iron as ferrous • This iron bound heme is an example of prosthetic group.

- A prosthetic group is a compound permanently associated with a protein and contributes to the protein's function.
- Prosthetic group is a tightly bound, specific non-polypeptide unit required for the biological function of some proteins. The prosthetic

group may be organic (such as a vitamin, sugar, or lipid) or inorganic (such as a metal ion), but is not composed of amino acids.



- Heme consists of a complex organic ring structure, protoporphyrin IX, to

which is bound a single iron atom in its ferrous (Fe 2+) state

- The iron atom has six bonds, • four to nitrogen atoms that are part of the flat porphyrin ring system • and two perpendicular to the porphyrin Negatively charged propionate (blue) groups on the porphyrin ring interact with arginine and histidine side chains from the hemoglobin, and the hydrophobic methyl(red) and vinyl(yellow) groups that extend out from the porphyrin ring interact with hydrophobic amino acid side chains from hemoglobin. All together, there are approximately 16 different interactions between myoglobin.

FIGURE 5–1 Heme. The heme group is present in myoglobin, hemoglobin, and many other proteins, designated heme proteins. Heme consists of a complex organic ring structure, protoporphyrin IX, with a bound iron atom in its ferrous (Fe 2+) state. • Porphyrins, of which protoporphyrin IX is only one example, consist of four pyrrole rings linked by methene bridges, with substitutions at one or more of the positions denoted X. (b, Two representations of heme (derived from PDB ID 1CCR). The iron atom of heme has six coordination bonds: four in the plane of, and bonded to, the flat porphyrin ring system, and (d) two perpendicular to it. Negatively charged propionate groups on the porphyrin ring interact with;
Arginine and
Histidine side chains from the hemoglobin. The hydrophobic; methyl and vinyl groups

• interact with hydrophobic amino acid side chains from hemoglobin.

• 78 Reversible binding of a protein 2 (conti...)

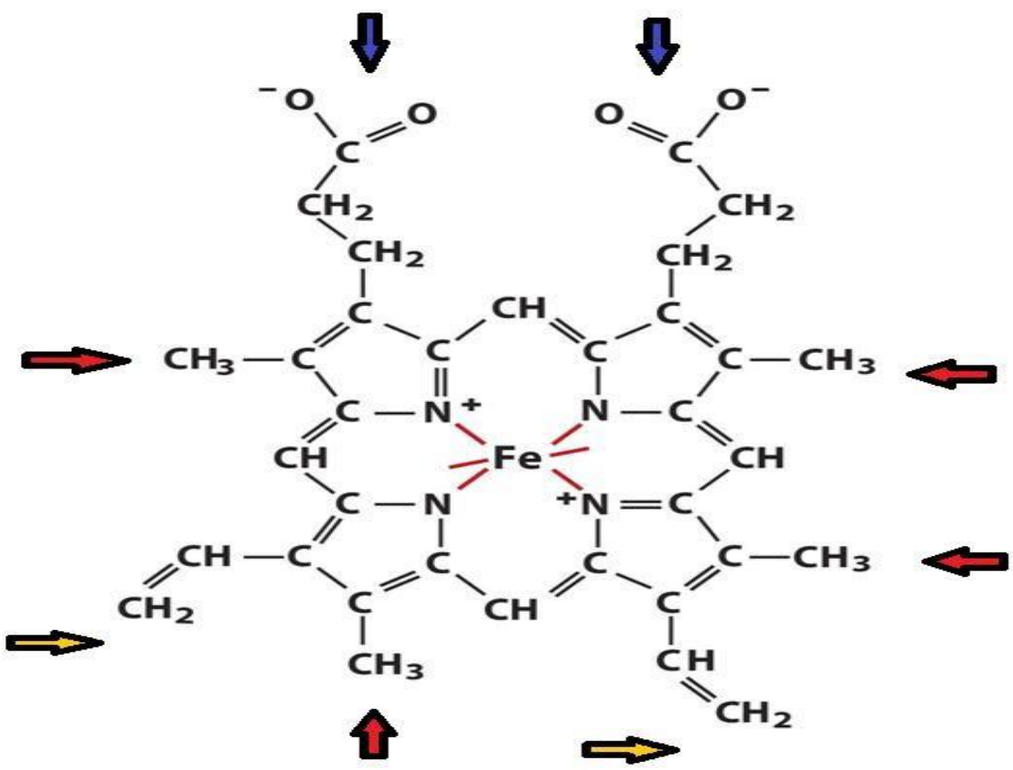
• Iron in the Fe 2+ state binds oxygen reversibly

• But in the Fe 3+ (Ferric) state it does not bind oxygen • The iron atom of heme has six coordination bonds four in the plane of, and bonded to, the flat porphyrin ring system with Nitrogen • Where as two perpendicular to it.

• A coordinate bond (also called a dative covalent bond) is a covalent bond (a shared pair of electrons) in which both electrons come from the same atom. Negatively charged propionate (blue) groups on the porphyrin ring interact with arginine and histidine side chains from the hemoglobin, and the hydrophobic methyl(red) and vinyl(yellow) groups that extend out from the porphyrin ring interact with hydrophobic amino acid side chains from hemoglobin. All together, there are approximately 16 different interactions between myoglobin.

Heme:
protoporphyrin

• Therefore free heme molecules (heme not bound to protein) leave Fe 2+ with two "open“ coordination bonds



Reversible binding of a protein

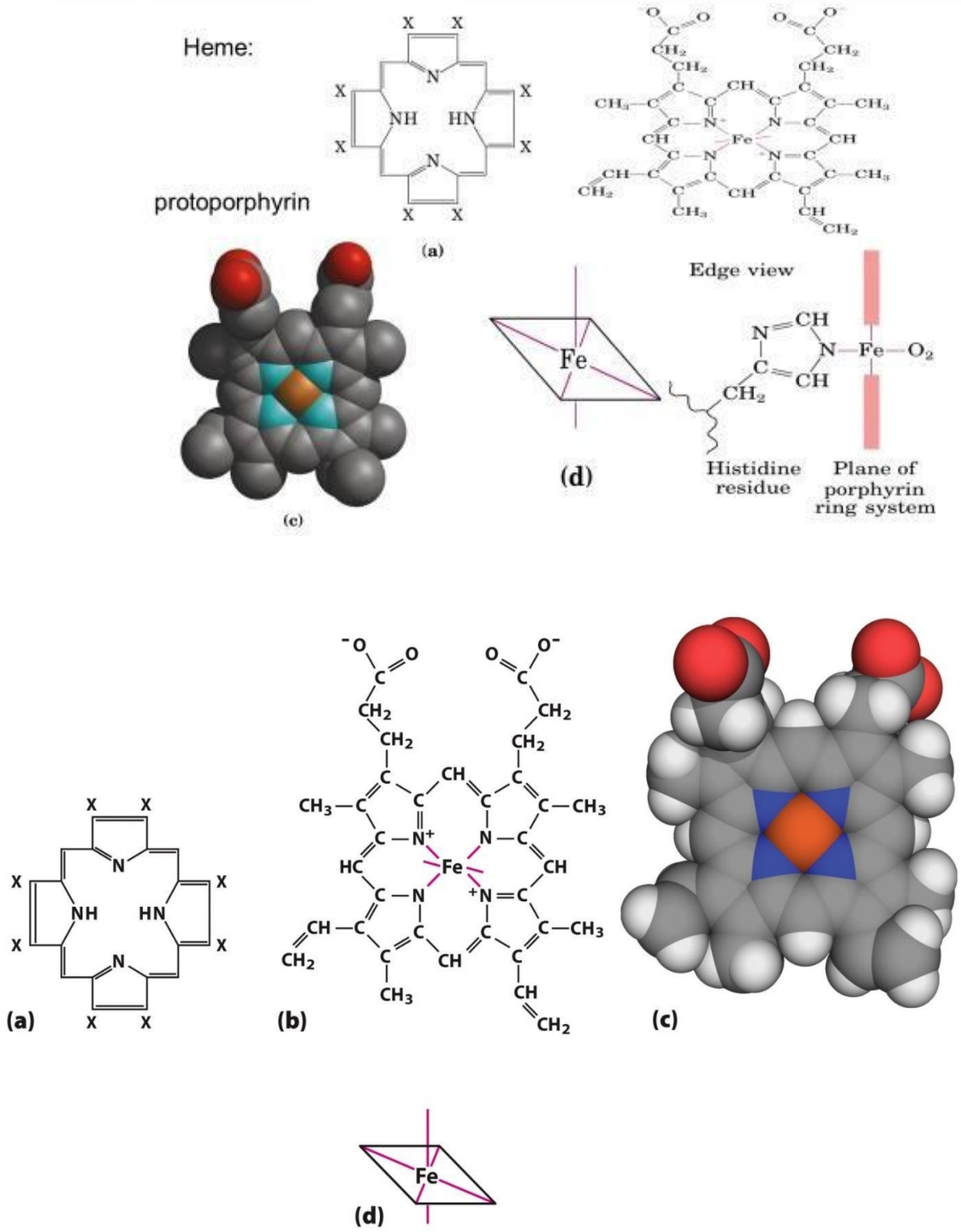


Figure 5-1
Lehninger Principles of Biochemistry, Sixth Edition

• One of these two coordination bonds is occupied by a side chain nitrogen of a His residue • The other is the binding site for molecular oxygen (O) 2

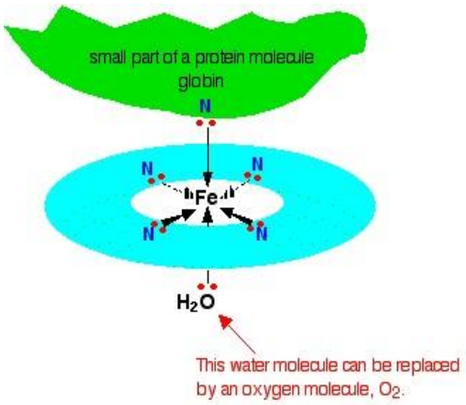
• One of these two coordination bonds is occupied by a side chain nitrogen of a His residue • The other is the binding site for molecular oxygen (O) 2

Negatively charged propionate (blue) groups= arginine and histidine side

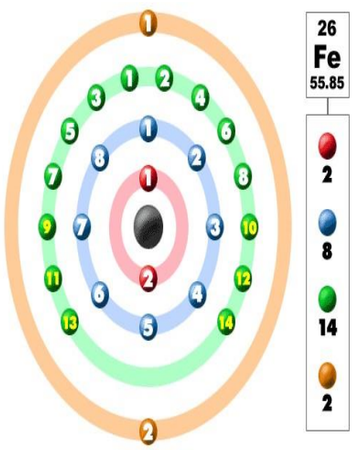
79 Reversible binding of a protein 3 (conti...)

- **Iron in the *Fe^{2+} state binds oxygen reversibly***
- **But in the Fe^{3+} (Ferric) state it does not bind oxygen**

- **The coordinated nitrogen atoms (which have an electron-donating character) help in preventing the conversion of the heme iron to the ferric (Fe^{3+}) state**



- **The conversion Fe^{2+} to Fe^{3+} is much more likely in *free* heme,**
- **Sequestering each heme deep with in the protein structure further helps to prevent this reaction.**
- **Iron is a transition metal & because its orbitals are so close energy wise they tend to give up either 2 or 3 electrons at time**



Problem of Octet rule

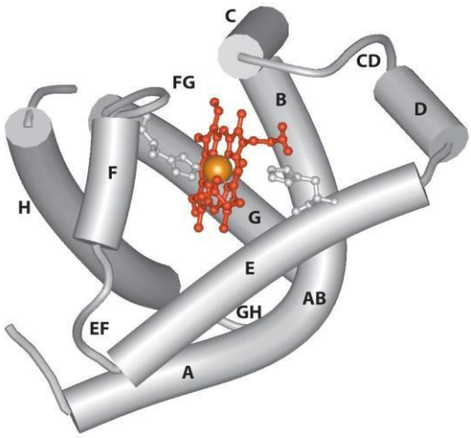
- **Octet rule cannot be used for type II cations (most of the transition and inner transition elements).**
- **The physiological oxidation state of an iron atom has a positive two charge (ferrous ion) instead of three charge (ferric ion)**
- **And it is too large to fit into the plane of protoporphyrin**
- **However, when iron oxidized from ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}), there is loss of one extra electron, forces between protons and electrons increases so that the electron cloud will penetrate more towards to the nucleus**
- **As a result, the ferric ion (Fe^{3+}) has a smaller size then ferrous ion (Fe^{2+}) and fits into the protoporphyrin plane**
- **This deep impregnation of Fe^{3+} in the protoporphyrin results in inability of Fe^{3+} to bind with oxygen.**

- **Some small molecules, such as carbon monoxide (CO) and nitric oxide (NO), coordinate to heme iron with greater affinity than does O_2**
- **When a molecule of CO is bound to heme, O_2 is excluded, that is why CO is highly toxic to aerobic organisms**

- By surrounding and sequestering heme, oxygen-binding proteins regulate the access of CO to the heme iron.

80 Reversible binding of a protein 4 (conti..)

- About 78% of the amino acid residues in the myoglobin are found in α helices with bends in between.
- The helical segments are named A through H.(eight)
- The bends are designated AB, CD, EF, and so forth.
- An individual amino acid residue is designated either by its position in the amino acid sequence
- or by its location in the sequence of a particular α-helical segment
- For example, the His residue coordinated to the heme in myoglobin, His^{93} (the 93rd residue from the amino-terminal end of the myoglobin polypeptide sequence), is also called His F8 (the 8^{th} residue in a helix F)

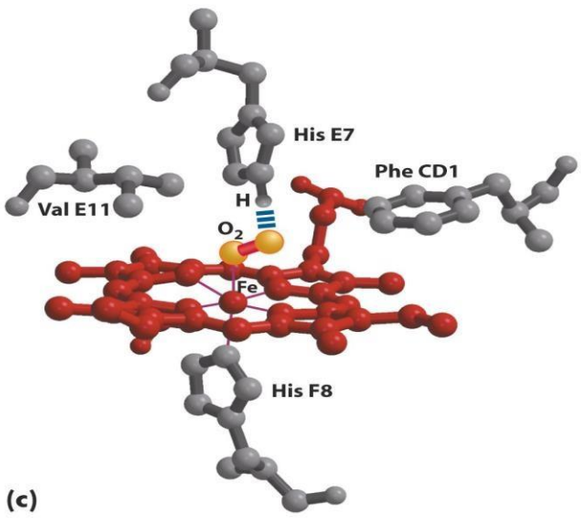


- Oxygen stored in red muscle myoglobin is released during O_2 deprivation (eg, severe exercise) for use in muscle mitochondria for aerobic synthesis of ATP.
- The surface of myoglobin is polar, important for interacting with polar aqueous environment of cytosol.
- The interior contains only nonpolar residues such as
- Leu,
- Val,
- Phe, and
- Met.
- with two notable exceptions—
- The exceptions are His E7 and His F8, the seventh and eighth residues in helices E and F,
- Which lie close to the heme iron, where they stabilize heme and help in O_2 binding. • Histidine is a generally considered to be a *polar* amino acid

81 Reversible binding of a protein 5 (conti..)

- The cervice (Pocket)created by nonpolar amino acids in the interior of myoglobin create a binding pocket for heme.
- The proximal histidine F8, binds directly to the iron of heme

- **Whereas distal histidine E7, does not directly interact with the heme, but helps stabilize the binding of oxygen to the ferrous iron.**
- **In myoglobin and hemoglobin, heme is covalently linked with histidine F8(eighth residue of F helix). because of covalent bond this histidine is closer to heme iron and named as proximal histidine (closer histidine),**
- **While other key histidine which responsible for stabilization of oxygen in E7**
  (seventh residue of E helix) is far from heme iron so named as distal
- **The protein, or globin portion of myoglobin thus creates a special microenvironment for the heme that permits the reversible binding of one oxygen molecule (oxygenation)**



(c)

- **The physiological importance of oxygen stabilization of the distal histidine (E7) lies in the fact that it prevents the binding of CO which is a low level product of cellular metabolism.**

82 Reversible binding of a protein 6 (Conti....)

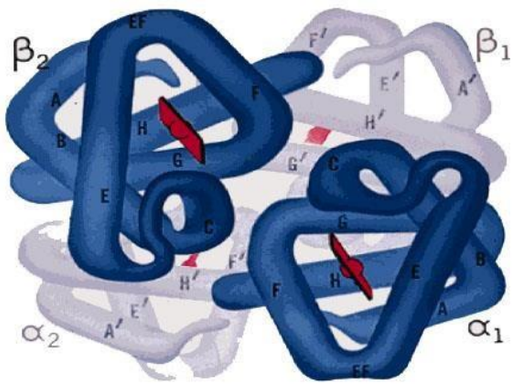
Structure and function of hemoglobin

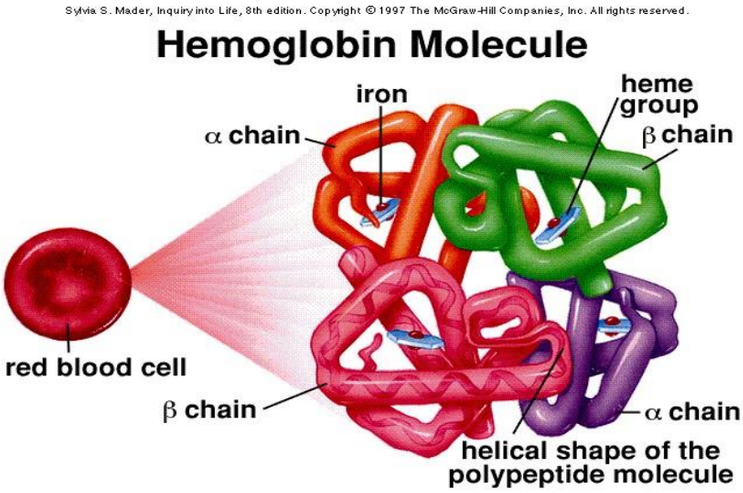
- **Hemoglobin is found exclusively in red blood cells, where its main function is to transport oxygen from the lungs to the capillaries of the tissues**
- **Hemoglobin A, the major hemoglobin in adults, is composed of four polypeptide chains**
- **Two alpha (α) chains**
- **Two beta (β) chains**

HbF: In infants, made up of 2 alpha chains and 2 gamma chains. HbA2: ($alpha_{2}delta_{2}$)

- **Which can be seen as a protein of two identical dimers $(\alpha\beta)_1$ and $(\alpha\beta)_2$**
- **The two polypeptide chains within each dimer are held tightly together, primarily by hydrophobic interactions**
- **Inter-chain hydrophobic interactions form strong associations between α-subunits and β-subunits in the dimers**
- **Ionic and hydrogen bonds also occur between the members of the dimer**
- **In contrast, the two dimers are able to move with respect to each other, being held together primarily by polar bonds**
- **Each subunit has stretches of α-helical structure,**

- And a binding pocket similar to that described for myoglobin
- Each subunit has stretches of α-helical structure,
- And a binding pocket similar to that described for myoglobin

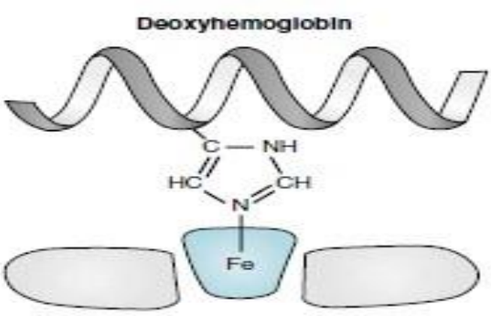




- However, the tetrameric hemoglobin molecule is structurally and functionally more complex than myoglobin
- For example, hemoglobin can transport CO_2 from the tissues to the lungs, and carry four molecules of O_2 from the lungs to the cells of the body

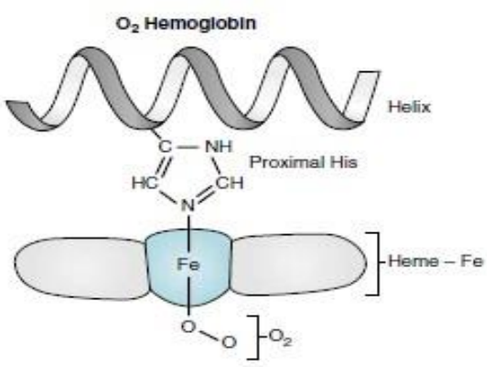
83 Reversible binding of a protein 7 (Conti....)

- The proximal His (F8 histidine)of myoglobin and hemoglobin is sterically repelled by the heme porphyrin ring.
- Thus, when the His binds to the Fe^{2+} in the middle of the ring, it pulls the Fe^{2+} above the plane of the ring.
- The interior contains only nonpolar residues such as Leu,Val, Phe, Met.with exceptions of His (polar)

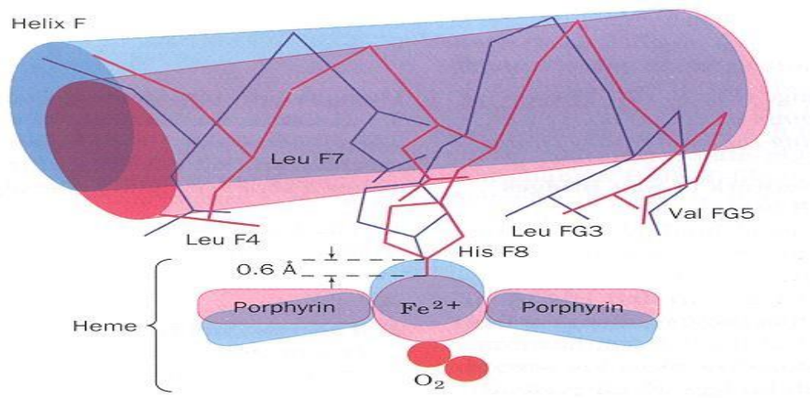


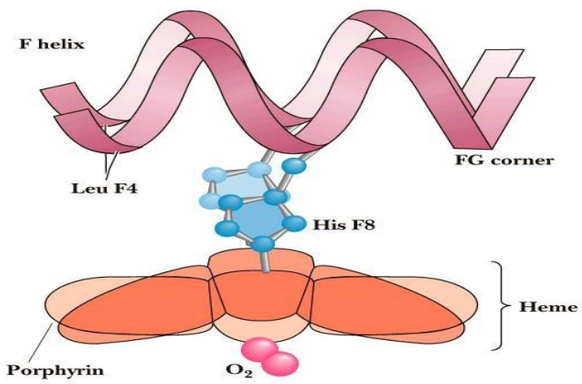
- When oxygen binds on the other side of the ring, it pulls the Fe^{2+} back into the plane of the ring.

- **The pull of O_2 binding moves the proximal histidine toward the porphyrin ring, which moves the helix containing the proximal histidine.**

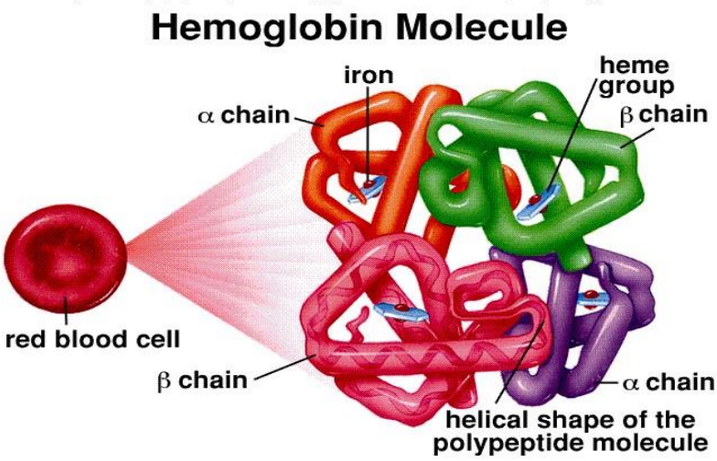


- **This conformational change has no effect on the function of myoglobin.**
- **However, in Hemoglobin this change is imperative for co-operative binding of oxygen to different subunits of hemoglobin.**
- **Shifting of amino acids by the oxygenation of one of the heme groups in the protein, (due to pull of porphyrin through His), alters the structure of the interfaces between the four subunits. This results in the change of shape of the whole protein.**
- **In the new shape, it is easier for the other three heme groups to become oxygenated.**
- **Thus, the binding of one molecule of O_2 to hemoglobin enhances the ability of hemoglobin to bind more O_2 molecules.**
- **This property of hemoglobin is known as "cooperative binding."**



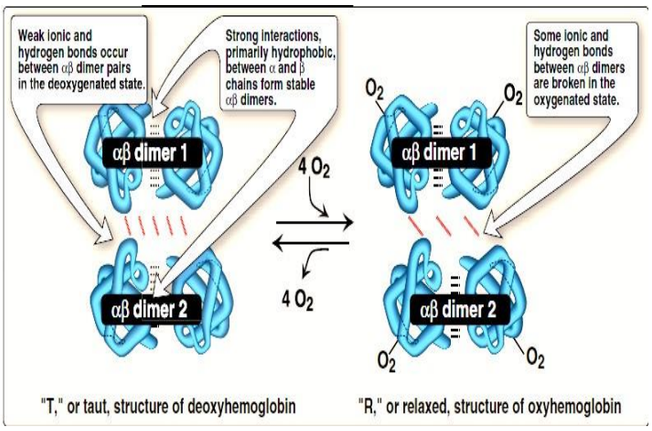


- **Cooperative binding of oxygen means that the binding of oxygen to one heme group enables an oxygen binding to the second heme group of the same hemoglobin molecule.**
- **Conversely, the oxygen release from one heme group facilitates the release of oxygen from other heme groups in the same hemoglobin molecule.**

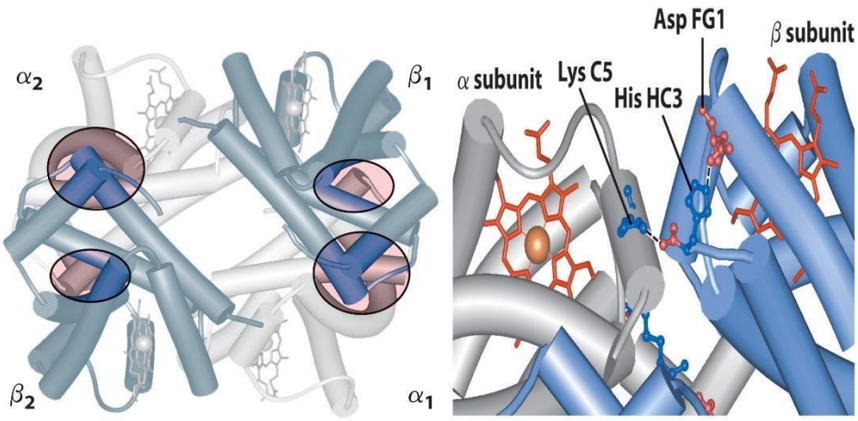


84 Reversible binding of a protein 8 (Conti....)

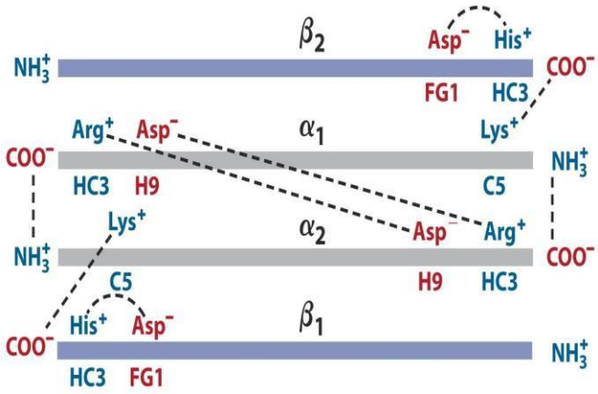
- **In hemoglobin, the movement of one helix after oxygen binding leads to the movement of other helices in that subunit, including one in a corner of the subunit that is in contact with a different subunit through ionic interactions.**
- **The loss of these ionic interactions, then induces conformational changes in all other subunits.**
- **And all four subunits may change in a concerted manner from their original conformation to a new conformation structure bringing cooperativity in oxygen binding in hemoglobin.**



As described previously, the binding of oxygen pulls the heme, which causes conformational change in the Polypeptide change, resulting in the loss of some of the ionic interactions in the different subunits of Hb thus increasing the affinity of the Hb for the further O2 addition.

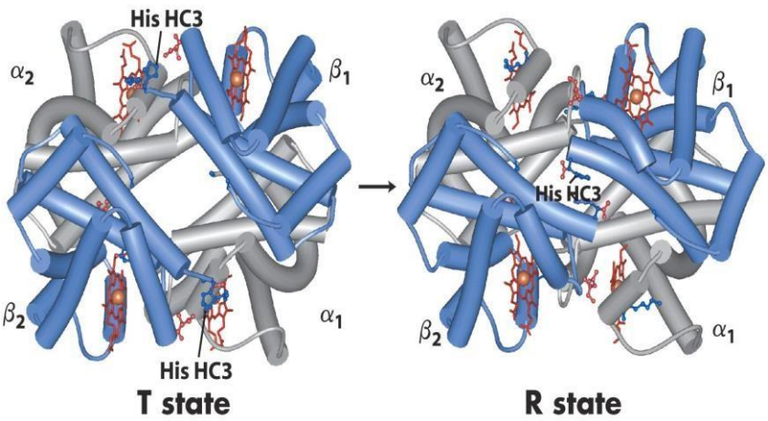


Left: Hemoglobin Structure Showing Inter chain Contact Points Right : One of the contact points magnified, showing Lys, Asp and His.



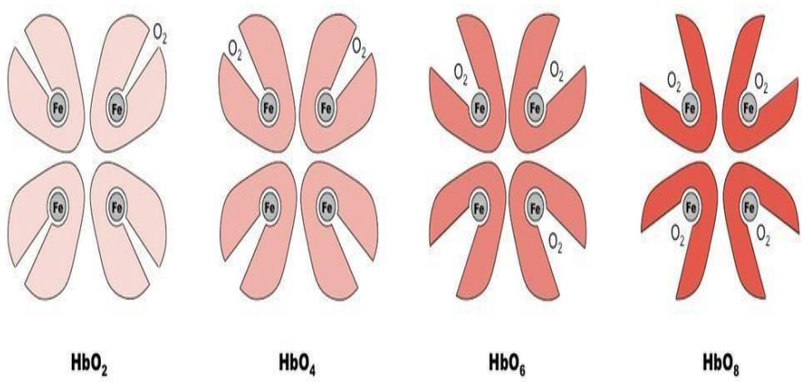
Contact Points in Primary Structure: These amino acids which are far apart from each other in primary sequence become close in the final folding and give rise to characteristic interactions In oxygenated and unoxygenated states.

- **The conformational change of hemoglobin is usually described as changing from a T (tense) state with low affinity for O_2 to an R (relaxed) state with a high affinity for O_2.**

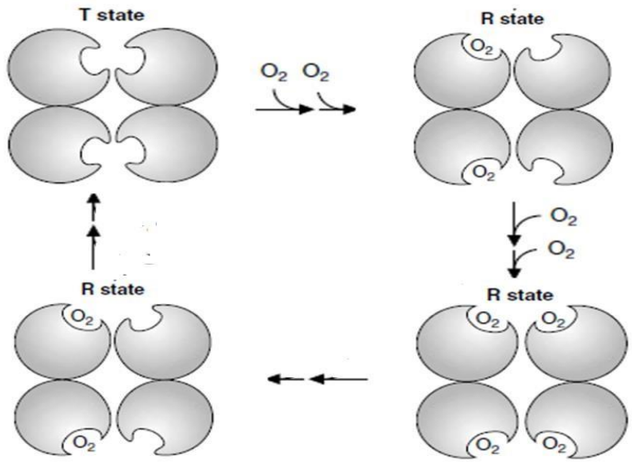


In the T subunits, the binding sites are hindered •

And in the R state the binding sites are open.



- **Each successive addition of O_2 shifts the equilibrium further toward the R state, thus addition of oxygen in the lungs.**
- **Similarly, removal of one oxygen facilitates the removal of subsequent oxygen molecule in the tissues.**



85 Reversible binding of a protein 9 (Conti....)

Comparison of oxygen binding to myoglobin and hemoglobin

- **Myoglobin can bind only one molecule of oxygen.**
- **Because it contains only one heme group**
- **In contrast, hemoglobin can bind four oxygen molecules-one at each of its four heme groups**

The degree of saturation (Y) of these oxygen-binding sites on all myoglobin or hemoglobin molecules can vary between zero (all sites are empty) and 100 percent

Oxygen dissociation curve

- **A plot of degree of saturation (Y) measured at different partial pressures of oxygen (pO_2) is called *the oxygen dissociation curve***
- **The curves for myoglobin and hemoglobin show important differences**
- **This graph illustrates that myoglobin has a higher oxygen affinity than does hemoglobin**

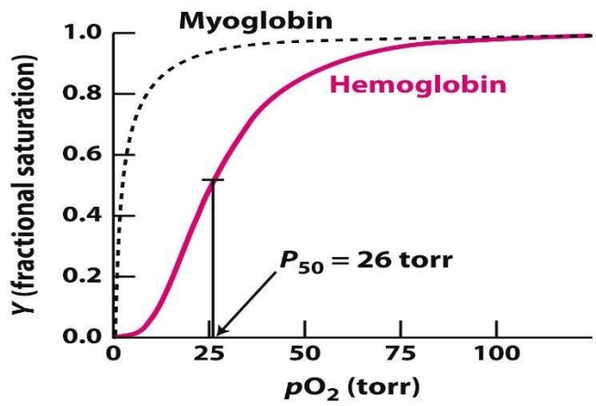


Figure 7.8
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1 torr= 1mmHg

- **Furthermore, the hyperbolic versus sigmoid curve, in myoglobin and hemoglobin respectively, also shows that binding of oxygen is co-operative in hemoglobin as compared to myoglobin.**
- **The *partial pressure of oxygen needed to achieve half-saturation of the binding sites is called (P_{50}).***
- ***P_{50}* is approximately 1 mm Hg for myoglobin and 26.6mm Hg for hemoglobin**
- **The partial pressure of oxygen in the blood at which the hemoglobin is 50% saturated, is known as the P_{50}.**
- **The P_{50} is a conventional measure of hemoglobin affinity for oxygen.**

- Values of P_{50} are negatively correlated with substrate affinity, with lower values of P_{50} corresponding to high affinity and *vice versa*.
- This means that the higher the oxygen affinity (that is the more tightly oxygen binds), the lower the P_{50}

86 Reversible binding of a protein 10 (Conti....)

Myoglobin

The oxygen dissociation curve for myoglobin has a hyperbolic shape

- This reflects the fact that myoglobin reversibly binds a single molecule of oxygen

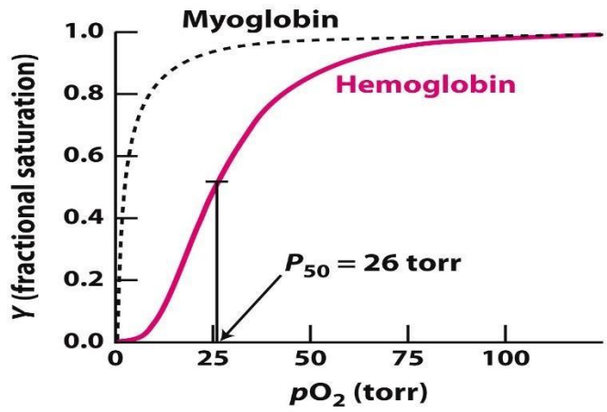

Figure 7.8
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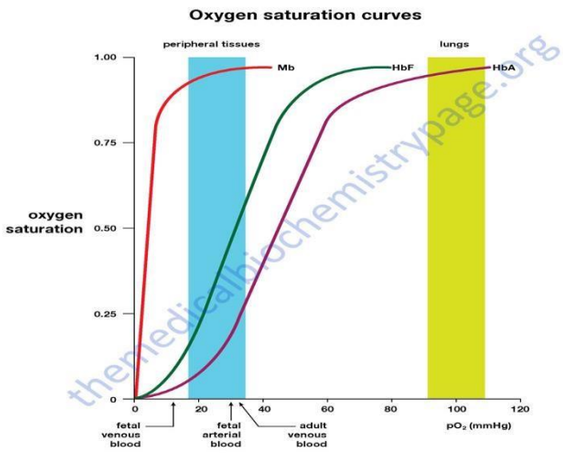
1 torr= 1mmHg Myoglobin

- Myoglobin is designed to release and bind oxygen at very low partial pressure.
- Oxygenated (MbO_2)and deoxygenated (Mb) myoglobin exist in a simple equilibrium:

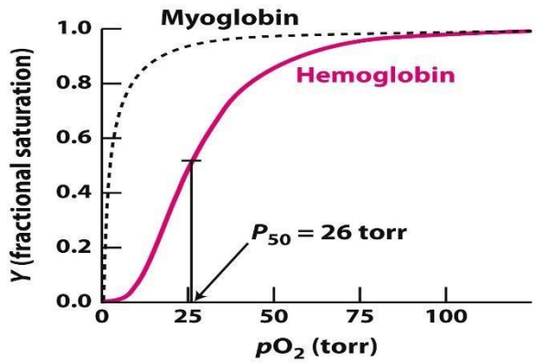
Mb + O2 MbO2

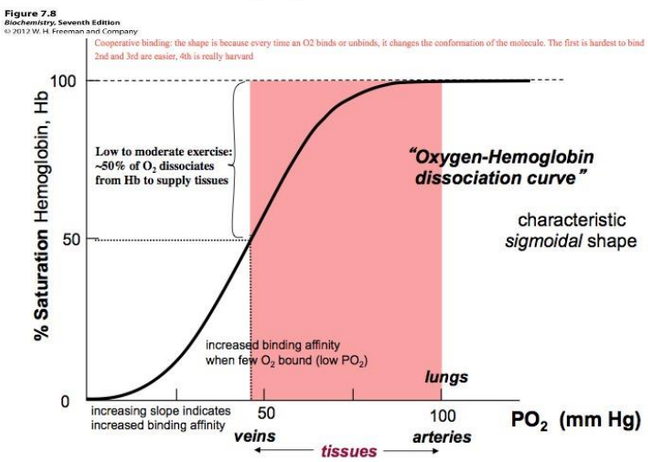
- The equilibrium is shifted to the right or to the left as oxygen is added to or removed from the system
- Myoglobin is designed to bind oxygen released by hemoglobin at the low pO_2 found in muscle
- Myoglobin, in turn, releases oxygen within the muscle cell in response to in oxygen demand during strenuous exercise.
- When strenuous exercise lowers the pO_2 of muscle tissue to about 5 mm Hg, myoglobin releases O_2 for mitochondrial synthesis of ATP, permitting continued muscular activity
- Myoglobin can load oxygen readily at the pO_2 of the lung capillary bed (100 mm Hg)
- However, since myoglobin releases its bound O_2 only at the pO_2 values typically encountered in active muscle (20 mm Hg), it represents an ineffective vehicle for delivery of O_2 to tissues ($P\ O_2$ = 40mmHg)



87 Reversible binding of a protein 11 (Conti....)

- **Hemoglobin Dissociation Curve**
- **Hemoglobin must bind oxygen efficiently in the lungs, where the pO_2 is about 100 mmHg,**
- **And release oxygen in the tissues, where the p O_2 is about 35 to 40 mmHg**

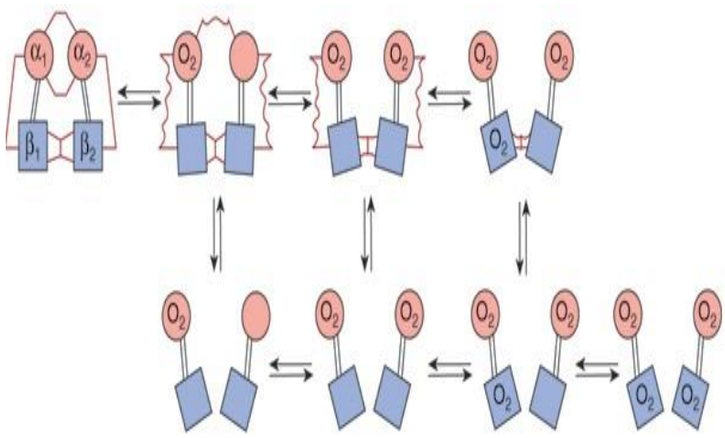




- **It is this sigmoidal loading and unloading of hemoglobin that makes it functionally useful as a on oxygen transporter in blood.**
- **Sigmoidal or sigmoid, literally means S-shaped**
- **The sigmoidal binding curve of Hemoglobin for oxygen is possible due to multisubunit structure of hemoglobin. i.e**
- **two alpha (α) and**
- **two beta (β) chains**

- **As described earlier binding of one O_2 molecule induces conformatory change in hemoglobin structure resulting in transition of hemoglobin from the low-affinity T**
  (taut) state to the high-affinity R (relaxed) state

- **These changes significantly increase the affinity of the remaining unoxygenated hemes for O_2, as subsequent binding require the disruption of fewer ionic interactions.**
- **The transition from T to R does not take place after a fixed number of oxygen molecules have been bound**
- **But becomes more probable as each successive oxygen binds**

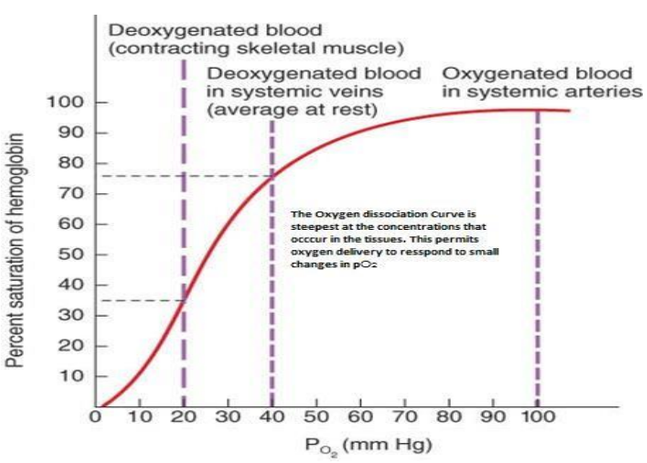


Every oxygen molecule that binds to Hb increases its affinity for the binding of further oxygen molecules.

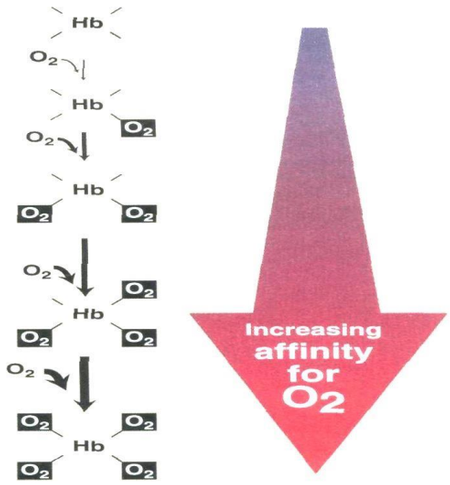
- **This effect is referred to as heme-heme interaction**

88 Reversible binding of a protein 12 (Conti....)

- **Hemoglobin Dissociation Curve**
- **Although it is more difficult for the first oxygen molecule to bind to hemoglobin.**
- **The subsequent binding of oxygen occurs with high affinity, as shown by the steep upward curve in the region near 20 to 30 mm Hg**



- **Cooperative binding of oxygen by the four subunits of hemoglobin means that the binding of an oxygen molecule at one heme group increases the oxygen affinity of the remaining heme groups in the same hemoglobin molecule**



- **The net effect is that the affinity of hemoglobin for the last oxygen bound is approximately 300 times greater than its affinity for the first oxygen bound**
- **In Summary,**
- **Hb must bind O_2 efficiently in the lungs (pO_2= 100 mm Hg), and release O_2 in the tissues (pO_2= 40 mm Hg).**
- **Hb solves the problem by undergoing a transition from a low-affinity state (the T state) to a high-affinity state (the R state) as more O_2 molecules are bound. •**

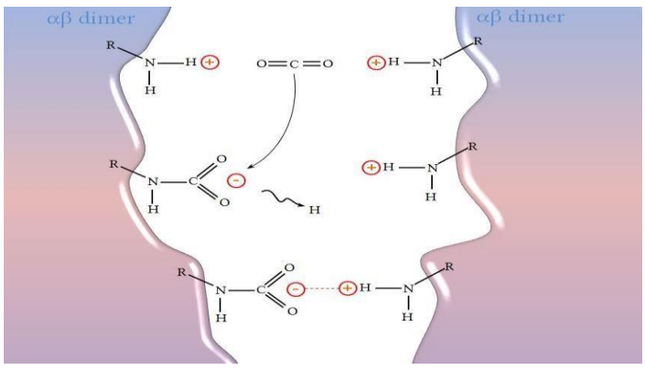
  Hb has a hybrid S-shaped, or sigmoid, binding curve for O_2

89 Reversible binding of a protein 13 (Conti....)

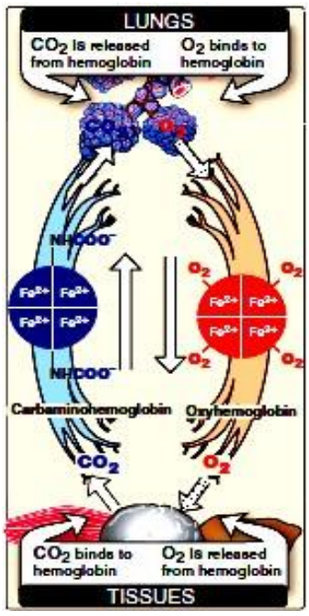
Allosteric effects

- **Apart from Oxygen, ability of hemoglobin to reversibly bind oxygen is affected by**
  - **the pH of the environment**
  - **the pCO_2 ; and**
  - **the availability of 2,3-bisphospho-phoglycerate**
- **These are collectively called Allosteric (other site) effectors**
- **because their interaction at one site on the hemoglobin molecule affects the binding of oxygen to heme groups at other locations on the molecule**
- **In Greek, Allo means other and Steric means Site.**
- **The binding of oxygen to myoglobin is not influenced by the allosteric effectors of hemoglobin**
- **In addition to transporting O_2 from the lungs to the peripheral tissues,**
- **hemoglobin transports CO_2, the by product of respiration,**
- **And protons from peripheral tissues to the lungs**
- **Transport of Carbon Dioxide**
- **Hemoglobin carries CO_2 as carbamates formed with the amino terminal nitrogens of the polypeptide chains.**

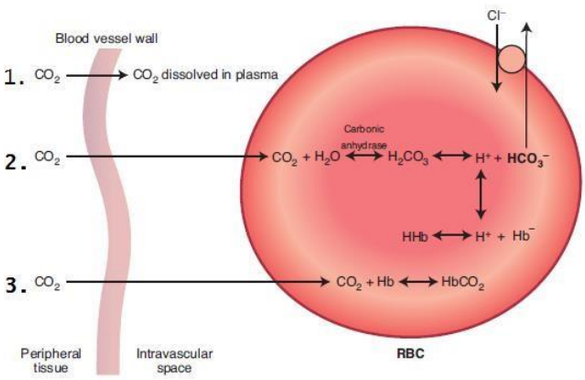
$$CO_2 + Hb{-}NH_3^+ \rightleftharpoons 2H^+ + Hb{-}\overset{\overset{H}{|}}{N}{-}\overset{\overset{O}{\|}}{C}{-}O^-$$



- **Carbamates change the charge on amino terminals from positive to negative, favoring ionic interaction formation between the α and β chains**
- **Hence, favoring the stabilization of taut form of deoxyhemoglobin**
- **Hemoglobin carbamates account for about 15% of the CO_2 in venous blood**



- **The remainder of the CO_2 is transported as dissolved**
- **HCO_3^- (80 to 85%) and**
- **dissolved CO_2 (<5%)**

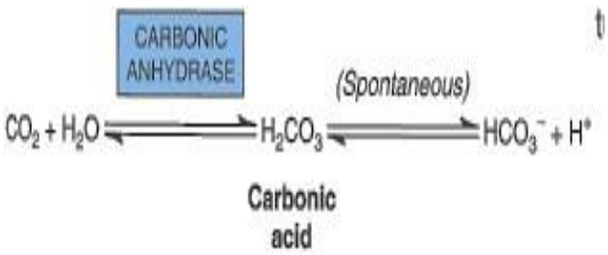


- **Modes of CO2 transport**
- **1: Dissolved CO2; 2: Dissolved bicarbonate 3: carbamate form**

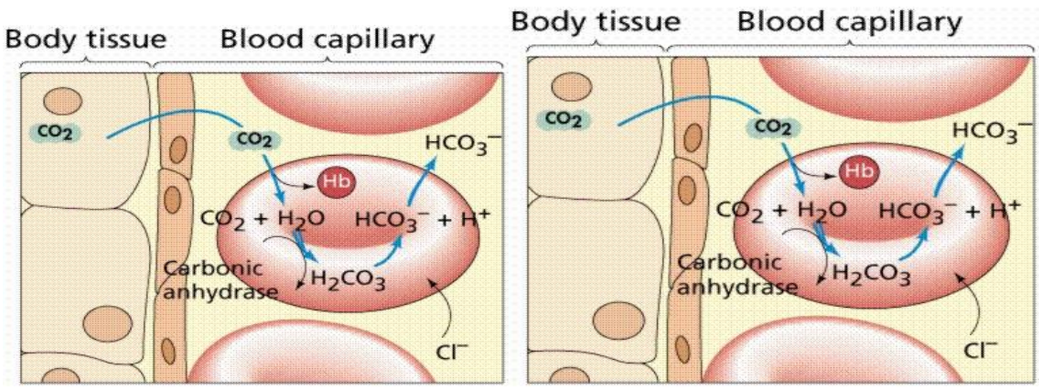
90 Reversible binding of a protein 14 (Conti....)

- **Transport of Carbon Dioxide (cont.)**

- Much of the remaining CO_2 is carried as bicarbonate.
- (80 to 85%)
- Bicarbonate is formed in erythrocytes by the hydration of CO_2 to carbonic acid (H_2CO_3)
- This process is catalyzed by carbonic anhydrase



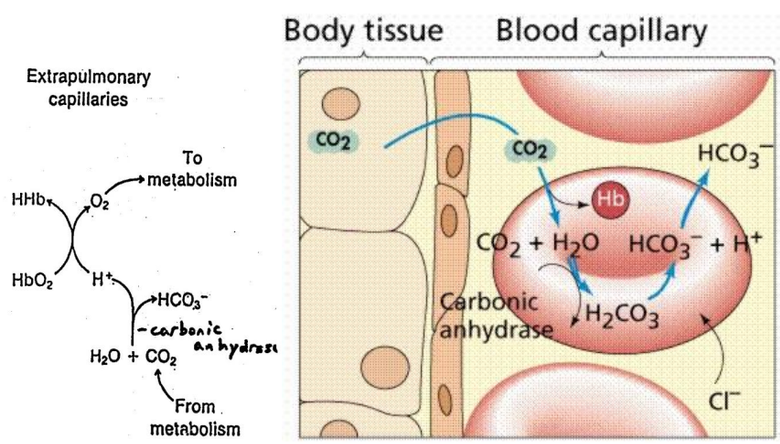
- At the pH of venous blood, H_2CO_3 dissociates into;
- bicarbonate and
- a proton

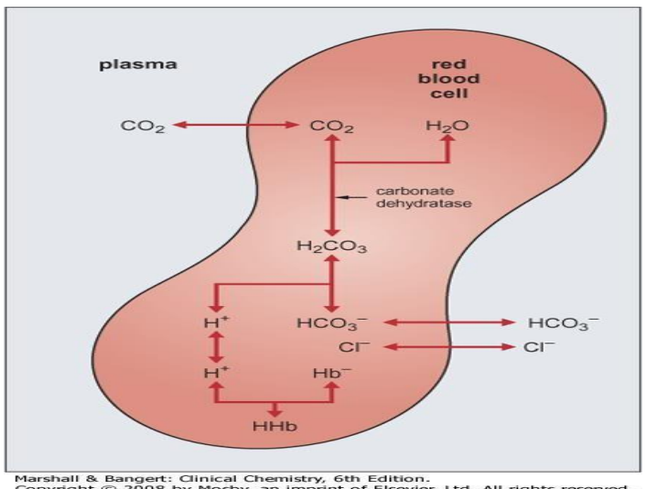


- Deoxyhemoglobin binds one proton for every two O_2 molecules released, contributing significantly to the buffering capacity of blood
- Oxygen and H^+ are not bound at the same sites in hemoglobin.
- However binding of proton, helps stabilize deoxyhemoglobin in the T state.
- The somewhat lower pH of peripheral tissues, aided by carbamation, stabilizes the T state and thus enhances the delivery of O_2
- This reciprocal coupling of proton and O_2 binding is termed the Bohr effect

91 Reversible binding of a protein 15 (Conti....)

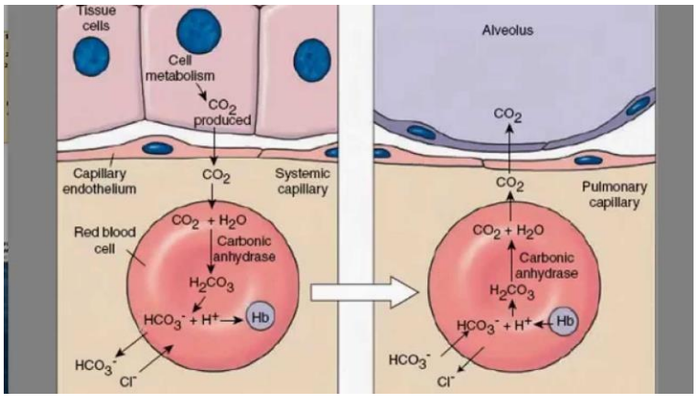
- This reciprocal coupling of proton and O_2 binding is termed the Bohr effect

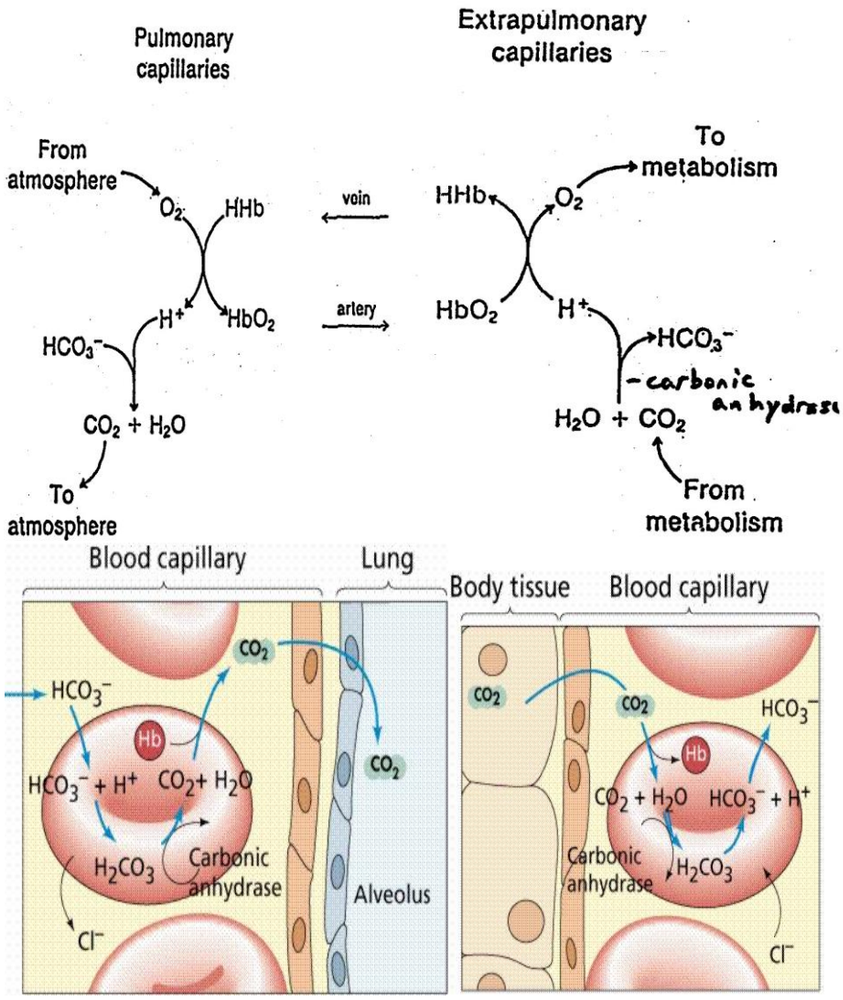




Chloride Shift: When bicarbonate is formed, chloride ions move into the RBCs, to maintain
electroneutrality This is referred to as Chloride shift.

- **In the lungs, the process reverses**
- **As O_2 binds to deoxyhemoglobin, protons are released and combine with bicarbonate to form carbonic acid**
- **Dehydration of H_2CO_3 catalyzed by carbonic anhydrase forms CO_2, which is exhaled**





- **Binding of oxygen thus drives the exhalation of CO_2**

92 Reversible binding of a protein 16 (Conti....)

- **Bohr effect**
- **Hemoglobin transports about 40% of the total H^+**
- **And 15% to 20% of the CO2 formed in the tissues to the lungs and the kidneys.**
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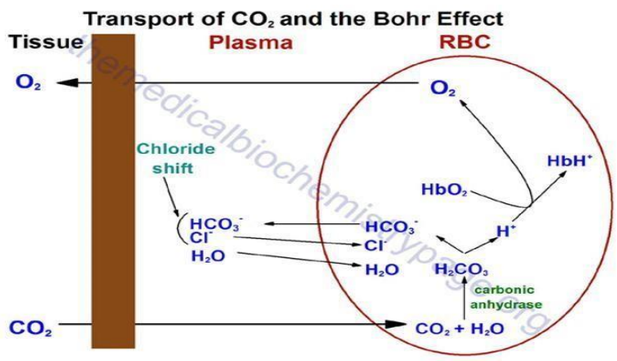


Bohr effect

- **The binding of H^+ and CO_2 is inversely related to the binding of oxygen.**

Bohr effect

- **At the relatively low pH and high CO_2 concentration of peripheral tissues, the affinity of hemoglobin for oxygen decreases as H^+ and CO_2 are bound, and O_2 is released to the tissues.**

Bohr effect

- **Conversely, in the capillaries of the lung, as CO_2 is exhaled**

Bohr effect

- **And the blood pH consequently rises, the affinity of hemoglobin for oxygen increases and the protein binds more O_2 for transport to the peripheral tissues.**

Bohr effect

- **This effect of pH and CO_2 concentration on the binding and release of oxygen by hemoglobin is called the Bohr effect, after Christian Bohr, the Danish Physiologist who discovered it in 1904**

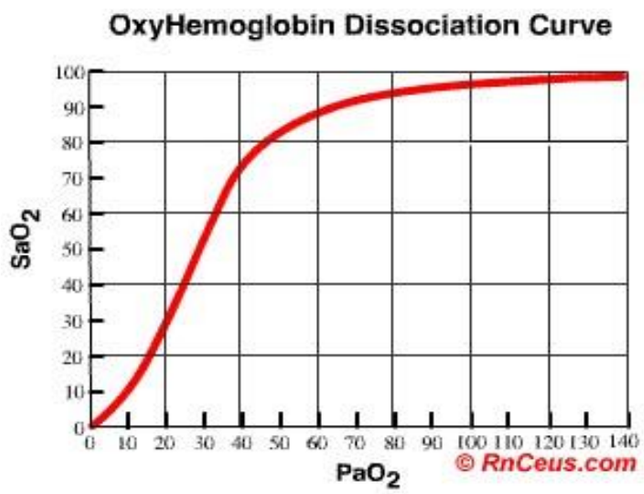
93 Reversible binding of a protein 17 (Conti....)

- **OxyHemoglobin Dissociation Curve**
- **Bohr effect decreased affinity of hemoglobin for oxygen caused by an increase of carbon dioxide pH etc.**

- **OxyHemoglobin Dissociation Curve**
- **Therefore oxyhemoglobin dissociation curve is displaced to the right because of higher partial pressure of carbon dioxide and lower pH.**

- **OxyHemoglobin Dissociation Curve**
- **This curve describes the relationship between available oxygen and amount of oxygen carried by hemoglobin.**
- **The horizontal axis is PaO_2, or the amount of oxygen available.**

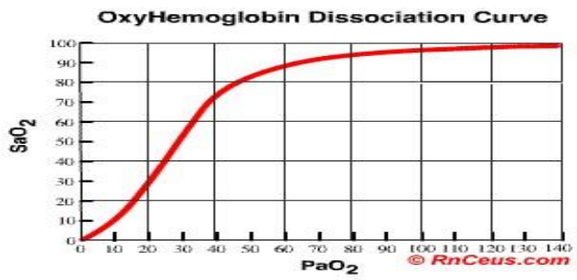
- **The vertical axis is SaO_2, or the amount of hemoglobin saturated with oxygen.**

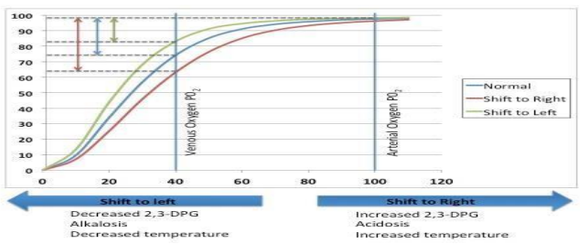


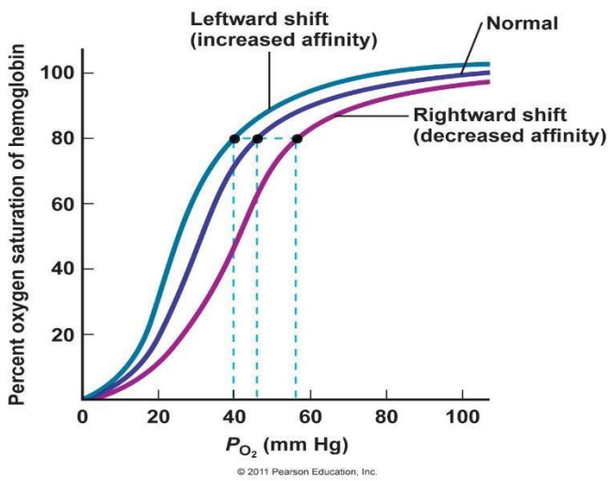
- **Once the PaO_2 reaches 60 mm Hg the curve is almost flat, indicating there is little change in saturation above this point.**
  - **So, PaO_2 of 60 or more is usually considered adequate.**
  - **But, at less than 60 mm Hg the curve is very steep, and small changes in the PaO_2 greatly reduce the SaO_2.**

- **The term "affinity" is used to describe oxygen's attraction to hemoglobin binding sites. Affinity changes with:**
  - **variation in pH,**
  - **temperature,**
  - **CO_2 and,**
  - **2,3,-BPG**
- **These are the metabolic by-products which competes with O_2 for binding sites.**
- **Traditionally the curve starts with:**
  - **pH at 7.4,**
  - **temperature at 37 Centigrade, and –**

  PaO_2 at 40.
- **Changes from these values are called "shifts**



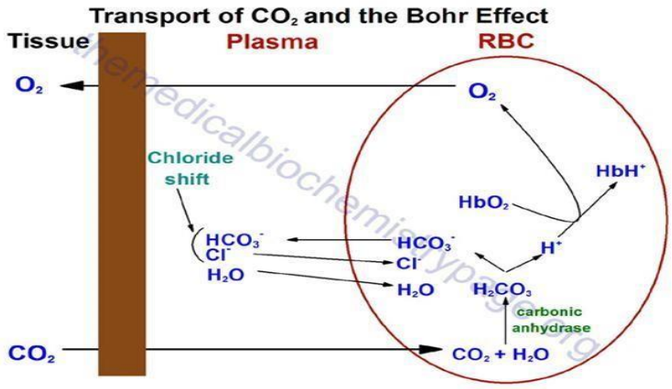




94 Reversible binding of a protein 18 (Conti....)

- **Bohr effect**
- **Hemoglobin transports about 40% of the total H^+**
- **And 15% to 20% of the CO2 formed in the tissues to the lungs and the kidneys.**
- **Bohr effect**
- **The remainder of the H^+ is absorbed by the plasma's bicarbonate buffer**

- And the remainder of the CO_2 is transported as dissolved HCO_3^- and CO_2.



Bohr effect

- The binding of H^+ and CO_2 is inversely related to the binding of oxygen.

Bohr effect

- At the relatively low pH and high CO_2 concentration of peripheral tissues, the affinity of hemoglobin for oxygen decreases as H^+ and CO_2 are bound, and O_2 is released to the tissues.

Bohr effect

- Conversely, in the capillaries of the lung, as CO_2 is exhaled

Bohr effect

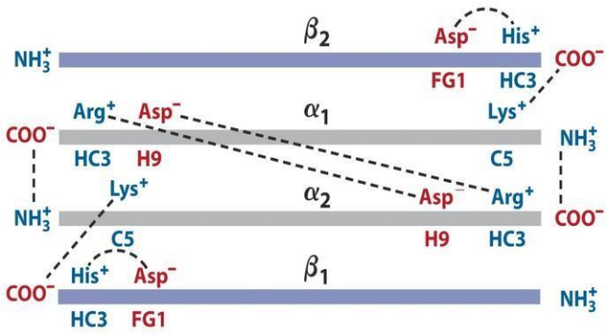
- And the blood pH consequently rises, the affinity of hemoglobin for oxygen increases and the protein binds more O_2 for transport to the peripheral tissues.

Bohr effect

- This effect of pH and CO_2 concentration on the binding and release of oxygen by hemoglobin is called the Bohr effect, after Christian Bohr, the Danish Physiologist who discovered it in 1904

95 Reversible binding of a protein 19 (Conti....)

- Proton Binding (Cont.)
- In the tissues, as the concentration of H^+ rises, (i.e. decrease in pH), protonation of His HC3 occurs.
- This protonation promotes release of oxygen by favoring a transition to the T state, subsequently resulting in oxygen unloading.
- In the lungs,
- the pH of blood is higher because CO_2 is being exhaled,
- And hemoglobin is being oxygenated due to higher partial pressure of oxygen in alveoli.
- The increase in pH,
- exhalation of CO_2
- and oxygenation of Hb;
- all result in; deprotonation of His HC3 of Hb.
- This disrupts the ionic interactions that stabilize the taut state
- And a new stabilized state of relaxed Hb is formed, resulting in oxygen loading.

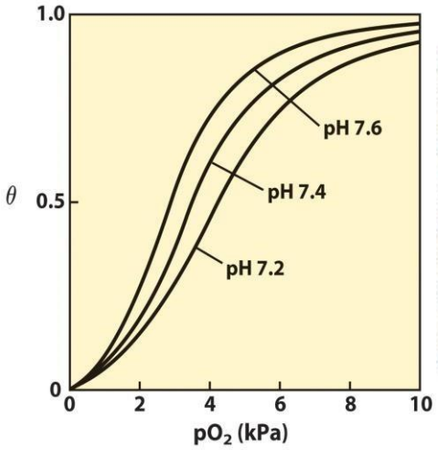


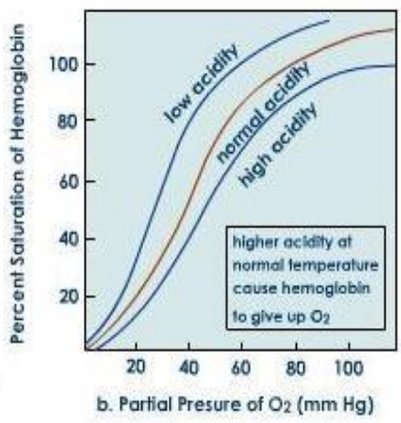
Some ion pairs that stabilize the T state of deoxyhemoglobin: Interactions between the ion pairs His HC3 and Asp FG.1 of the Beta subunit and between Lys C5 of the alpha Subunit and His HC3 of the Beta subunit are shown with dashed line.

- **Binding of O_2, protons and CO_2 to the Hb is a dynamic process, in which relative concentrations of each of the compounds in the different compartments of the body dictate the binding of each other.**
- **Thus we have seen that the four polypeptide chains of hemoglobin communicate with each other about not only O_2 binding to their heme groups**
- **But also H^+ binding to specific amino acid residues**

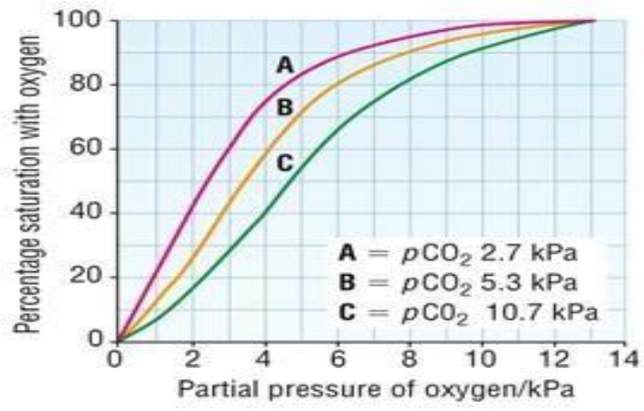
96 Reversible binding of a protein 20 (Conti....)

- **Proton Binding (cont.)**
- An increase in protons and\or a higher pCO_2 shifts the equilibrium to the right
- Therefore favoring deoxyhemoglobin T form.
- Whereas a decrease in pCO_2 and\or a decrease in protons shifts the equilibrium to the left
- Conversely, an increase in pO_2 promotes proton release





Decreased pH and increased CO2 shift the curve to right, i.e. . *increased* O2 unloading : Increased pH and decreased CO2 shift the curve to left i.e. *decreased* O2 unloading.



Decreased pH and increased CO2 shift the curve to right, i.e. . *increased* O2 unloading : Increased pH and decreased CO2 shift the curve to left i.e. *decreased* O2 unloading.

- The differential pH gradient (lungs having a higher pH, tissues a lower pH) favors the unloading of oxygen in the peripheral tissues,
- And the loading of oxygen in the lung
- In Summary,
- Binding of oxygen to facilitate other molecule of oxygen is called positive co-operativity.
- Whereas, binding of carbon dioxide and proton to release oxygen is called negative allostery, in which;
- increased CO_2 and H^+ ion favor oxygen unloading.
- Thus we see that the four polypeptide chains of hemoglobin communicate with each other about not only O_2 binding to their heme groups

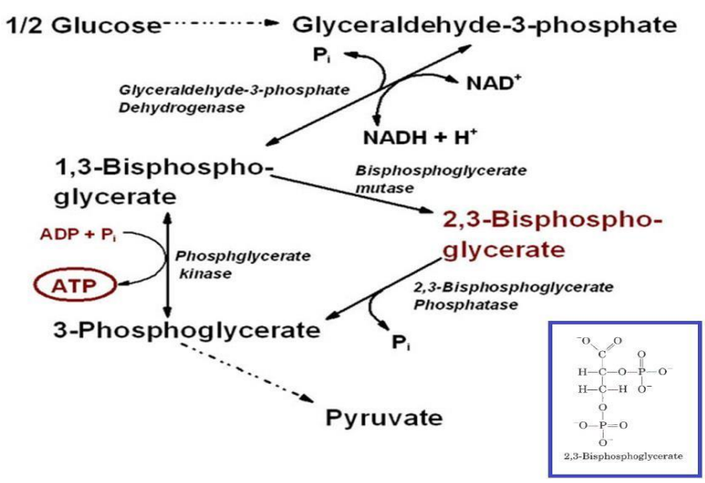
But also H^+ binding to specific amino acid residues and CO_2 binding to amino terminal.

It is the capacity to communicate ligand binding information from one polypeptide subunit to the others that makes the hemoglobin molecule so beautifully adapted to integrate the transport of O_2, CO_2 and H^+ by RBCs.

- And there is still more to the story.
- Many more interactions of different amino acids, are being identified as new techniques are employed to study these effects, which are out of the scope of this course.

97 Reversible binding of a protein 21 (Conti....)

- **2,3 bisphosphoglycerate**
- **The interaction of 2,3 bisphosphoglycerate (BPG) with hemoglobin further refines the function of hemoglobin**
- **and provides example of heterotropic allosteric modulation.**
- **2, 3 BPG is an alternate product of glycolysis and its concentration increases in RBCs in states of low oxygen delivery to the tissues.**
- **It is a negatively charged molecule which increases the stability of taut form of hemoglobin.**



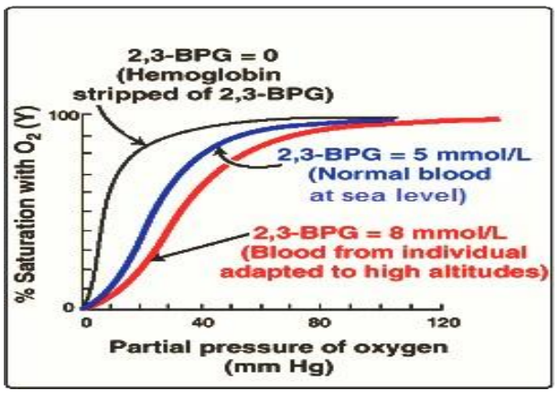
- **2,3-Bisphosphoglycerate is known to greatly reduce the affinity of hemoglobin for oxygen.**

-
-
- There is an inverse relationship between the binding of O_2 and the binding of BPG.
- BPG binds at a site distant from the oxygen-binding site
- And therefore regulates the O_2-binding affinity of hemoglobin in relation to the pO_2 in the lungs.

BPG is important in the physiological adaptation to the lower pO_2 at high altitudes

At sea level, the binding of oxygen to hemoglobin is regulated such that the amount of oxygen delivered to the tissues is nearly 40% of the maximum that could be carried by the blood

- At high altitude of hills and mountains, where the pO_2 is considerably lower (due to low atmospheric pressure).
- The delivery of oxygen to the tissues is now reduced.
- However, after just a few hours at the higher altitude, the BPG concentration in the blood begins to rise, leading to a decrease in the affinity of hemoglobin for oxygen.
- This adjustment in the BPG level has only a small effect on the binding of oxygen in the lungs
- But a considerable effect on the release of oxygen in the tissues shown by increased oxygen unloading at the peripheral tissues.



Increase in BPG concentration in the RBC shifts the dissociation curve to the right, i.e. increased O_2 unloading.

BPG concentration also increases in those conditions in which there is decreased O_2 delivery to tissues, such as
in anemia and respiratory diseases.

- As a result, the delivery of oxygen to the tissues is restored to nearly 40% of the oxygen that can be transported by the blood.

98 Reversible binding of a protein 22 (Conti....)

2,3 bisphosphoglycerate

(Count.)

- The site of BPG binding to Hb is the cavity between the beta subunits.
- This cavity is lined with positively charged amino acid residues that interact with the negatively charged groups of BPG.

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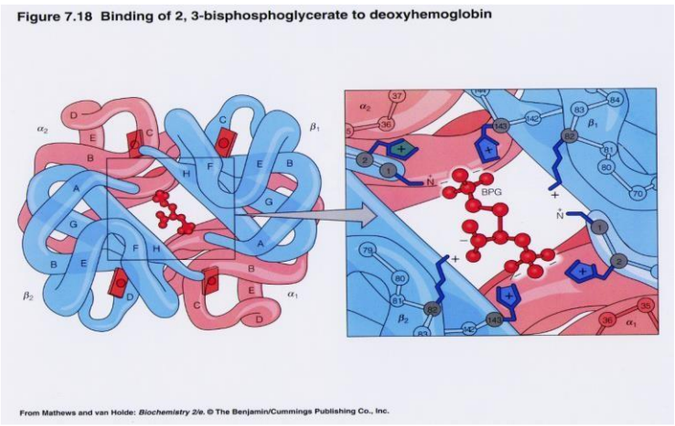
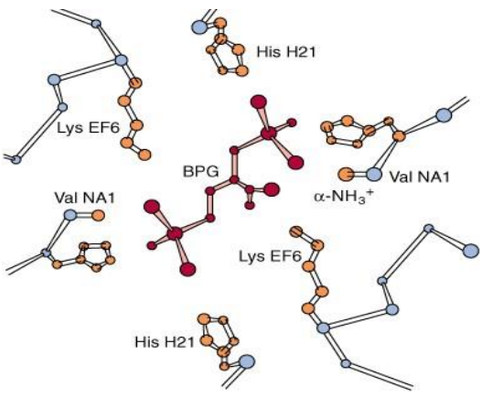


- **BPG forms ionic interactions with the terminal amino groups of both chains via Val NA1 and with Lys EF6 and His H21.**
- **NA is a non-helical portion between the start of amino terminal and start of A helix of beta chain.**
- **Val NA1 is the first amino acid in this non-helical portion.**



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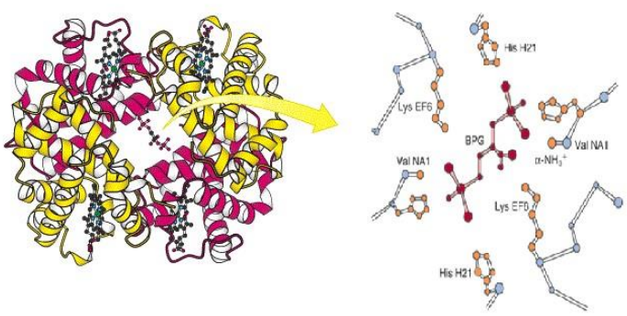
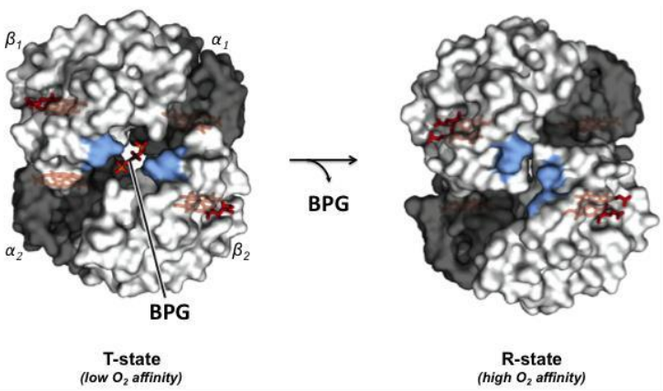
Val NA1 is the first amino acid in this non-helical portion.



- **BPG therefore stabilizes deoxygenated (T state) hemoglobin by forming additional ionic interactions that must be broken prior to conversion to the R state.**
- **Unlike oxygen, only one molecule of BPG is bound to each hemoglobin tetramer**
- **BPG lowers hemoglobin's affinity for oxygen by stabilizing the T state**
- **The transition to the R state narrows the binding pocket.**
- **Therefor BPG, precluding BPG binding.**



- **Residue H21 of the γ subunit of HbF is Ser rather than His which cannot form ionic interactions with BPG**
- **Therefore, BPG binds more weakly to HbF than to HbA.**
- **The lower stabilization afforded to the T state by BPG accounts for HbF having a higher affinity for O_2 than HbA**

99 Reversible binding of a protein 23 (Conti....

Sickle Cell Anemia

- **Sickle Cell Anemia is a molecular disease of hemoglobin.**
- **It demonstrates the importance of amino acid sequence in hierarchical structure of globular proteins, and thus their biological functions**

Sickle cell anemia is a genetic disorder caused by a single nucleotide alteration (a point mutation) in the β-globin gene.

- **This results in the production of altered hemoglobin.**
- **The point mutation in the DNA sequence result in the replacement of;**
- **Glutamate residue with Valine residue**
- **At position 6 of the β chain of hemoglobin.**

HBB Sequence in Normal Adult Hemoglobin (Hb A):

Nucleotide	CTG	ACT	CCT	GAG	GAG	AAG	TCT
Amino Acid	Leu	Thr	Pro	Glu	Glu	Lys	Ser
	3			6			9

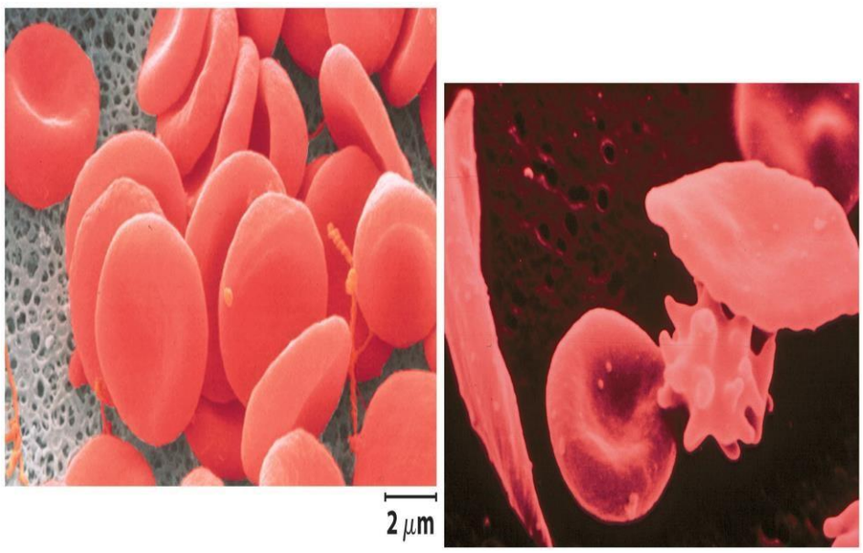
HBB Sequence in Mutant Adult Hemoglobin (Hb S):

Nucleotide	CTG	ACT	CCT	GTG	GAG	AAG	TCT
Amino Acid	Leu	Thr	Pro	Val	Glu	Lys	Ser
	3			6			9

- **Because humans generally have two copies of β-globin gene.**
- **An individual may have two copies of mutant allele (i.e. homozygous)**
- **or one copy of each of normal and mutant allele**

(i.e. heterozygous)

- **Sickle-cell anemia occurs in individuals who inherit the allele for sickle-cell hemoglobin from both parents.**
- **The resulting hemoglobin $\alpha_2\beta^s_2$, is referred to as HbS**
- **The RBCs of these individuals are fewer and also abnormal.**
- **In addition to an unusually large number of immature cells, the blood contains many long, thin, sickle shaped RBCs.**



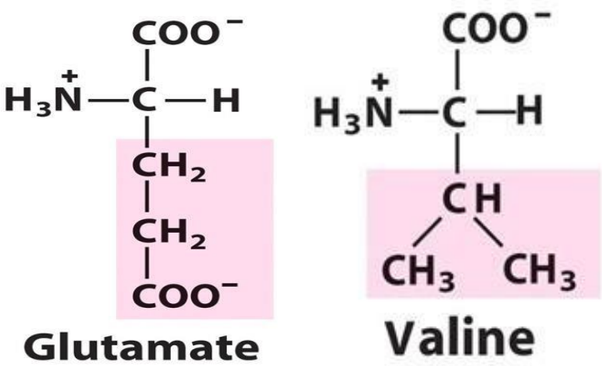
- When HbS is deoxygenated, it becomes insoluble and forms polymers that aggregate into tubular fibers.
- The insoluble fibers of deoxygenated hemoglobin S cause the deformed, sickle shape of the RBCS.
- The proportion of sickled cells increases greatly as blood is deoxygenated.
- Normal hemoglobin (hemoglobin A) remains soluble on deoxygenation.

100 Reversible binding of a protein 24

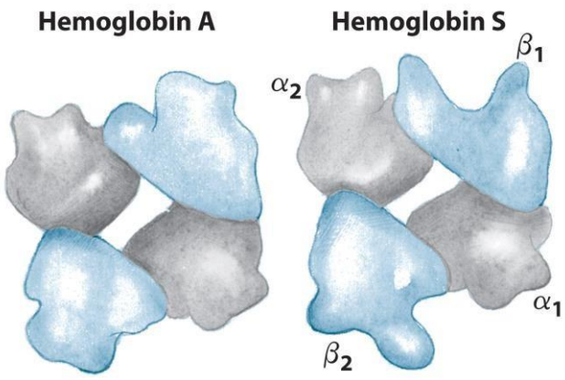
Sickle Cell Anemia

(Count.)

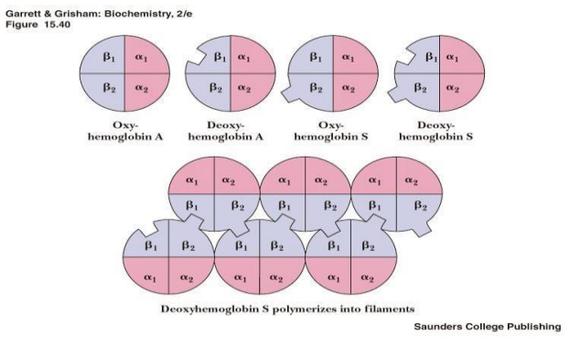
- The altered properties of hemoglobin S result from the replacement of Val instead of a Glu residue at position 6 in the two β chains.
- The R group of valine is neutral, whereas glutamate has a negative charge at pH 7.4.
- Hemoglobin S therefore has two fewer negative charges than hemoglobin A.



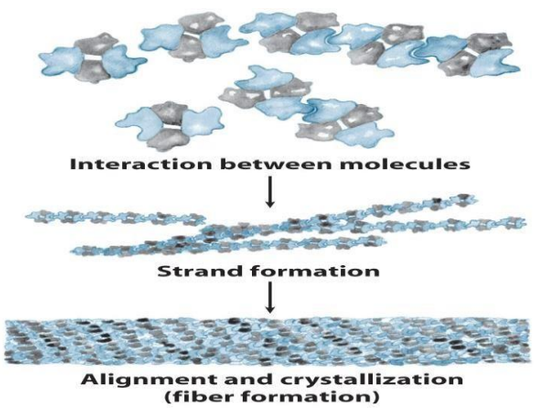
- Replacement of the Glu residue by Val creates a "sticky" hydrophobic contact point at position 6 of the β chain, which is on the outer surface of the molecule



- These sticky spots cause deoxyhemoglobin S molecules to associate abnormally with each other,
- Therefore forming the long, fibrous aggregates characteristic of this disorder.



- The polymerization of Hb S inside the cytosol of RBCs make RBCs sickle shaped and fragile..

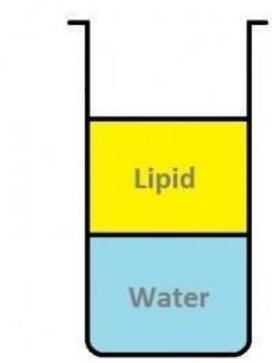


- Normal Hb A does not have this sticky hydrophobic pocket and therefore is soluble in the cytosol.
- The lifetime of an RBC homozygous for HbS is approximately 20 days, compared with 120 days for normal red blood cells.
- Sickled cells are very fragile and rupture easily; this results in anemia.
- Moreover, these cells have less ability to squeeze through small blood vessels resulting in the occlusion of micro blood vessels in the body, i.e. capillaries.
- Sickle-cell anemia, occurs in individuals homozygous for the HbS allele,
- Individuals who receive the HbS allele from only one parent (i.e. heterozygous) experience a milder condition called sickle-cell trait.
- Only about 1% of their RBCs become sickled on deoxygenation.

101 Lipid Chemistry

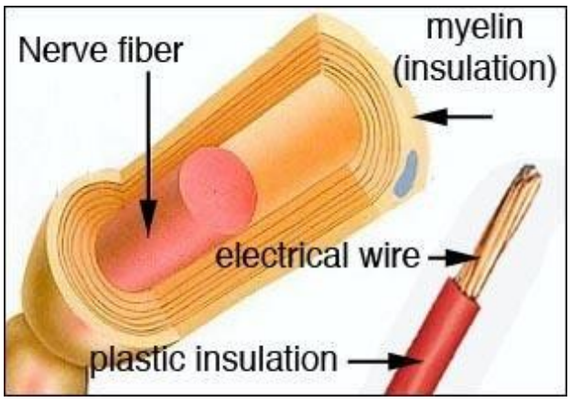
- The lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes,
- their derivatives
- and precursors,
- that are related more by their physical than by their chemical properties.
- They have the common property of being
- (1) Insoluble in water

- **(2) Soluble in nonpolar solvents such as ether and chloroform.**



Lipids are characterized by their hydrophobicity and their immiscibility in water

- **The biological functions of the lipids are as diverse as their chemistry.**
- **Fats and oils are the principal stored form of energy in many organisms.**
- **Phospholipids and sterols are major structural elements of biological membranes.**
- **Other lipids, present in relatively small quantities, play crucial roles as; enzyme cofactors, electron carriers, hydrophobic anchors for proteins, emulsifying agents in the digestive tract, hormones, and cellular messengers**
- **Adipose tissue Fat also serves as a thermal insulator in the subcutaneous tissues and around certain organs.**
- **Nonpolar lipids act as electrical insulators, allowing rapid propagation of depolarization waves along myelinated nerves**



Myelination with FAT greatly reduce the loss of charges from nerve fibre, resulting in a High speed transmission of nerve impulse.

- **The fat-soluble vitamins serve as co enzymes for many important reactions in the body.**
- **Combinations of lipid and protein (lipoproteins) serve as the means of transporting lipids in the blood**
- **Importantly, lipids provide the hydrophobic barrier that permits partitioning the aqueous contents of cells and subcellular structures as; phospholipids and sterols are the major structural elements of biological membranes.**

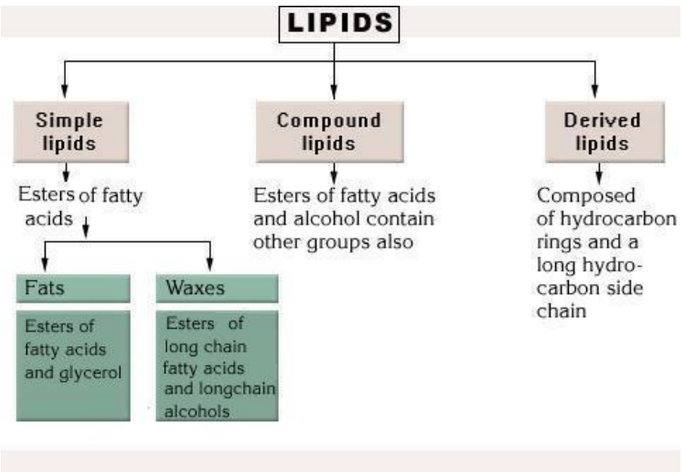
102 Lipid Chemistry Classification of lipids

CLASSIFICATIONS OF LIPIDS

- **As mentioned previously, lipids are characterized by their hydrophobicity.**
- **The precursors and derivatives of such compounds are included in classification of lipids.**

- **Lipids are classified in following three major groups:**

1. **Simple lipids**
2. **Complex Lipids and**
3. **Precursor and Derived Lipids**



1. Simple lipids:
Esters of fatty acids with various alcohols
These contain:

a. **Fats (and Oils) and**
b. **Waxes.**
c. **a. Fats: Esters of fatty acids with glycerol (Oils are fats in the liquid state)**
d. **b. Waxes: Esters of fatty acids with higher molecular weight monohydric alcohols.**
e. **(having one OH group)**

2. Complex lipids: Esters of fatty acids containing groups in addition to an alcohol and a fatty acid.

- **These include:**

a) **Phospholipids**
b) **Glycolipids**
c) **Other Complex lipids**
d) **a. Phospholipids: Lipids containing, in addition to fatty acids and an alcohol, a phosphoric acid residue**
e) **Phospholipids frequently have nitrogen containing bases and other substituent:**
f) **Glycerophospholipids the alcohol is glycerol.**
g) **Sphingophospholipids the alcohol is sphingosine.**

b. Glycolipids (glycosphingolipids):

- **Lipids containing a;**
- **fatty acid,**
- **sphingosine, and**
- **carbohydrate**

C. Other complex lipids: Lipids such as

- **sulfolipids**

- aminolipids
- lipoproteins may also be placed in this category

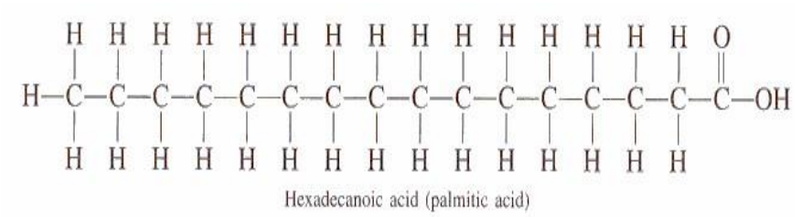
3.Precursor and derived lipids:

- **These include**
  - fatty acids
  - glycerol
  - steroids
  - other alcohols
  - fatty aldehydes
  - ketone bodies
  - lipid-soluble vitamins

103 Lipid Chemistry Fatty Acids

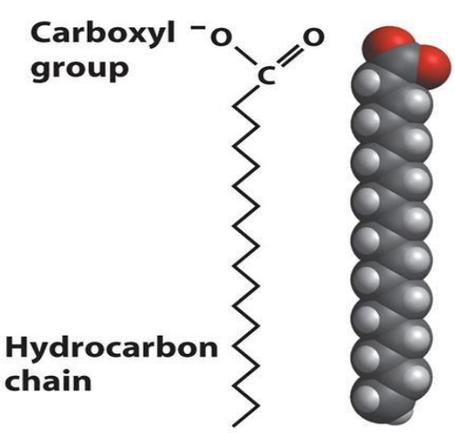
Fatty Acids

- **The fats and oils used almost universally as stored forms of energy in living organisms are derivatives of fatty acids**
- **Fatty acids are aliphatic carboxylic acids with hydrocarbon chains.**



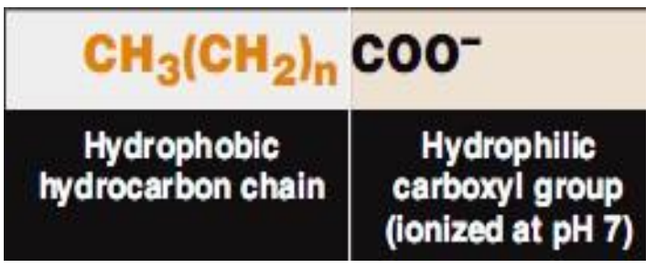
Basic structure of Fatty Acid: Long chain of hydrocarbon terminating in the carboxylic group.

Hexa: 6; Deca: 10 **Hexadecanoic acid: 16 carbon Fatty acid.**

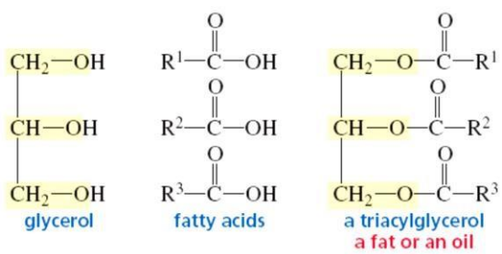


- **Fatty acids occur in the body mainly as esters in natural fats and oils,**
- **But are found in the unesterified form as free fatty acids, a transport form in the plasma.**
- **Fatty acids that occur in natural fats usually contain an even number of carbon atoms ranging from 4 to 36 carbons (C_4 to C_{36})**

- Fatty acids are named after the corresponding hydrocarbons.
- A fatty acid consists of
- a hydrophobic hydrocarbon chain with
- a terminal carboxyl group that has a pKa of about 4.8
- At physiologic pH, the terminal carboxyl group (-COOH) ionizes, becoming $-COO^-$



- This anionic group has an affinity for water, giving the fatty acid its amphipathic nature
- (having both a hydrophilic and a hydrophobic region)
- However, for long-chain fatty acids (LCFA), the hydrophobic portion is predominant
- These molecules are highly water-insoluble, and must be transported in the circulation in association with protein.
- More than ninety percent of the fatty acids found in plasma are in the form of;
- fatty acid esters (primarily triacylglycerol, cholesteryl esters, and phospholipids) contained in circulating lipoprotein particles



Triacylglycerol: An example of Fatty acid containing compound, R denotes the hydrocarbon chain.

- Unesterified fatty acids are transported in the circulation in association with albumin.
- Low levels of free fatty acids occur in all tissues,
- But substantial amounts sometimes can be found in the plasma, particularly during fasting.

104 Lipid Chemistry Classification Of Fatty Acids

Classification Of Fatty Acids:

- Fatty Acids are classified on the basis of :
- Hydrocarbon Chain Length
- Degree of Saturation
- Dietary Requirement
- Classification on the basis o dietary requirement include essential and non-essential fatty acids.

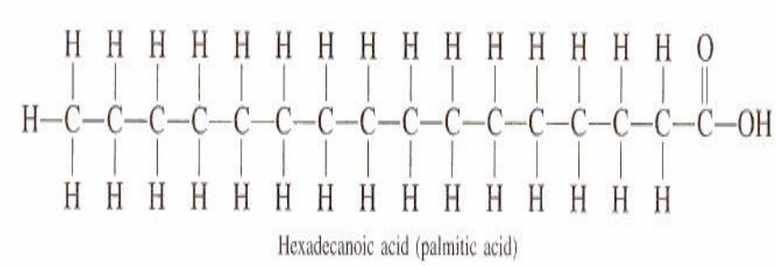
- **Classification On The Basis Of Hydrocarbon Chain Length**

These include

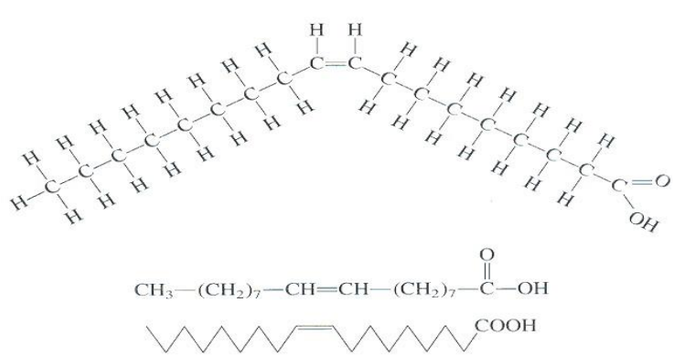
- **Short Chain Fatty Acids (2-4 C)**
- **Medium Chain Fatty Acids (6-12 C)**
- **Long Chain Fatty Acids (14-18 C)**
- **Very Long Chain Fatty Acids (18 and more C)**

Saturation of fatty acids

- **Fatty acid chains may contain**
- **no double bonds, that is be saturated, or**
- **contain one or more double bonds that is, be mono- or polyunsaturated**

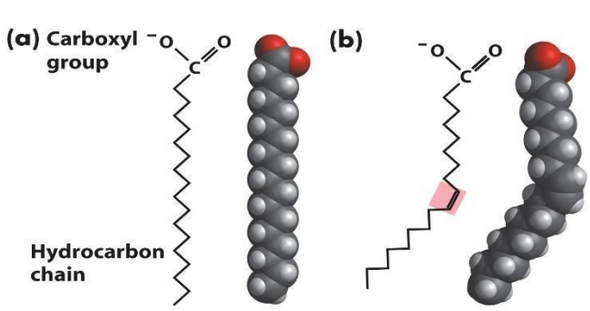


Hexadecanoic acid (palmitic acid)

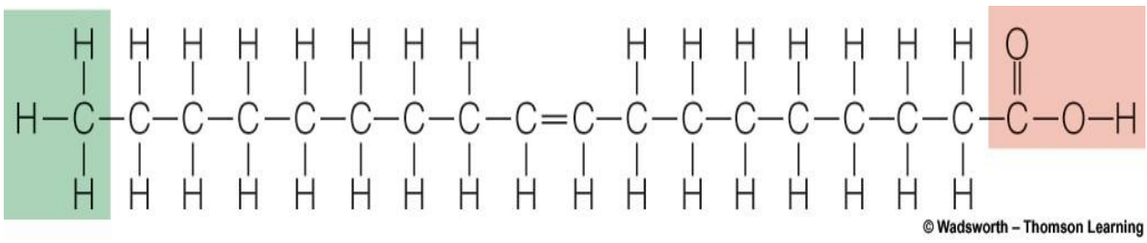


Oleic acid – 18-carbon

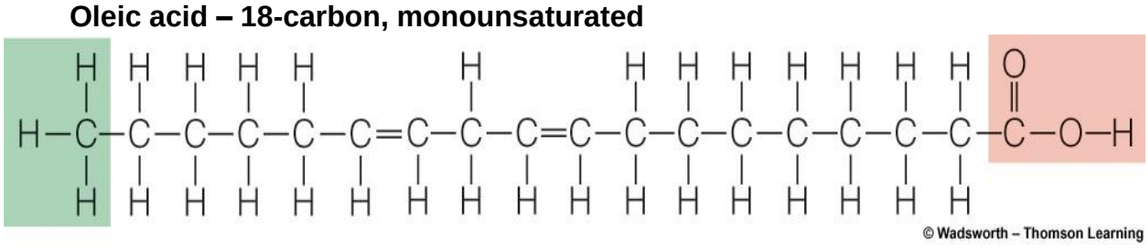
- **When double bonds are present, they are nearly always in the cis rather than in the trans configuration.**



- **If the fatty acid has two or more double bonds they are always spaced at three carbon intervals.**



Oleic acid – 18-carbon, monounsaturated



Linoleic acid – 18-carbon, polyunsaturated

- **Classification of Fatty Acid on Dietary Basis**
- **Fatty acids that are required by the human body but**
- **cannot be synthesized in the body, and**
- **therefore must be obtained from food, are called essential fatty acids.**
- **In contrast to amino acids, there are only two essential Fatty Acids:**
- **Linoleic Acid**
- **Alpha Linolenic Acid**
- **Humans lack the ability to introduce double bonds in fatty acids beyond carbons 9 and 10 as counted from the carboxylic acid side.**
- **Linoleic Acid has two double bonds at C9 and C12.**
- **Similarly, α-linolenic acid has three double bonds at C 9, 12 and 15.**
- **Therefore, both of these fatty acids can not be synthesized in the body and must be obtained in the diet.**
- **The rest of the Fatty Acids fall into the category of non-essential fatty Acids.**

105 Lipid Chemistry Nomenclature of Fatty Acids

Nomenclature

- **The most frequently used systematic nomenclature names the fatty acid after the hydrocarbon with the same number and arrangement of carbon atoms, with**

-oic being substituted for the final –e

- **Thus, saturated acids end in –anoic. Octane becomes Octanoic Acid.**
- **Unsaturated acids with double bonds end in -enoic, e.g. octadecenoic acid (C18) also called oleic acid.**
- **A simplified nomenclature (more aptly called as symbolic nomenclature) for unbranched fatty acids specifies**
- **the chain length and**
- **number of double bonds,**
- **separated by a colon.**

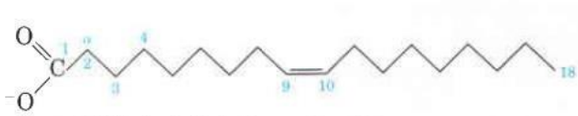
- The carbon atoms are numbered, beginning with the carboxyl carbon as C 1
  - The number before the colon indicates the total number of carbons in the chain,
- Those after the colon indicate the numbers and positions of double bonds.
- The 16-carbon saturated palmitic acid is abbreviated

16:0,

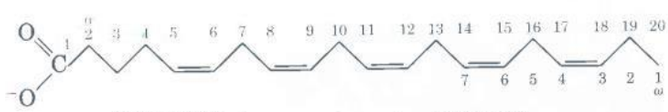
- The 18-carbon oleic acid, with one double bond, is

18:1

- The carbon to which the carboxyl group is attached, carbon 2 is also called the αcarbon,
- carbon 3 is the β-carbon, and
- carbon 4 is the γ-carbon
- The positions of any double bonds are specified relative to the carboxyl carbon by superscript numbers following Δ (delta)
- A 20-carbon fatty acid with two double bonds between C-9 and C-10 (C-1 being the carboxyl carbon) and another between C-12 and C-13 is designated • $20:2\ \Delta^{9,12}$



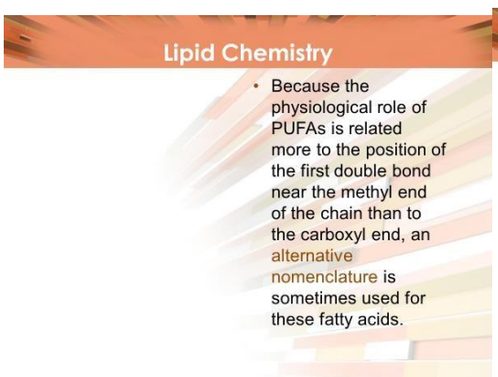
(a) 18:1(Δ^9) *cis*-9-Octadecenoic acid

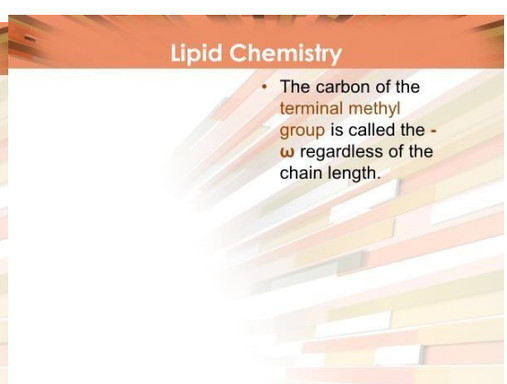


(b) 20:5($\Delta^{5,8,11,14,17}$) Eicosapentaenoic acid (EPA), an omega-3 fatty acid

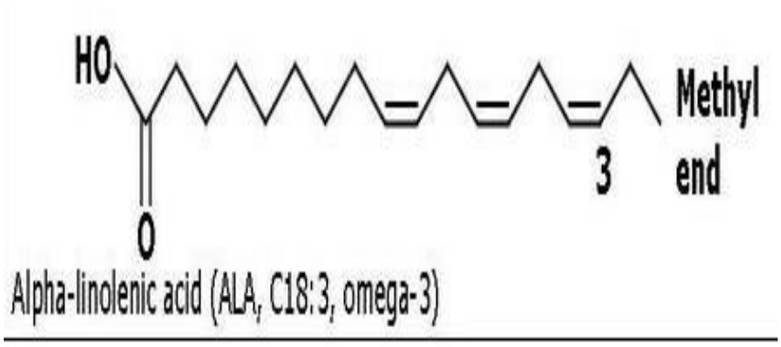
106 Lipid Chemistry Nomenclature of Fatty Acids (Conti....)

- The family of polyunsaturated fatty acids (PUFAs) with a double bond between the third and fourth carbon from the methyl end of the chain are of special importance in human nutrition





- Arachidonic acid $20:4\Delta^{5,8,11,14}$ is referred to as an ω-6 fatty acid because the closest double bond to the ω end begins six carbons from that end.
- The essential fatty acid linoliec acid, $18:2\ \Delta^{9,12}$ is ω-6 fatty acid.
- In contrast, α-linolenic acid, $18:3\Delta^{9,12,15}$ is an ω-3 fatty acid



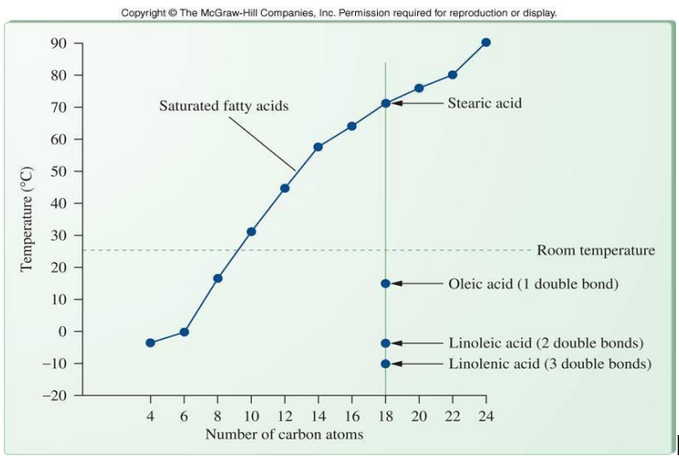
Alpha-linolenic acid (ALA, C18:3, omega-3)

107 Lipid Chemistry Physical and Chemical Properties of Fatty Acids

- Physical and Chemical Properties of Fatty Acids
- These are dictated by
- length of hydrocarbon chain and
- degree of saturation and unsaturation of Fatty Acids.
- The melting points T_m of even-numbered carbon fatty acids increase with increasing chain length and
- decrease with increasing unsaturation.
- A triacylglycerol containing three saturated fatty acids of 12 carbons or more is solid at body temperature, whereas
- if the fatty acid residues are 18:2, i.e. unsaturated it is liquid to below 0°C

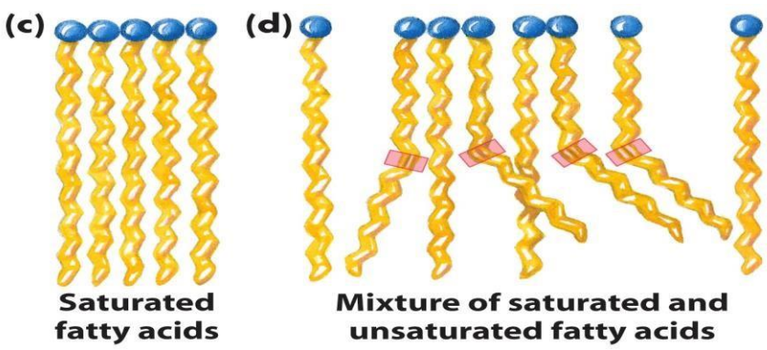
Carbon Atoms: Double Bonds	Common Name	Melting Point (°C)
Saturated Fatty Acids		
12:0	Lauric acid	44
14:0	Myristic acid	58
16:0	Palmitic acid	63
18:0	Stearic acid	70
20:0	Arachidic acid	77
Unsaturated Fatty Acids		
16:1	Palmitoleic acid	1
18:1	Oleic acid	16
18:2	Linoleic acid	-5
18:3	Linolenic acid	-11
20:4	Arachidonic acid	-49

- At room temperature (25^{0}C), the saturated fatty acids from 12:0 to 24:0 have a waxy consistency, whereas
- unsaturated fatty acids of these lengths are oily liquids.



Increasing chain length increases the melting temp. Stearic acid, Oleic acid, Linoleic acid and Linolenic acid all have 18 C, but with increasing no of Double bonds, which decreases the Tm

- **This difference in melting points is due to different degrees of packing of the fatty acid molecules due to;**
- **different proportions of saturated and unsaturated Fatty Acids**
- **Saturated fatty acids are more tightly packed as compared to**
- **Unsaturated Fatty Acids; which have a less compact packing.**



- **The more compact packing;**
- **the more attractive molecular forces between Fatty Acid molecules and vice versa.**
- **Hence, saturated Fatty Acids have high Tm and**
- **unsaturated Fatty Acids have low Tm.**
- **Therefore saturated and Unsaturated Fatty Acids help to Shape Foods**

108 Lipid Chemistry Physical and Chemical Properties of Fatty Acids (Conti....)

- **Physical and Chemical Properties of Fatty Acids (Contd.)**
- **The melting points (Contd.)**
- **The loose packing of unsaturated fatty acids is due to;**
- **cis isomerism of unsaturated fatty acids.**
- **Unsaturated fatty acids show**
- **Geometric isomerism, i.e.**
- **They are either**
- **cis or**
- **trans**

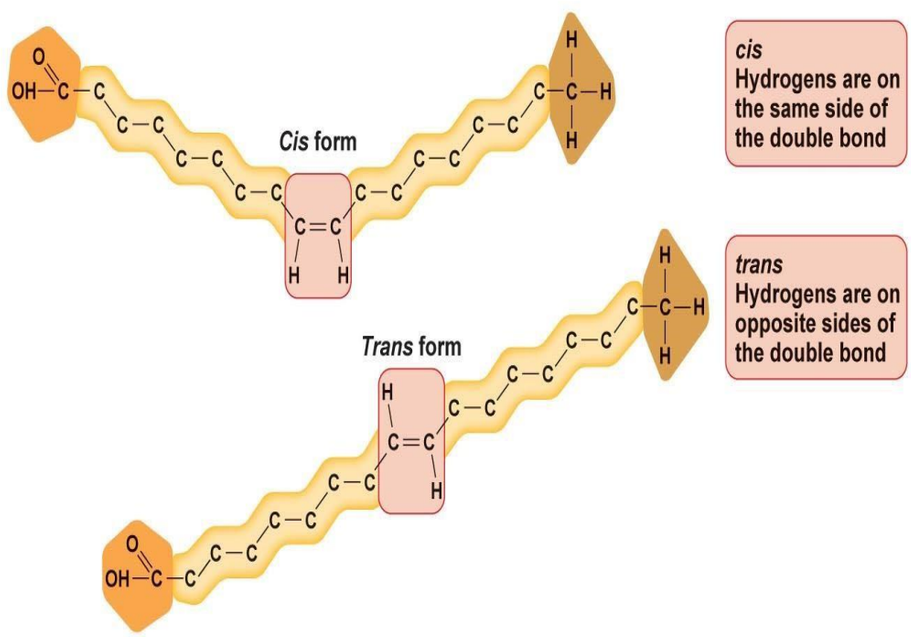
- **When double bonds are present, they are nearly always in the cis rather than in the trans configuration.**
- **A cis configuration means that the two hydrogen atoms adjacent to the double bond stick out on the same side of the chain.**
- **Unsaturated fatty acids can be cis with bulky groups on same side of C=C.**
- **Unsaturated fatty acids can be cis with bulky groups on same side of C=C.**
- **CH3– (CH2)5 (CH2)7– COOH *cis***
-
- **C=C**
- **H H**
- **trans have bulky groups on opposite sides of C=C.**

CH3– (CH2)5 H

-

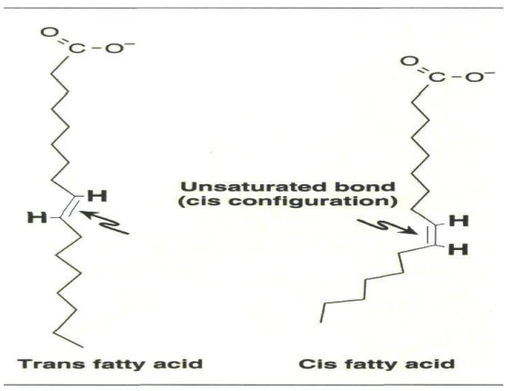
C=C *trans*

- **H $(CH_2)_7$– COOH**
- **Cis configuration leads to kink, which causes loose packing, have low Tm.**

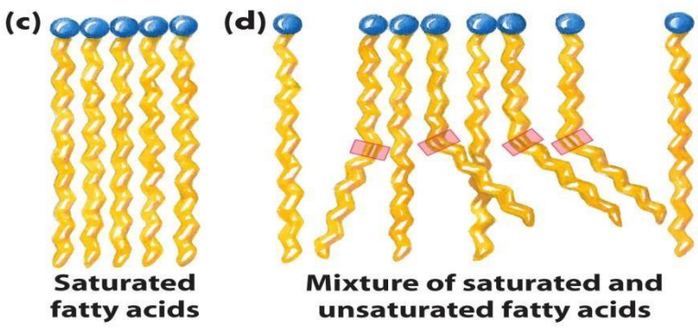


cis configuration,the molecules bent120 degrees at the doublebond.Trans 110 degree

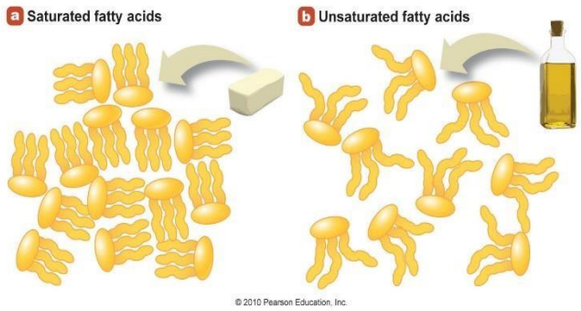
In unsaturated fatty acids, a cis double bond forces a • kink in the hydrocarbon chain



- Fatty acids with such kinks
- cannot pack together as tightly as fully saturated fatty acids,
- resulting in weak interaction with each other.



- In practice, natural acylglycerols contain a mixture of fatty acids tailored to suit their functional roles
- The membrane lipids, which must be fluid at all environmental temperatures, are more unsaturated than storage lipids.



Saturated; All the carbons on the fatty acid are bound to hydrogen. Solid at room tmp.
Higher melting point

- *Trans* fatty acids are present in certain foods, arising as a by-product of the saturation of fatty acids during hydrogenation, or "hardening," of natural oils in the manufacture of margarine.
- An additional small contribution comes from the ingestion of ruminant fat that contains *trans* fatty acids arising from the action of microorganisms in the rumen.

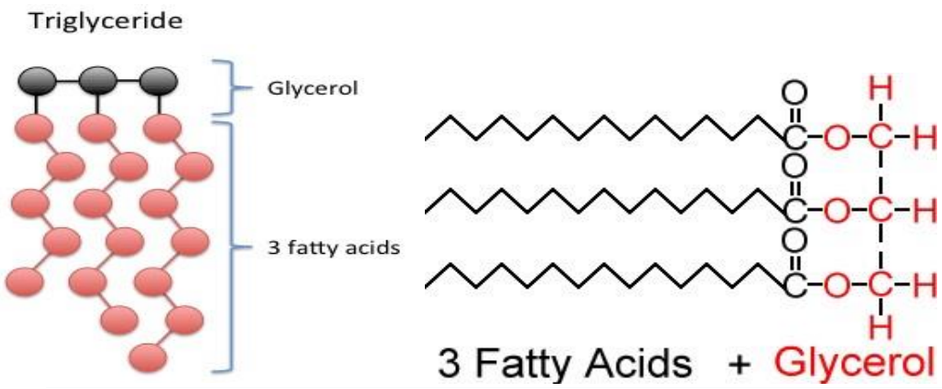
109 Lipid Chemistry Physical and Chemical Properties of Fatty Acids 1(Conti....)

- Physical and Chemical Properties of Fatty Acids (Contd.)
- Water Solubility

- **The presence of nonpolar hydrocarbon chain results in the poor water solubility of fatty acids.**
- **The larger the fatty acyl chain**
- **and the fewer the double bonds,**
- **the lower is the solubility in water.**
- **The carboxylic acid group is polar and**
- **ionized at neutral pH and**
- **accounts for the slight solubility of**
- **short-chain fatty acids in water**

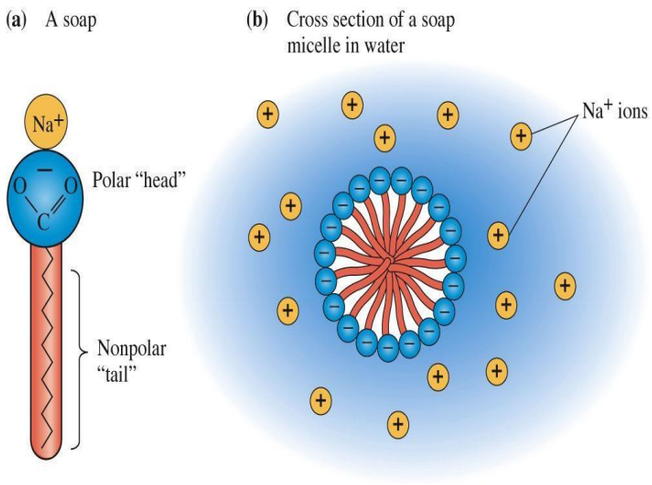
Melting Points and Solubility in Water of Fatty Acids

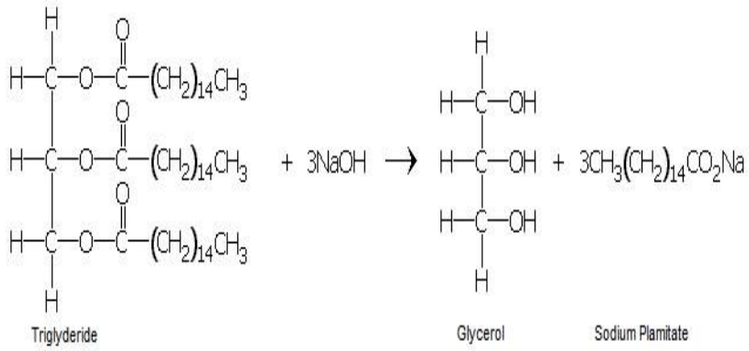
- **Formation of Esters**
- **In combination with alcohol Fatty Acids form Esters.**
- **For example, fatty acids react with glycerol to form mono, di and tri glycerides.**



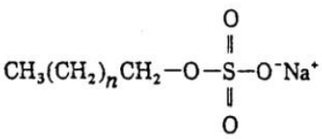
110 Lipid Chemistry Physical and Chemical Properties of Fatty Acids 2 (Conti....)

- **Physical and Chemical Properties of Fatty Acids (Contd.)**
- **Formation of Salts**
- **Fatty Acids forms salts with alkali metals and alkaline earth metals.**
- **Salts of Na, K, Ca and Mg are called soaps.**
- **A soap micelle: nonpolar (hydrophobic) hydrocarbon chains cluster in the inside and polar (hydrophilic) carboxylate groups lie on the surface.**

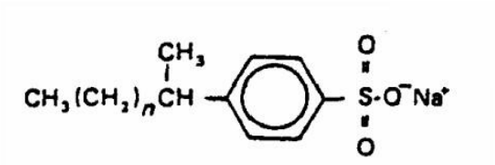




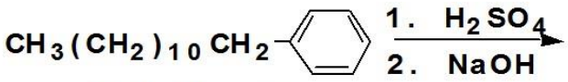
- **The Na and K salts are water soluble,**
- **Whereas, Ca and Mg salts are insoluble.**
- **K salts are too soluble and are used in liquid soap preparations.**
- **Formation of Detergents**
- **Reduction of the carboxyl group of fatty acids produce alkyl alcohols**
- **which can be sulfated (OSO_3H) or sulfonated (SO_3H) to produce detergents.**



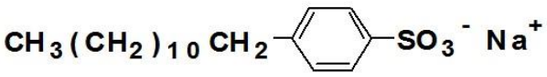
a sodium alkyl sulfate



a sodium alkylbenzene sulfonate

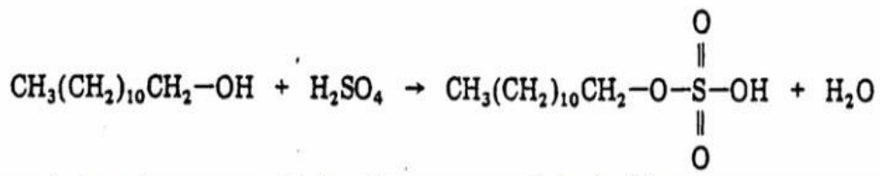


Dodecylbenzene



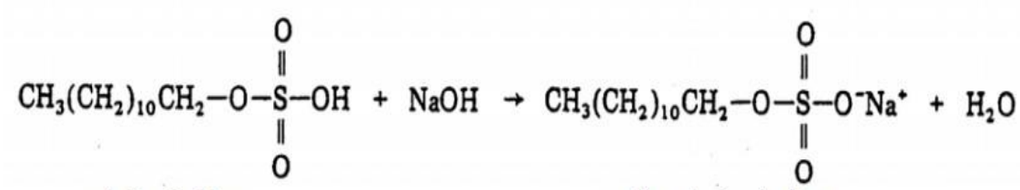
Sodium 4-dodecylbenzenesulfonate
(an anionic detergent)

A synthetic detergent, a sodium alkyl sulfate called sodium dodecylsulfate, can be prepared by reacting dodecyl alcohol (dodecanol) with sulfuric acid.



dodecanol sulfuric acid dodecylsulfate

The resulting dodecylsulfate is converted to the sodium salt by a reaction with sodium hydroxide.

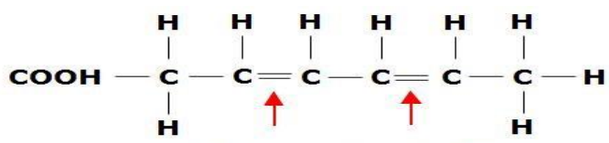


dodecylsulfate sodium dodecylsulfate

Lauric acid or dodecanoic acid, the saturated fatty acid **with a 12-carbon atom chain, thus falling into the**medium chain fatty acids

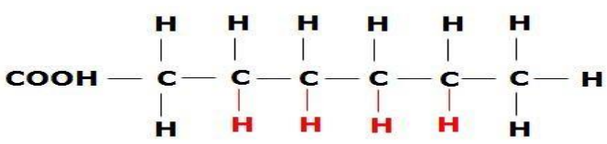
111 Lipid CHemistry Special Reactions of Unsaturated Fatty Acids

- **Special Reactions of Unsaturated Fatty Acids**
- **Hydrogenation**
- **It is the addition of Hydrogen at the**
- **double bonds of unsaturated fatty acids.**



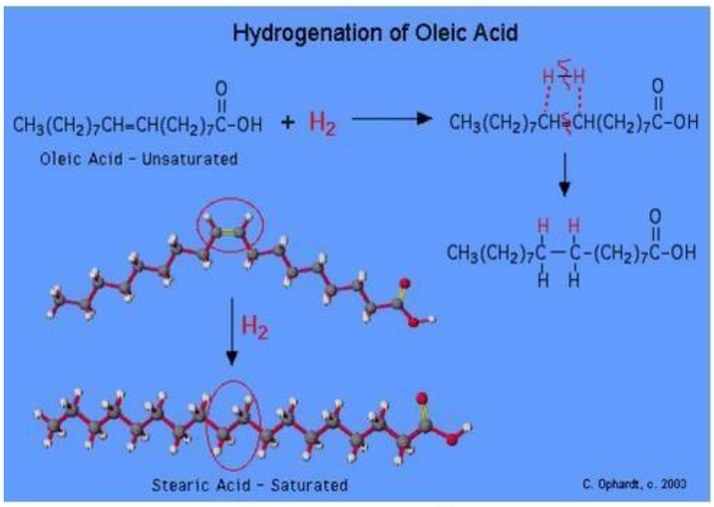
Polyunsaturated Fat

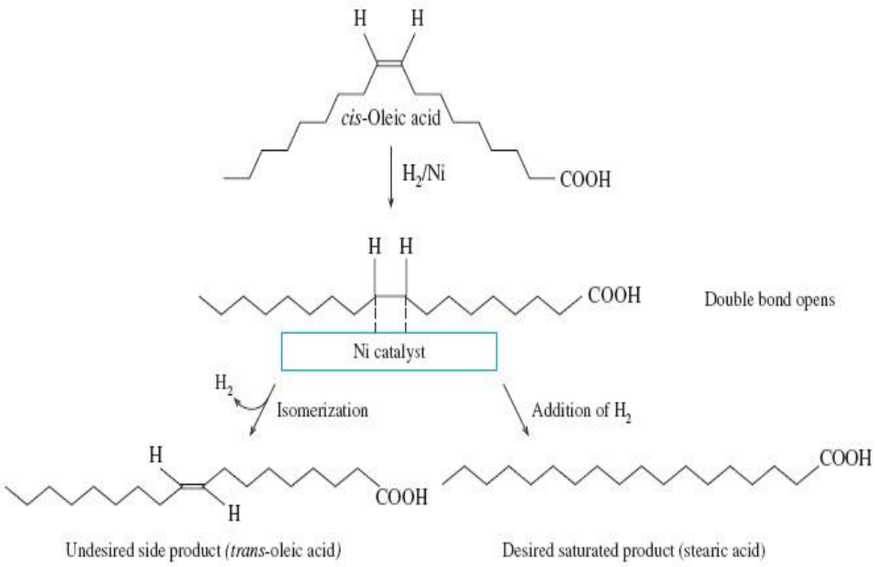
Hydrogenation



Hydrogenated Fat

- **Hydrogenation converts**
- **Unsaturated fatty acids to • Saturated fatty acids.**





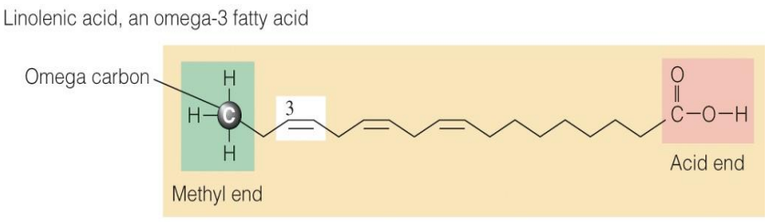
- **Margarines are vegetable oils**
- **treated with partial hydrogenation to**
- **form semi- solid consistency 'butter'**
- **Hydrogenated fats are used by many commercial food producers to**
- **Provide rich texture**
- **Increase shelf live**
- **Increase melting point**
- **Resistance to oxidation and flavour deterioration**
- **Partial hydrogenation has an undesirable, effect:**
- **some cis double bonds are converted**
- **to trans double bonds.**
- **There is a strong evidence that dietary intake of trans fatty acids**
- **(often referred to as "trans fats")**
- **leads to a higher incidence of cardio-vascular disease,**
- **therefore these fats should be avoided in the diet.**
- **Halogenation**
- **Similar to hydrogen,**

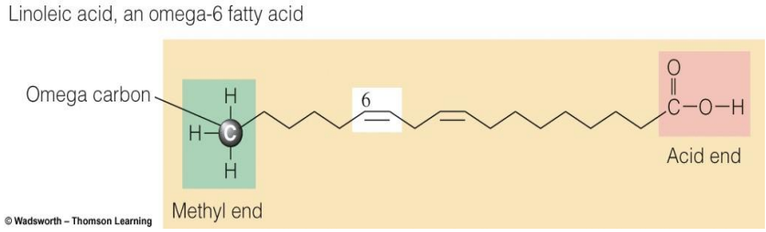
- **Halogens such as chlorine, bromine and iodine can also be added to double bonds in**
- **unsaturated fatty acids.**
- **Degree of halogenation is a**
- **good index of**
- **degree of unsaturation of Fatty Acids**
- **The number of grams of iodine which will be absorbed by 100 grams of a fat is termed its iodine number.**

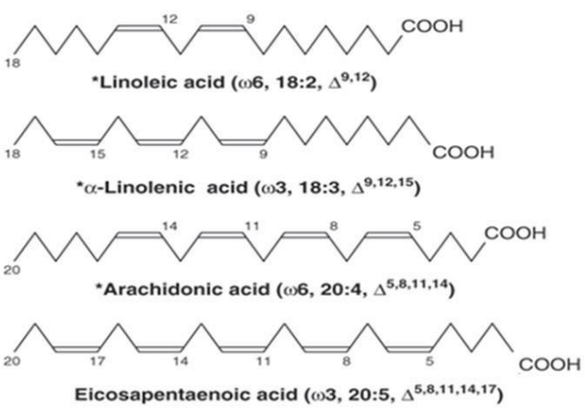
112 Polyunsaturated Fatty Acids of Biological Importance

Lipid Chemistry

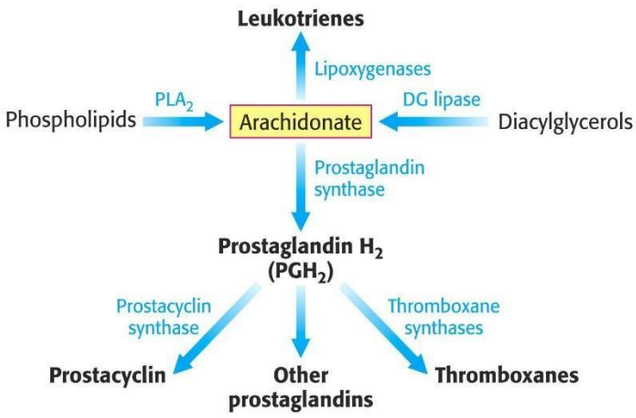
- **Polyunsaturated Fatty Acids Of Biological Importance • Polyunsaturated fatty acids contain more than one double bond**
- **These include:**
- **Linoleic acid (18: 2$\Delta^{9, 12}$)**
- **α-Linolenic acid (18 : 3$\Delta^{9, 12, 15}$)**
- **Arachidonic acid series (20 : 4$\Delta^{5, 8, 11, 14}$)**
- **Of these**
- **Linoleic acid and**
- **α-Linolenic acid are essential fatty acids.**
- **Whereas, arachidonic acid is synthesized from both Linoleic acid and α-Linolenic acid.**



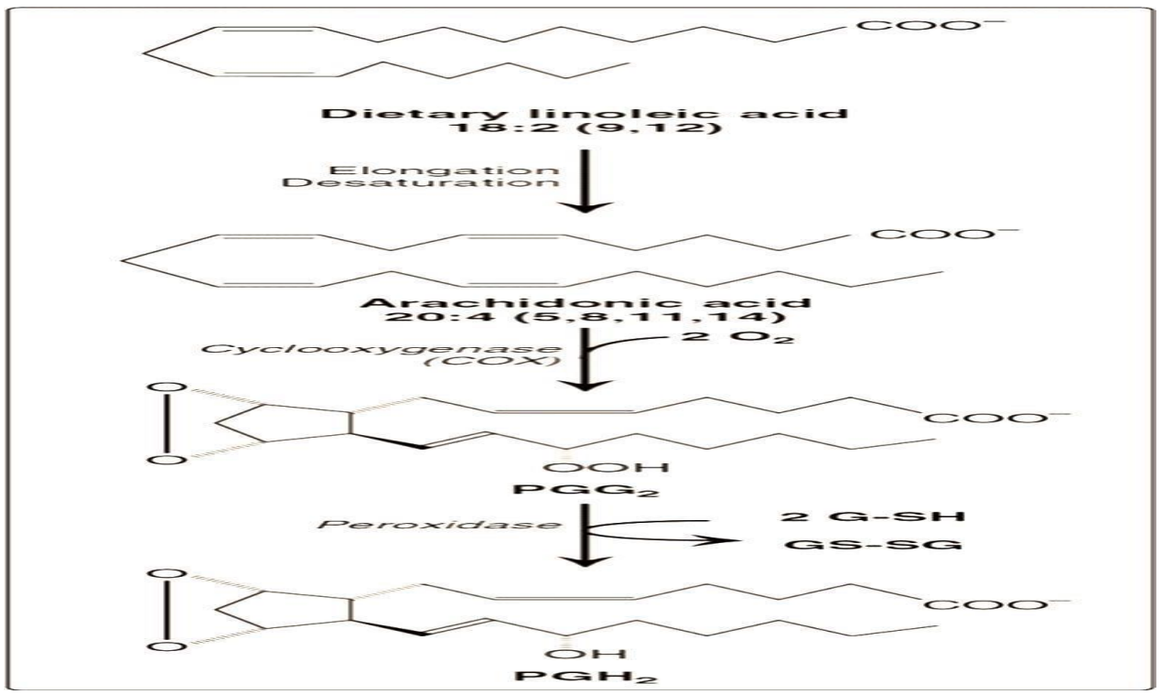


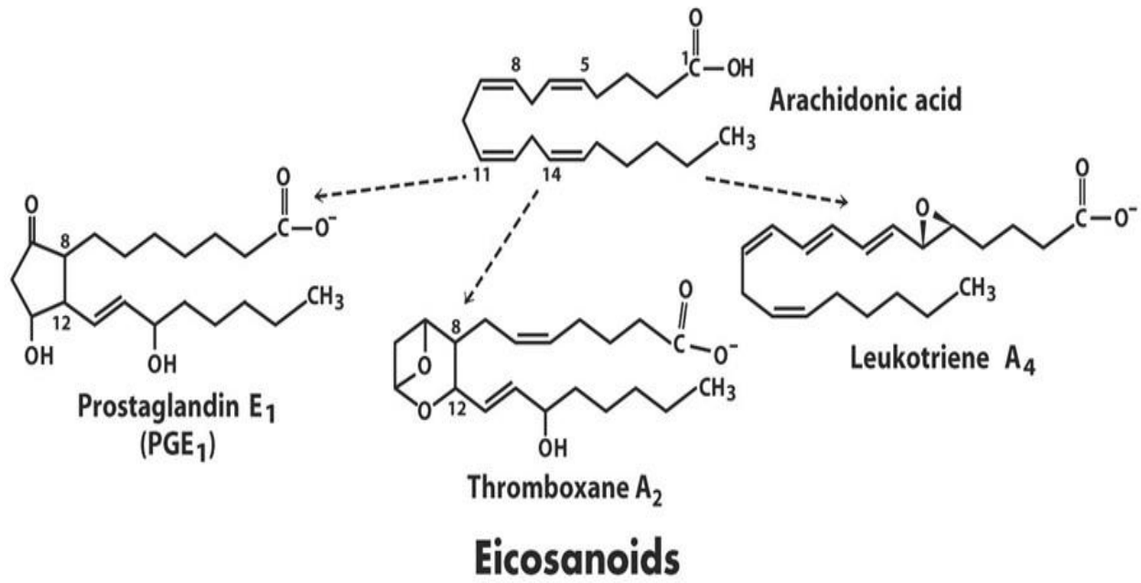


- **Arachidonic acid is the precursor of paracrine hormones called Eicosanoids.**
- **Eicosanoids are chemical messengers which act over a short distance and include:**
- **Prostaglandins**
- **Leukotrienes and**
- **Thromboxanes**



- **These eicosanoids are semi cyclic structures.**
- **They exert complex control over many bodily systems; in**
- **inflammation**
- **immunity, and as**
- **messengers in the central nervous system.**





Eicosanoids

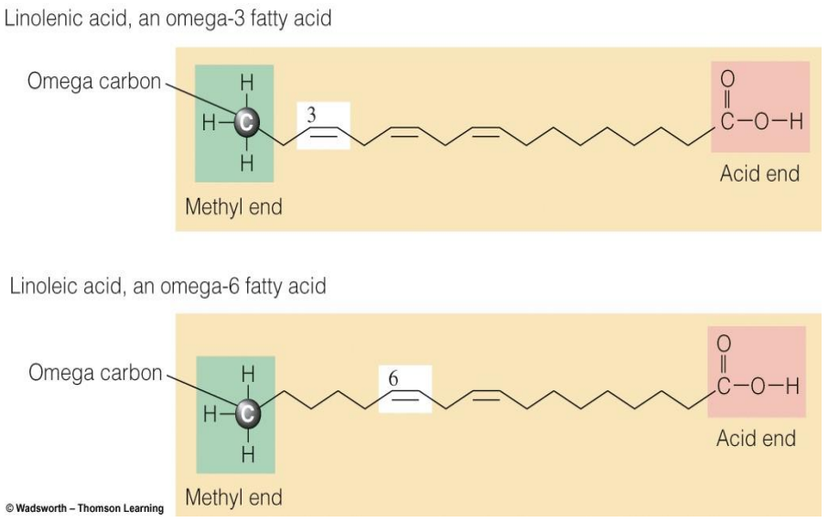
FINAL TERM 113 TO 225

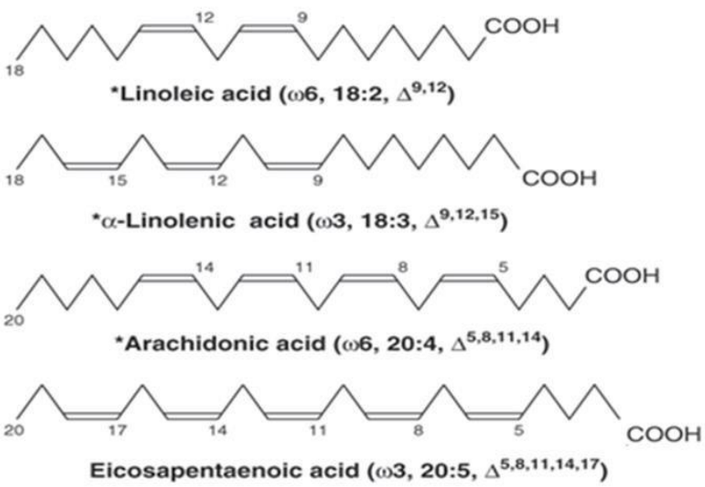
BIO202 - Biochemistry -I

114 Polyunsaturated Fatty Acids of Biological Importance

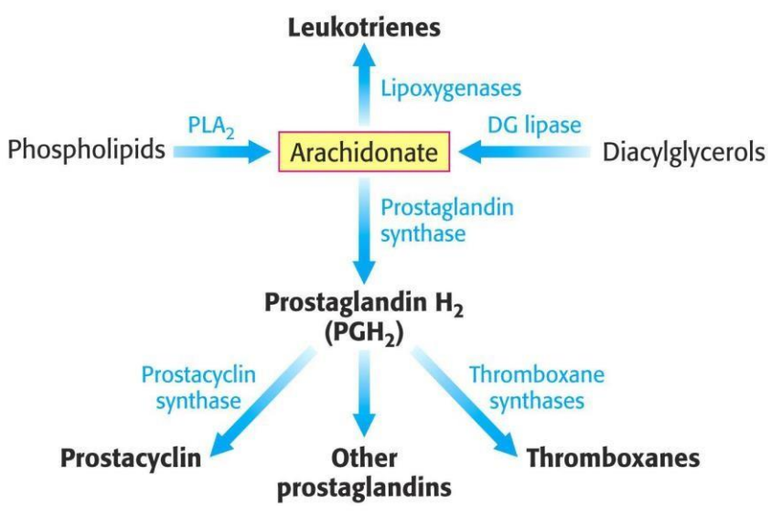
- Polyunsaturated Fatty Acids Of Biological Importance • Polyunsaturated fatty acids contain more than one double bond
- These include:
- Linoleic acid (18: $2\Delta^{9, 12}$ **)**
- α-Linolenic acid (18 : $3\Delta^{9, 12, 15}$)
- Arachidonic acid series (20 : $4\Delta^{5, 8, 11, 14}$)
- Of these

- Linoleic acid and α-Linolenic acid are essential fatty acids.
- Whereas, arachidonic acid is synthesized from both Linoleic acid and α-Linolenic acid.
- Arachidonic acid becomes essential if linoleic acid is deficient in the diet





- Arachidonic acid is the precursor of paracrine hormones called **Eicosanoids.**
- Eicosanoids are chemical messengers which act over a short distance and include:
- Prostaglandins
- Leukotrienes and • Thromboxanes



- These eicosanoids are semi cyclic structures.

- They exert complex control over many bodily systems; in
- inflammation
- immunity, and as
- messengers in the central nervous system.

115 Polyunsaturated Fatty Acids Of Biological Importance Contd.

- **Polyunsaturated Fatty Acids Of Biological Importance** (Cont.)
- Eicosanoids are derived from either omega-3 (ω-3) or omega-6 (ω-6) fatty acids.
- In general, the ω-6 eicosanoids are **pro-inflammatory.**

Figure 2. Classes of Essential Fatty Acids

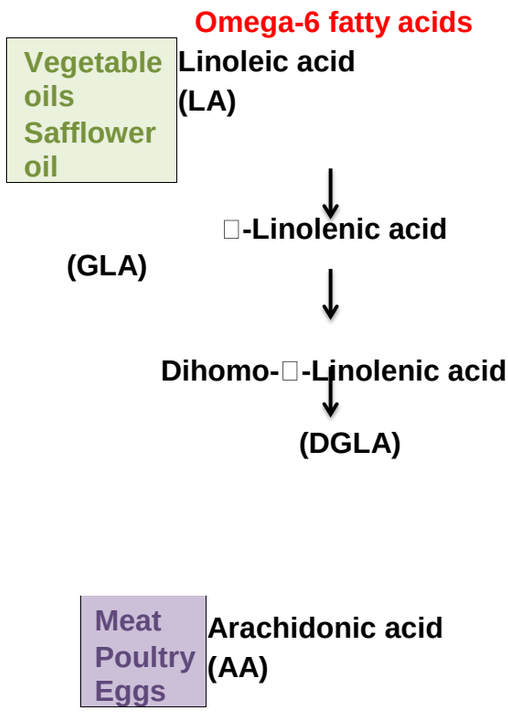
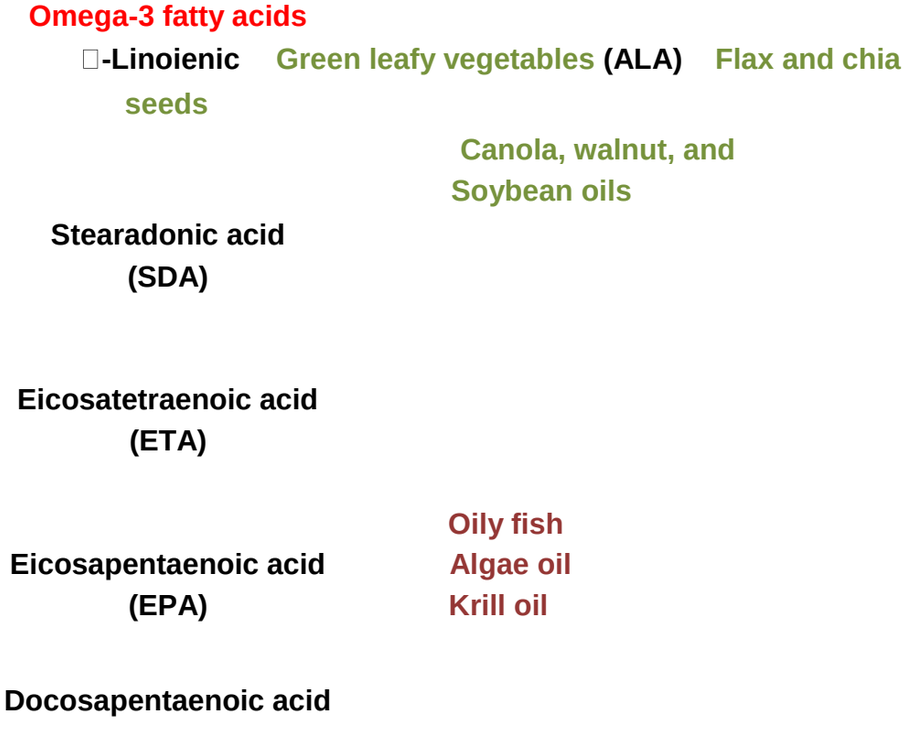


Figure 2. Classes of Essential Fatty Acids



↓

↓

(DPA)

↓

↓

↓

Oily fish

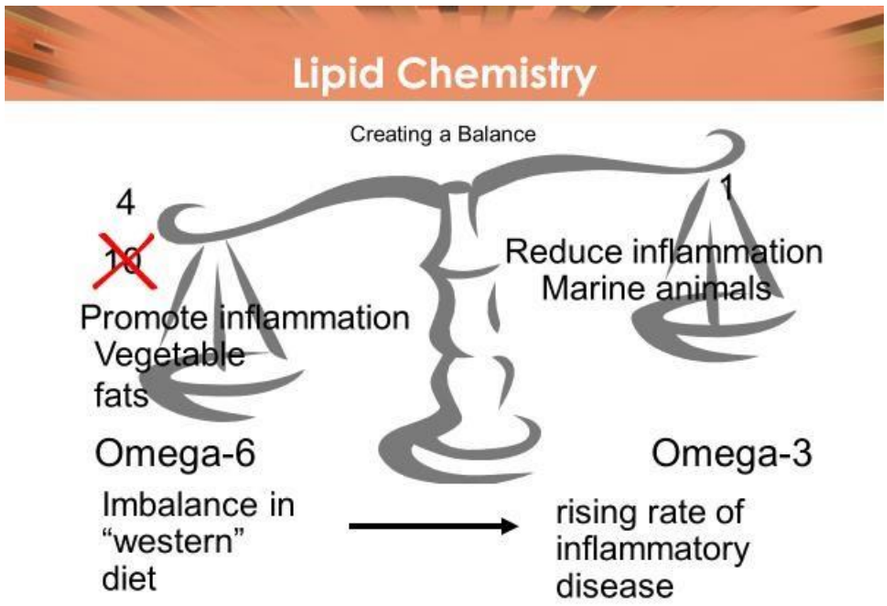
Krill oil

Algea oil

Docosahexaenoic acid

(DHA)

- **Imbalance of omega-6 and omega-3** PUFAs in the diet is associated with:
  - an increased risk of cardiovascular disease.



  -
  - Long chain omega-3 fatty acids such as alpha-linolenic acid and their derivatives such as
  - eicosapentaenoic acid (EPA) and
  - docosahexaenoic acid (DHA) have
  - anti-inflammatory effects
  - In addition, docosahexaenoic acid (DHA) is selectively incorporated into
  - retinal cell membranes and
  - postsynaptic neuronal cell membranes
  - suggesting it plays important roles in vision and nervous system function.
  - Current evidence suggests that diet rich in omega 3 fatty acids are beneficial particularly for
  - cardiovascular disease and also to some extent for
  - Alzheimer's disease, – Cancer and
  - Rheumatoid Arthritis.
  - This disease preventing role is due to the
  - anti-inflammatory action of these
  - omega-3 derivatives.
  - Classically, ω-3 PUFAs mediate some of these effects by
  - antagonizing ω-6 PUFA (arachidonic acid)-induced pro-inflammatory prostaglandin E_2 (PGE_2) formation

Nutritional Significance

Number of C Atoms and Common Double Bonds	Family	Common Name	Systematic Name	Occurrence
Monoenoic acids (one double bond)				
16:1;9	ω7	Palmitoleic	*cis*-9-Hexadecenoic	In nearly all fats.
18:1;9	ω9	Oleic	*cis*-9-Octadecenoic	Possibly the most common fatty acid in natural fats; particularly high in olive oil.

Number of C Atoms and Common Double Bonds	Family	Common Name	Systematic Name	Occurrence
Monoenoic acids (one double bond)				
18:1;9	ω9	Elaidic	*trans*-9-Octadecenoic	Hydrogenated and ruminant fats.

Number of C Atoms and Common Double Bonds	Family	Common Name	Systematic Name	Occurrence
Dienoic acids				
18:2;9,12	ω6	Linoleic	all-*cis*-9,12-Octadecadienoic	Corn, peanut, cottonseed, soy bean, and many plant oils.

Number of C Atoms and Common Double Bonds	Family	Common Name	Systematic Name	Occurrence
Trienoic acids				
18:3;6,9,12	**ω6**	**γ-Linolenic**	**all-*cis*-6,9,12-Octadecatrienoic**	**Some plants, eg, oil of evening primrose, borage oil; minor fatty acid in animals.**
18:3;9,12,15	**ω3**	**α-Linolenic**	**all-*cis*-9,12,15-Octadecatrienoic**	**Frequently found with linoleic acid but particularly in linseed oil.**

Linseed .Alsi borage oil: Gao Zaban primrose: Gul e Fanjani (used in skin care products)

Number of C Atoms and Common Double Bonds	Family Omega	Common Name	Systematic Name	Occurrence
Tetraenoic acids (four double bonds)				
20:4;5,8,11,14	ω 6	Arachidonic	all-*cis*-5,8,11,14-Eicosatetraenoic	Found in animal fats; important component of phospholipids in animals.

Number of C Atoms and Common Double Bonds	Family Omega	Common Name	Systematic Name	Occurrence
Pentaenoic acids (five double bonds)				
20:5;5,8,11,14,17	ω 3	Timnodonic	all-*cis*-5,8,11,14,17-Eicosapentaenoic	Important component of fish oils, e.g. cod liver, salmon oils and other fish

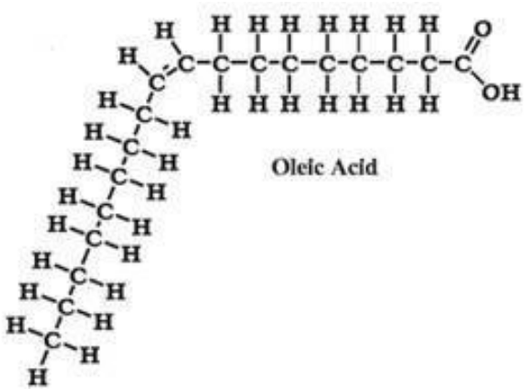
Number of C Atoms and Common Double Bonds	Family Omega	Common Name	Systematic Name	Occurrence
Hexaenoic acids (six double bonds)				
22:6;4,7,10,13,16,19	ω3	Cervonic	all-*cis*-4,7,10,13,16,19-Docosahexaenoic	Fish oils, phospholipids in brain.

117 Lipid Chemistry-Other Common Fatty Acids

Other Common Fatty Acids

- Other Fatty acids commonly found in body which are worth knowing are:
- Palmitic Acid (16:0)
- Stearic Acid (18:0) and Oleic acid (18:1)
- Palmitic Acid(16:0) Palmitic acid, or hexadecanoic acid, is the
- most common saturated Fatty Acid found in animals, plants and microorganisms •
  Palmitic acid mainly occurs as its ester in triglycerides (fats), especially palm oil.
- It is also found in high amounts in
- Butter,
- Cheese,
- milk and
- meat
- Excess carbohydrates in the body are converted to palmitic acid.
- Palmitic acid is the first fatty acid produced during fatty acid synthesis and the precursor to longer fatty acids
- As a consequence, palmitic acid is a major body component of fats found in the animals.
- Stearic Acid (18:0)

- As its ester, stearic acid is one of the most common saturated fatty acids found in nature following palmitic acid.
- Fats and oils rich in stearic acid are
- more abundant in animal fat (up to 30%)
- than in vegetable fat (typically <5%)
- Oleic Acid(18:1)
- Possibly the most common fatty acid in adipose tissue • It is particularly high in olive oil



- Oleic acid in olive oil accounts for the blood pressure lowering effect of olive oil. • In addition, because of cis configuration and the kink produced due to the
- cis configuration.
- It is also highly abundant in membrane lipids, providing
- fluidity to membrane.
- Saturated Fatty Acids

Common name	Number of carbons	
Acetic	2	Major end product of carbohydrate fermentation by rumen organisms
Butyric	4	In certain fats in small amounts (especially butter). An end product of carbohydrate fermentation by rumen organisms. Also formed in the cecum of herbivores and to a lesser extent in the colon of humans.
Valeric	5	
Caproic	6	

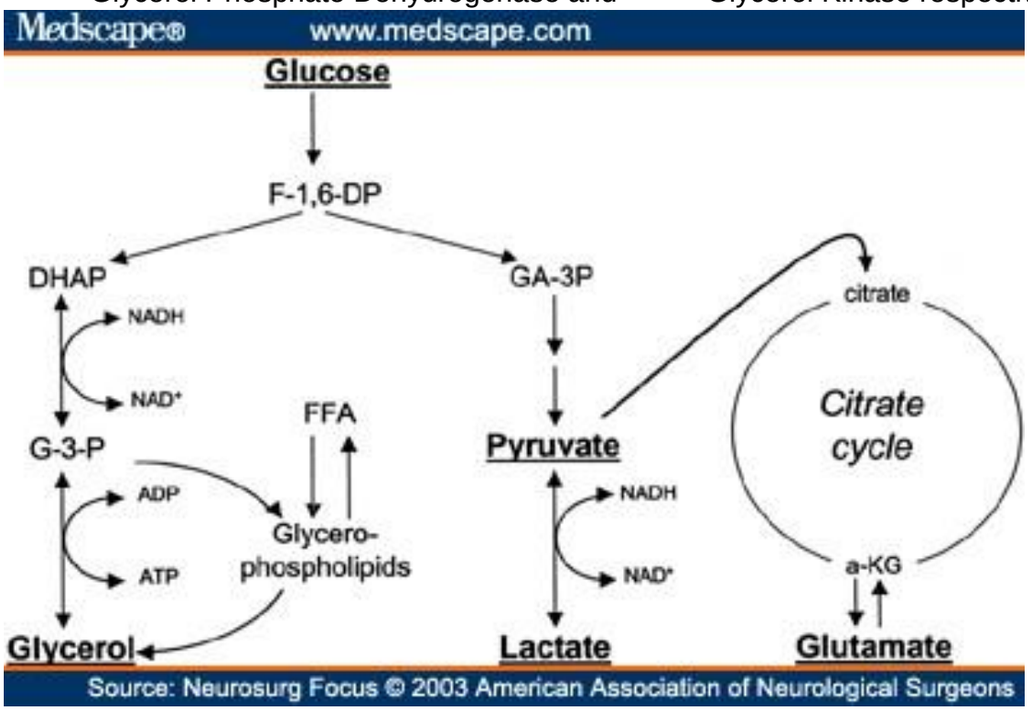
Saturated Fatty Acids

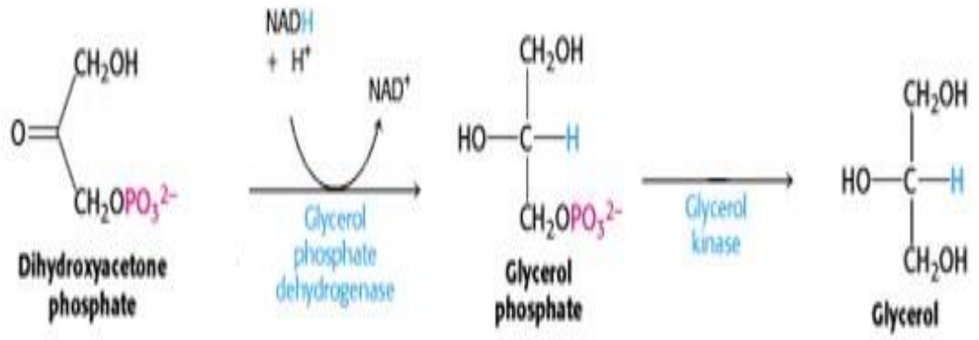
Common name	Number of carbons	
Lauric	12	Spermaceti, cinnamon, palm kernel, coconut oils, laurels, butter
Myristic	14	Nutmeg, palm kernel, coconut oils, myrtles, butter
Palmitic	16	Common in all animal and plant fats
Stearic	18	

- Spermaceti by the sperm whale. Palm saeed. evergreen tree *(Laurus nobilis)* of the
- Mediterranean region valued for its aromatic ovate leaves. Nutmeg Jorenk, Jefal

118 Lipid Chemistry-Glycerol and Sphingosine

- Glycerol and Sphingosine are the two types of alcohols most commonly found in lipids.
  • Glycerol
- It is a simple poly hydroxy alcohol (also called polyol or sugar alcohol)
- and part of a class of lipids: glycolipids
- It contains 3 carbons and 3 hydroxyl (OH) groups.
- Glycerol is synthesized from
- Dihydroxyacetone Phosphate (an intermediate of the glycolytic pathway)
- Dihydroxyacetone phosphate is acted upon by two enzymes to form glycerol, namely
- Glycerol Phosphate Dehydrogenase and • Glycerol Kinase respectively.





- Glycerol is a precursor for synthesis of triacylglycerols and of phospholipids in the liver and adipose tissue.
- When the body uses stored fat as a source of energy, glycerol and fatty acids are released into the bloodstream.
- And enter the glycolysis pathway directly

119 Lipid Chemistry: Sphingosine

- Sphingosine is an amino alcohol,
- which is a component of the class of lipids known as sphingolipids • Sphingosine is synthesized in the body in the form of ceramide,
- to which different moieties are added to form sphingolipids.
- Serine and palmitoyl CoA condense to form a product (ketosphinganine) that is reduced.
- A very long-chain fatty acid forms an amide with the amino group.
- a double bond is generated, and ceramide is formed.

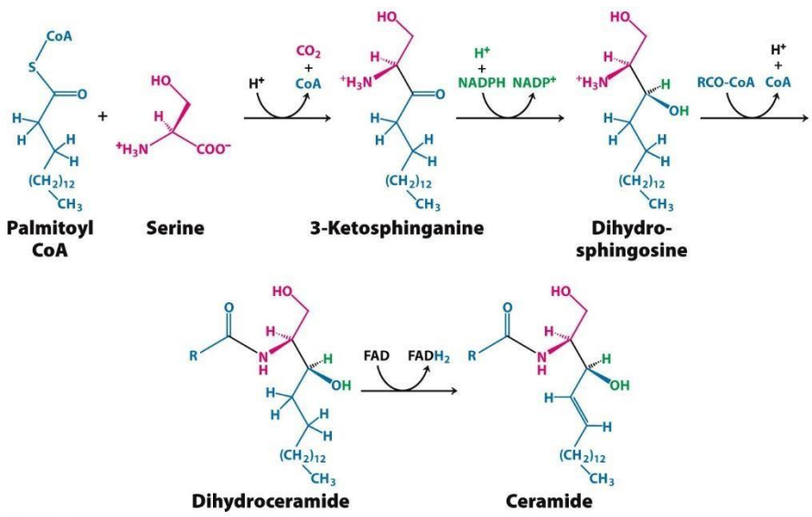
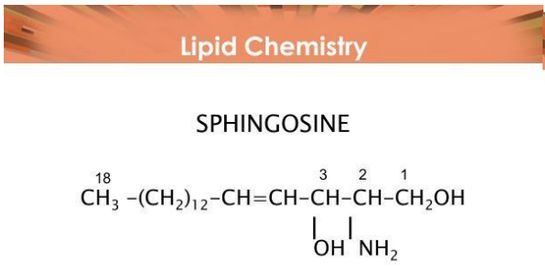
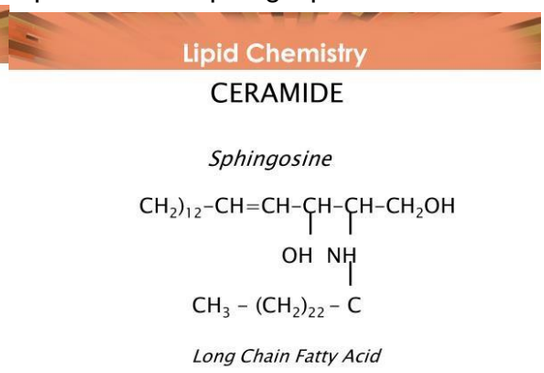
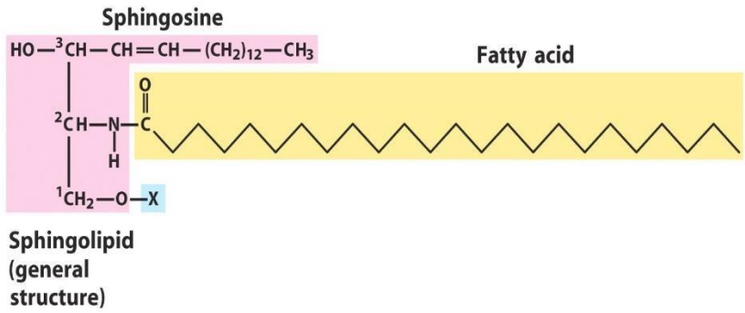


Figure 26.3
Biochemistry, Seventh Edition

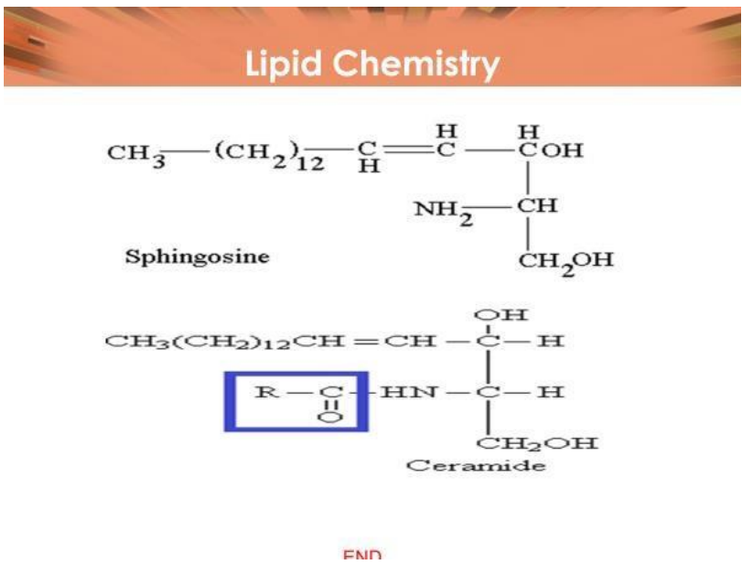
- There is no direct route of synthesis from sphinganine (dihydrosphigosine) to sphingosine;
- it has to be acylated
- first to dihydroceramide, which is then dehydrogenated to ceramide.
- Sphingosine is formed via degradation of sphingolipid in the lysosome.
- Therefore ceramide is the structural parent of all sphingolipids.







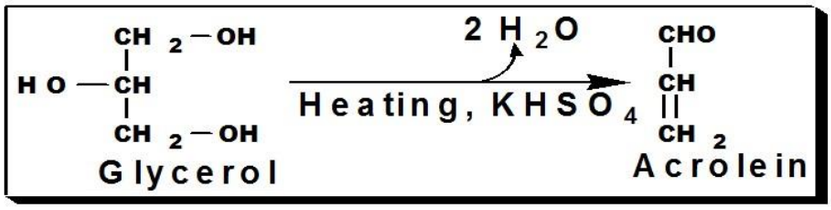
- In short,
- Serine+ Palmitate
- Sphingosine
- Sphingosine +
- FA = ceramide



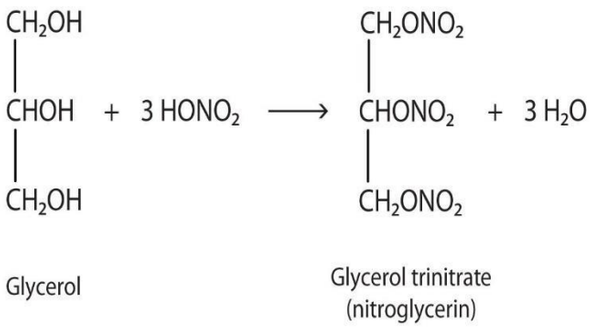
120 Lipid Chemistry-Properties of Glycerol

Popularly known as glycerin,

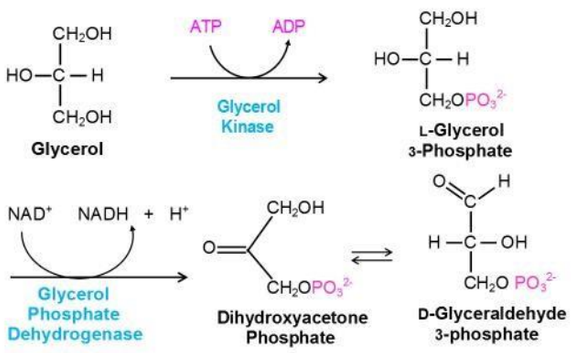
- Glycerol is widely used in pharmaceutical and cosmetic preparations.
- It has the following properties:
- Colorless Viscous oily liquid with sweet taste.



-
- Acrolein Test
- On heating with sulfuric acid or KHSO4 (dehydration)
- it gives acrolein that has a bad odor.
- used for detection of free glycerol or any compound containing glycerol.
- In contrast to glycerol
- Sphingosine does not show positive acrolein test.
- Therefore glycerolipids and shingolipids can be differentiated on the basis of acrolein test.
- Glycerol combines with three molecules of nitric acid to form Glycerol trinitrate that is
- used as explosive and vasodilator

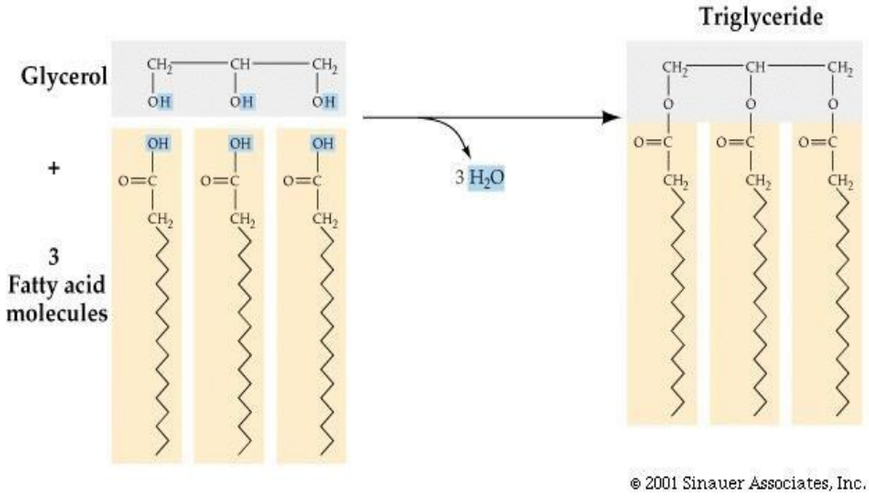


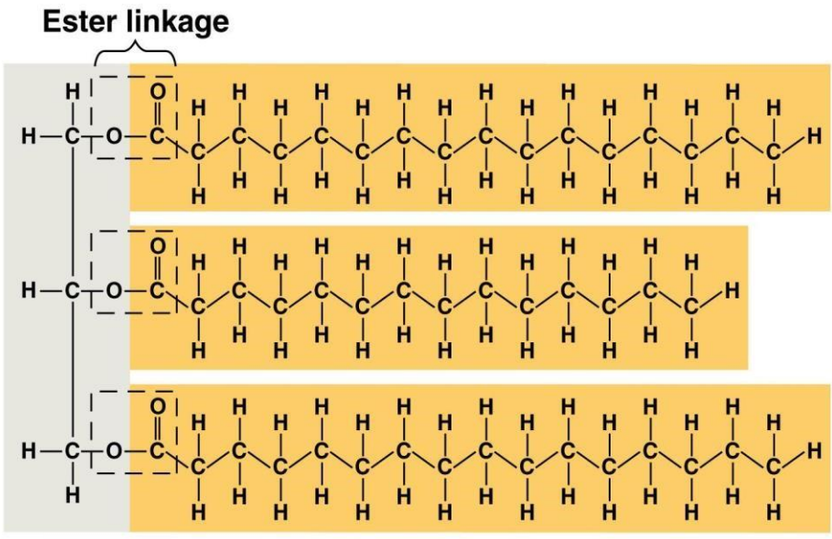
-
- On esterification with fatty acids it gives:
- monoacylglycerol: one fatty acid + glycerol.
- diacylglycerol: two fatty acids + glycerol.
- triacyl-glycerol: three fatty acids + glycerol.
- Gluconegenic substrate Glycerol can form dihydroxyacetone phosphate which can convert into glucose or glyceraldehyde phosphate to enter into gluconeogenic pathway.



121 Lipid Chemistry-Simple lipids

- Esters of fatty acids with various alcohols
- These contain:
- Fats (and Oils) and Waxes.
- Fats: Esters of fatty acids with glycerol (Oils are fats in the liquid state)
- Waxes: Esters of fatty acids with higher molecular weight monohydric alcohols.
- (having one OH group)
- Triacylglycerols (TAGs)
- The simplest lipids constructed from fatty acids are the triacylglycerols,
- Also referred to as;
- triglycerides,
- fats, or neutral fats or storage lipids.
- Triacylglycerols are composed of three fatty acids in ester linkage with a single glycerol

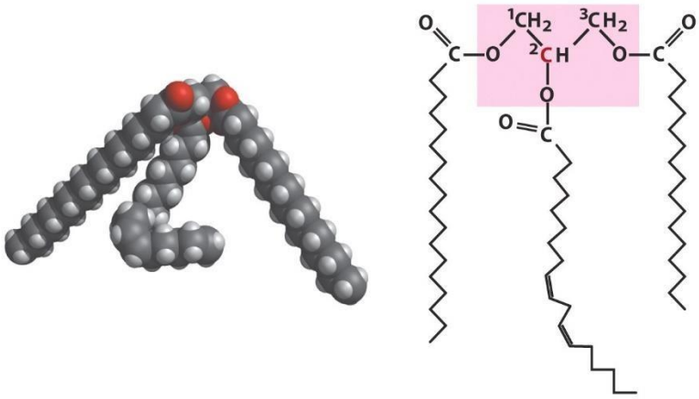




(b) Fat molecule (triacylglycerol)

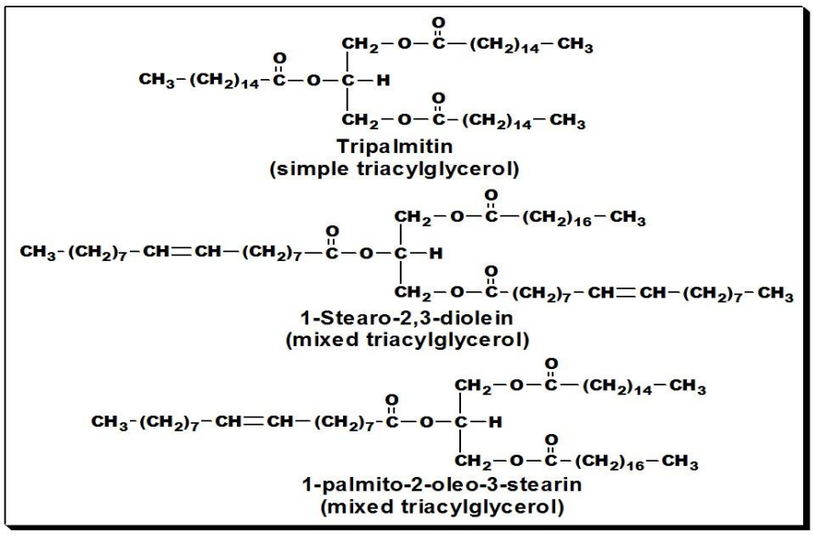
Structure of triacylglycerols

- The three fatty acids esterified to a glycerol molecule are usually not of the same type
- The fatty acid on carbon 1 is typically saturated,
- Whereas that on carbon 2 is unsaturated, • and that on carbon 3 can be either

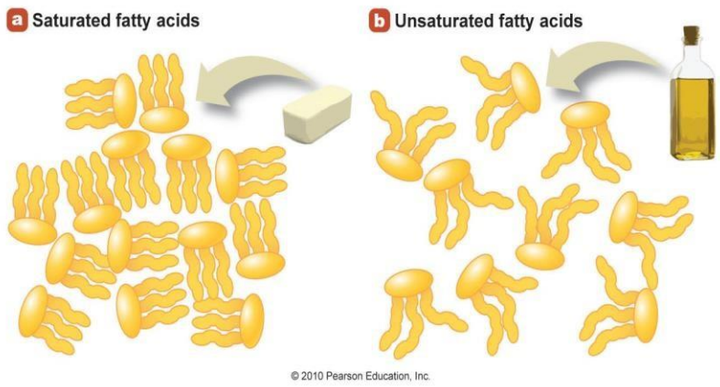


1-Stearoyl, 2-linoleoyl, 3-palmitoyl glycerol, a mixed triacylglycerol

- Simple triglycerides:
- Fatty acids connected to glycerol are of the same type
- e.g., tripalmitin.
- Mixed triglycerides:
- Fatty acids are of different types, e.g., • stearo-diolein and palmito-oleo-stearin.



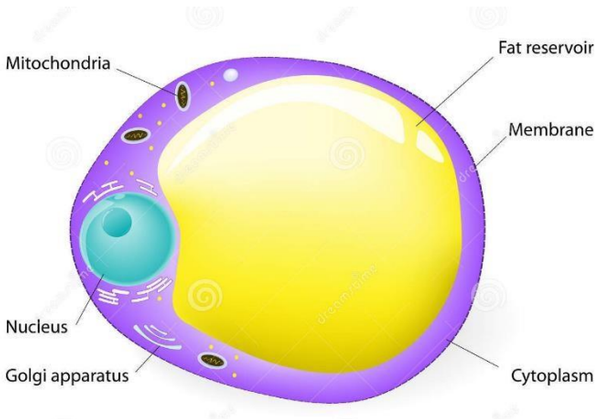
- The main difference between fats and oils is for oils being liquid at room temperature, whereas, fats are solids.
- This is mainly due to presence of larger percentage of unsaturated fatty acids in oils than fats that has mostly saturated fatty acids.

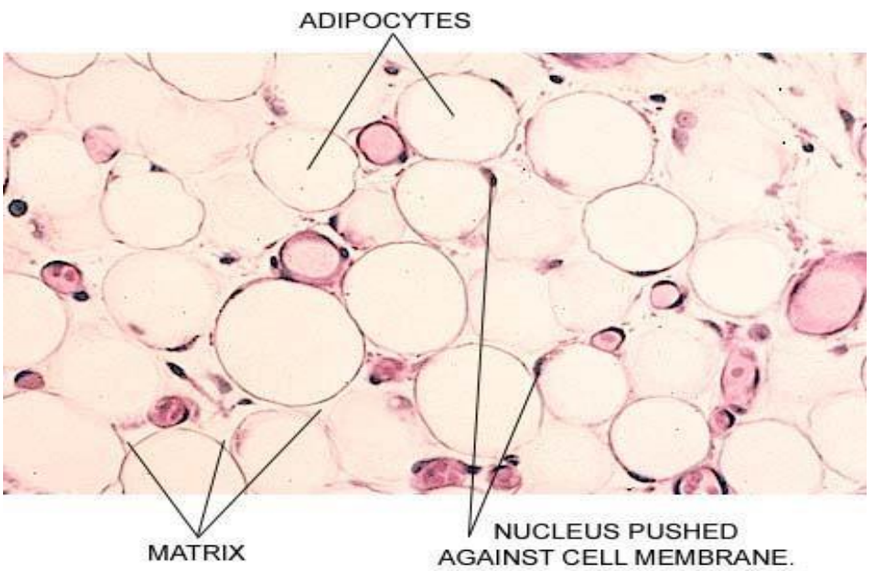


- TAGs containing saturated fatty acids are solid at room temperature such as butter whereas
- TAGs containing unsaturated fatty acids are liquid at room temperature such as olive oil.(Olic acid, 18:1,9)

122 Lipid Chemistry-Triacylglycerols

- Because the polar hydroxyls of glycerol and the polar carboxylates of the fatty acids are bound in ester linkages
- Therefore the triacylglycerols are nonpolar, hydrophobic molecules, essentially insoluble in water
- Triacylglycerols provide
- Stored Energy and Insulation.
- In vertebrates, specialized cells called adipocytes, or fat cells, store large amounts of triacylglycerols as fat droplets that nearly fill the cell





- Triacylglycerols are also stored as oils in the seeds of many types of plants.
- Thus providing energy and biosynthetic precursors during seed germination

- Adipocytes and germinating seeds contain lipases, enzymes that catalyze the hydrolysis of stored triacylglycerols.

  Therefore releasing fatty acids for export to sites where they are required

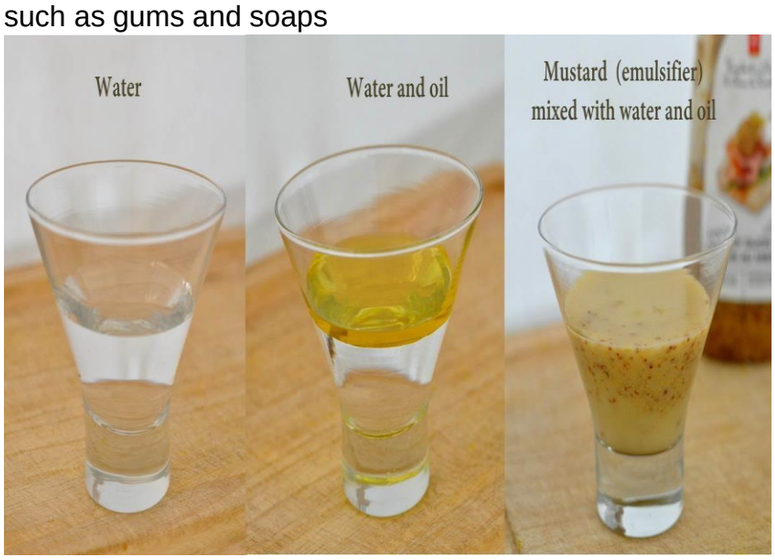
123 Lipid Chemistry-Triacylglycerols (TAGs) Cont.

- There are two significant advantages to using triacylglycerols as stored fuels, rather than polysaccharides such as glycogen and starch.
- First, the carbon atoms of fatty acids are more reduced than those of sugars, and oxidation of triacylglycerols yields more than twice as much energy, as the oxidation of carbohydrates .Second, because triacylglycerols are hydrophobic and therefore unhydrated.
- The organism that carries fat as fuel does not have to carry the extra weight of water of hydration that is associated with stored polysaccharides (2 g per gram of polysaccharide)
- Moderately obese people with 15 to 20 kg of triacylglycerols deposited in their adipocytes could meet their energy needs for months by drawing on their fat stores
- In contrast, the human body can store less than a day's energy supply in the form of glycogen (the polymer of glucose)
- In some animals, triacylglycerols stored under the skin serve as insulation against low temperatures. Seals, penguins, bears and other warm-blooded polar animals are amply padded with triacylglycerols

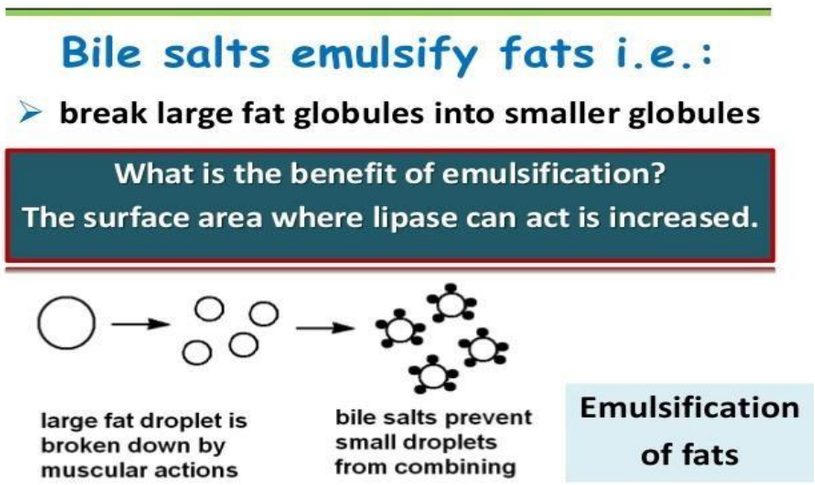
124 Lipid Chemistry-Properties of TAGs

Physical properties

- Neutral fats are
- colourless, odorless and tasteless substances • Solubility:
- They are insoluble in water but soluble in organic fat solvents(e.g., ether, benzene, acetone, chloroform)
- 5. Specific gravity:
- The specific gravity of all fats is less than 1.0, consequently all fats float in water
- 6. Emulsification:
- Emulsions of fat may be made by shaking vigorously in water and by emulsifying agents such as gums and soaps



- The emulsification of dietary fats in intestinal canal, brought about by bile salts, is a prerequisite for digestion and absorption of fats.
- The bile salts, act to break apart the fat globules in the small intestines and allow them to become more "soluble" for absorption.
- The hydrophobic fat molecules will clump together into globules in the watery mixture in the digestive system.
- The emulsifiers break them down to smaller "globules" and allow them to become more soluble.



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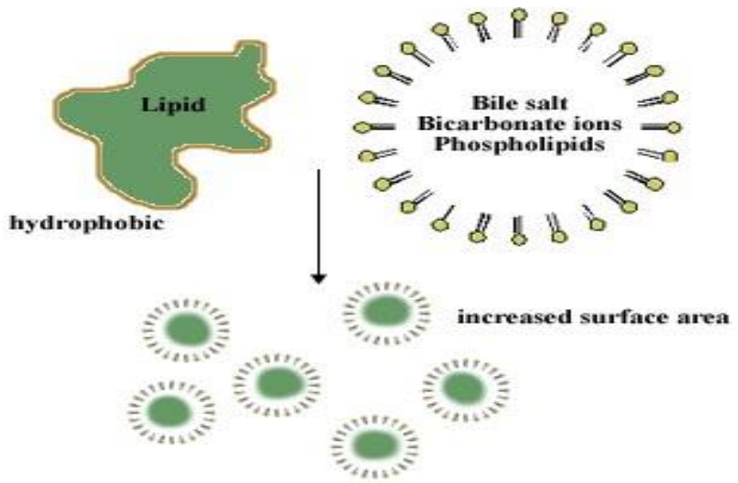
- In Mustard variety of chemicals in the mucilage surrounding the seed hull act as emulsifiers



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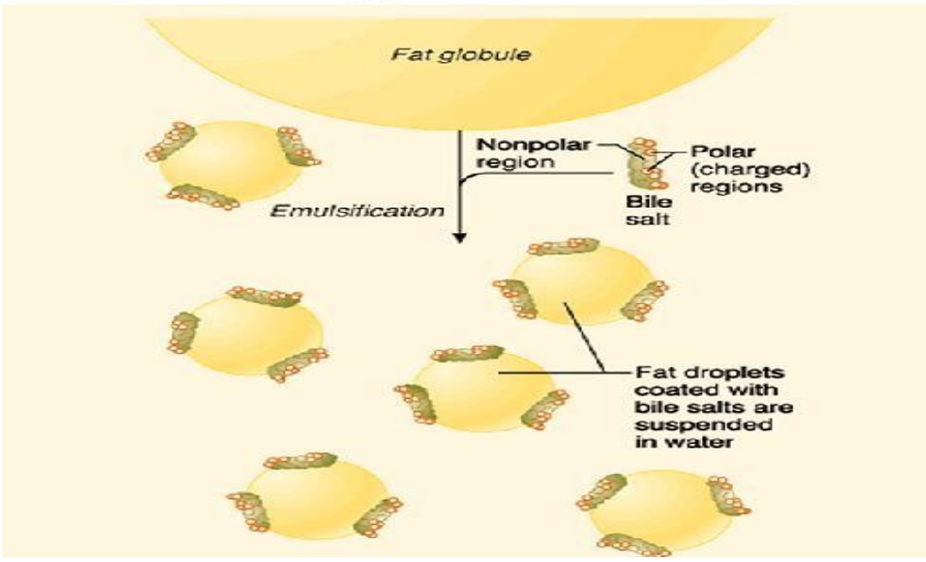

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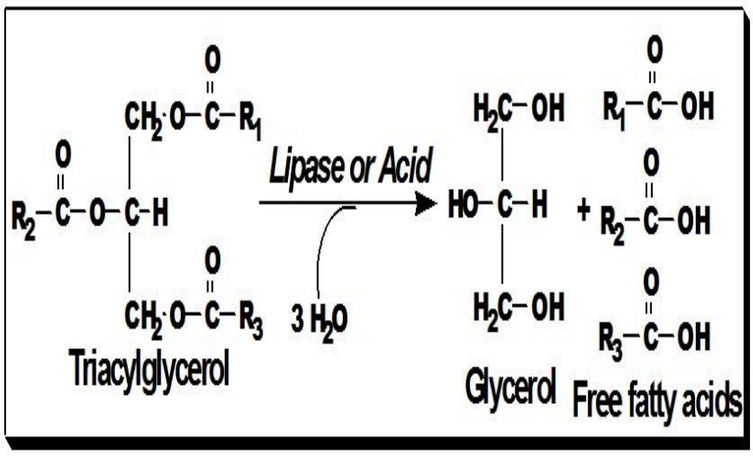
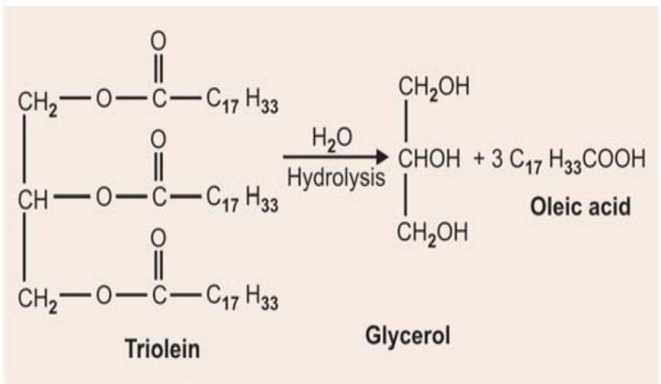
125 Lipid Chemistry-Chemical Properties of TAGs

Chemical Properties of TAGs

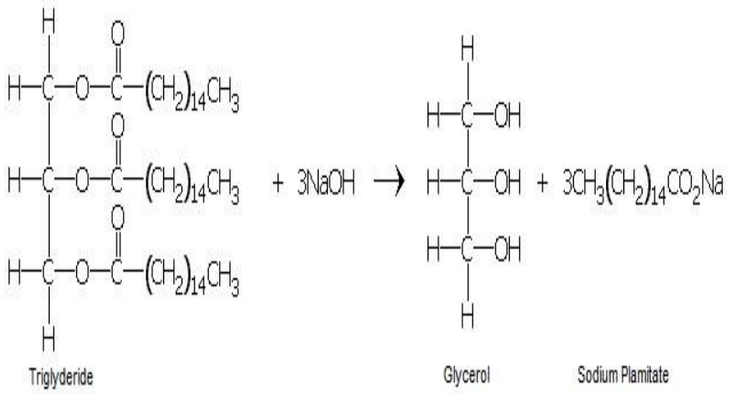
- Hydrolysis

  The fats may be hydrolysed with super heated steam, by acids, or alkalies, by the specific fat splitting enzymes lipases to – free fatty acids – glycerol





- Saponification
- Hydrolysis of a fat by an alkali is called saponification
- The resultant products are;
- glycerol and
- the alkali salts of the fatty acids, which are called “soaps”



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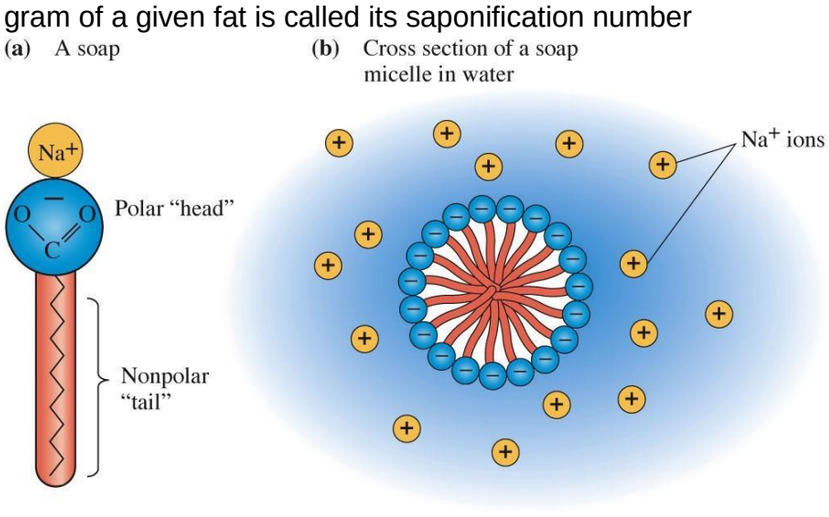
- The number of mgs of NaOH/KOH required to saponify the free and combined FA in one gram of a given fat is called its saponification number



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The amount of alkali needed to saponify a given quantity of fat will depend upon the number of carboxylic (–COOH) group present

- Thus fats containing short chain fatty acids will have more –COOH groups per gram than long- chain fatty acids and this will take up more alkali • And hence will have higher saponification number

Lipid properties

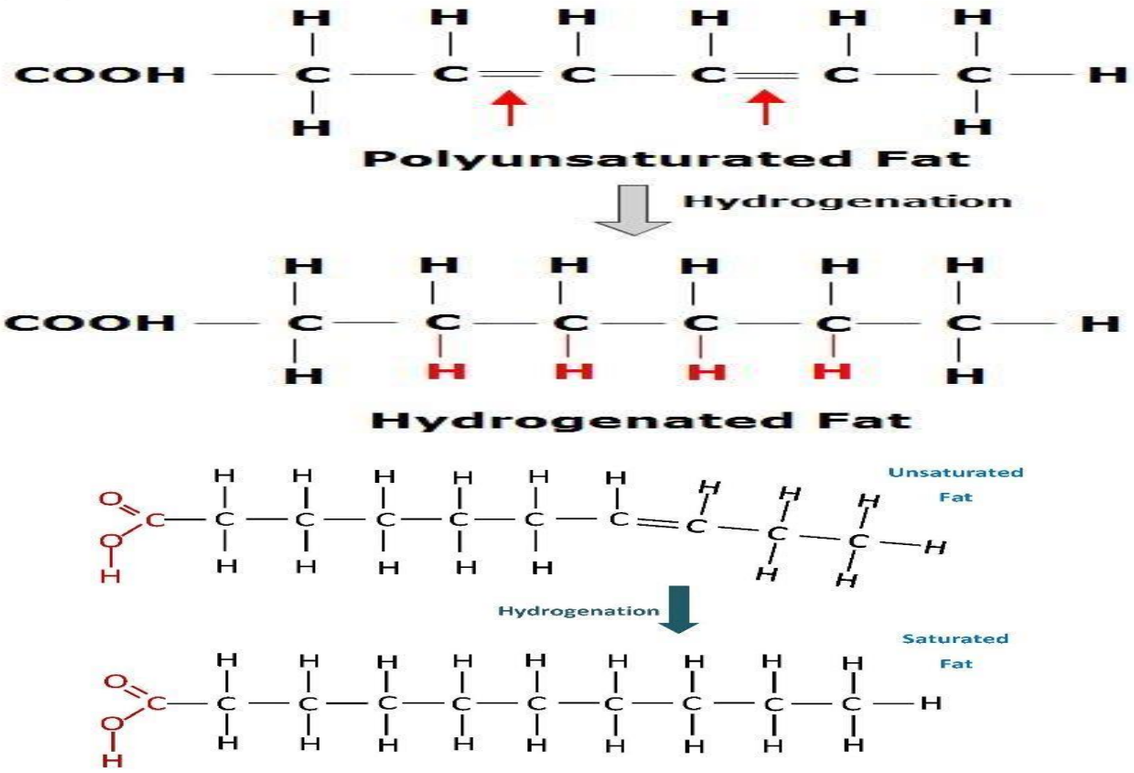
Table 1. Examples saponification and iodine numbers

Fat or oil	Saponification #	Iodine #
Beef tallow	194 – 200	34 – 43
Cocoa butter	192 – 198	32 – 42
Coconut oil	245 – 262	6 – 10
Cottonseed oil	192 – 196	103 – 112
Lard	193 – 200	50 – 80
Milk fat	210 – 233	26 – 35
Peanut oil	186 – 194	89 – 98

-
- Butter containing a larger proportion of short- chain fatty acids, such as butyric (C4) acid and caproic (C6) acid etc.
- Therefore it has relatively high saponification number from 220 to 230 • In contrast, Olive Oil (which contain Oleic acid (C18), a longer chain FA), • has saponification number of 195 or less.
- Triolein is another example of simple TAG.
- Tallow is the fat obtained from cattle used in soap industry.

126 Lipid Chemistry-Chemical Properties of TAGs Contd

- 2. Additive Reactions
- The unsaturated fatty acids present in neutral fat exhibits all the additive reactions, i.e. – hydrogenation, – halogenation.
- Hydrogenation



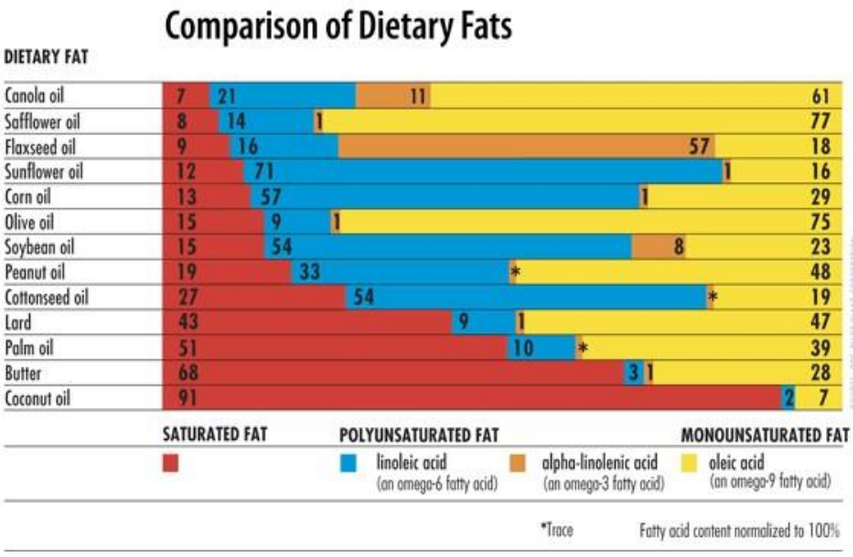
- A hydrogenation reaction involves conversion of a carbon-carbon double bond to a carbon-carbon single bond through the addition of hydrogen

-
- Hydrogenation – As you continue to hydrogenate your molecule Melting point increases
- Fat becomes more solid at room temp
- Hydrogenation
- Oils which are liquid at ordinary room temperature, on hydrogenation become solidified
- This is the basis of Banaspti ghee manufacturing.
- Where inedible and cheap oils like cotton seed oil are hydrogenated and converted to edible solid fats.
- The hydrogenation is done
- under high pressure of hydrogen and is catalyzed by finely divided nickel or copper and heat. • It is the base of hardening of oils (margarine manufacturing), e.g. • change of oleic acid of fats (liquid)
- into stearic acid (solid).
- unsaturated fats have lower melting points,stearic (SFA) melts at 70

127 Lipid Chemistry-Chemical Properties of TAGs Contd.

Halogenation

- Similar to hydrogenation,
- Halogens such as chlorine, bromine and iodine can also be added to double bonds in
- unsaturated fatty acids.
- It is a very important property to determine the degree of unsaturation of the fat or oil that determines its biological value.
- The degree of unsaturation is reflected by Iodine number.
- Iodine number is defined as the number of grams of iodine absorbed by 100 gm of fat.
- The more the iodine number, the greater the degree of unsaturation.
- Fats rich in saturated fatty acids have low iodine numbers,
- while fats rich in unsaturated fatty acids have high iodine numbers
- The determination of iodine number is useful to the chemist in determining the quality of an oil or its freedom from adulteration
- Iodine number of cotton seed oil varies from 103 to 111.
- That of olive oil from 79 to 88,
- And that of linseed oil from 175 to 202
- A commercial lot of olive oil which has iodine number higher than 88 might have been adulterated with cotton seed oil
- The higher is the iodine number, the more reactive, less stable, more susceptible to oxidation and rancidification is the oil or fat.



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Oil/Fat	16:0	16:1	18:0	18:1	18:2	18:3	20:1	22:1	24:0	Average Unsaturation Per Triglyceride
soybean	11	0.1	4	23.4	53.2	7.8				4.6
palm	44.4	0.2	4.1	39.3	10	0.4				1.8
rapeseed	3	0.2	1	13.2	13.2	9	9	49.2	1.2	3.8
sunflower	6		5	20	60					1.4
tallow	27	11	7	48	2					0.6
cottonseed	21.6	0.6	2.6	18.6	54.5	0.7				3.9
olive	13.7	1.2	2.5	71.1	10	0.6				2.8
corn	10.9	0.2	2	25.4	59.6	1.2				4.5
canola	4.1	0.3	1.8	60.9	21	8.8	1	0.7	0.2	3.9
linseed	5.5		3.5	19.1	15.3	56.6				6.6

128 Lipid Chemistry-Chemical Properties of TAGs Cont.

Chemical Properties (Contd.)

- Rancidity
- The chemical deterioration of fats.
- When lipid-rich foods are exposed too long to the oxygen in air, they may spoil and become foul smelling.
- Rancidity
- Definition:
- It is a physico-chemical change in the natural properties of the fat leading to the development of
- unpleasant odor or
- taste or
- abnormal color
- It occurs particularly
- on aging
- after exposure to atmospheric oxygen,
- light,
- moisture,
- bacterial or fungal contamination
- and/or heat.
- Saturated fats resist rancidity more than unsaturated fats that have unsaturated double bonds.
- Rancidity is due to
- Oxidation • Hydrolysis • Oxidative Rancidity
- Oxidation of the fat molecules give rise to some short • chain aldehydes, ketones and dicarboxylic acids
- which have objectionable taste and odor.
- The unpleasant taste and smell associated with rancidity result from the oxidative cleavage of double bonds in unsaturated fatty acids
- The oxygen of the air is necessary for this type of • rancidity. This can be prevented by addition of anti-
- oxidants such as vitamin E to foods.
- II. Hydrolytic Rancidity
- It is due to the slow hydrolysis of fats,
- which in case of fats like butter results in the
- liberation of short chain fatty acids which are volatile and have rancid taste and odor.

129 Lipid Chemistry-Waxes

Waxes

- A second group of neutral lipids that are of physiological importance.

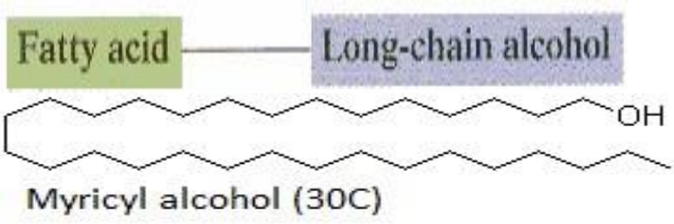
- Although they are a minor component of biological systems.
- Properties of waxes

Waxes are insoluble in water, but

- soluble in fat solvents and are negative for acrolein test. very resistant to rancidity.
- Waxes are not easily hydrolyzed as the fats and are indigestible by lipases (enzymes responsible for fat digestion in body) •

Thus they are of no nutritional value • Waxes are of two types:

- True waxes • Other Waxes or Non true waxes or
- Wax-like compounds
- True
- Waxes are solid simple lipids containing a monohydric alcohol (with a higher molecular weight than glycerol



- esterified to long-chain fatty acids.
-
-
- **Triacontanol** is a fatty alcohol of the general formula $C_{30}H_{62}O$, also known as **melissyl alcohol** or **myricyl alcohol.**
- It is found in plant cuticle waxes and in beeswax.
-
- Waxes are widely distributed in nature such as the secretion of certain insects as;
- Bees-wax, • Spermaceti of the sperm whale
- Waxes also form protective coatings of the skins and furs of animals and • leaves and fruits of plants.
- Triacontanol is a fatty alcohol of the general formula C30H62O, also known as
- melissyl alcohol or myricyl alcohol.
- It is found in plant cuticle waxes and in beeswax.
- The name cetyl derives from the whale oil (Latin: cetus) from which it was first isolated
- Cetyl Alcohol can be found in mooisturizer,facial moisturizer,conditioner,antiaging,hair color,hair bleaching,facial cleanser. hand cream,shampoo,lipstic,eye cream.

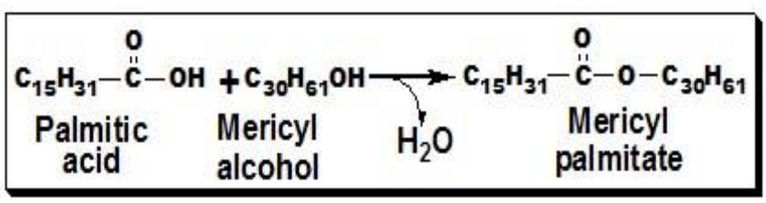
130 Lipid Chemistry-True Waxes

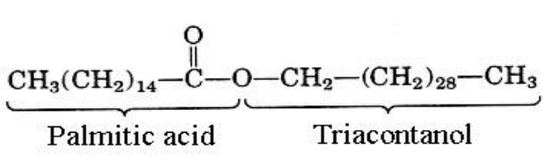
1.True Waxes

Bees-wax is secreted by the honeybees that use it to form the combs. It is a mixture of waxes

chief constituent is myricyl palmitate

(30C) (16C)





- 2.Spermaceti

- is a wax that is most often found in the head cavities of the sperm whale.
- Fatty esters are formed essentially of
- cetyl palmitate and
- cetyl myristate.
- It was used in cosmetics, pharmacy and also in candles
- recent international regulation concerning whale captures, has stopped its use.
- It is now replaced by synthetic cetyl palmitate. • 2.Other Waxes or Non true waxes • include esters of:
- Cholesterol
- Vitamin A
- Vitamin D
- Cholesterol esters: Lanolin (or wool fat) is secreted by sheep sebaceous glands • and It contains both free and esterified cholesterol, e.g., cholesterol-palmitate
- Lanolin secretion helps sheep in reducing water evaporation from the skin.
- It is used as industrial lubricant and in
- cosmetics

TABLE 15.2 Some Typical Waxes

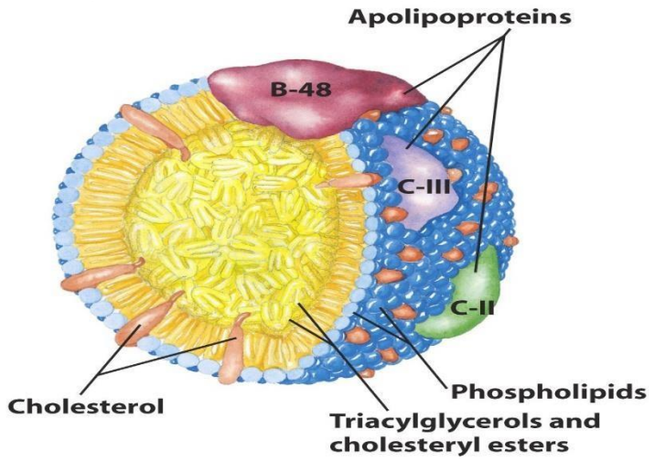
Type	Structural Formula	Source	Uses
Beeswax	$CH_3(CH_2)_{14}-\overset{\overset{O}{\|}}{C}-O-(CH_2)_{29}CH_3$	Honeycomb	Candles, shoe polish, wax paper
Carnauba wax	$CH_3(CH_2)_{24}-\overset{\overset{O}{\|}}{C}-O-(CH_2)_{29}CH_3$	Brazilian palm tree	Waxes for furniture, cars, floors, shoes
Jojoba wax	$CH_3(CH_2)_{18}-\overset{\overset{O}{\|}}{C}-O-(CH_2)_{19}CH_3$	Jojoba	Candles, soaps, cosmetics

.

131 Lipid Chemistry-Lipids and Nutrition

- Dietary fats strongly influence the incidence of coronary heart disease (CHD).
- In the past, dietary recommendations emphasized decreasing the total amount of fat in the diet.
- Research now indicates that the type of fat is more important than the total amount of fat consumed
- To gauge the effect of these dietary fats on CHD, we measure different biochemical parameters, which include:
- LDL-Cholesterol
- HDL-Cholesterol
- Total Cholesterol
- TAG
- LDL and HDL stand for
- Low Density Lipoprotein and
- High Density Lipoprotein respectively
- they are included in the class of complex lipids and serve to
- transport lipids in the blood. • Serum Cholesterol and TAGs are a risk factor for CHD among others.
- The risk increases progressively with higher values for serum total cholesterol and that of LDL cholesterol.
- Association of TAGs is weaker than that of LDL cholesterol with CHD.
- A much stronger correlation exists between the levels of blood LDL cholesterol and heart disease.
- In contrast, high levels of HDL cholesterol have been associated with a decreased risk for heart disease
- In order to understand the influence of these parameters on CHD, we must first understand biochemical role of Lipoproteins:

- Lipoprotein particles are spherical aggregates with hydrophobic lipids at the core and hydrophilic protein side chains and lipid head groups at the surface.
- These proteins are called apolipoproteins.



- Lipoproteins function is to keep their component lipids soluble as they transport them in the aqueous environment of plasma.
- Due to imbalanced metabolism of these lipoproteins there can be a gradual deposition of lipid—especially cholesterol—in tissues, which in arteries of the heart, can lead to CHD.

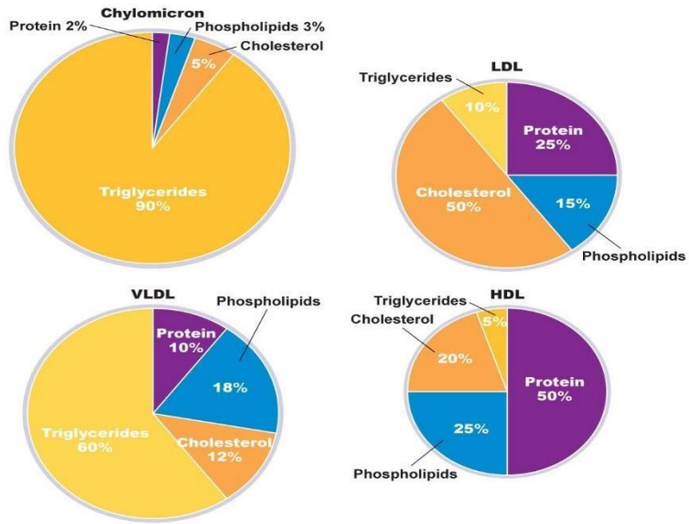
132 Lipid Chemistry-Lipoproteins

- The lipoprotein particles include
- chylomicrons (CM),
- very-low-density lipoproteins (VLDL), • low-density lipoproteins (LDL), and
- high-density lipoproteins (HDL).
- They differ in • lipid and protein composition,
- size, • density and • site of origin
- Different combinations of lipids and proteins produce particles of different densities
- ranging from chylomicrons to high-density lipoproteins
- these particles can be separated by ultracentrifugation and HPLC

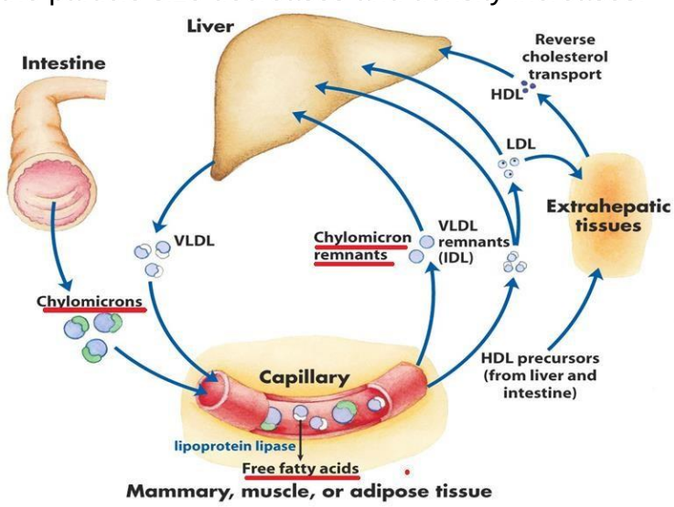
TABLE 21-2 Major Classes of Human Plasma Lipoproteins: Some Properties

		Composition (wt %)				
Lipoprotein	*Density (g/mL)*	*Protein*	*Phospholipids*	*Free cholesterol*	*Cholesteryl esters*	*Triacylglycerols*
Chylomicrons	<1.006	2	9	1	3	85
VLDL	0.95-1.006	10	18	7	12	50
LDL	1.006-1.063	23	20	8	37	10
HDL	1.063-1.210	55	24	2	15	4

Source: Modified from Kritchevsky, D. (1986) Atherosclerosis and nutrition. *Nutr. Int.* **2**, 290-297.



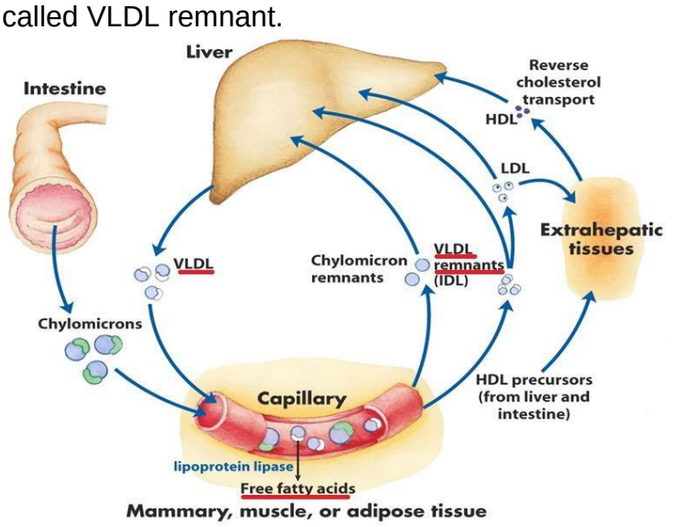
- Chylomicrons are formed and assembled in intestinal mucosal cells after a fatty meal
- And carry dietary triacylglycerol, cholesterol, and cholesteryl esters to the peripheral tissues. • As the chylomicron circulates and most of its dietary TAG are degraded and taken up by peripheral tissues in the form of fatty acids,
- the particle size decreases and density increases.



- The remaining particle, called Chylomicron Remnant, is removed from the circulation by the liver.

133 Lipid Chemistry-Lipoproteins Contd

- VLDLs are assembled in the liver.
- composed predominantly of TAGs synthesized in liver and
- contain some cholesterol and cholesteryl esters
- As VLDL pass through the circulation, TAG is degraded and taken up by peripheral tissues in the form of fatty acids,
- causing the VLDL to decrease in size and become denser,
- called VLDL remnant.

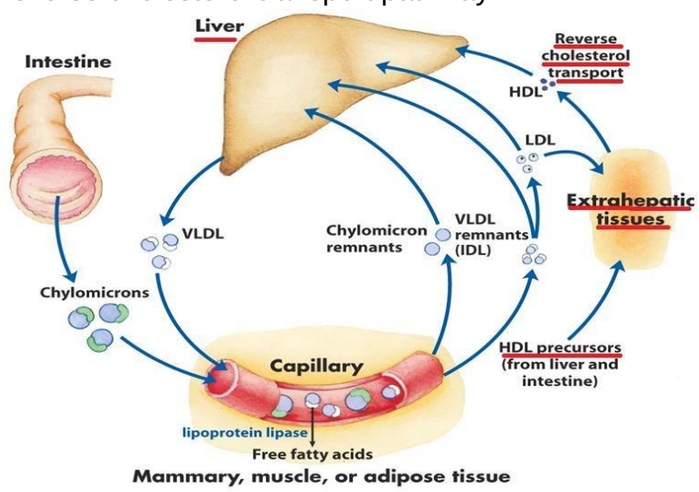


- further removal of
- from these remnants produces low-density lipoprotein (LDL)
- LDL particles contain much less triacylglycerol than their VLDL predecessors,
- and have a high concentration of cholesterol and cholesteryl esters
- LDLs contain apoB-100 as their major apolipoprotein
- they carry cholesterol to extra hepatic tissues that have specific plasma membrane receptors that recognize apoB-100.
- apoB-100 receptors internalize the LDL into the cell.
- Remaining LDL is endocytosed by Liver cells.
- Oxidized LDL can also accumulate in the macrophage cells lining the arteries
- resulting in the formation of atherosclerosis.

134 Lipid Chemistry-Lipoproteins Cont.

HDL The fourth major lipoprotein type, high-density lipoprotein,

- originates in the liver and small intestine
- as small, protein-rich particles that contain little cholesterol and no cholesteryl esters
- They take up cholesterol from non-hepatic (peripheral) tissues and return it to the liver as cholesteryl esters
- When cholesterol is taken up by HDL, it is immediately esterified and becomes hydrophobic and
- which is sequestered in the core of the HDL,
- This transforms the nascent HDL to a mature HDL particle.
- Mature HDL then returns to the liver, where the cholesterol is unloaded in a process called reverse cholesterol transport pathway



- This is the basis for the inverse relationship seen between plasma HDL concentration and atherosclerosis, and for HDL's designation as the "good" cholesterol carrier.
- A much stronger correlation exists between the levels of blood LDL cholesterol and heart disease
- In contrast, high levels of HDL cholesterol have been associated with a decreased risk for heart disease
- Abnormal levels of plasma lipids (dyslipidemias) act in combination with smoking, obesity, sedentary lifestyle, insulin resistance, and other risk factors to increase the risk of CHD
- Clinical studies have demonstrated that dietary or drug treatment of hyper - cholesterolemia is effective in decreasing LDL, increasing HDL, and reducing the risk for cardiovascular events.

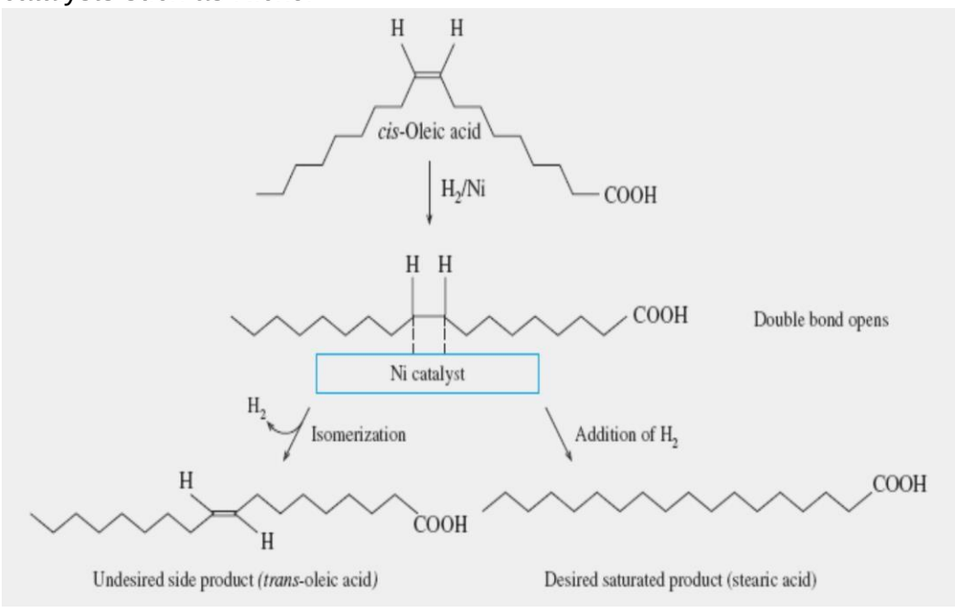
135 Lipid Chemistry-

136 Lipid Chemistry-Role of Dietary Lipids

Role of dietary Lipids (Contd.)

- Unhealthy Fat contains
- Trans fat
- Saturated Fat
- Increased cholesterol content

- Trans fat
- elevate serum LDL (but not HDL),
- Therefore they increase the risk of CHD
- Fatty acids of trans configuration in our food come from two different sources:
- industrially produced partially hydrogenated fat (IP-TFA) and
- ruminant produced Trans Fatty acid (RP-TFA)
- Industrially produced trans fatt (IP-TFA)
- These are made by partial hydrogenation of vegetable fat, and to a lesser extent, of fish oils by heating to about 400°C under high pressure and with the addition of different catalysts such as Nickel



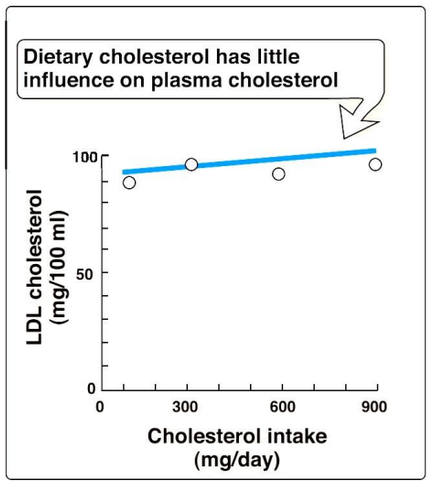
- Ruminant produced Trans Fatty acid (RP-TFA)
- are made by bacterial metabolism of polyunsaturated fatty acids in the rumen of ruminants, such as cow & sheep
- And are consequently present in all fats from these animals
- The concentration of IP-TFA in partially hydrogenated fat may be as high as 60%.
- Whereas the maximum content of RP-TFA in ruminant fat is about 6%.
- In milk, RP-TFA is 4–6% of the fat.
- Recent studies suggest that with equal amounts of intake
- IP-TFA is more harmful than RP-TFA when compared on a gram-to-gram basis.
- The deleterious effects of trans fats occur at intakes of 2 to 7 g/day
- A single serving of French fries in a restaurant may contain this amount of trans fatty acids!
- Therefore, to reduce the harmful effects of trans fats, one should
- limit intake of foods prepared in IP-TFA containing oils such as baked goods and fast foods etc
- . • instead of limiting fat intake from dairy sources.

137 Lipid Chemistry-Role of Dietary Lipids Contd

Role of dietary Lipids (Contd.)

- Saturated fats —
- Consumption of saturated fats is associated with high levels of
- total plasma cholesterol and • LDL cholesterol. • Saturated fats —
- butter, • hard cheeses, • whole milk, • animal fats, • palm oil, and • coconut oils.
- Among the SFAs, stearic acid (18:0) appears to have a neutral effect on LDL-C.
- While lauric (12:0), myristic (14:0), and palmitic (16:0) acids are considered to be hypercholesterolemic
- Saturated FAs increase plasma LDL-C by;
- increasing the formation of LDL in the plasma compartment • and by decreasing LDL turnover.
- The lowering of plasma LDL-C observed with PUFAs is likely due to;

- redistribution of cholesterol between plasma and tissue pools
- and up regulation of the LDL receptor
- In contrast, monounsaturated fatty acids (MUFAs) and PUFAs of the (n-6) family have been shown to;
- decrease plasma cholesterol concentrations in clinical studies • Dietary cholesterol: Cholesterol is found only in animal products.
- dietary cholesterol has little effect on plasma cholesterol.

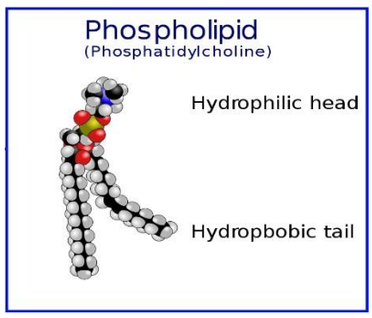


- Therefore, effect of dietary cholesterol on plasma cholesterol is less important than the amount and types of fatty acids consumed.
- A further reduction in dietary cholesterol seems to be unnecessary in those people;
- who have already reduced their intake of saturated fat
- and increased the ratio of polyunsaturated to saturated fatty acids
- The Mediterranean Diet
- Mediterranean cultures, show a low incidence of coronary heart disease
- The Mediterranean diet is an example of a • diet rich in monounsaturated fatty acids (from olive oil) and ω-3 fatty acids (from fish oils and some nuts),
- but low in saturated fat
- The Mediterranean diet contains
- seasonally fresh food, with
- an abundance of plant material,
- low amounts of red meat, and
- olive oil as the principal source of fat
- The Mediterranean diet is associated with decreased serum total cholesterol
- and LDL cholesterol—but
- little change in HDL cholesterol—when compared with a typical Western diet higher in saturated fats

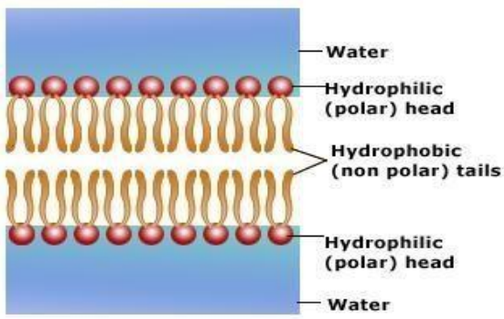
138 Lipid Chemistry-Structure of Phospholopids

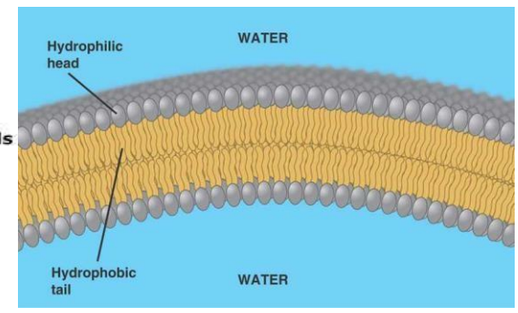
STRUCTURE OF PHOSPHOLIPIDS

- There are two classes of phospholipids:
- those that have glycerol as a backbone: glycerophospholipids • those that contain sphingosine:
- sphingphospholipids
- Phospholipids are the predominant lipids of cell membranes
- Membrane lipids are amphipathic i.e. one end of the molecule is hydrophobic, the other hydrophilic

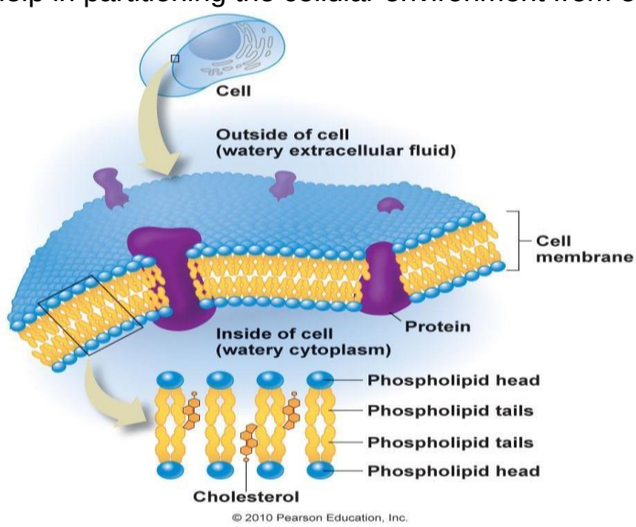


- Their hydrophobic interactions with each other and their
- hydrophilic interactions with water direct their
- packing into sheets called membrane bilayers • The formation of membrane bilayers





- help in partitioning the cellular environment from extracellular environment.

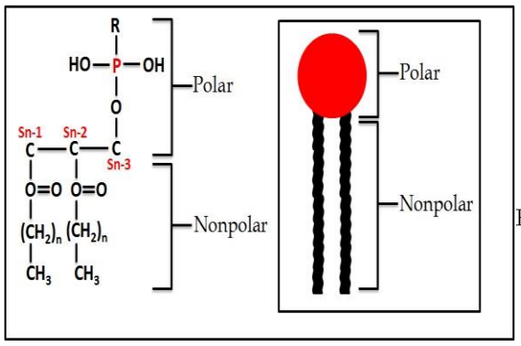


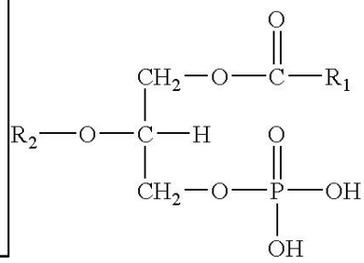
- Non-membrane-bound phospholipids serve additional functions in the body, for • example, as components of lung surfactant
- essential components of bile.
- In contrast to triacylglycerol which is essentially synthesized only in liver, adipose tissue, lactating mammary glands, and intestinal mucosal cells
- essentially all cells except mature erythrocytes can synthesize phospholipids.

139 Lipid Chemistry-Glycerophosholopids

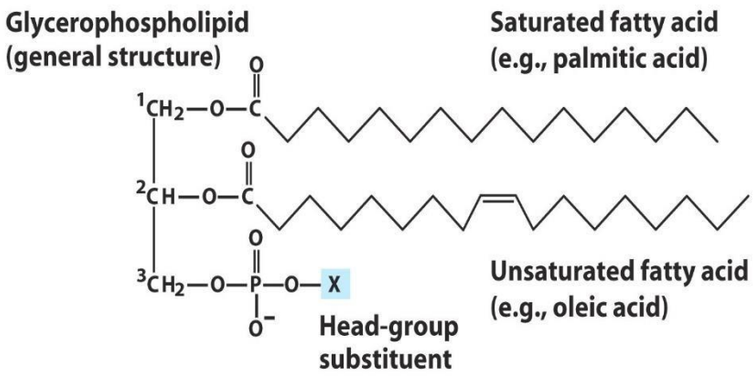
Glycerophospholipids

- Phosphatidic acid
- It is the simplest phosphoglyceride,
- It is a diacylglycerol with a phosphate group on the third carbon of glycerol.





- Phosphatidic acid is the precursor of the other members of this group.
- Further esterification with a low-molecular weight alcohol gives a glycerophospholipid
- In general, glycerophospholipids contain
- a C16 or C18 saturated fatty acid at C-1 and
- a C18 or C20 unsaturated fatty acid at C-2 in addition to • a phosphate group on C-3



- The phosphate group on phosphatidic acid (PA) can be esterified to another compound containing a hydroxyl group
  - Serine + PA → phosphatidylserine – Ethanolamine + PA → phosphatidylethanolamine – Choline+ PA → phosphatidylcholine (lecithin)
  - Inositol + PA → phosphatidylinositol

–

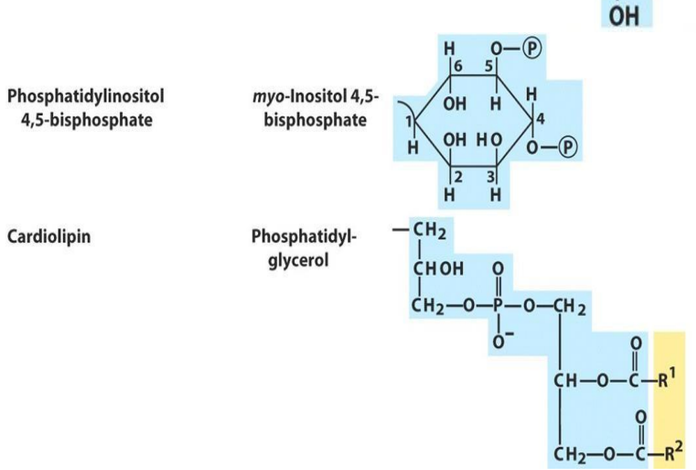
Name of glycerophospholipid	Name of X	Formula of X
Phosphatidic acid	—	—H
Phosphatidylethanolamine	Ethanolamine	$-CH_2-CH_2-\overset{+}{N}H_3$
Phosphatidylcholine	Choline	$-CH_2-CH_2-\overset{+}{N}(CH_3)_3$
Phosphatidylserine	Serine	$-CH_2-\underset{COO^-}{CH}-\overset{+}{N}H_3$
Phosphatidylglycerol	Glycerol	$-CH_2-\underset{OH}{CH}-CH_2-OH$

–

- The molecule is both a primary amine and primary alcohol

a

- The fatty acids in



glycerophospholipids can be any of a wide variety, so a given phospholipid (phosphatidylcholine, for example) may consist of several molecular species, each with its unique complement of fatty acids.

- For instance, Dipalmatoyl phosphatidyl choline and Palmitoyl-oleylphosphatidylcholine
- are two different examples of Lecithins.

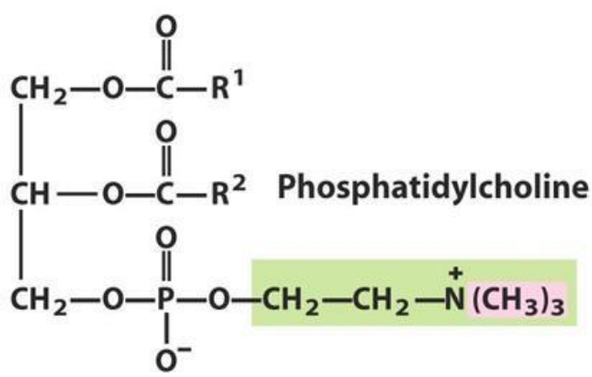
140 Lipid Chemistry-Phosphotidylcholines

Glycerophospholipids

(Contd.)

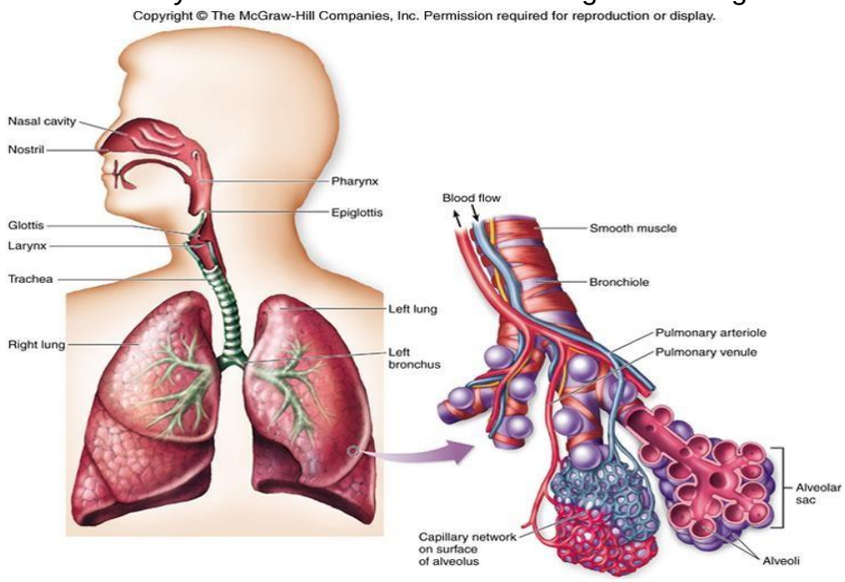
Phosphatidylcholines (Lecithins)

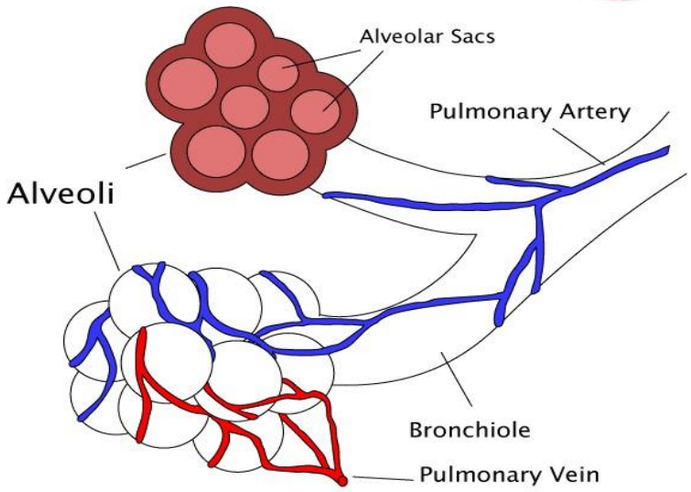
- the most abundant phospholipids of the cell membrane
- represent a large proportion of the body's store of choline- important in nervous transmission, as acetylcholine.



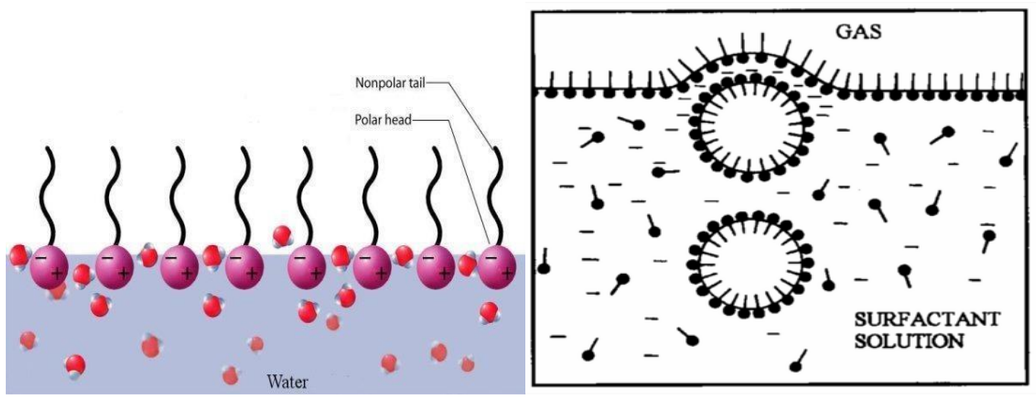
- Dipalmitoyl phosphatidyl choline (DPPC or dipalmitoylecithin), is also the major lipid component of lung surfactant
- Alveoli are the structural and functional unit of respiratory system in which gaseous exchange takes place.

- A thin fluid layer lines the alveoli for efficient gas exchange.





- When the water forms a surface with air, the water molecules on the surface (in contact of air) are strongly attracted to each other trying to reduce the surface area of contact. This is called "surface tension".
- surface tension might result in alveolar collapse
- it requires a certain inflation pressure to maintain expanded alveoli
- the higher the surface tension, the more pressure required to inflate the bubble, especially in small alveoli.
- made and secreted by lung cells, surfactant serves to decrease the surface tension of this fluid layer.
- It scatters among the fluid molecule decreasing the attraction between them.

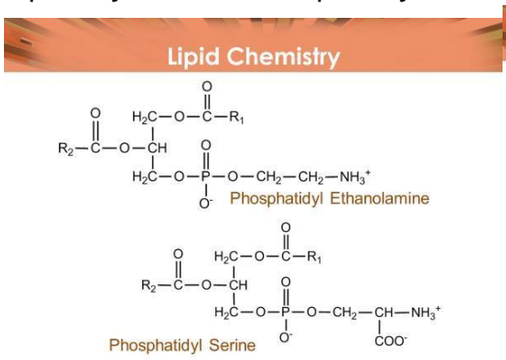


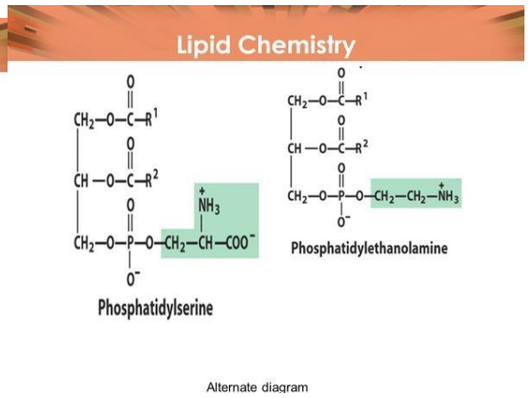
- surfactant have both hydrophilic and hydrophobic regions
- by adsorbing to the air-water interface of alveoli hydrophilic head groups in the fluid and the hydrophobic tails facing towards the air (DPPC) reduces surface tension.

141 Lipid Chemistry-Phosphotidyl Ehanol Amine

Glycerophospholipids (Contd.)

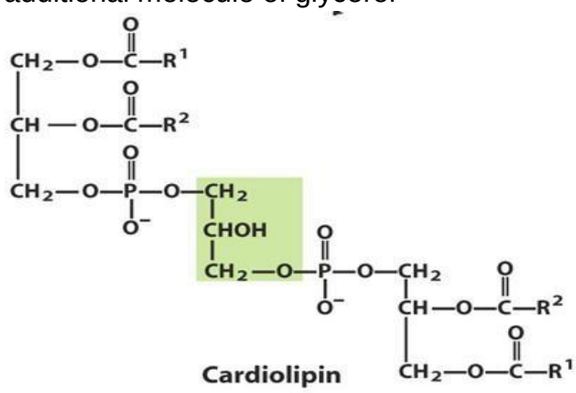
- Phosphatidyl ethanol amine and phosphatidylserine
- are also found in cell membranes and
- differ from phosphatidylcholine only in that ethanolamine or serine, respectively, replaces choline
- Serine is a standard amino acid
- which on decarboxylation produces ethanolamine:
- a primary alcohol and a primary amine.





Alternate diagram

- Phosphatidylserine also plays a role in apoptosis (programmed cell death)
- Cardiolipin
- **diphosphatidylglycerol**
- Two molecules of phosphatidic acid esterified through their phosphate groups to an additional molecule of glycerol

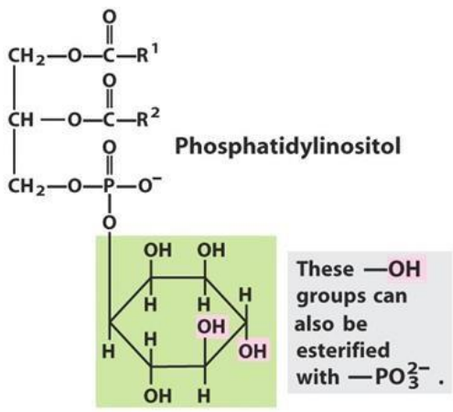


- In eukaryotes, cardiolipin is virtually exclusive to the inner mitochondrial membrane, where it appears to be required for the maintenance of certain respiratory complexes of the electron transport chain.
- Decreased cardiolipin levels or alterations in its structure or metabolism cause mitochondrial dysfunction in aging and in pathological conditions including heart failure
- Cardiolipin is antigenic, and is recognized by antibodies raised against Treponema pallidum, the bacterium that causes syphilis
- The VDRL test measures immunoglobulin G (IgG) and IgM antibodies to lipoidal material released from damaged host cells as well as to lipoprotein-like material
- and possibly cardiolipin released from the treponemes
- Anti-cardiolipin antibodies may also be found non-specifically in certain auto- immune diseases.

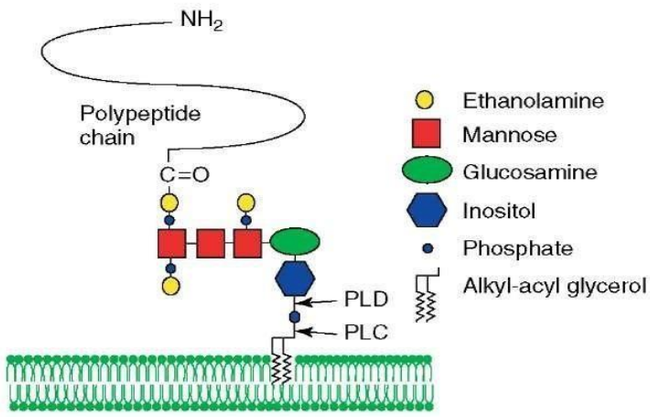
142 Lipid Chemistry-Phosphotidylinositol Glycerophospholipids (Contd.)

Phosphatidylinositol

- consists of phosphatidic acid and
- Inositol in an
- ester linkage



- Inositol is a polyol synthesized in the body from glucose
- and have the same chemical formula as glucose
- Phosphatidylinositol (PI) is an unusual phospholipid in that it often contains stearic acid on carbon 1 and arachidonic acid on carbon 2 of the glycerol
- PI, therefore, as a reservoir of arachidonic acid, serves as precursor for prostaglandin synthesis
- Specific proteins can be covalently attached via a carbohydrate bridge to membrane-bound PI.

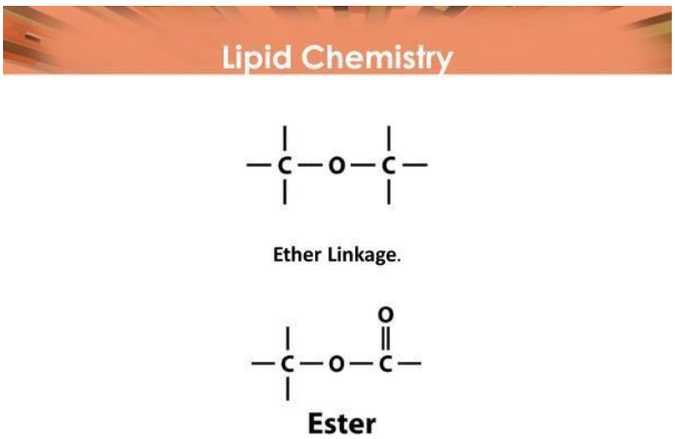


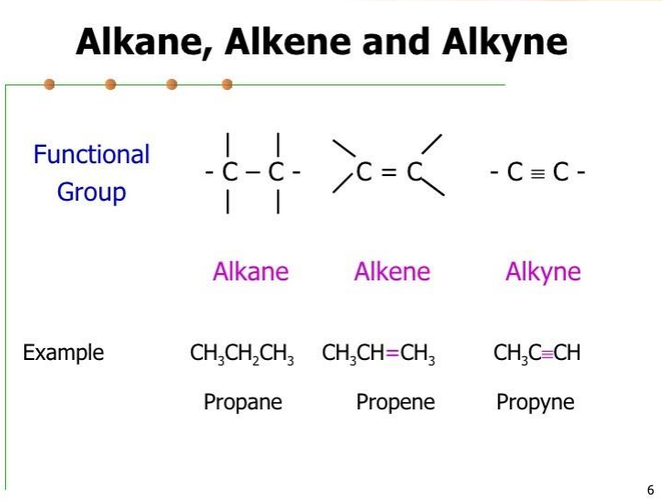
- • Phosphatidylinositol is a precursor of second messengers
- The phosphorylation of membrane-bound phosphatidylinositol produces phosphatidylinositol 4,5-bisphosphate (PIP2)
- The degradation of PIP2 by phospholipase C occurs in response to the binding of a variety of neurotransmitters, hormones, and growth factors to receptors on the cell membrane
- The products of this degradation are
- inositol 1,4,5 trisphosphate (IP3) and
- diacylglycerol (DAG)
- These products mediate the
- mobilization of intracellular calcium and
- the activation of protein kinase C, respectively, which act synergistically to evoke specific cellular responses

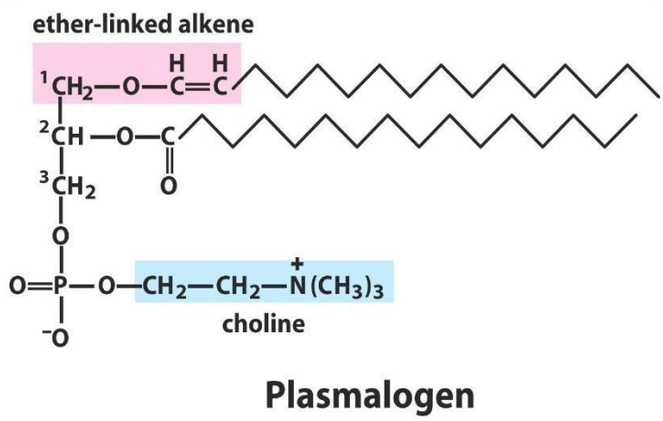
143 Lipid Chemistry-Ether Lipids

- **Ether lipids**

- They are type of Glycerophospholipids,
- in which one acyl chains is attached to glycerol in ether linkage, rather than ester linkage
- Ether lipids with an unsaturated group (alkenyl) at the 1st position on the glycerol chain are called Plasmalogens

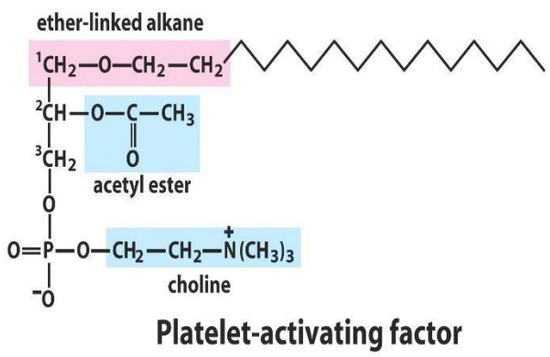






- In some instances serine, or inositol may be substituted for ethanolamine or choline
- These compounds constitute as much as 10% of the phospholipids of brain and muscle
- Vertebrate heart tissue is uniquely enriched in ether lipids
- About half of the heart phospholipids are plasmalogens
- The membranes of halophilic bacteria, ciliated protists, and certain invertebrates also contain high proportions of ether lipids.

- The functional significance of ether lipids in these membranes is unknown;
- perhaps their resistance to the phospholipases that cleave ester-linked fatty acids from membrane lipids is important in some roles
- Platelet-activating factor (PAF)
- This is an unusual ether glycero phospholipid, with a saturated alkyl group in an ether link to carbon 1 and an acetyl residue (rather than a fatty acid) at carbon 2 of the glycerol backbone

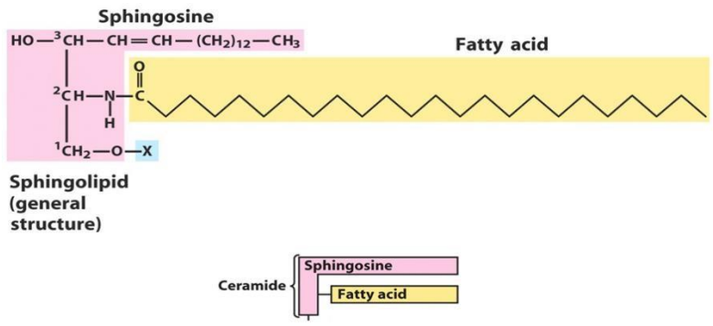


- PAF is synthesized and released by a variety of cell types
- It binds to surface receptors, triggering potent thrombotic and acute inflammatory events
- It causes platelets to aggregate and degranulate (required for clotting), and neutrophils and alveolar macrophages to generate superoxide radicals (required for microbial killing)

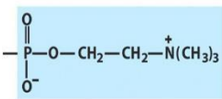
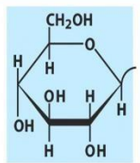
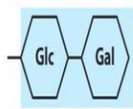
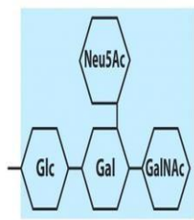
144 Lipid Chemistry-Sphingolipids

SPHINGOLIPIDS

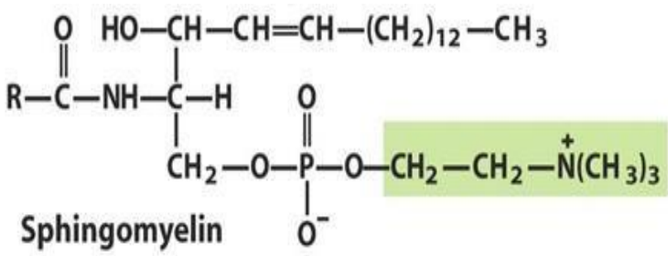
- Sphingolipids, like other membrane lipids, are composed of a
- hydrophobic portion, (ceramide) and
- a polar head group



-
- The first three carbons at the polar end of sphingosine are analogous to the three carbons of glycerol in glycerophospholipids
- The amino group at C-2 bears a fatty acid in amide linkage • The fatty acid is usually saturated or monounsaturated, with
- 16, 18, 22, or 24 carbon atoms.
- Ceramide is the parent compound for this group
- **SPHINGOLIPIDS**
- There are two subclasses of sphingolipids:
- Sphingomyelins
- Sphingoglycolipids
- **Sphingomyelins**
- contain phosphocholine or phosphoethanolamines as their polar head group and are therefore classified along with glycerophospholipids as phospholipids
- **Sphingoglycolipids**
- are molecules that contain both
- carbohydrate and
- lipid (in the form of ceramide) components

Name of sphingolipid	Name of X	Formula of X
Ceramide	—	—H
Sphingomyelin	Phosphocholine	
Neutral glycolipids Glucosylcerebroside	Glucose	
Lactosylceramide (a globoside)	Di-, tri-, or tetrasaccharide	
Ganglioside GM2	Complex oligosaccharide	

- When the alcohol group at carbon 1 of sphingosine is, esterified to **phosphorylcholine**, **sphingomyelin,** the only significant sphingophospholipid in humans, is produced

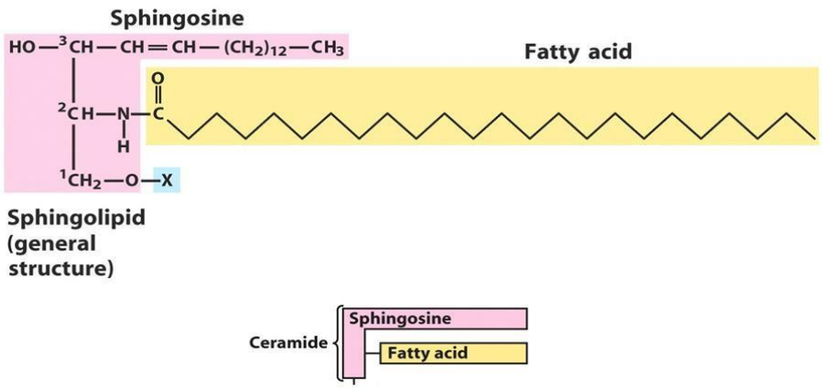


- **Sphingomyelins** are
- present in the plasma membranes of animal cells and are especially prominent in nerve tissue including myelin, -thus the name "sphingomyelins
- Sphingomyelin of the **myelin sheath** contains predominant longer-chain fatty acids such as lignoceric acid and nervonic acid (24 carbon)
- whereas **gray matter of the brain** has sphingomyelin that contains primarily stearic acid(18 carbon)

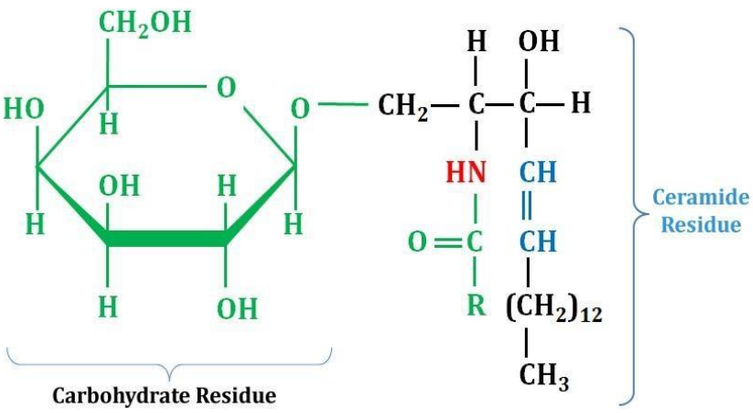
145 Lipid Chemistry-Glycosphingolipids

Glycosphingolipids are molecules that contain both

- carbohydrate and lipid components
- Like the phospholipid sphingomyelin, they are derivatives of ceramide
- They are also an important component of membrane bilayers.
- They occur largely in the outer face of plasma membranes have polar head groups with one or more sugars connected directly to the -OH at C-1 of the ceramide moiety by an O-glycosidic bond

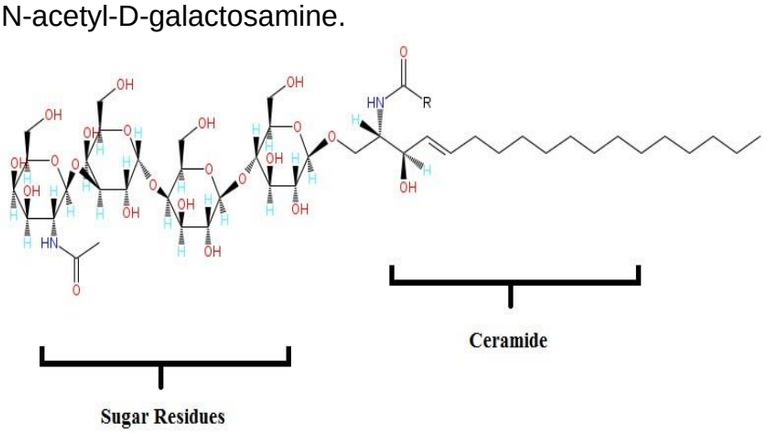


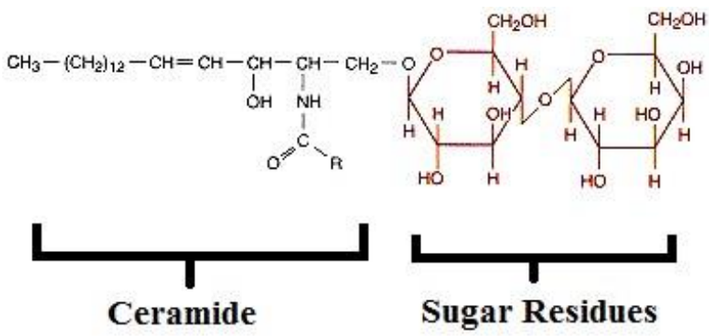
- **Cerebrosides**
- have a single sugar linked to ceramide;
- galactocerebrosides are found in the plasma membranes of neural cells, • glucocerebrosides in the plasma membranes of non neural cells



Cerebroside

- Galactosylceramide is a major glycosphingolipid of brain and other nervous tissue,
- It contains a number of characteristic C24 fatty acids,
- eg, cerebronic acid
- **Globosides**
- are glycosphingolipids
- with two or more sugars, usually
- D -glucose,
- D- galactose, or
- N-acetyl-D-galactosamine.

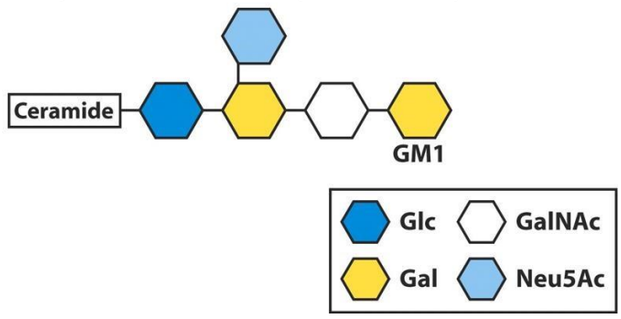




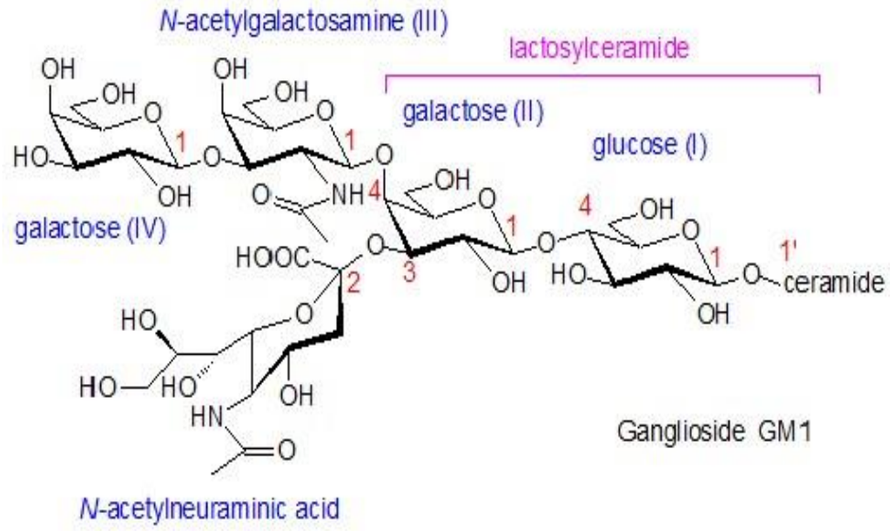
-
- A glycosphingolipid that has only one sugar as the side chain is called a cerebroside
- Globosides are highly abundant in RBCs.
- Globosides are also found in human serum, spleenand liver.
-

146 Lipid Chemistry-Glycosphingolipids Contd

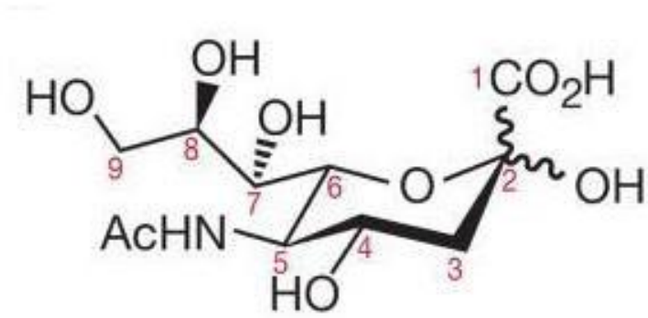
- **Gangliosides**
- They have oligosaccharides as their polar head groups and one or more residues of N-acetylneuraminic acid (a sialic acid), at the termini.



-
- D -glucose,
- D- galactose, or
- N-acetyl-D-galactosamine. • *N*-acetylneuraminic acid

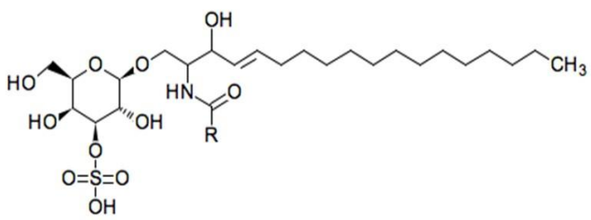


-
- Sialic acids are acidic sugars with a nine-carbon backbone, of which the most common is
- N-acetylneuraminic acid
- Sialic acid gives gangliosides the negative charge at pH 7



N-Acetylneuraminic acid
(Neu5Ac)

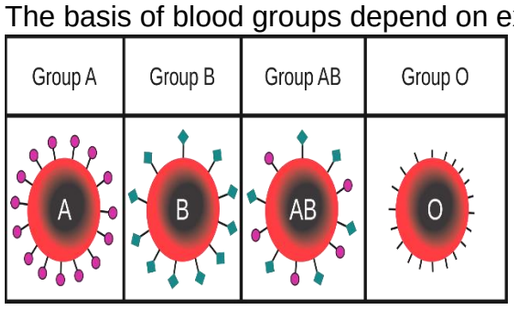
-
- Gangliosides with one sialic acid residue are in the GM (M for mono-) series,
- those with two are in the GD (D for di-) series,
- and so on (GT three sialic acid residues; GQ, four)
- About 6% of brain lipids are gangliosides and were first isolated from the ganglion of brain cells
- They act as specific receptors for glycoprotein hormones in the cells.
- Some gangliosides also serve as receptors for some bacterial protein toxins e.g.
- Cholera toxin binds to the GM1 gangliosides on the surface of target cells
- Gangliosides also help in cell-cell recognition and thus have a significant role in growth and differentiation of tissues and also in carcinogenesis
- Sulfatides
- Sulfoglycosphingolipids (sulfatides) are cerebrosides that contain sulfated galactosyl residues, and are therefore negatively charged at physiologic pH



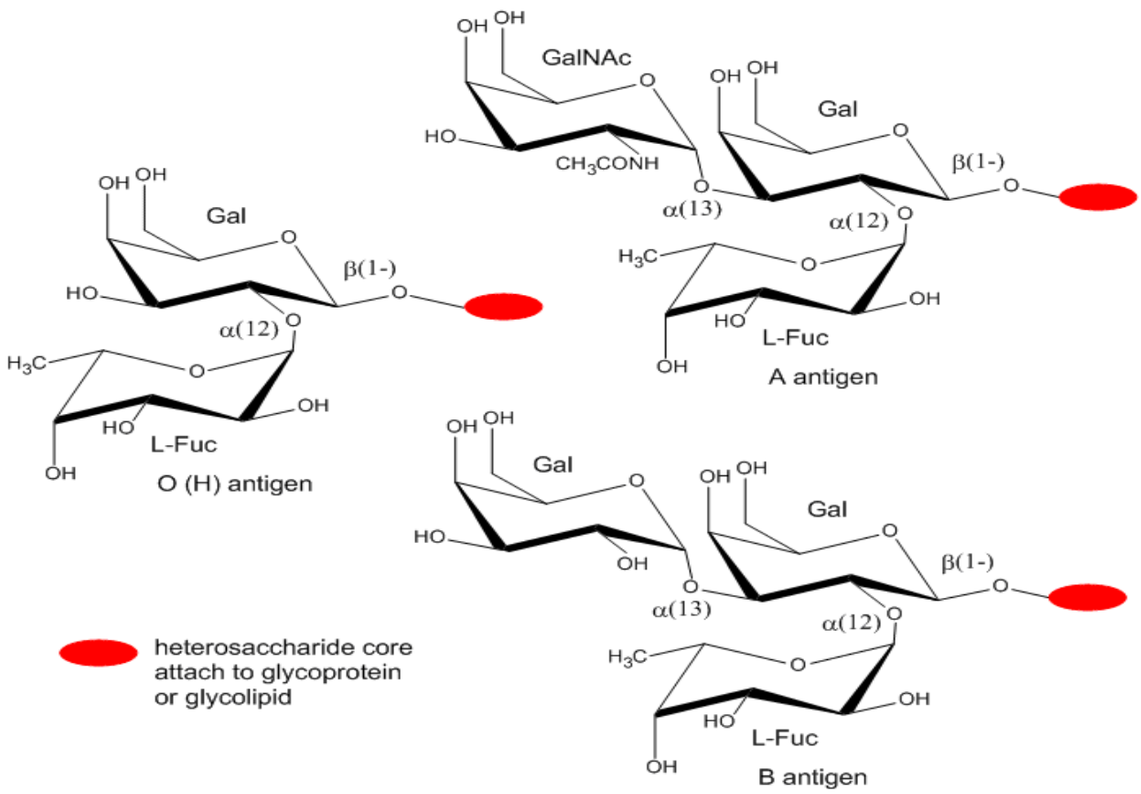
-
- • Sulfatides are found predominantly in nerve tissue and kidney

147 Lipid Chemistry-ABO Blood Groups and Glycosphingolipids

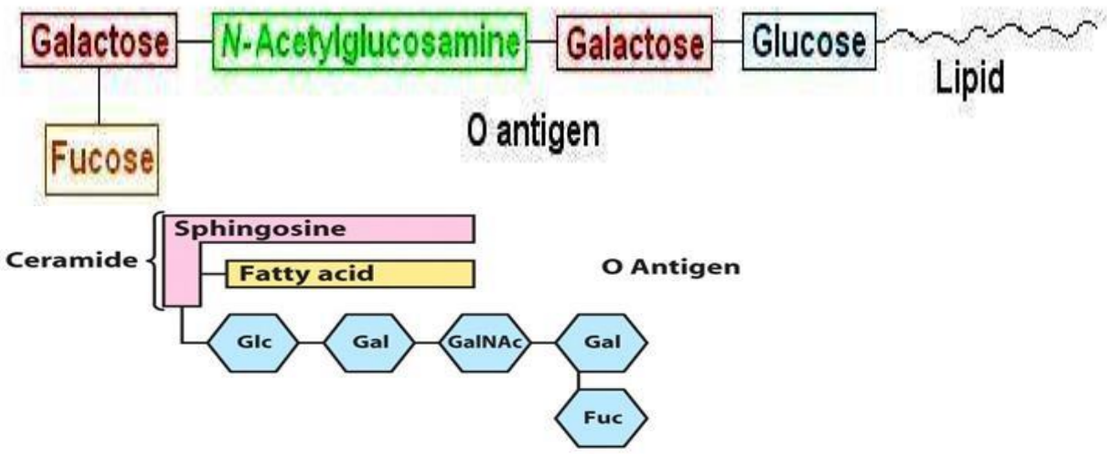
- ABO Blood Groups and Glycosphingolipids
- The basis of blood groups depend on expression of different antigens on RBCs.



-
- These antigens are found in cell membranes as oligosaccharide components of
- glycosphingolipids
- glycoproteins
- Different compositions of carbohydrate (oligosaccahride chains) in different antigens.
- These oligosaccharides are attached to proteins through a serine or threonine residue
- or to ceramide lipid intermediate



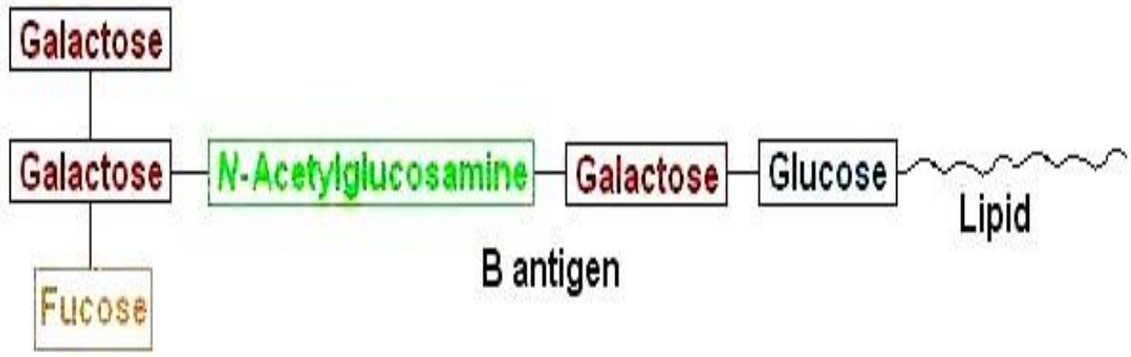
-
- The precursor to the ABO blood group antigens, present in people of all common blood

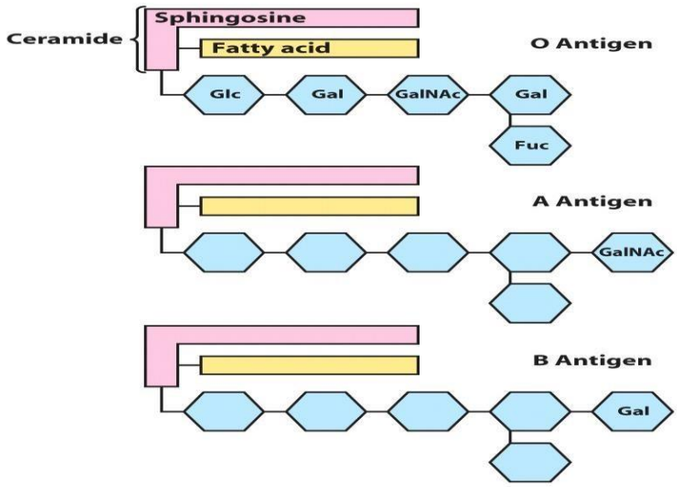


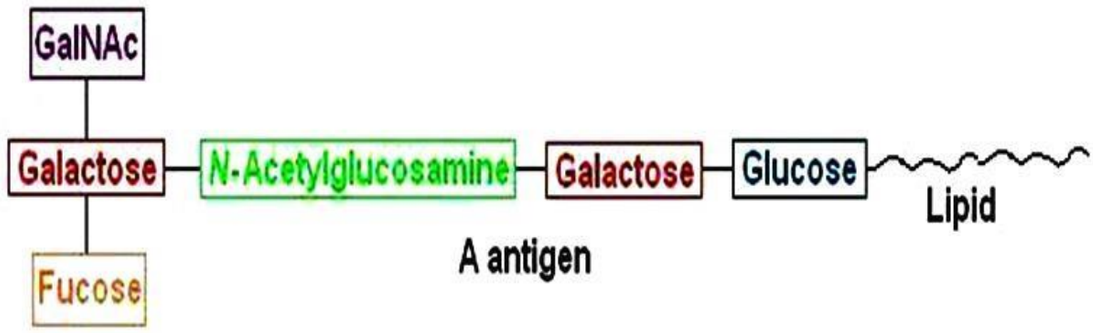
types, is the O antigen (also referred to as H antigen.)

- Modification of this O antigen then

- results in A, B or AB blood groups respectively.







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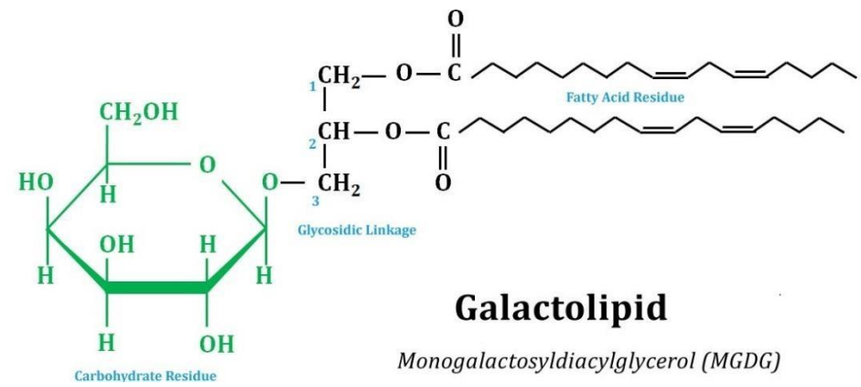
-
- These antigens are present on other cells of the body as well.

148 Lipid Chemistry-Glycerophospholipids

Glyceroglycolipids

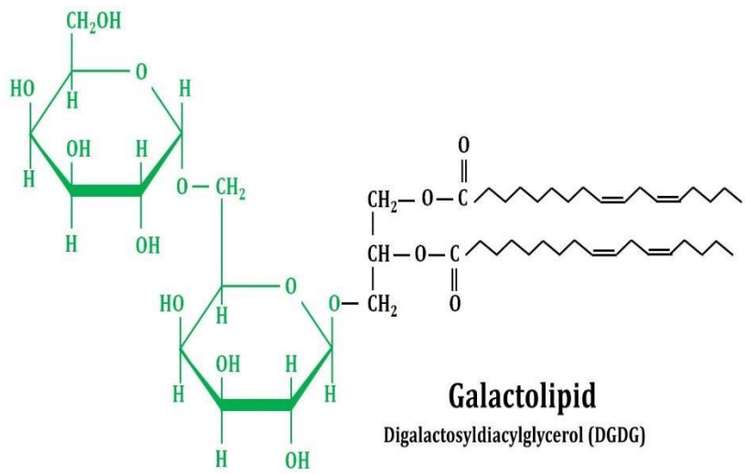
- These are the predominant membrane lipids of the plants, such as those of chloroplast.
- They include
- Galactolipids and

- Sulfolipids
- As the name indicates these glycolipids contain glycerol instead of sphingosine as their backbone.
- Galactolipids
- In galactolipids, the C3 of the glycerol moiety is connected to one or more galactose residues by glycosidic linkages.



Galactolipid

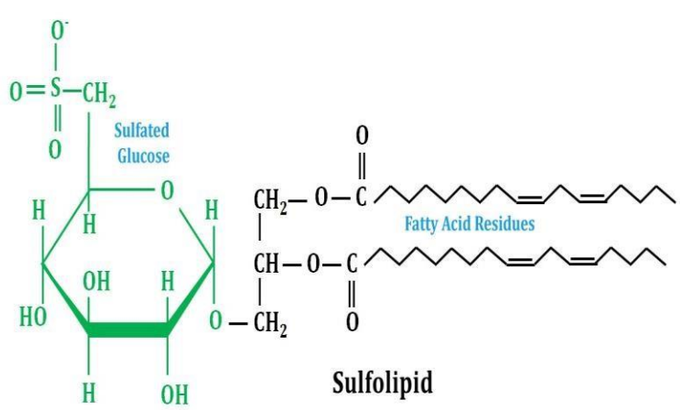
Monogalactosyldiacylglycerol (MGDG)



Galactolipid

Digalactosyldiacylglycerol (DGDG)

- The head groups of these galactolipids are uncharged but polar.
- Galactolipids are localized in the internal membranes of chloroplast • They constitute about 70% to 80 % of plant membrane lipids and
- thus are the most abundant lipids in the biosphere.
- Sulfolipids
- They are membrane glycolipids with sulfur containing functional
- groups Sulfonated glucose is joined to the C3 of diacylglycerol in • glycosidic linkage



Sulfolipid

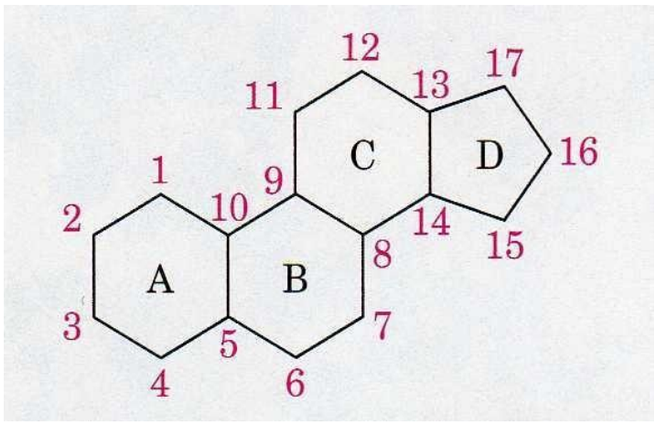
149 Lipid Chemistry-Steroids and Cholesterol

Steroids and Cholesterol

• A steroid is a lipid whose structure is based on the tetracyclic (four-ring) structure consists of

• 3 cyclohexane rings.

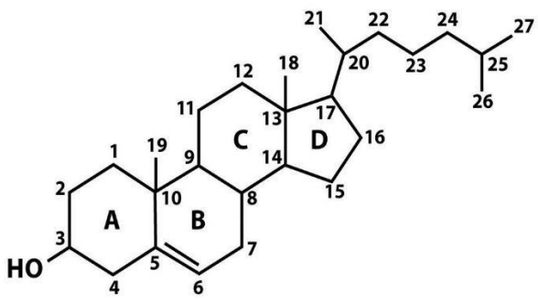
• 1 cyclopentane ring.



• Steroids with eight to ten carbon atoms in the side chain at C-17 and a hydroxyl group at C-3 are classified as sterols

• Cholesterol is the major sterol in animal tissues

• Cholesterol has an eight carbon branched hydrocarbon chain attached to C-17 of the D ring • Ring A has a hydroxyl group at C-3, and ring B has a double bond between C-5 and C-6



Cholesterol

• Cholesterol is an amphipathic lipid

• Cholesterol is a structural component of all cell membranes, modulating their fluidity

• Cholesterol is a precursor of bile acids steroid hormones vitamin D

All tissues containing nucleated cells are capable of cholesterol synthesis, which occurs in the endoplasmic reticulum and the cytosol

• Cholesterol is present in tissues and in plasma either as free cholesterol or combined with a long-chain fatty acid as cholesteryl ester

• In plasma, both forms are transported in lipoproteins

• in esterified form, with a FA attached at C-3, • the structure becomes more hydrophobic than free cholesterol

• Cholesteryl esters are not found in membranes, and are normally present only in low levels in most cells

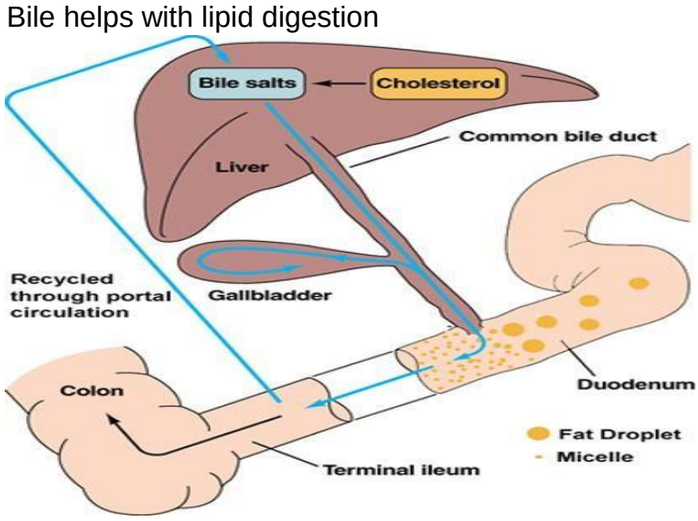
• The ring structure of cholesterol cannot be metabolized to CO_2 and H_2O in humans

• Therefore humans cannot utilize cholesterol for energy.

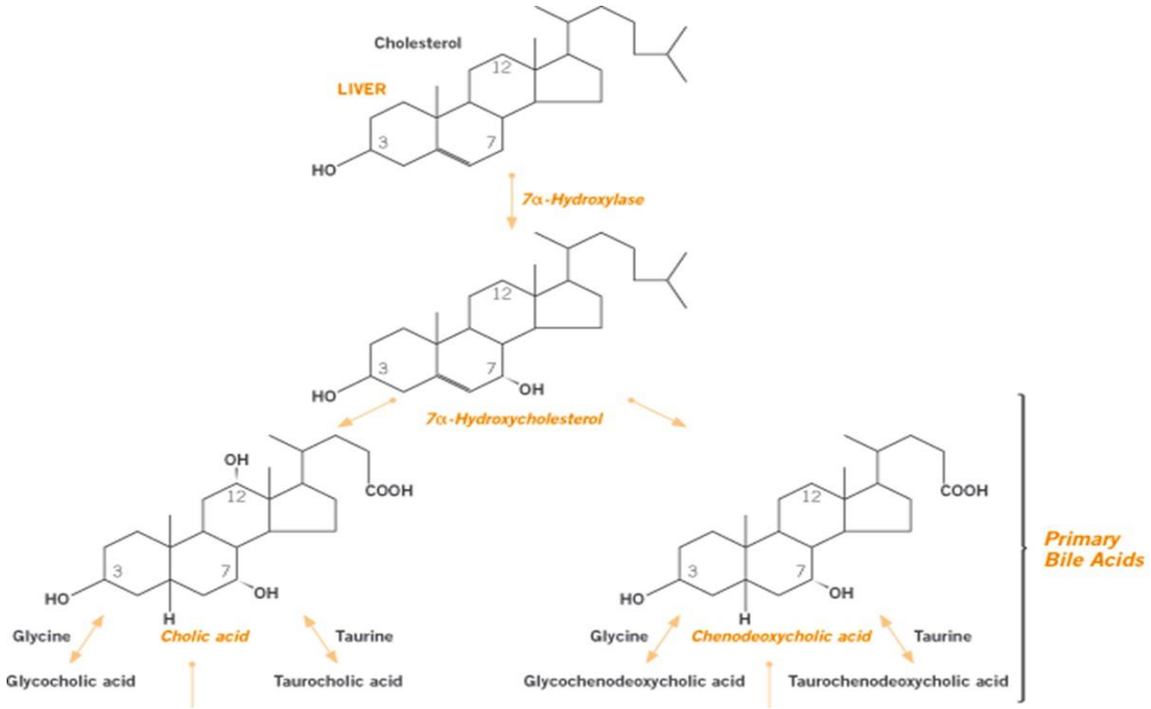
• Cholesterol is excreted from the body via the bile either in the unesterified form or after conversion into bile acids in the liver

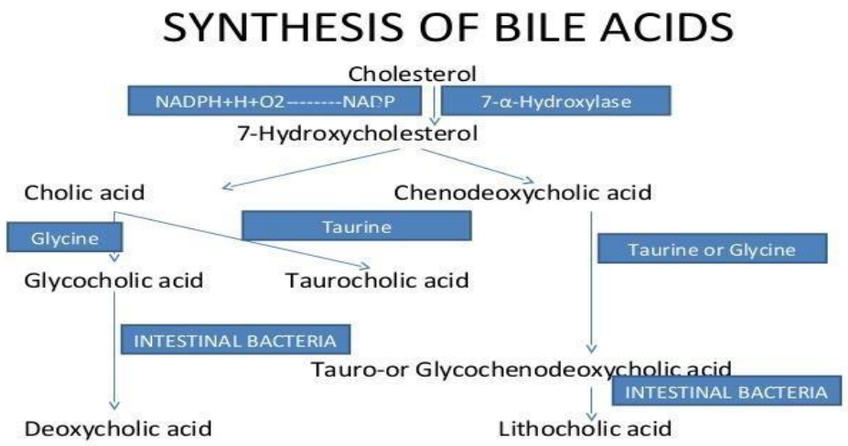
150 Lipid BILE ACIDS AND BILE SALTS

- Bile is a fluid that is made and released by the liver and stored in the gallbladder • Bile helps with lipid digestion

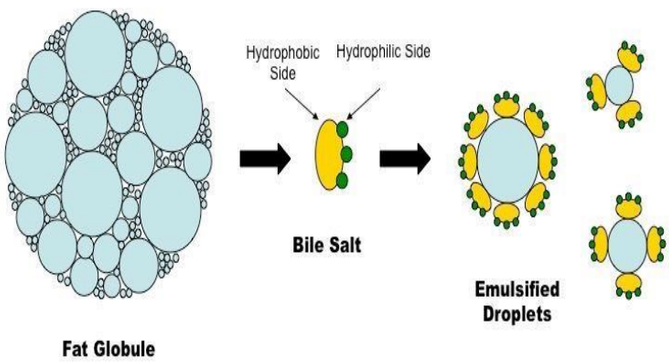


- Bile can either pass directly from the liver into the duodenum, or
- be stored in the gallbladder when not immediately needed for digestion
- Bile consists of a watery mixture of organic and inorganic compounds
- Phosphatidylcholine (lecithin) and
- bile salts (conjugated bile acids) are quantitatively the most important organic components of bile
- The primary bile acids are synthesized in the liver from cholesterol
- These are cholic acid and • chenodeoxycholic acid





- The bile acids contain 24 carbons, with two or three hydroxyl groups and a side chain that terminates in a carboxyl group
- The carboxyl group has a pKa of about 6 and, is not fully ionized at physiologic pH- hence the term "bile acid"
- The bile acids are amphipathic molecules
- They therefore can act as emulsifying agents in the intestine



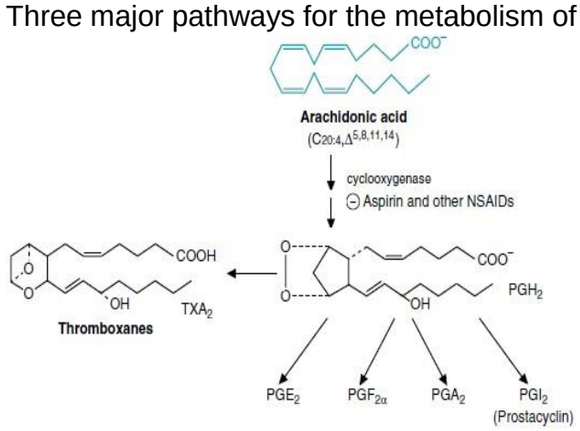
- They emulsify
- dietary triacylglycerol and other complex lipids • for degradation by pancreatic digestive enzymes.

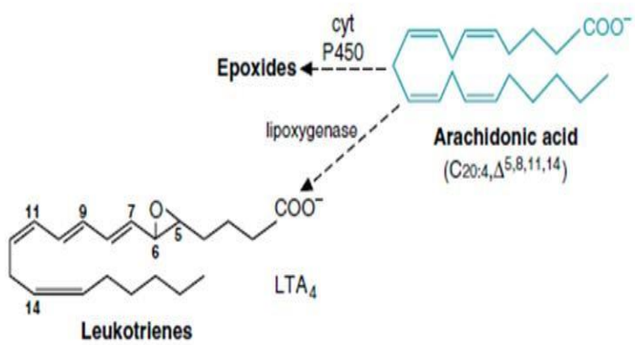
151 Lipid Chemistry-Eicosanoids

- Eicosanoids are a large group of lipid messengers with potent effects on every tissue in the body
- **Eicosanoids** are derived from metabolism of 20-carbon, polyunsaturated fatty acids (eicosanoic acids) • Eicosanoids include (but not limited to)
- **Prostanoids** consisting of
- Prostaglandins
- Prostacyclins
- Thromboxanes

1. **Leukotrienes**
2. **Lipoxins**
3. **Epoxides**

- These extremely potent compounds acting through their specific receptors
- elicit a wide range of physiologic and pathologic responses.
- particularly important in eliciting inflammatory response that occurs after infection or injury an
- produce symptoms such as pain, swelling, and fever.
- they also control bleeding through forming blood clots
- Eicosanoids are derived from either omega-3 (ω-3) or omega-6 (ω-6) fatty acids.
- Arachidonic acid is the most common precursor of the eicosanoids.
- Three major pathways for the metabolism of arachidonic have been discovered so far.



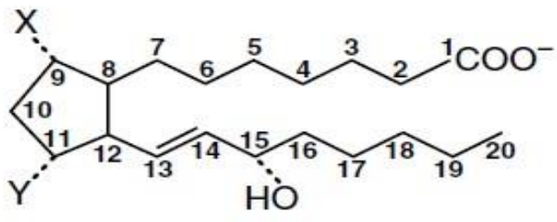


- Cyclooxygenase pathway; prostaglandins and thromboxanes.
- lipoxygenase pathway; leukotrienes, HETEs, and lipoxins.
- cytochrome P450 pathway epoxides and HETEs
- There are different series of eicosanoids
- depending on the precursor 20 C FA. derived from the essential fatty acids linoleic acid and linolenic, OR
- directly from dietary arachidonic acid and eicosapentaenoic acid
- Depending on the precursor different numbers of double bonds are present in these eicosanoids reflecting the parent 20C FA.

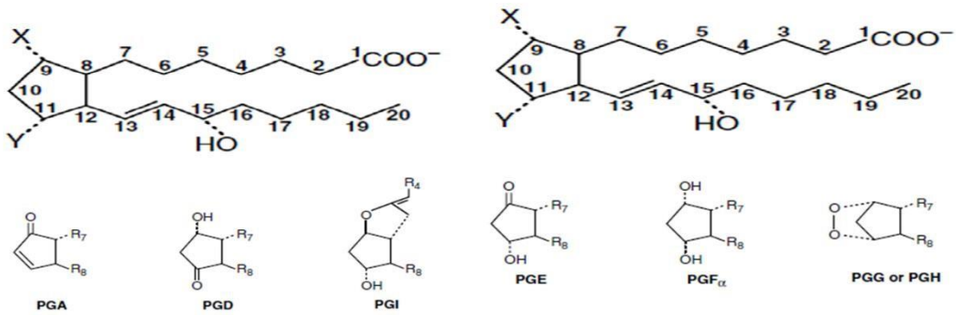
152 Lipid Chemistry- Cyclooxygenase Pathway

- Prostaglandins

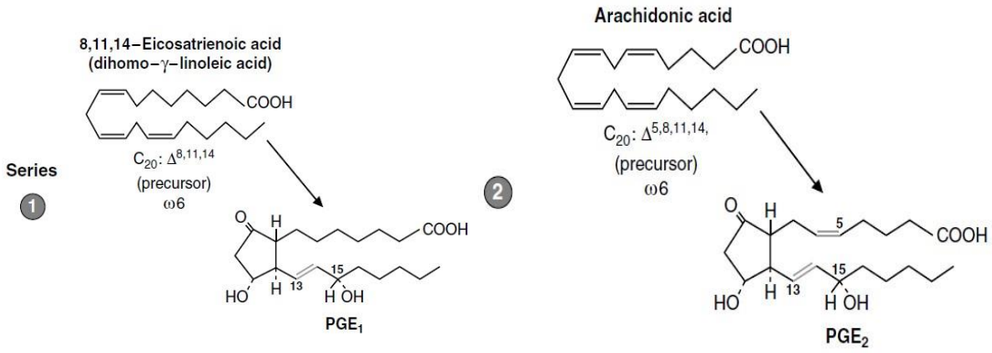
- Prostaglandins are fatty acids containing 20 carbon atoms, including • an internal 5-carbon ring.



-
- Prostaglandins have
- a hydroxyl group at C 15,
- a double bond between C 13 and C 14, and
- various substituents on 5 membered ring at C9 and C11
-
- Nomenclature of prostaglandins (PGs) involves the
- assignment of a capital letter (PGE), • a numeral subscript (PGE1), and
- for the PGF family, a Greek letter subscript (PGF2α).
- The capital letter refers to the ring substituents at positions X and Y.



-
- The numeral subscript that follows the capital letter(e.g. PGE1) refers to the PG series 1, 2, or 3, determined by the number of unsaturated bonds present in the linear portion of the hydrocarbon chain.

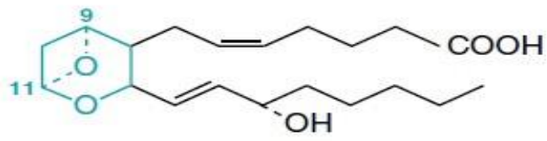


-
- It does not include double bonds in the internal ring.
- The double bonds between carbons 13 and 14 are trans; the others are cis
- The Greek letter subscript, found only in the F series, refers to the position of the hydroxyl group at carbon 9.
- This hydroxyl group primarily exists in the α position, i.e. it lies below the plane of the ring.
- Prostaglandins have a hydroxyl group at C 15, a double bond between C 13 and C 14, and various substituents on 5 membered ring at C9 and C11 Double bonds also may be present between carbons 5 and 6 and between carbons 17 and 18 in case of series 2 and 3

153 Lipid Chemistry-Cyclooxgenase Pathway (Contd.)

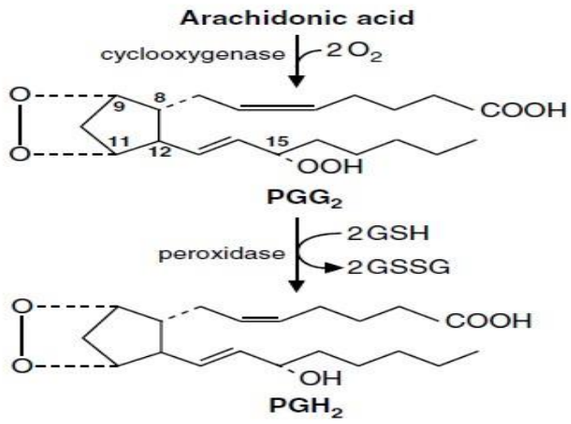
CYCLOOXYGENASE PATHWAY (Contd.)

- Thromboxanes (TX)
- also formed via the cyclooxygenase pathway,
- differ from PGs in that they contain a 6-membered ring that includes an oxygen atom



Thromboxane A_2 (TXA_2)

-
- Biosynthesis of the PGS and TX
- Those derived from arachidonic acid, the 2-series, (such as PGE2,TXA2), are described here • because the 1-series and the 3-series are present in very small amounts in humans.
- The initial step, which is catalyzed by a cyclooxygenase (COX), forms the fivemembered ring and
- adds four atoms of oxygen (two between C 9 and C11, and two at C 15) to
- form the unstable PGG2.
- The hydro-peroxy group at carbon 15 is
- quickly reduced to a hydroxyl group by a peroxidase to form PGH2 • PGH2 is the precursor of all other PGs and TXAs



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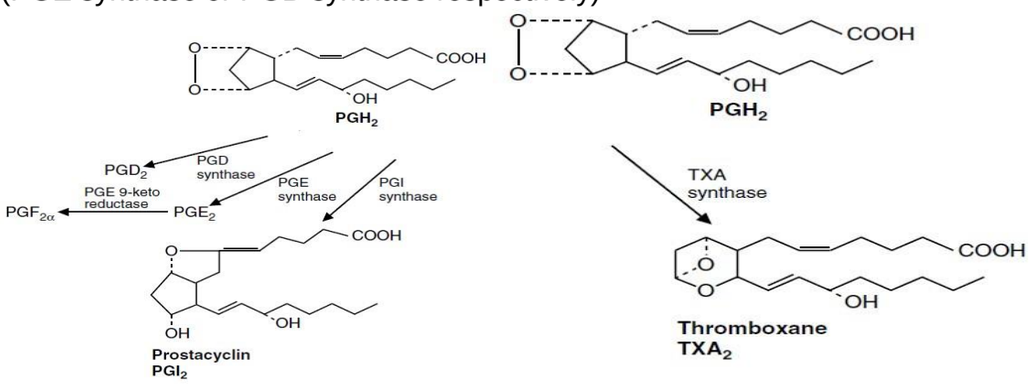
- The next step is tissue specific
- For example, PGH2 may be reduced to PGE2 or PGD2 by specific isomerases (PGE synthase or PGD synthase respectively)



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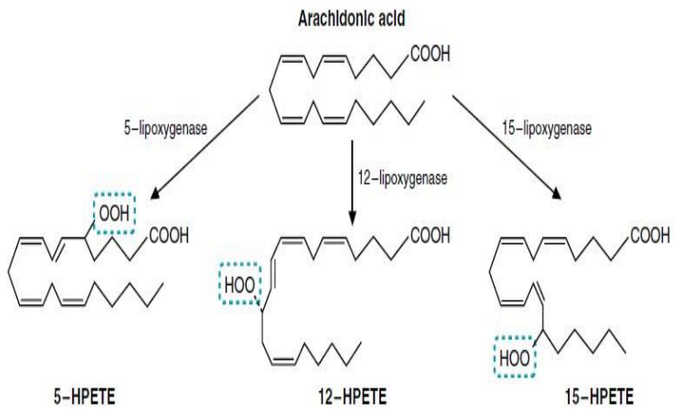
- . • For example, TXA Synthase is present in high concentration in platelets and forms TXA2.
- In the vascular endothelium, however, PGH2 is converted to the PGI2 by action of Prostacyclin Synthase.
- The beneficial effect of cold water fish (e.g., salmon), with a high content of eicosa-pentaenoic(TX3) acid (EPA), and docosa-hexaenoic acid (DHA) comes from the fact that they lead to formation of more TXA3 relative to TXA2.
- TXA3 is less effective in stimulating platelet aggregation than its counterpart in the 2-series, TXA2.
- Platelet aggregation is the culminating step in the cardiovascular diseases due to atherosclerosis.

154 Lipid Chemistry-Lipoxygenase Pathway

Lipoxygenase Pathway:

Synthesis of the Leukotrienes, and Lipoxins (Count.)

- In addition to serving as a substrate for the cyclooxegenase pathway,
- arachidonic acid and other 20 C FAs also act as substrate for the lipoxygenase pathway.
- In contrast to products of the cyclooxegenase pathway which are cyclical.
- Products of lipoxygenase pathway are linear. • Similar nomenclature rules are followed for Leukotrienes and Lipoxins, • except that there are no series of 1 and 2.
- The series starts from 3.
- the eicosanoids synthesized in lipoxygenase pathway from;
- Eicosa-tri-enoic acid (ETA) have 3 double bonds
- from; Arachidonic acid have 4 double bonds
- From; Eicosa-pentaenoic acid (EPA) have 5 double bonds
- . • The lipoxygenase enzymes catalyze the incorporation of an oxygen molecule on to a carbon of one of several double bonds of arachidonic acid, forming a hydro-peroxy (–OOH) group at these positions



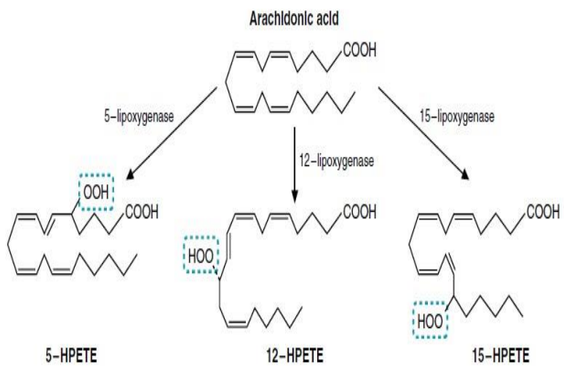
-
- The double bonds at which oxygen is added are between C5 & C6, between C11 and C12 and between C14 and C15.

155 Lipid Chemistry-Lipoxygenase Pathway (Contd)

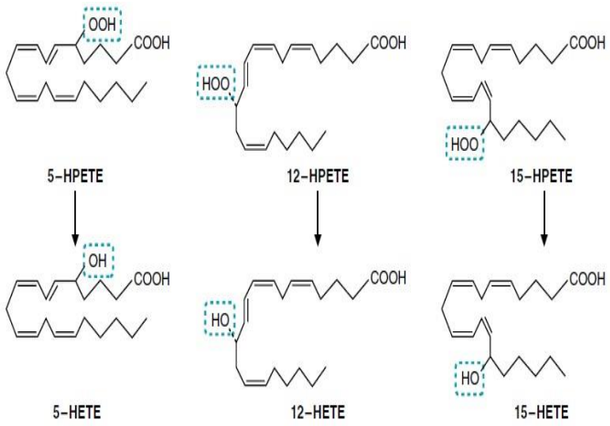
Lipoxygenase Pathway:

Synthesis of the Leukotrienes, and Lipoxins (Contd.)

- When the oxygen is added, to the double bond it isomerizes to a position one carbon away from the hydro-peroxy group and
- is transformed from the *cis* to the *trans* configuration



- As a result of this isomerization, double bond
- between C5 & C6 moves to C6 & C7,
- between C11 & C12 moves to C10 & C11
- between C14 & C15 moves to C13 & C14
- The hydro-peroxy group is unstable and
- can be converted to the more stable hydroxy group to form HETEs • HETEs themselves act as messenger molecules

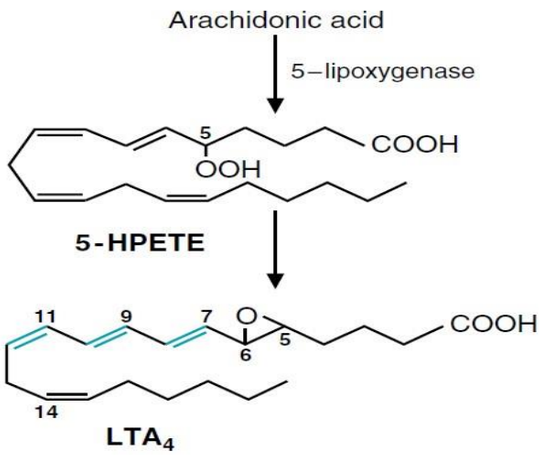


- The other alternate is the conversion of HPETEs to leukotrienes(LTs) and Lipoxins(LXs),
- Which are more potent and have more defined physiological roles.
- The leukotrienes(LTs) and Lipoxins(LXs),
- Have 3 to 5 double bonds as described earlier, in comparison to
- prostaglandins which have 1 to 3 double bonds.

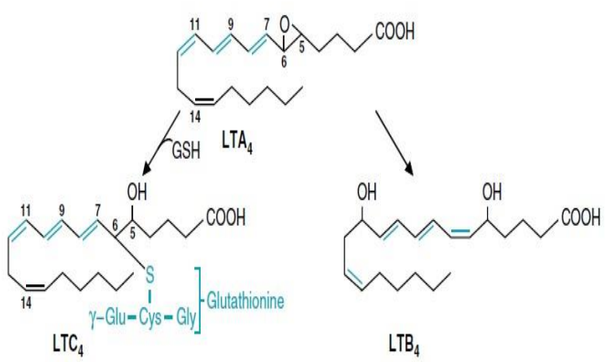
156 Lipid Chemistry-Lipoxygenase Pathway (Contd.)

Lipoxygenase Pathway:

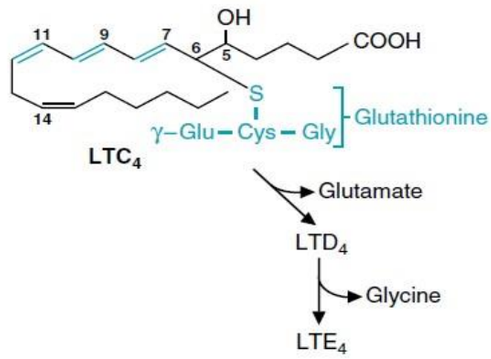
- HPETEs (Hydro-per-oxy-eicosa-tetra-enoic acids) are the precursors of leukotrienes and lipoxins
- The major leukotrienes are produced by 5-lipoxygenase • 5-HPETE is converted to leukotriene A4 (LTA4).



- Other functional leukotrienes are formed from LTA4 for example, • LTA4 is converted to LTB4, as a 5,12-dihydroxy derivative.
- the addition of reduced glutathione to carbon 6 forms LTC4



- Removal of glutamate residue from LTC4 forms LTD4 • LTD4 on removal of glycine becomes LTE4

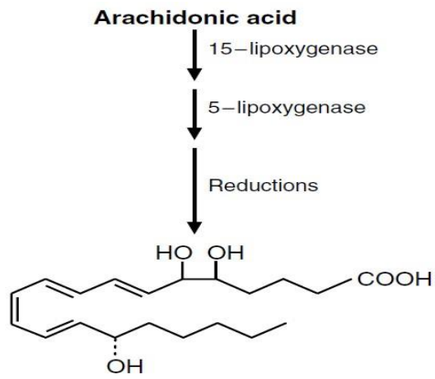


-
- Leukotrienes were so named because they were first discovered in leukocytes(white blood cells)

157 Lipid Chemistry-Lipoxygenase Pathway (Cont)

Synthesis of the Leukotrienes, and Lipoxins (Contd.)

- The lipoxins are formed through the action of 15-lipoxygenase followed by the action of 5-lipoxygenase on arachidonic acid.
- A series of reductions of the resultant hydro-per-oxy groups leads to the formation of tri-hydroxy derivatives of arachidonic acid known as the lipoxins.

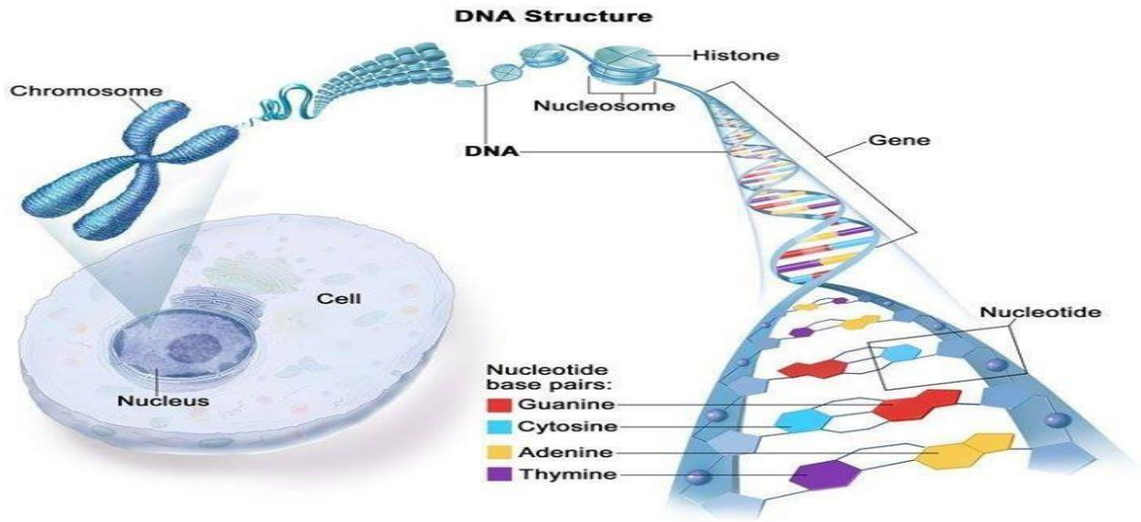


- **Lipoxin A_4**
- Lipoxins induce chemotaxis and stimulate superoxide radicals for killing of microorganisms
- Prostaglandins, thromboxanes, leukotrienes and lipoxins have very short half lives and rapidly degraded in the body.
- In summary, Eicosanoids are derived from C20 (eicosanoic) fatty acids synthesized from the essential fatty acids and make up important groups of physiologically active compounds.

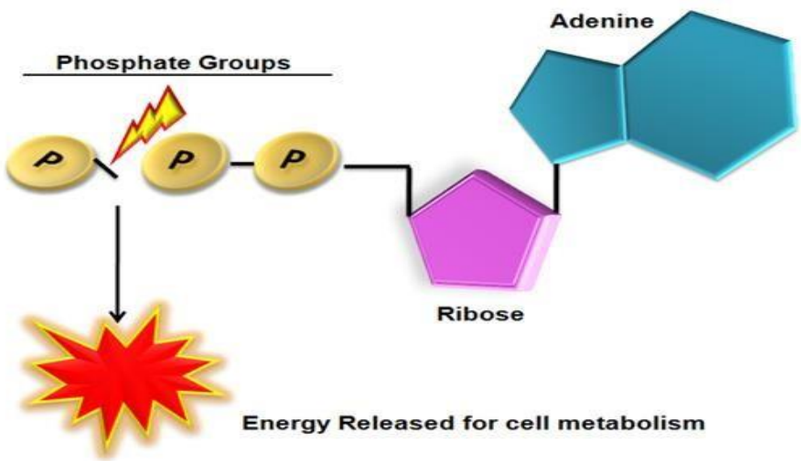
158 Nucleotides and Nucleic Acids

Biomedical importance of nucleotides

- **Precursors of nucleic acids**: Nucleotides are the building blocks of nucleic acids.
- Without them, DNA or RNA can not be produced.



- Transmission of genetic information;
- This gives them the ability to store and transmit genetic information from;
- one generation to the next which is a fundamental condition for life
- Protein synthesis,
- the ultimate expression of this information, is therefore dependent on nucleotides.
- Energy currency: Nucleotides play an important role as "energy currency" in the cell.
- Nucleoside tri- and diphosphates such as ATP and ADP are the principal donors and acceptors of phosphoryl group in metabolism.
- By doing this, they play a key role in the energy transduction.

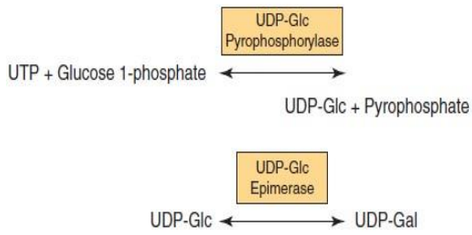


-
- This energy is used in almost every energy requiring process in the body, such as;
- Muscle contraction, Transmission of nerve impulse, Transports of nutrients across cell membrane Motility of spermatozoa And many more energy dependent processes

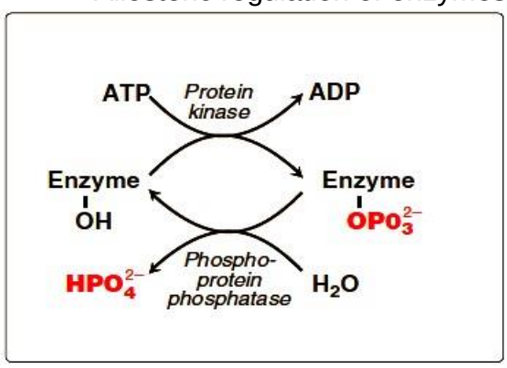
159 Nucleotides and Nucleic Acids- Biomedical importance of nucleotides

Carriers of intermediates: Nucleotides also serve as carriers of activated intermediates in the synthesis of some carbohydrates, lipids, and proteins.

- The sugar derivatives UDP-glucose and UDP-galactose participate in sugar inter conversions
- And in the biosynthesis of starch and glycogen
- Similarly, nucleoside-lipid derivatives such as CDP acylglycerol are intermediates in lipid biosynthesis.



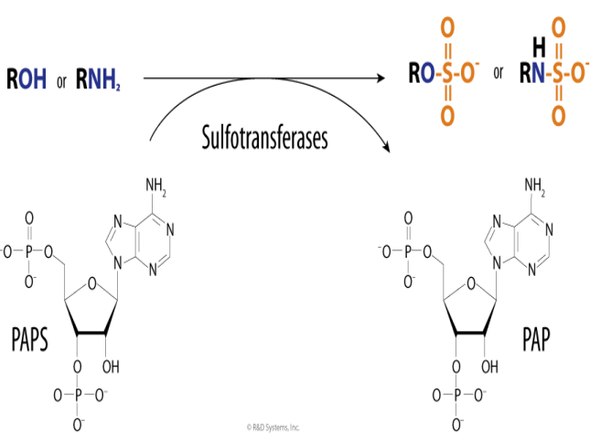
- **Co-enzymes:**
- When linked to vitamins nucleotides are structural components of several essential coenzymes, for example,
- coenzyme A,
- FAD, (Flavin Adenine Dinucleotide) • **Co-enzymes:**
- NAD^+ (Nicotinamide adenine Dinucleotide) and
- $NADP^+$ (Nicotinamide adenine Dinucleotide Phosphate)
- **Regulatory compounds:** Nucleotides are important regulatory compounds for many of the pathways of intermediary metabolism, inhibiting or activating key enzymes.
- Roles that nucleotides perform in metabolic regulation include:
- ATP-dependent enzyme phosphorylation in key metabolic reactions.
- Allosteric regulation of enzymes by ATP, AMP, and CTP



- Phosphorylation can either activate or inhibit ATP- dependent enzyme depending on the type of enzyme.
- Similar is the case with dephosphorylation.

160 Nucleotides and Nucleic Acids- Biomedical importance of nucleotides (Contd)

- Sulfate group donor: Adenosine 3'-phosphate-5'-phosphosulfate is the sulfate donor for
- sulfated proteoglycans • sulfate conjugates of drugs.



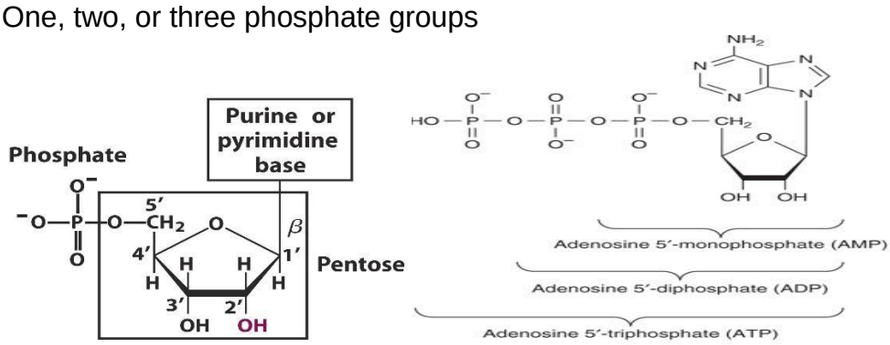
- Methyl group donor:
- S-adenosylmethionine is a methyl group donor • e.g Nor-adrenaline Adrenaline by methylation.

- • Second messengers: Nucleotides, such as cyclic AMP (cAMP) and cyclic GMP (cGMP),
- • serve as second messengers in signal transduction pathways.
  Signal Transduction: GTP and GDP play key roles in activating or inhibiting proteins in various cellular signaling cascades.
- • Medical applications Specifically medical applications include the use of synthetic purine and pyrimidine analogs that contain halogens, thiols, or additional nitrogen atoms;
- There use include chemotherapy for cancer
- as suppressors of the immune response during organ transplantation.
- as anti-viral drugs such as in the treatment of AIDS

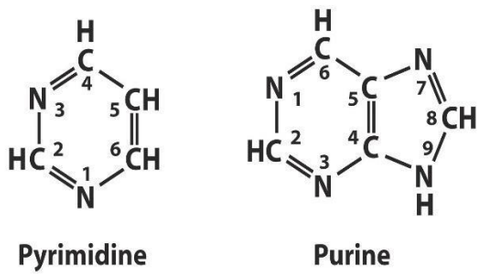
161 Nucleotides and Nucleic Acids-Composition of Nucleotides

Nucleotides are composed of

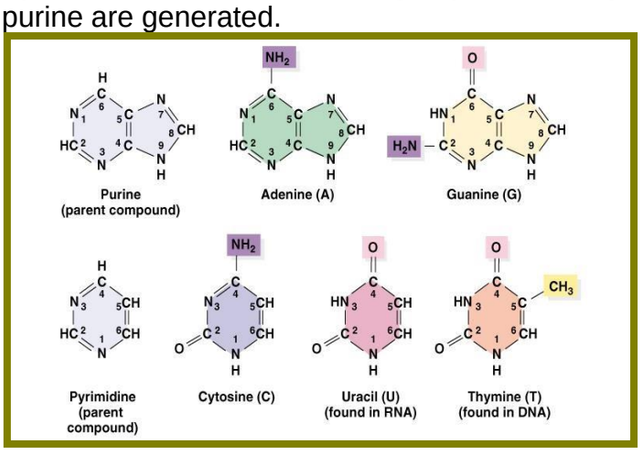
- A nitrogenous base (purine or pyrimidine)
- A pentose monosaccharide
- One, two, or three phosphate groups



- Nitrogenous Bases
- **The nitrogen-containing bases belong to two families of compounds:**
- Purines
- Pyrimidines



- By the attachment of different groups to the rings, different types of pyrimidine and purine are generated.

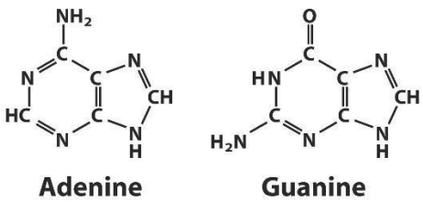


- The suffix “ine” in these bases denotes the presence of nitrogen (amine) in the ring.
- However, there are some exceptions such as naming of uracil
- The utility of these nitrogen-containing ring structures lies in the ability of the nitrogen to form hydrogen bonds and to

- accept and donate electrons while still part of the ring.

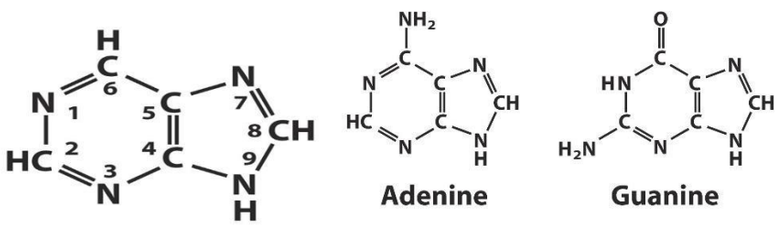
162 Nucleotides and Nucleic Acids- Composition of Nucleotides (Contd.)

- **Purines**
- Both DNA and RNA contain the same purine bases:
- Adenine (A)
- Guanine (G)



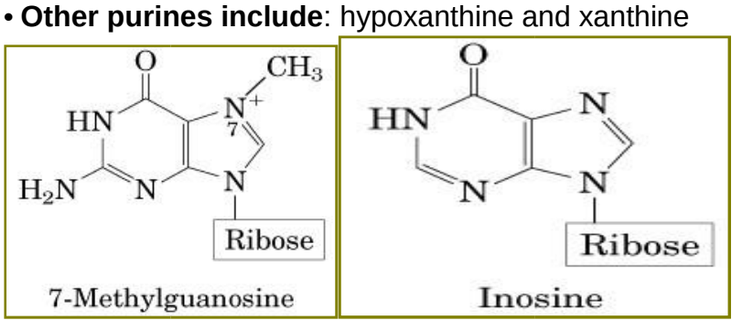
Purines

- Adenine when combines with pentose the structure is known as Adenosine or deoxyadenosine
- Guanine when combines with pentose the structure is known as Guanosine or deoxyguanosine
- Adenine is 6-aminopurine
- Guanine is 2-amino,6-hydroxypurine



Purines

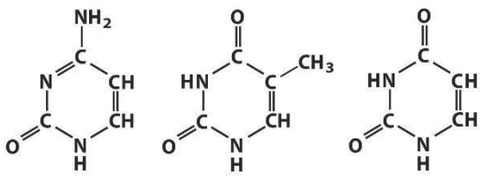
- **Minor Purine Bases:** Inosine (I) & methyl guanine (7mG)
- Unnatural: Mercaptopurine, Allopurinol & 8-Azaguanine
- **Other purines include**: hypoxanthine and xanthine



- Uric acid is the catabolic end product of purines in human beings.

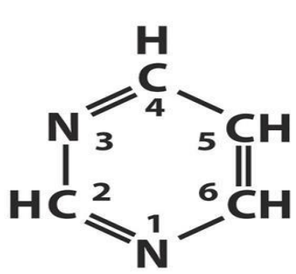
163 Nucleotides and Nucleic Acids- Composition of Nucleotides (Cont.)

- **Pyrimidines:**
- **Pyrimidines include:** • Cytosine (C)—in both DNA and RNA
- Thymine (T)—only in DNA
- Uracil (U) —only in RNA

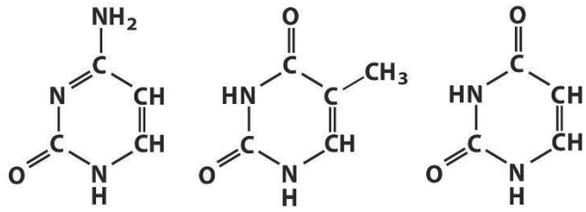


Pyrimidines

- Cytosine when combines with pentose it becomes deoxycytidine and cytidine
- Thymine becomes thymidine and deoxythymidine
- Uracil (U) becomes uridine and deoxyuridine depending on the type of sugar.
- Cytosine is 2-oxy-4-amino-pyrimidine
- Thymine is 2,4-dioxy-5-methyl-pyrimidine • Uracil is 2,4-dioxy-pyrimidine

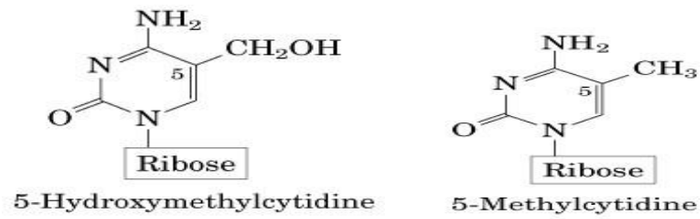


Pyrimidine



Pyrimidines

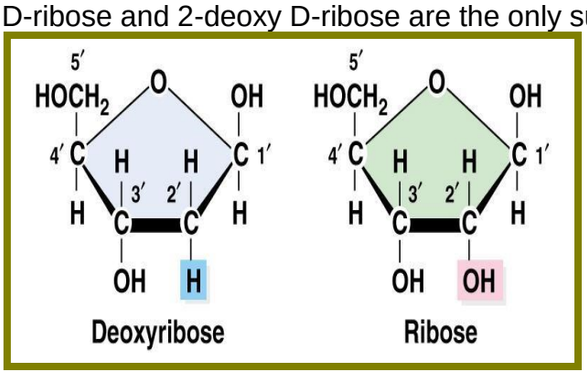
- T and U differ by only one methyl group, which is present on T but absent on U
- **Minor Pyramidine Bases:** • Dihydrouridine (DHU) ,
- 5-Methyl Cytadine & • 5-Hydroxy-Methyl Cytadine
- **Unnatural Pyramidine Bases:**
- Fluorouracil (5FU) &
- 6-Aza Cytosine (AZC)



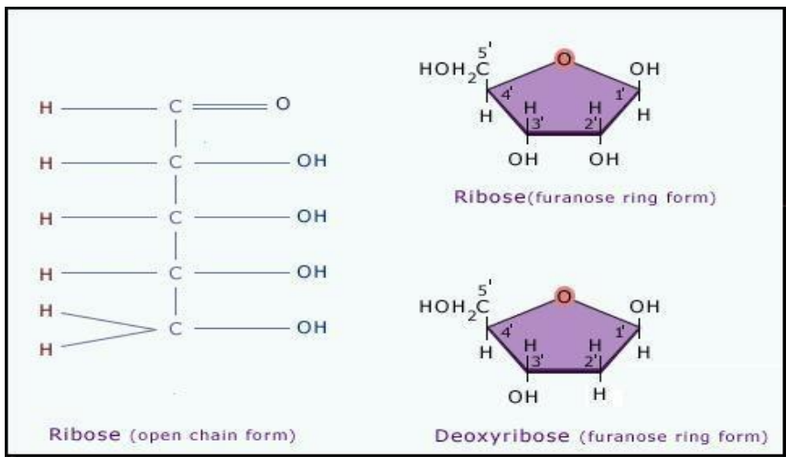
- Unlike the purine ring, which is not cleaved in human cells, the pyrimidine ring is opened and degraded
- to highly soluble products, β-alanine and β-amino-iso-butyrate,
- with the production of NH3 and CO2.

164 Nucleotides and Nucleic Acids- Composition of Nucleotides (Contd)

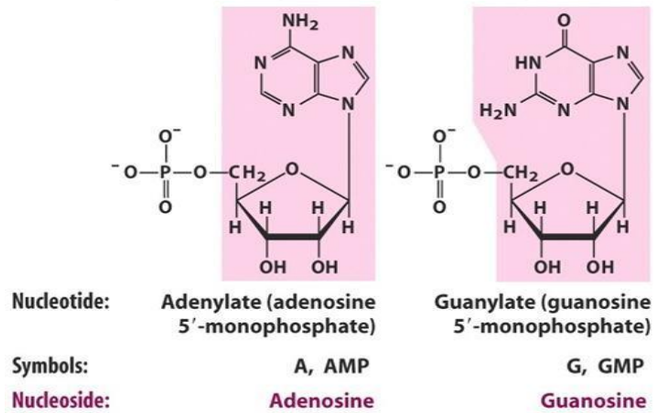
- **Pentose Sugar**
- D-ribose and 2-deoxy D-ribose are the only sugars so far found in the nucleic acids.



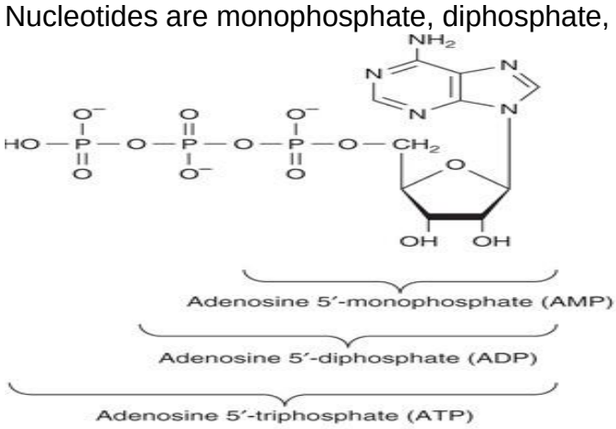
- These aldo pentoses belong to D-family
- They are present as Furanose (ring) in the form of β-Anomer



- The addition of a pentose sugar to a base produces a nucleoside
- If the sugar is D-ribose, a ribonucleoside is produced
- If the sugar is 2-deoxy D- ribose, a deoxyribonucleoside is produced



- **Phosphate group**
- There may be one, two, or three phosphate groups present in nucleotides.
- Nucleotides are monophosphate, diphosphate, or triphosphate esters of nucleosides

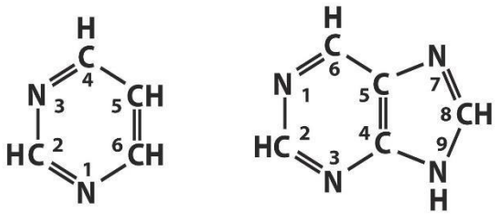


- These phosphate groups give an over all negative charge to the nucleotides.

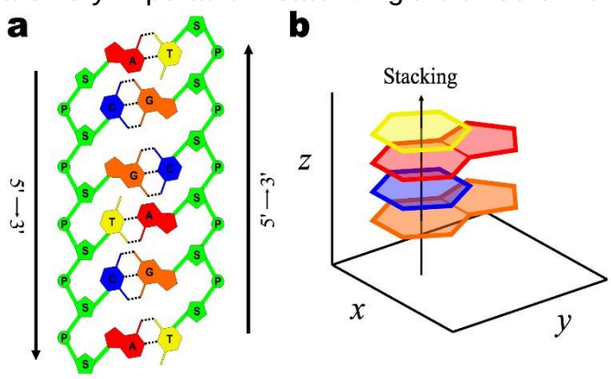
165 Nucleotides and Nucleic Acids- Properties of Nitrogenous Bases

Properties of Nitrogenous Bases

- **Aromatic:** The Nitrogen containing bases are aromatic i.e. they have alternate double bonds • **Heterocyclic**:
- They are heterocyclic i.e. structures that contain other atoms in addition to carbon, such as nitrogen in the ring structure
- The six-atom rings of purines and pyrimidines are numbered in opposite directions.

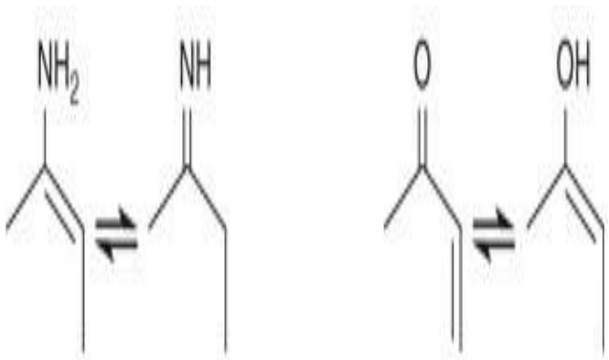


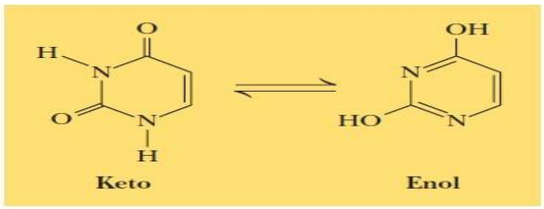
- **Weak Bases:** Purines or pyrimidines with an –NH2 group are weak bases
- **Functional Groups:** The most important functional groups of pyrimidines and purines are
- ring nitrogens
- carbonyl groups
- exocyclic amino groups
- **Hydrophobicity:** • The purine and pyrimidine bases are hydrophobic and relatively insoluble in water at the near-neutral cell pH
- **Stacking Interaction**: Hydrophobic stacking interactions in which two or more bases are positioned with the planes of their rings parallel (like a stack of coins) are one of two important modes of interaction between bases in nucleic acids.
- Base stacking helps to minimize contact of the bases with water, and these interactions are very important in stabilizing the three-dimensional structure of nucleic acids.



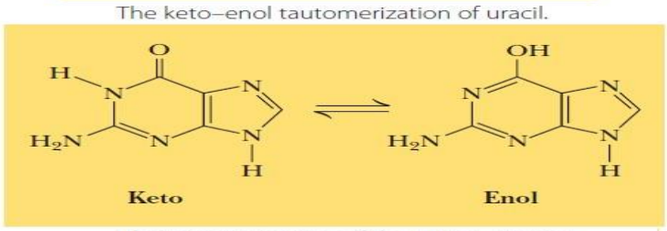
166 Nucleotides and Nucleic Acids- Properties of Nitrogenous Bases (Contd)

- **UV light absorbance:** The conjugated double bonds of purine and pyrimidine derivatives absorb ultraviolet light.
- Nucleic acids are characterized by a strong absorption at wavelengths near 260 nm.
- The mutagenic effect of ultraviolet light is due to its absorption by nucleotides that results in chemical modifications in DNA.
- This property is also utilized in quantitative and qualitative analysis of nucleotides and nucleic acids.
- **Tautomerism:** • All these bases can exist in keto-enol or amine-imine form.
- At physiologic pH keto and amine form is predominant.





The keto–enol tautomerization of uracil.



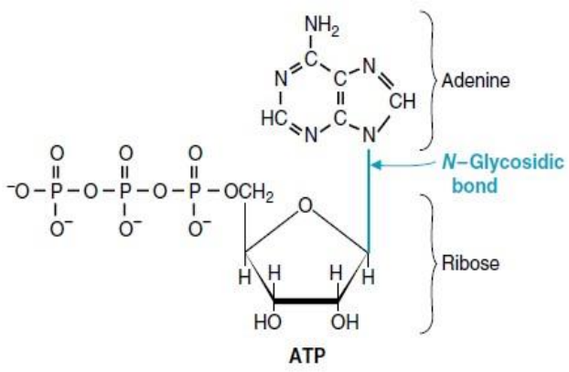
The tautomerization of the purine guanine.

- Note that the smaller pyrimidine molecule has the longer name and • the larger purine molecule the shorter name.

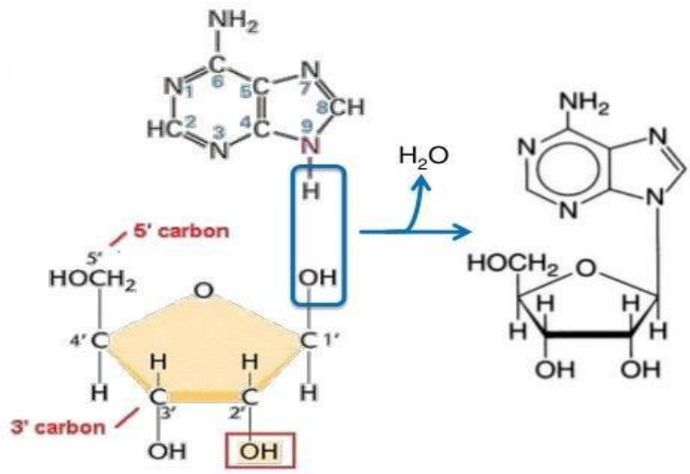
167 Nucleotides and Nucleic Acids- N-glycosidic bond

N-glycosidic bond

- Sugars are linked to the heterocycle by a β -N-glycosidic bond, almost always to the
- N-1 of a pyrimidine
- N-9 of a purine



- The N-glycosyl bond is formed by removal of the elements of water
- a hydroxyl group from the pentose and
- hydrogen from the base

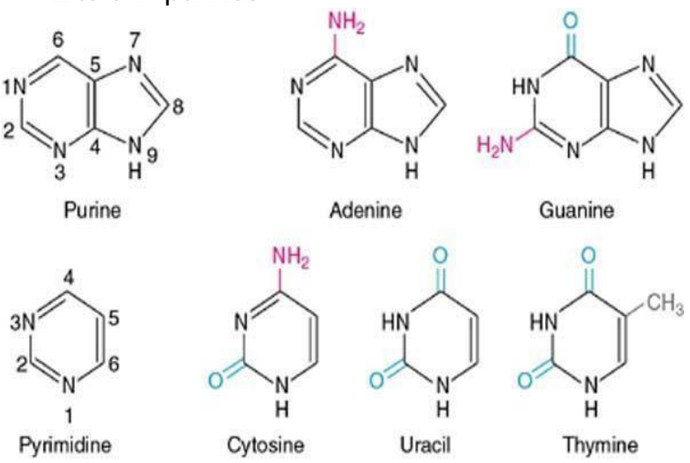


- Thus it is a condensation reaction.
- Similar to O-glycosidic bond formation in carbohydrates
- However, N-glycosidic bonds, have Nitrogen atom instead of oxygen linking the two residues. • the addition of the glycosidic bond to nitorgenous base is indicated by the name change
- such as from adenine to adenosine for the glycosidic bond

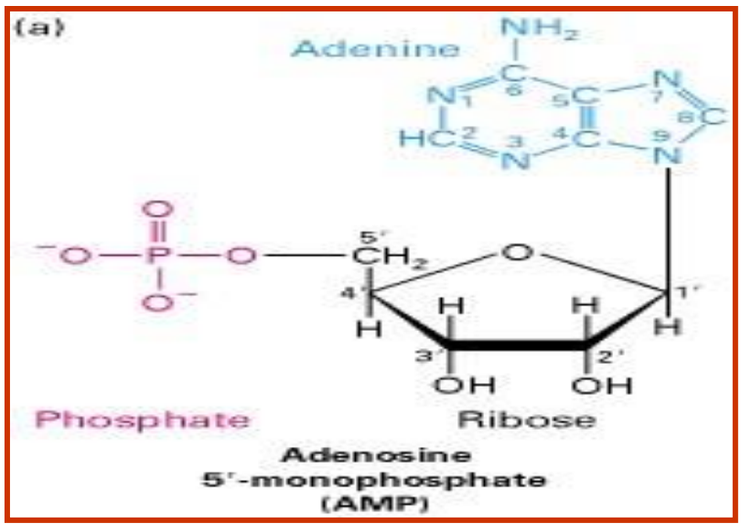
168 Nucleotides and Nucleic Acids-Numbering of Carbon and Nitrogen Atoms

Numbering of Carbon and Nitrogen Atoms

- The carbon and nitrogen atoms in the rings of the base and the sugar are numbered separately
- . • The atoms in the rings of the bases are numbered
- 1 to 6 in pyrimidines &
- 1 to 9 in purines



- In the pentoses of nucleotides and nucleosides the carbon numbers are given a prime (') designation to distinguish them from the numbered atoms of the nitrogenous base.
- The carbons in the pentose are numbered 1' to 5 '.
- Numerals with a prime (e.g., 2' or 3') distinguish atoms of the sugar from those of the heterocycle.

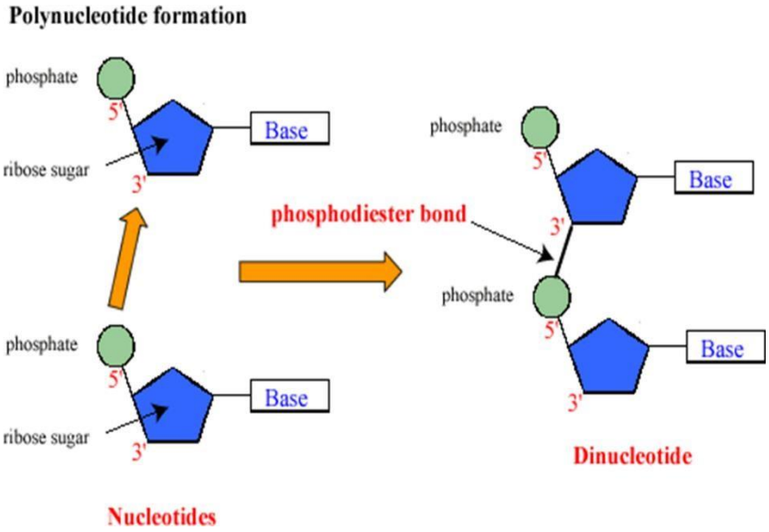


- Thus, when the 5'-carbon of a nucleoside (or nucleotide) is referred to, a carbon atom in the pentose, rather than an atom in the base, is being specified.

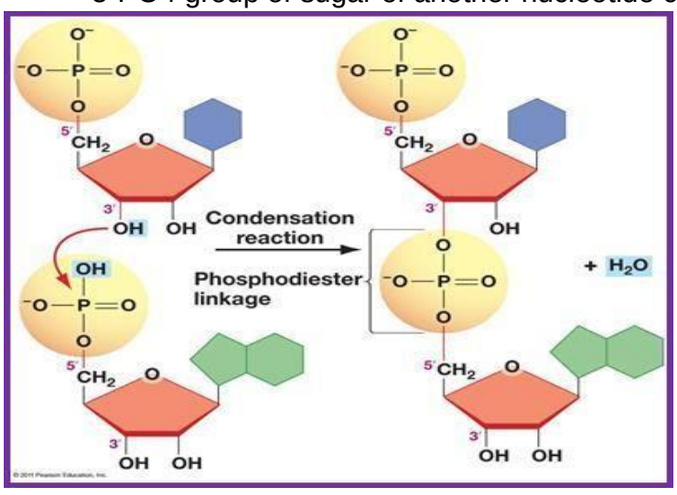
169 Nucleotides and Nucleic Acids- Phosphodiester Bond

Phosphodiester Bond

- When two or more nucleotides combine together a phosphodiester bond is formed.



- This bond is formed mainly between the 3´OH group of sugar of one nucleotide and 5´PO4 group of sugar of another nucleotide creating a phosphodiester linkage.



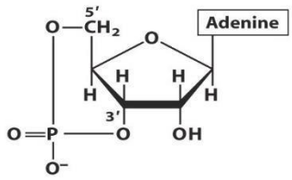
- By definition, the 5´ end lacks a nucleotide at the 5´ position and the 3´ end lacks a nucleotide at the 3´ position.
- The sugar and phosphate group is called the backbone of the nucleic acid.
- The backbones of both DNA and RNA are hydrophilic.
- The hydroxyl groups of the sugar residues form hydrogen bonds with water.

- The phosphate groups, are completely ionized and negatively charged at pH 7 The negative charges are generally neutralized by ionic interactions with positive charges which are present on proteins, metal ions, and polyamines.
- Depending on the number of nucleotides
- mono- di-, tri-, oligo- and polynucleotides are formed
- as a result of phosphodiester bond formation.

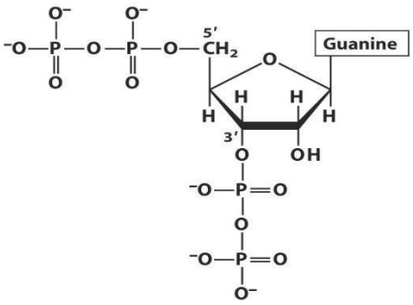
170 Nucleotides and Nucleic Acids-Cyclic Nucleotides

Cyclic Nucleotides

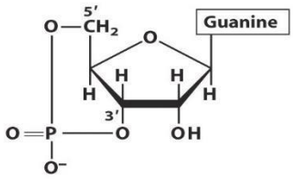
- There are two important cyclic nucleotides:
- Cyclic AMP cAMP
- Cyclic GMP cGMP
- Cyclic AMP is a cyclic nucleotide
- cAMP is synthesized in tissues from ATP • chemically it is 3´-5´ adenosine monopohosphate.



Adenosine 3′,5′-cyclic monophosphate
(cyclic AMP; cAMP)



Guanosine 5′-diphosphate, 3′-diphosphate
(guanosine tetraphosphate)
(ppGpp)



Guanosine 3′,5′-cyclic monophosphate
(cyclic GMP; cGMP)

Functions of c-AMP

- Acts as second messenger in the cell
- It has role in glycogen metabolism
- cAMP, glycogenolysis
- cAMP TAG metabolism
- cAMP lipolysis
- It decreases cholesterol synthesis
- It causes activation of protein kinases which in turn;
- activate or deactivate other enzymes.
- It regulates the cell membrane permeability, by increasing permeability of cell membrane
- to H2O, Na +, K+& Ca +2
- Moreover, it regulates
- insulin secretion,
- catecholamine biosynthesis & Melatonin synthesis
- Cyclic GMP is synthesized from GTP
- It serves as a second messenger in response to nitric oxide during relaxation of smooth muscle (especially blood vessels) so it has role in smooth muscle relaxation and vasodilatation.
- **It also has role in**
- Protein phosphorylation
- Neurotransmission
- Insulin action
- Regulation of sodium channels

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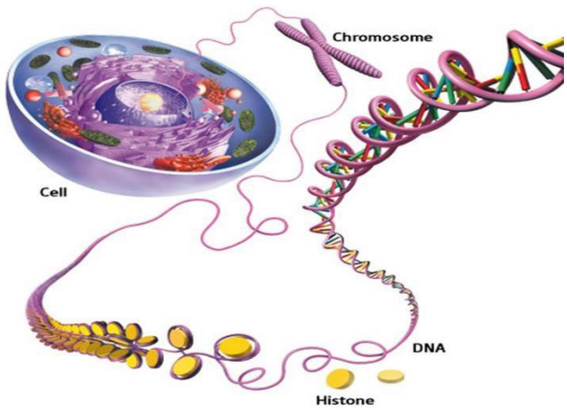
171 Nucleotides and Nucleic Acids-DNA

DNA

- It stands for Doeoxyribonucleic acid
- DNA is present in nuclear chromosomes of eukaryotes
  mitochondria • chloroplasts and • plasmids of prokaryotes •
  DNA is a polymer of deoxyribonucleoside monophosphates
- covalently linked by 3’ 5’-phosphodiester bonds.
- DNA is a repository of genetic information
- In eukaryotic cells, DNA is present in the chromosomes in the nucleus.
- It is found associated with basic proteins HISTONES and also various other proteins present in nucleus (nucleoproteins)



- Prokaryotic cells lack nuclei, and have a single chromosome.
- The protein-DNA complex is present in a non membrane bound region known as nucleoid.
- It also contain non-chromosomal DNA in the form of plasmids.
- Nucleic acid structure can be described in terms of hierarchical levels of complexity (primary, secondary, tertiary)
- The primary structure of a nucleic acid is its • covalent structure and • nucleotide sequence.
- Any regular, stable structure taken up by some or all of the nucleotides in a nucleic acid can be referred to as secondary structure
- In DNA double helix, the two strands of DNA are held together by hydrogen bonds.
- The nucleotides on one strand base pairs with the nucleotide on the other strand.
- The secondary structure is responsible for the shape that the nucleic acid assumes.
- The complex folding of large chromosomes within eukaryotic chromatin and bacterial nucleoids is generally considered tertiary structure

172 Nucleotides and Nucleic Acids-DNA Interactions

- The purine and pyrimidine bases are hydrophobic and relatively insoluble in water at the near-neutral pH of the cell.
- Hydrophobic stacking interactions in which bases are positioned with the planes of their rings parallel
- Hydrophobic stacking are an important interaction between bases in nucleic acids.
- The stacking also involves a combination of van der Waals and dipole-dipole interactions between the bases.
- Base stacking helps to minimize contact of the bases with water. • Therefore base-stacking interactions are very important in stabilizing the three-dimensional structure of nucleic acids.
- The most important functional groups of pyrimidines and purines are • ring nitrogens, • carbonyl groups, and • exocyclic amino groups.

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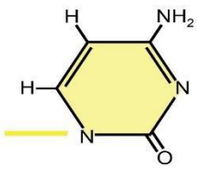
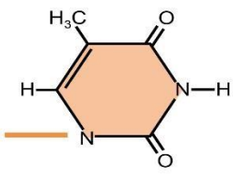
Cytosine

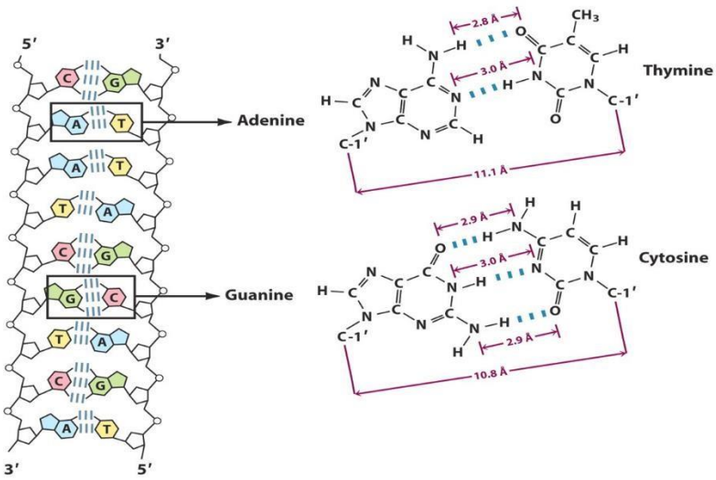


Thymine
(DNA Only)



Hydrogen bonds involving the amino and carbonyl groups are the second important mode of interaction between bases in nucleic acid molecules.

- The most important hydrogen-bonding patterns are those defined by James D. Watson and Francis Crick, in which • A bonds specifically to T (or U) and • G bonds to C.



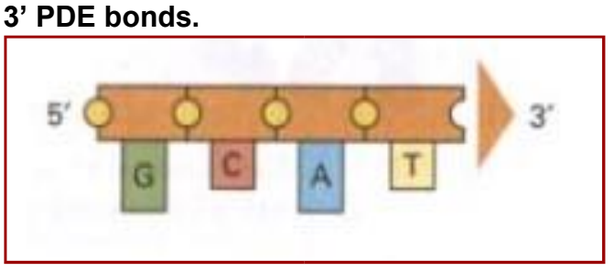
- These two types of base pairs predominate in double-stranded DNA and RNA.

173 Nucleotides and Nucleic Acids-DNA Primary Structure

The primary structure of a nucleic acid is its covalent structure and nucleotide sequence.

- The back bone of the primary structure is the linear strand made by sugar phosphate residues, linked together, while the bases project laterally. This way a long, un-branched chain is formed.

Primary structure is a huge linear polymer of dNTPs that are joined to each other by 5'-3' PDE bonds.



- The resulting long, un-branched chain has polarity.
- Both 5'-end and 3'-end are free. at 5'-end there is a free phosphate. at 3'-end there is a free OH that are not attached to other nucleotides.
- Purines and pyrimidines project laterally from the backbone and forms a variable part. • The variable part is concerned with the expression of genetic information.

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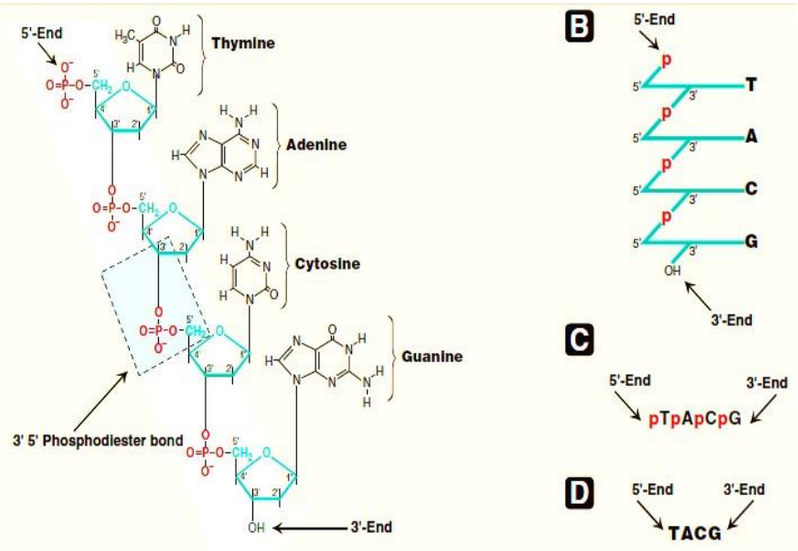


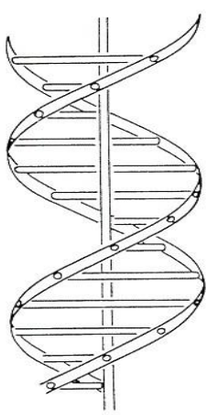
By convention, the structure of a single strand of nucleic acid is always written with the 5´ end at the left and the 3´ end at the right • that is, in the 5´ to 3´ direction

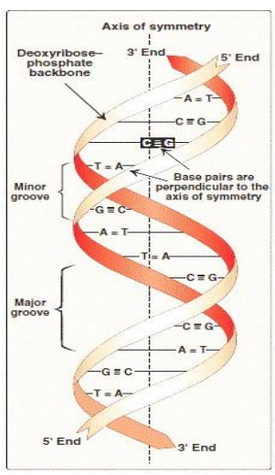
. • Some simpler representations of this penta deoxy ribonucleotide are • pA-C-G-T-A OH, • pApCpGpTpA, and finally 5´-ACGTA-3´.

174 Nucleotides and Nucleic Acids-DNA Secondary Structure

DNA Secondary Structure

- Any regular, stable structure taken up by some or all of the nucleotides in a nucleic acid can be referred to as secondary structure
- DNA exist as double stranded molecule (double helix), except few viruses that contain single stranded molecule.
- This model was presented by Watson and Crick in 1953.
- The two polydeoxyribonucleotide strands are coiled around a common axis called axis of symmetry**.**
- The chains are paired in anti-parallel manner, i.e., the 5'-end of one strand is paired with the 3'-end of the other strand.
- In the DNA helix
- Deoxyribose-phosphate backbone is hydrophilic and thus it is on the outside of the molecule.
- The bases are hydrophobic and are thus stacked inside the molecule.





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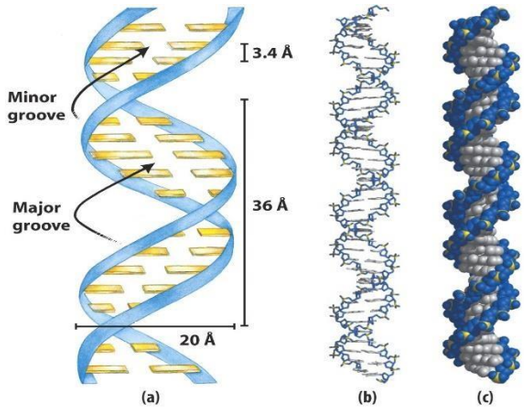
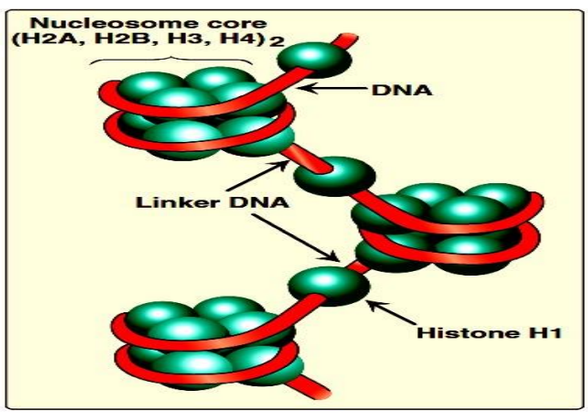
- The overall structure resembles a twisted ladder.

- Grooves: The spatial relationship between the two strands in the helix creates
- a major (wide) groove
- a minor (narrow) groove.
- 

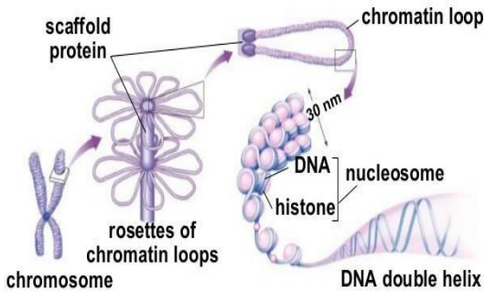
- These grooves provide access for the binding of regulatory proteins to their specific recognition sequences along the DNA

175 Nucleotides and Nucleic Acids-DNA Tertiary Structure

DNA tertiary Structure

- The complex folding of large chromosomes within eukaryotic chromatin and bacterial nucleoids is generally considered tertiary structure
- Eukaryotic DNA is associated with tightly bound basic proteins, called histones.
- These serve to order the DNA into fundamental structural units, called nucleosomes.
- There are five classes of histones, designated H1, H2A, H2B, H3, and H4.
- These are positively charged at physiologic pH as a result of their high content of lysine and arginine.
- Two molecules each of H2A, H2B, H3, and H4 form the structural core of the nucleosome.
- Around this core, a segment of the DNA double helix is wound nearly twiceapproximately 140bp
- 

- The DNA wrapped around the nucleosome core is continuous and joins one nucleosome core to the next -the linker DNA
- this 50 bp DNA is complexed with the fifth type of histone, H1.
- Nucleosomes can be packed more tightly to form a polynucleosome also called a nucleofilament or a 30-nm fiber.
- The fiber is organized into loops that are anchored by nuclear scaffolding proteins.



- Additional levels of organization lead to the final chromosomal structure

176 Nucleotides and Nucleic Acids-DNA Properties

DNA Properties

Template and Non-template Strands

- The term template strand refers to the sequence of DNA that is copied during the synthesis of mRNA.
- The opposite strand is called the Non Template or coding strand or the mRNA-like strand
- it has base sequence directly corresponding to the mRNA sequence
- the sequence corresponds to the codons that are translated into protein
- The 3´-5´strand is called Template strand while
- 5´-3´ strand is called Non Template (coding strand).

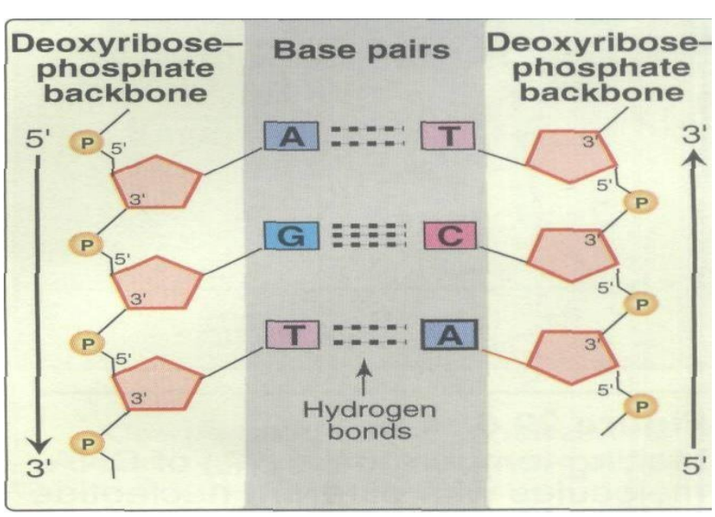
(5′)CGCTATAGCGTTT(3′) DNA nontemplate (coding) strand

(3′)GCGATATCGCAAA(5′) DNA template strand

(5′)CGCUAUAGCGUUU(3′) RNA transcript

Base Pairing

- The bases of one strand of DNA are paired with the bases of the opposite strand, so that
- Adenine is always paired with thymine
- Cytosine is always paired with guanine.



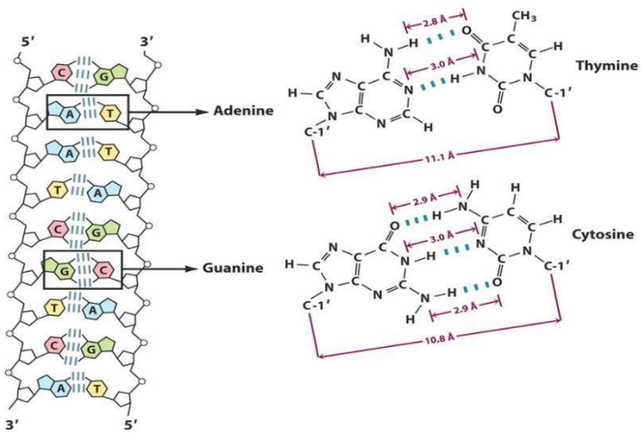
- One polynucleotide chain of DNA double helix is always complementary to the other

- Given the sequence of bases on one chain, the sequence of bases on the complementary chain can be determined

177 Nucleotides and Nucleic Acids-DNA Properties Contd

Chargaff's Rules

- Due to specific base pairing of DNA i.e A toT and G to C
- In any sample of double-stranded DNA the amount of adenine equals the amount of thymine.
- The amount of guanine equals the amount of cytosine
- the total amount of purines equals the total amount of pyrimidines ;
- i.e. A + G = T+ C.
- The base pairs are held together by hydrogen bonds
- Two between A and T
- Three between G and C

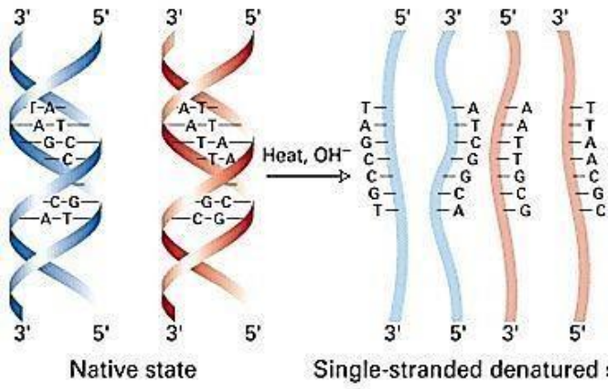


- These hydrogen bonds, plus the van der Waals and hydrophobic interactions between the adjacent stacked bases, stabilize the structure of the double helix. The base composition of DNA varies from one species to another, but is same if isolated from different tissues of same species. The base composition does not change with age, nutritional status and environment.

178 Denaturation of DNA

DNA Denaturation

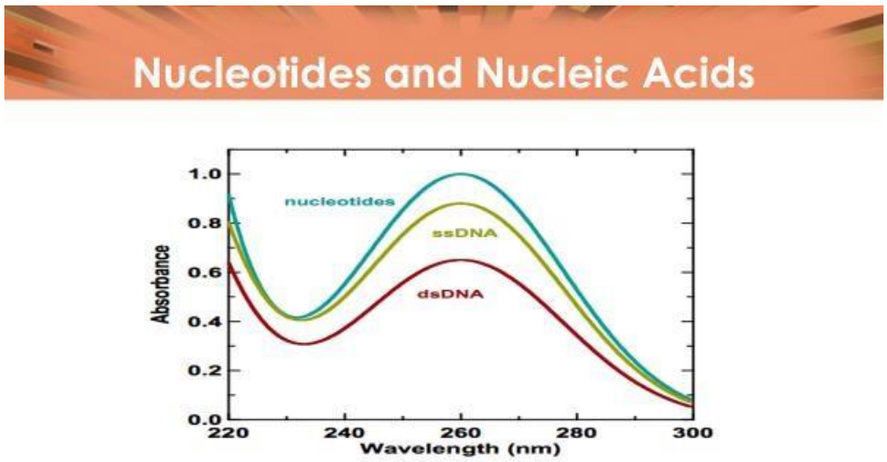
- Separation of the two strands of the double helix when hydrogen bonds between the paired bases are disrupted.
- **Disruption can occur in the laboratory if**
- the pH or the salt concentration of the DNA solution is altered
- if the solution is heated above 80°C
- Disruption of the hydrogen bonds between paired bases and of base stacking causes unwinding of the double helix to form two single strands complete separation of DNA stands along the entire length.
- No covalent bonds in the DNA are broken



- RNA duplexes are more stable than DNA duplexes.
- At neutral pH denaturation of a double helical RNA often requires temperatures 20° C or more higher than those required for denaturation of a DNA molecule with a comparable sequence.
- **T_m**: When DNA is heated, the temperature at which one half of the helical structure is lost is defined as the melting temperature.
- A specific sequence of DNA has a characteristic denaturation temperature, or melting point (T_m)
- Careful determination of the melting temperature of a DNA specimen, under fixed conditions of pH and ionic strength, can yield an estimate of a DNA base composition.

179 Nucleotides and Nucleic Acids-II

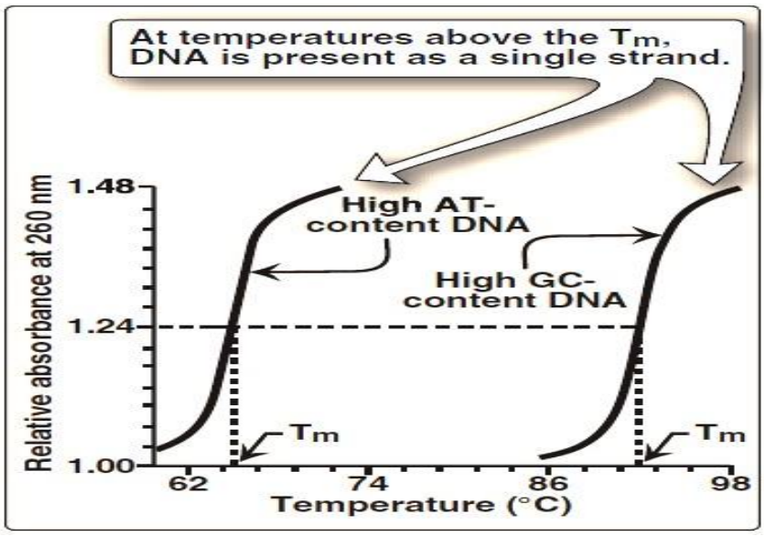
- **DNA Denaturation (Contd.)**
- Concomitant with this denaturation of the DNA molecule is an increase in the optical absorbance of the purine and pyrimidine bases—a phenomenon referred to as hyperchromicity of denaturation.



The absorbance of double-stranded DNA (dsDNA) at 260 nm is less than that of either single-stranded DNA (ssDNA) or the free bases. This is called "hyperchromism."

- So single-stranded DNA has a higher relative absorbance at this wavelength than does double-stranded DNA.
- Denaturation can be monitored by measuring its absorbance at 260 nm
- **Factors affecting Tm**
- The Tm is influenced by

- the base composition of the DNA
- the salt concentration of the solution
- The higher the content of GC base pairs, the higher the melting point of the DNA.
- This is because GC base pairs, with three hydrogen bonds, require more heat energy to dissociate than AT base pairs.

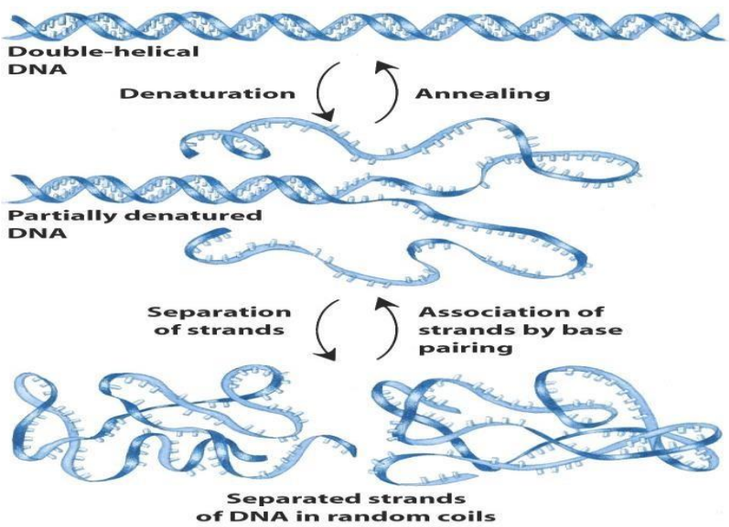


- An *increase* in salt concentration *increases* and a
- decrease in salt concentration decreases the Tm

180 Nucleotides and Nucleic Acids-DNA Renaturation

DNA Renaturation

- Under appropriate conditions *(temp. & salt concentration)*, separated strands of DNA will renature or reassociate and form the double helix by the process called renaturation (or reannealing).
- This reannealing process is also referred to as hybridization.
- When the temperature or pH is returned to the range in which most organisms live, the unwound segments of the two strands spontaneously rewind, or anneal, to yield the intact duplex



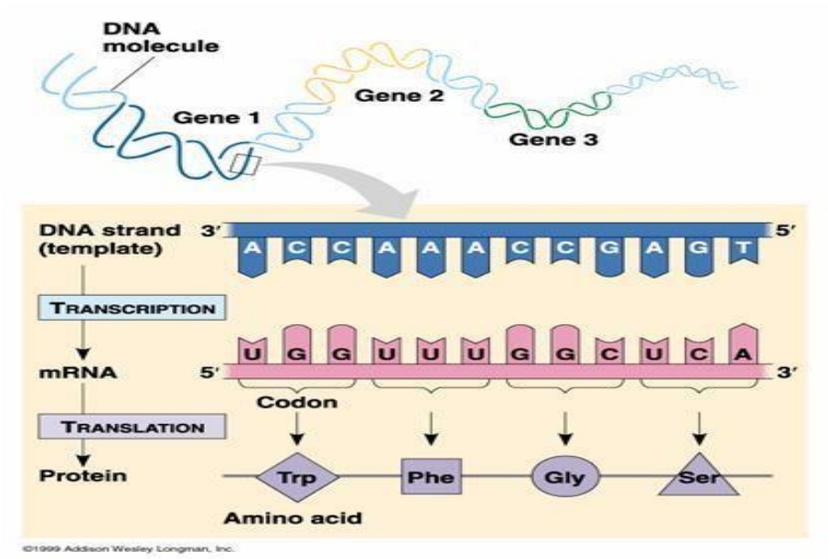
- Renaturation of a DNA molecule is a rapid one-step process.
- The rate of reassociation depends upon the concentration of the complementary strands.

- At a given temperature and salt concentration, a particular nucleic acid strand will associate tightly only with a complementary strand
- This property is utilized in analyzing nucleotide sequencing of a given nucleic acid.
- Under appropriate conditions DNA will form a hybrid with a complementary DNA or with a complementary RNA
- Hybridization is combined with gel electrophoresis techniques that separate nucleic acids by size,
- coupled with radioactive or fluorescent probe labeling to provide a detection of a nucleotide sequence.

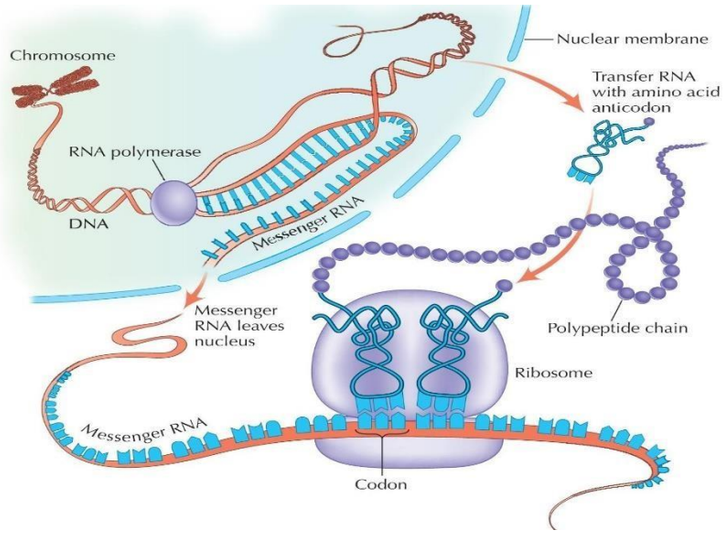
181 Nucleotides and Nucleic Acids- RNA

RNA

- The genetic master plan is contained in the nucleotide sequence of DNA.
- It is through the ribonucleic acid (RNA)—the "working copies" of the (DNA) — that the master plan is expressed



- RNA is a polymer of ribonucleotides of Adenine, Uracil, Guanine and Cytosine, joined together by 3´-5´phosphodiester bonds.
- RNA does not contain thymine except in rare cases.
- The pentose sugar of RNA is D-ribose.
- **Location:** RNA is found in the nucleolus, ribosomes, mitochondria, and cytoplasm.
- The genetic material for some animal and plant viruses is RNA rather than DNA.
- There is a wide variety of RNAs,
- messenger RNAs (mRNAs)- transfer genetic information from DNA to the proteinsynthesizing machinery.
- ribosomal RNAs (rRNAs)- contribute to the formation and function of ribosomes
- transfer RNAs (tRNAs)- adapter molecules that carry specific amino acids for protein synthesis

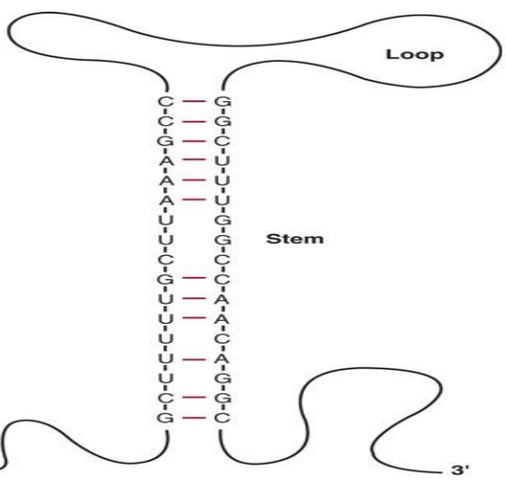


- small nuclear RNA (snRNA)- play pivotal roles in RNA processing, particularly mRNA processing
- ribozymes — some RNA molecules have intrinsic catalytic activity these RNA enzymes, are called ribozyme

182 Nucleotides and Nucleic Acids-Difference between RNA and DNA

Differences between DNA and RNA

- Although sharing many features with DNA, RNA possesses several specific differences.
- Size: They are considerably smaller than DNA
- Sugar**:** In RNA, the sugar moiety to which the phosphates and purine and pyrimidine bases are attached is ribose rather than the 2'-deoxyribose of DNA • Pyrimidine: The pyrimidine components of RNA differ from those of DNA.
- Instead of thymine, RNA contains the ribonucleotide of uracil.
- Thymine is present in the rare case of tRNA
- Single Strand**:**
- RNA typically exists as a single strand whereas DNA exists as a double-stranded helical molecule.
- However, given the proper complementary base sequence with opposite polarity, the single strand of RNA is capable of self- folding like a hairpin and thus acquiring doublestranded characteristics: G pairing with C, and A with U.



- Chargaff's Rules Do Not Apply:

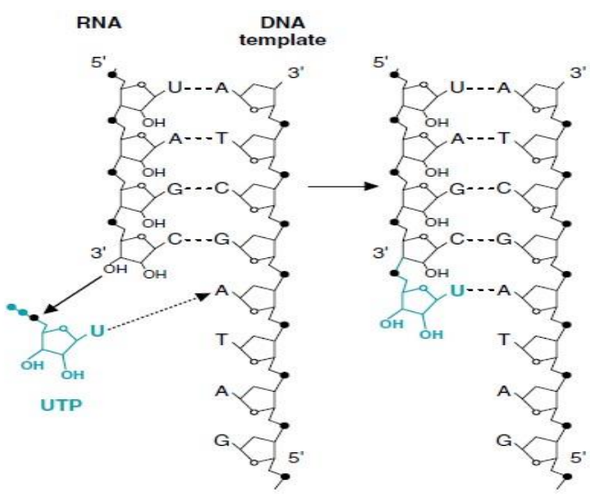
- Since the RNA molecule is a single strand complementary to only one of the two strands of a gene,
- G≠C
- A≠U
- Purines ≠ Pyrimidines
- Hydrolysis**:** RNA can be hydrolyzed by alkali to 2',3' cyclic diesters of the mononucleotides, compounds that cannot be formed from alkali-treated DNA because of the absence of a 2'-hydroxyl group.
- Location: In addition to nucleus, RNA is found in cytoplasm.
- Reverse Transcription**:** DNA forms RNA by transcription whereas the process by which RNA form DNA is called reverse transcription.

183 Nucleotides and Nucleic Acids- RNA Structural Hierarchy

- RNA Structural Hierarchy
- RNA has no simple, structural hierarchy that serves as a reference point, as does the double helix for DNA.
- The three-dimensional structures of many RNAs, like those of proteins, are complex and unique.

Primary Structure of RNA

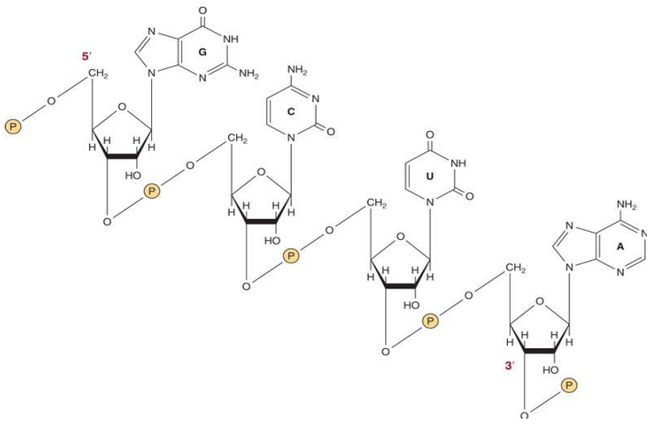
- It is defined as the number and sequence of ribonucleotides in the RNA chain.
- The sequence is complementary to the template strand of the gene from which it was transcribed.



template strand, the sequence of DNA that is copied.

Non Template (coding strand).

- The ribonucleotides are held together by 3´- 5´ phosphodiester bonds.
- 3´-OH group of one nucleotide is bound to 5´-PO_4 of the other nucleotide and form a linear strand.

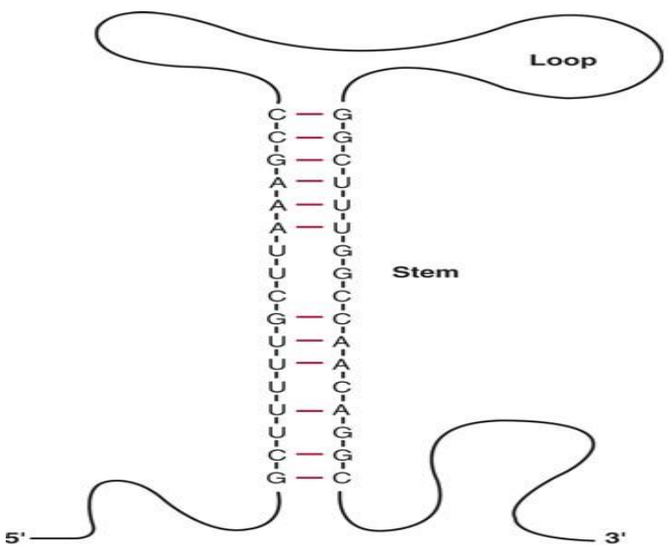


- The ribosyl moieties are attached to the nucleobases by *N*-glycosidic bonds •

  Similar to DNA RNA polymer also has polarity

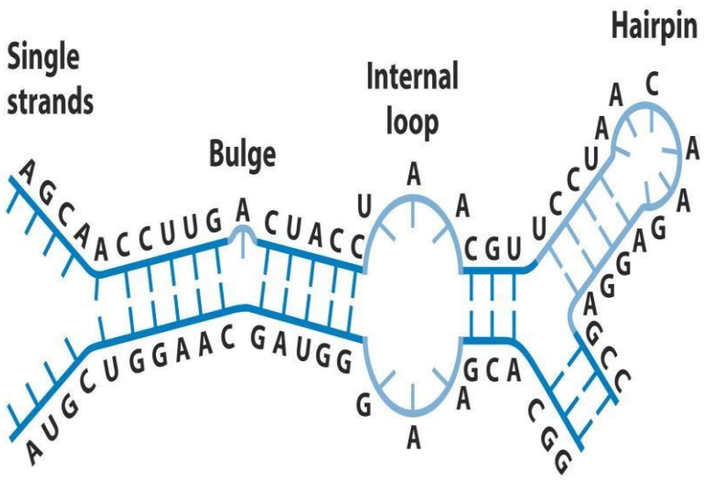
184 Nucleotides and Nucleic Acids- Secondary Structure of RNA

- **Secondary Structure of RNA**
- Secondary structure involves coil formation of the polyribonucleotide chain.
- The coiled structures are stabilized by Hydrophobic interactions between purine and pyrimidine bases.Intra-chain hydrogen bonds between G-C and A-U.



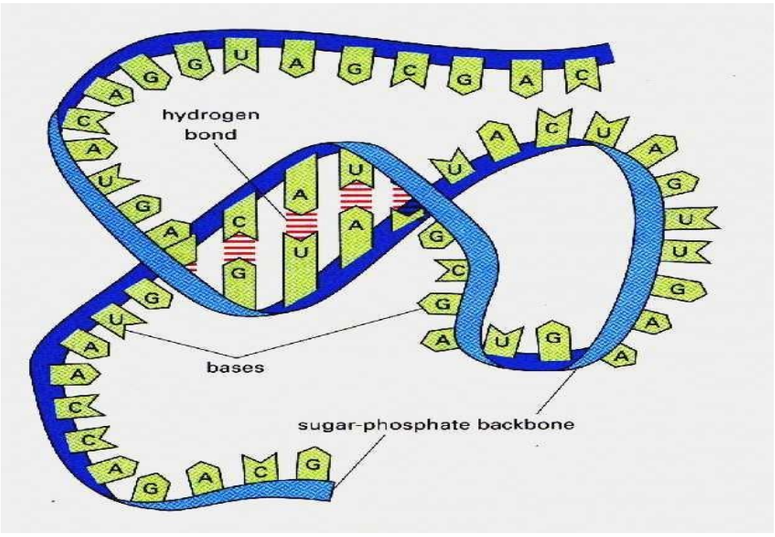
Note: Adenine pairs with Uracil in RNA

- Weak interactions, especially base-stacking interactions.
- Where complementary sequences are present, the predominant double-stranded structure is formed.
- Common RNA secondary structures
- **Internal loops:** a short series of unpaired bases in a longer paired helix and
- **bulges**: regions in which one strand of a helix has "extra" inserted bases with no counterparts in the opposite strand

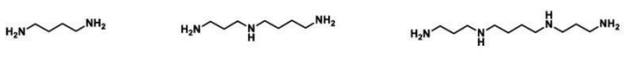


Tertiary Structure of RNA

- It is the folding of the molecule into three dimensional structure.
- cross-linking at various sites stabilized by hydrophobic and hydrogen bonds produces a compactly coiled globular structure.

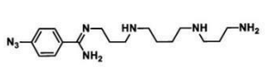


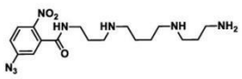
- The stacking of helices, together with specific helix–helix contacts or helix–loop interactions, lead to compact tertiary structure of the RNA assemblies, generally in the presence of divalent ions or polyamines

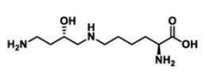

Putrescine

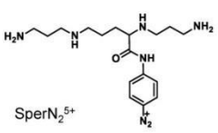
Spermidine

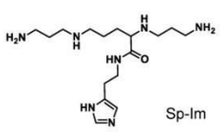
Spermine


ABA-spermine


ANB-spermine


Hypusine


$SperN_2^{5+}$

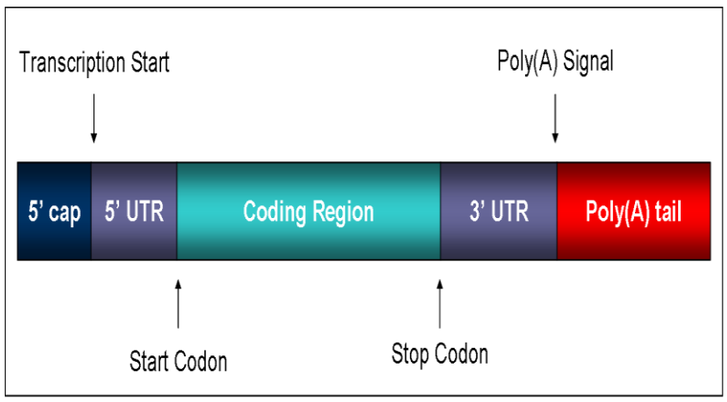

Sp-Im

185 Nucleotides and Nucleic Acids- Messenger RNA

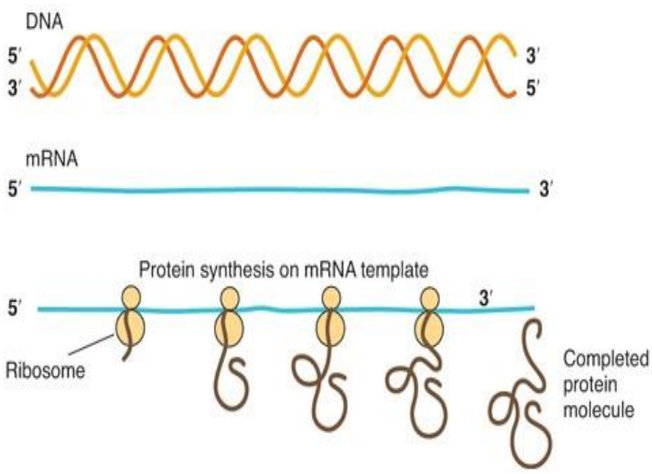
Messenger RNA (mRNA)

- This class is the most heterogeneous in
- Abundance

- Size (500-6000 nucleotides)
- base sequence
- Stability
- mRNA comprise about 2–5% of total cellular RNA
- mRNA molecules are formed with the help of DNA template strand (3´-5´) during the process called transcription.
- In addition to the protein coding regions in the mature eukaryotic mRNA that can be translated,
- there are untranslated regions at its 5´ and 3´ ends
- Moreover, there is a 5´ cap and
- a poly A tail at 3´ end



- **Function of mRNA**
- The members of this class function as messengers to convey the information in a gene to the protein synthesizing machinery.



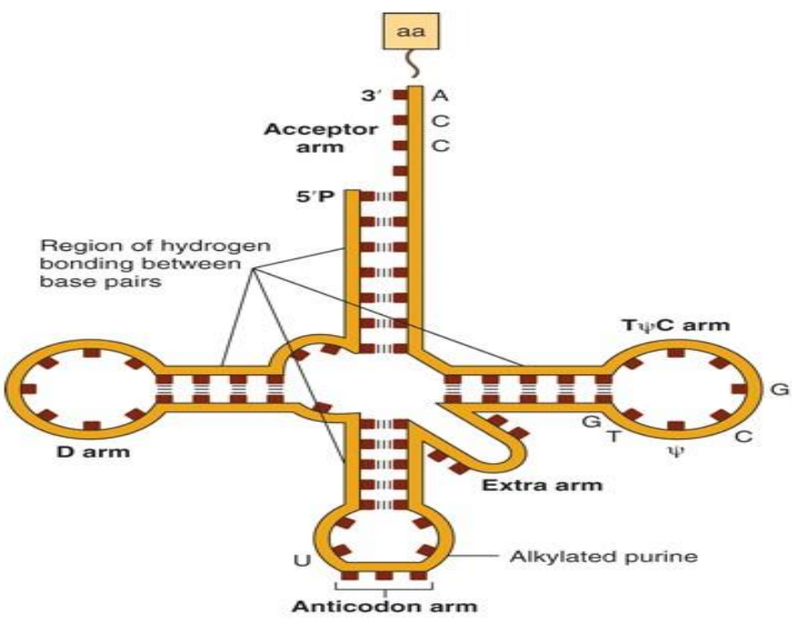
- The mRNA carries genetic information from the nuclear DNA to the cytosol, where it is used as a template for protein synthesis.

186 Nucleotides and Nucleic Acids- Transfer RNA

Transfer RNA (tRNA)

- t RNA is the smallest of the three major species of RNA (4S).
- They are single stranded globular molecules.
- They remain largely in cytoplasm.
- They are generated by nuclear processing of a precursor molecule.

- tRNAs compose roughly 20% of total cellular RNA
- There are at least 20 species of tRNA molecules in every cell.
- Although each specific tRNA differs from the others in its sequence of nucleotides, the tRNA molecules as a class have many features in common
- **Primary structure**
- t RNA molecules consist of 74-95 nucleotides in a particular sequence.
- The t RNA molecules contain not only the usual bases like adenine, guanine, cytosine, uracil but also contain **unusual bases**
- These unusual bases(also called modified bases) include
- Dihydrouracil • Pseudouridine
- Thymine.
- **Secondary Structure Pseudouridine**
- Each single stranded
- t RNA is folded extensively.
- Extensive intra chain base pairing which leads to a characteristic CLOVER-LEAF structure.



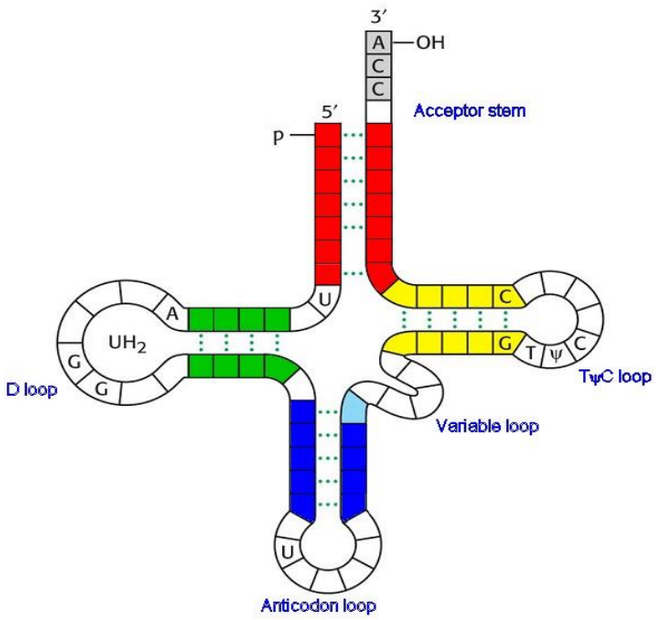
- These folds are stabilized by hydrogen bonds between complementary bases of the same strand.

187 Nucleotides and Nucleic Acids- Transfer RNA (continued..)

Arms or loops of tRNA

- All tRNA molecules contain 4 main arms or loops.
- **1-Acceptor arm:** This is made up of unpaired sequences of cytosine-cytosine-adenine (CCA) at the 3´end.
- The 3´OH group of adenine binds with the carboxylic group of a specific amino acid and carries it to ribosomes for protein synthesis.
- **2-Anticodon arm:** It is in the form of a loop and carries specific sequences of three bases which constitute the anticodon.
- The bases of anticodon are bonded with three complementary bases of codon on mRNA.

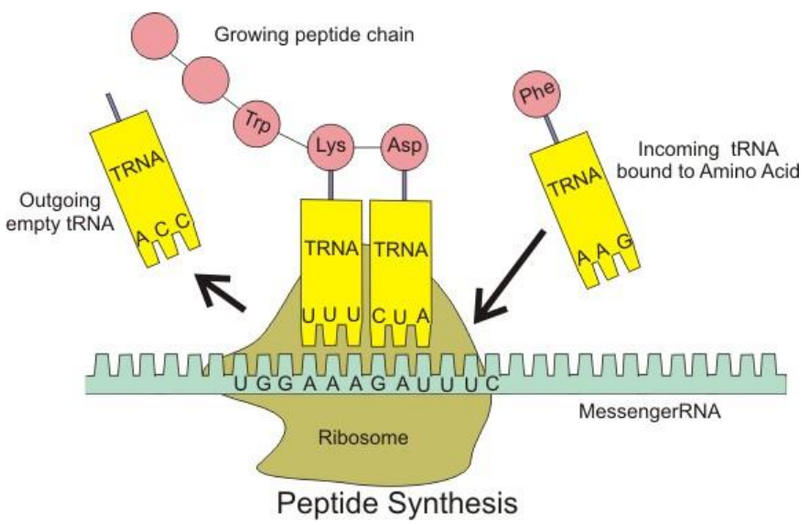
- **3-D arm:** It contains the base dihydrouridine.
- **4-TΨC arm:** It contains thymine, pseudouridine and cytosine.
- The extra arm and the TΨC arms help to define a specific tRNA.



D arm:contains dihydrouridine.**TΨC arm:** thymine, pseudouridine and cytosine.

Function of tRNA

- The t RNA molecules serve as ADAPTERS for the translation of information in the sequence of nucleotides of the mRNA into specific amino acids.



- There is at least one (and often several) specific type of tRNA molecule for each of the amino acids commonly found in proteins.
- Each t RNA carries its specific amino acid to the site of protein synthesis.
- There it recognizes the genetic code word on mRNA (codon) and this specifies the addition of its amino acids to the growing peptide chain.

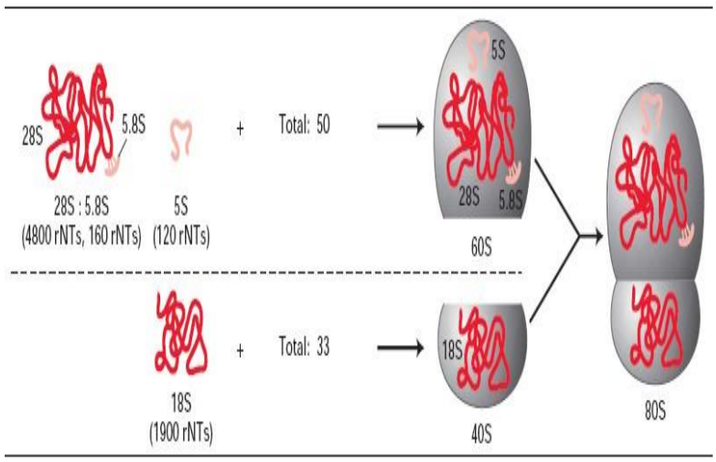
188 Nucleotides and Nucleic Acids- TRNA Continued

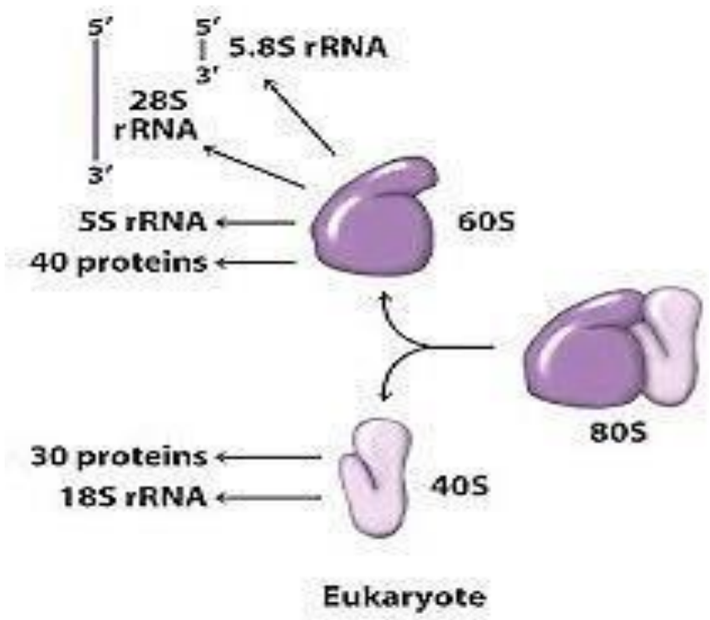
189 Nucleotides and Nucleic Acids- ribosomal RNA

Ribosomal RNA (rRNA)

- found in association with several proteins as a component of ribosomes--- a cytoplasmic nucleoprotein structure that acts as the machinery for the synthesis of proteins from the mRNA template.

- RNAs make up 80% of the total RNA in the cell.
- The ribosomal subunits are defined according to their sedimentation velocity in Svedberg units.
- Svedberg unit is related to the molecular weight and shape of the compound.
- The bases in r RNA are mainly adenine, guanine, cytosine and uracil and a few pseudouridine
- **Eukaryotic Ribosome**
- The mammalian ribosome contains two major nucleoprotein subunits:
- a larger one with 60S
- a smaller one with 40S.
- The **60S** subunit contains
- a 5S rRNA
- a 5.8S rRNA
- a 28S rRNA
- more than 50 specific polypeptides.
- The **40S** subunit is smaller and contains
- a single 18S rRNA
- Approx. 30 distinct polypeptide chains.

- 

- 

- In eukaryotes, all of the ribosomal RNA molecules except the 5S rRNA, which is independently transcribed, are processed from a single 45S precursor RNA molecule in the nucleolus- packed with the specific ribosomal proteins.
- The rRNA are necessary for ribosomal assembly and play a key role in the binding of mRNA to ribosomes and its translation
- In the cytoplasm, the ribosomes remain quite stable and capable of many translation cycles

190 Nucleotides and Nucleic Acids- small Nuclear RNA

Other types of RNA

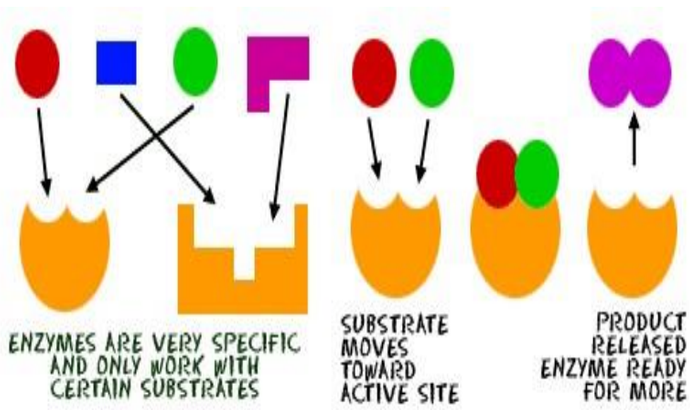
Small nuclear RNA (snRNA)

- Small nuclear RNA (snRNA) are large number of small stable RNA species found in eukaryotic cells.
- Most of them are complexed with proteins to form ribonucleoproteins.
- They are distributed in the nucleus, in the cytoplasm or in both.
- They are significantly involved in rRNA and mRNA processing and gene regulation.
- **Large & Small Noncoding Regulatory RNAs**
- One of the most exciting discoveries in the last decade of eukaryotic regulatory biology has been the identification and characterization of regulatory nonprotein coding RNAs (ncRNAs).
- NcRNAs exist in two general size classes,
- small consisting of microRNA (miRNAs) and silencing (siRNAs) and
- Large consisting of long noncoding RNAs (lncRNAs)

- The small ncRNAs termed microRNA (miRNAs) and silencing (siRNAs) typically inhibit gene expression at the level of specific protein production by
- targeting mRNAs through one of several distinct mechanisms.
- Both siRNAs and miRNAs typically hybridize, via the formation of RNA–RNA hybridization to their targeted mRNAs
- **long noncoding RNAs** (**lncRNAs).**
- LncRNAs, which as their name implies, do not code for protein (ie, the mRNA encoding genes).
- ncRNAs make up a significant portion of eukaryotic transcription
- ncRNAs play many roles ranging from contributing to structural aspects of chromatin to regulation of mRNA gene transcription by RNA polymerase II.

191 Enzymes

- Reaction catalysts of biological systems: the enzymes, the most remarkable and highly specialized proteins.
- Enzymes have extraordinary catalytic power, often far greater than that of synthetic or inorganic catalysts.
- With the exception of some catalytic RNA molecules all enzymes are proteins.
- Thus the primary, secondary, tertiary, and quaternary structures of protein enzymes are essential to their catalytic activity.
- Enzyme-catalyzed reactions have three basic steps:
- binding of substrate: **E+S↔ES**
- conversion of bound substrate to bound product: **ES ↔ EP**
- release of product : **EP ↔ E+P**
- They have a high degree of specificity for their substrates, they function in aqueous solutions under very mild conditions of temperature and pH.
- Enzymes do not invent new reactions; they simply make reactions occur faster.



- In addition to increasing the speed of reactions, enzymes provide means for regulating the rate of metabolic pathways in the body.
- The commonly used names for most enzymes describe the type of reaction catalyzed, followed by the suffix ***–ase*** *e.g.*
- **dehydrogen*ases*** remove hydrogen atoms

- **proteases** hydrolyze proteins
- Modifiers may precede the name to indicate, for example,
- the substrate (xanthine oxidase),
- the source of the enzyme (pancreatic ribonuclease),
- its regulation (hormone-sensitive lipase)
- Where needed, alphanumeric designators are added to identify multiple forms of an enzyme e.g,
- RNA polymerase III
- protein kinase C

192 Enzymes- classification

IUB Classification of Enzymes

- International Union of Biochemists (IUB) developed an unambiguous system of enzyme nomenclature in which each enzyme has a
- unique name and
- code number
- As an example, the formal systematic name of the enzyme (hexokinase) catalyzing the reaction
- ATP + D-glucose→ ADP + D-glucose-6 phosphate is
- ATP:glucose phosphotransferase,
- Its Enzyme Commission (E.C.) number is 2.7.1.1.
- (2) denotes the class name (transferase)
- (7) the subclass phosphotransferase
- (1) denotes a hydroxyl group as acceptor
- (1) D-glucose as the phosphoryl group acceptor.
- In the systematic naming system, enzymes are divided into six major classes each with numerous subgroups

CLASSIFICATION OF ENZYMES		
Group of Enzyme	**Reaction Catalysed**	**Examples**
1. Oxidoreductases	Transfer of hydrogen and oxygen atoms or electrons from one substrate to another.	Dehydrogenases Oxidases
2. Transferases	Transfer of a specific group (a phosphate or methyl etc.) from one substrate to another.	Transaminase Kinases
3. Hydrolases	Hydrolysis of a substrate.	Estrases Digestive enzymes
4. Isomerases	Change of the molecular form of the substrate.	Phospho hexo Isomerase, Fumarase
5. Lyases	Nonhydrolytic removal of a group or addition of a group to a substrate.	Decarboxylases Aldolases
6. Ligases (Synthetases)	Joining of two molecules by the formation of new bonds.	Citric acid synthetase

CLASSIFICATION OF ENZYMES		
Group of Enzyme	**Reaction Catalysed**	**Examples**
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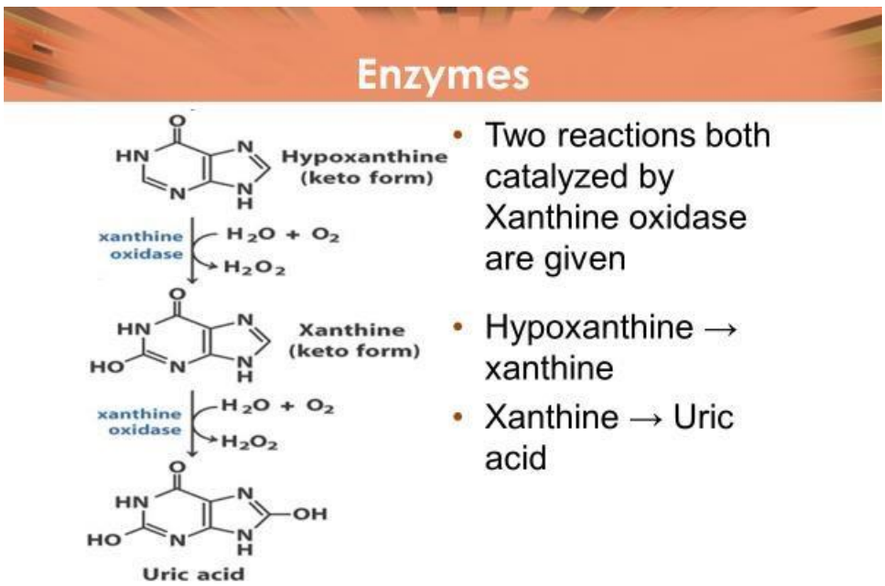
2. Transferases	**Transfer of specific group (a phosphate or methyl etc.) from one substrate to another.**	**Transaminase Kinases**
3. Hydrolases	**Hydrolysis of a substrate.**	**Estrases Digestive enzymes**

CLASSIFICATION OF ENZYMES		
Group of Enzyme	**Reaction Catalysed**	**Examples**
4. Isomerases	**Change of the molecular form of the substrate.**	**Phospho hexo Isomerase, Fumarase**
5. Lyases	**Nonhydrolytic removal of a group or addition of a group to a substrate.**	**Decarboxylases Aldolases**
6. Ligases (Synthetases)	**Joining of two molecules by the formation of new bonds.**	**Citric acid Synthetase**

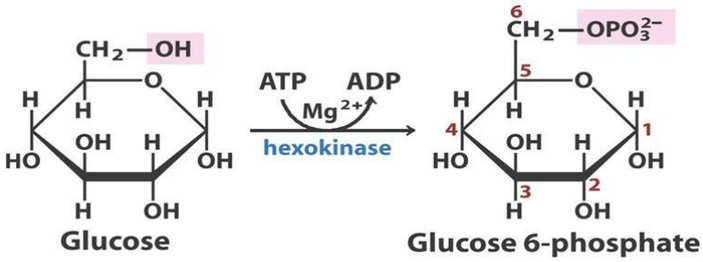
1) Oxidoreductases

- catalyze oxidation reduction reactions
- further divided into four subgroups;
- Oxidase,
- Dehydrogenases, • Hydroperoxidases
- Oxygenases.



2) Transferases

- These bring about a transfer of functional groups such as
- phosphate and
- amino group
- from one molecule to another molecule called donor and acceptor molecules respectively.
- The common examples of this group are
- Transminases
- Phosphotransfrases (Kinases)



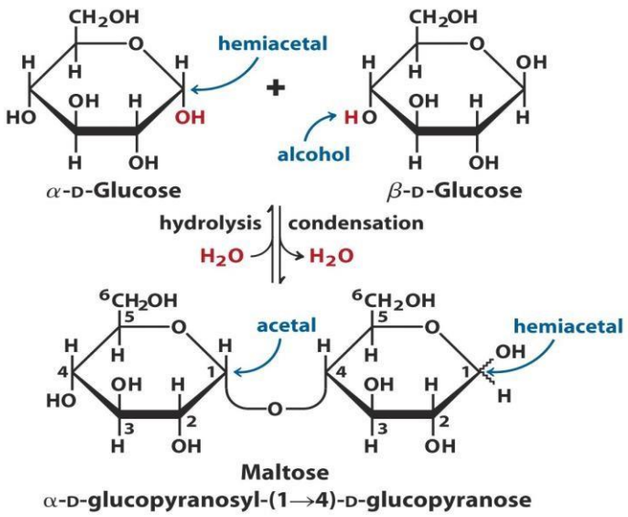
- Hexokinase is a phosphotransfease which catalyze the transfer of phosphate groups.
- Glucose + ATP → Glucose 6-phosphate + ADP.

193 Enzymes- IUB classification (continued..)

3) Hydrolases

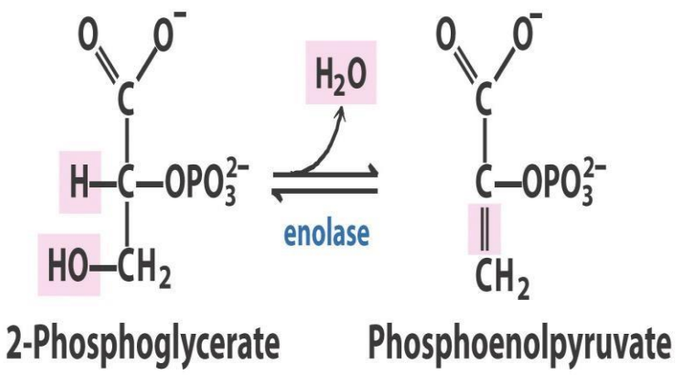
- These enzymes catalyze hydrolysis, i.e.
- add water molecule to the substrate which is simultaneously decomposed; the functional group of substrate is transferred to water.
- Common example of hydrolases are:
- Protein hydrolyzing Enzymes (peptidases).
- Carbohydrases
- Lipid hydrolyzing enzymes e.g. Lipases and

- Phospholipases.

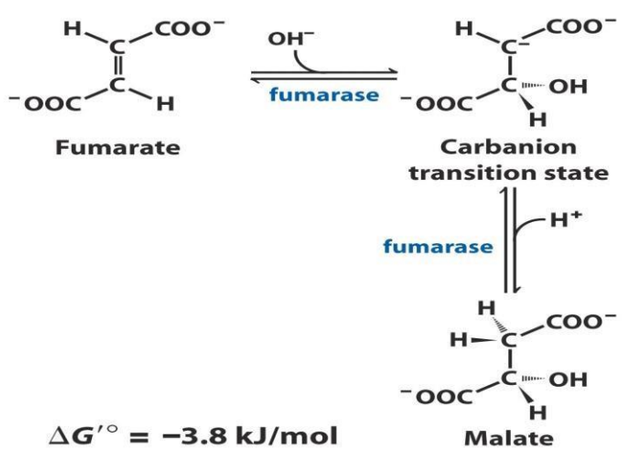


4) Lyases

- Theses enzymes catalyze the addition of
- NH_3,
- H_2O or
- CO_2 to double bonds or
- the removal of these groups leaving behind double bonds.



$\Delta G'^{\circ} = 7.5$ kJ/mol



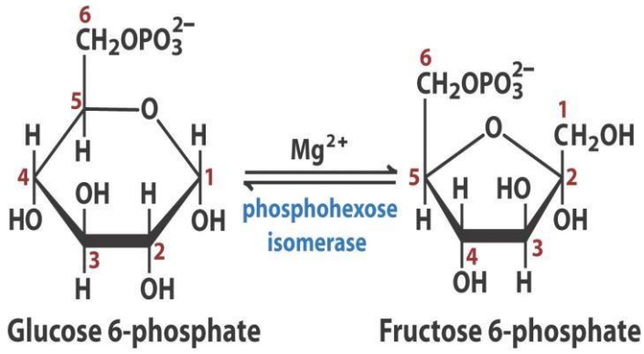
$\Delta G'^{\circ} = -3.8$ kJ/mol

- Lyases are included in a separate class because they catalyze these reactions by means other than hydrolysis or oxidation.

194 Enzymes- IUB Classification (Continued...)

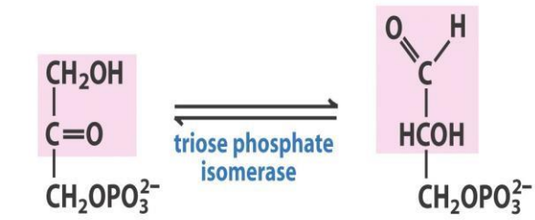
5) Isomerases

These enzymes catalyze the structural change within a single molecule by the transfer of groups within it, resulting in the formation of an isomeric form of substrate.



Glucose 6-phosphate — Fructose 6-phosphate

$\Delta G'^{\circ} = 1.7$ kJ/mol

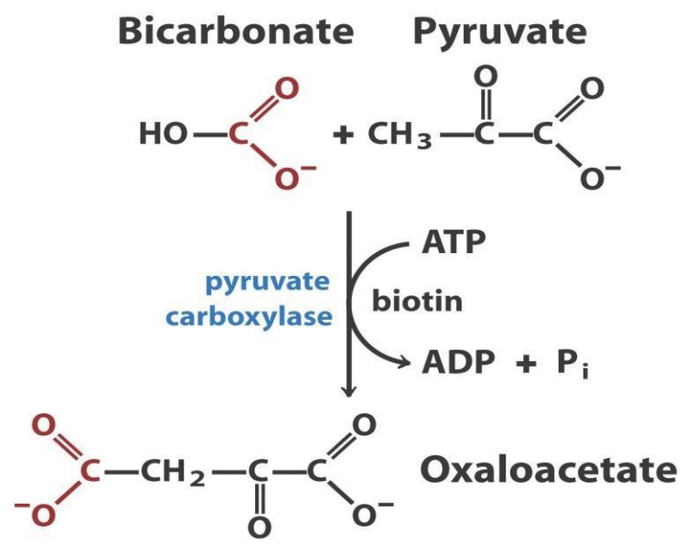


Dihydroxyacetone phosphate — Glyceraldehyde 3-phosphate

$\Delta G'^{\circ} = 7.5$ kJ/mol

6) Ligases

- These enzymes catalyze condensation reactions joining two molecules by forming
- C-O,
- C-S,
- C-N and
- C-C bonds.



- The energy for condensation is provided by cleavage of high energy phosphates, e.g. ATP, GTP etc.

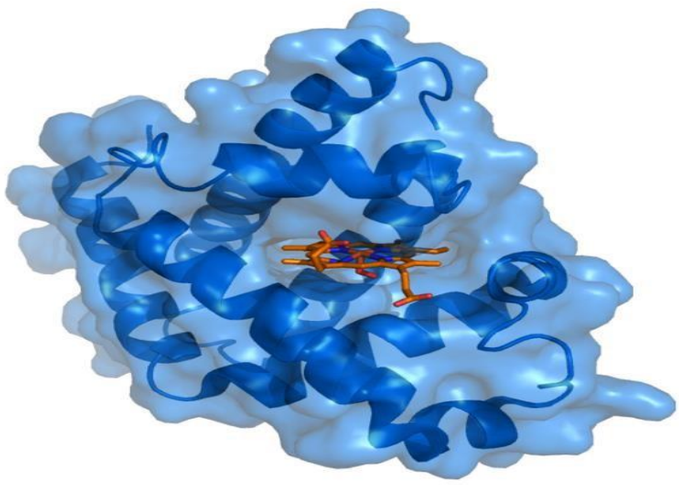
•

195 Enzymes- Ligand

Ligand

In biochemistry and pharmacology, a **ligand** (from the Latin *ligandum, binding*) is a substance (usually a small molecule),

- that forms a complex with a biomolecule to serve a biological purpose.



- In a narrower sense, it is a signal triggering molecule, binding to a site on a target protein
- A molecule bound reversibly by a protein is called a **ligand.**
- Ligands include substrates, inhibitors, activators, and neurotransmitters
- A ligand may be any kind of molecule, including another protein.
- A ligand binds at a site on the protein called the **binding site, binding site** is complementary to the ligand insize,shape ,charge, and hydrophobic or hydrophilic character.
- Furthermore, the interaction is **specific**: the protein can discriminate among the thousands of different molecules in its environment and selectively bind only one or a few.
- The binding of a protein and ligand is often coupled to a **conformational change** in the protein that makes the binding site more complementary to the ligand, permitting tighter binding called **induced fit.**
- A given protein may have **separate binding sites** for several different ligands.
- In a multi-subunit protein, a conformational change in one subunit often affects the conformation of other subunits.
- Interactions between **ligands** and proteins may be regulated, through interactions with additional ligands.
- These other ligands may cause conformational changes in the protein that affect the binding of the **first ligand**.

196 Enzymes- Mechanism of enzyme action

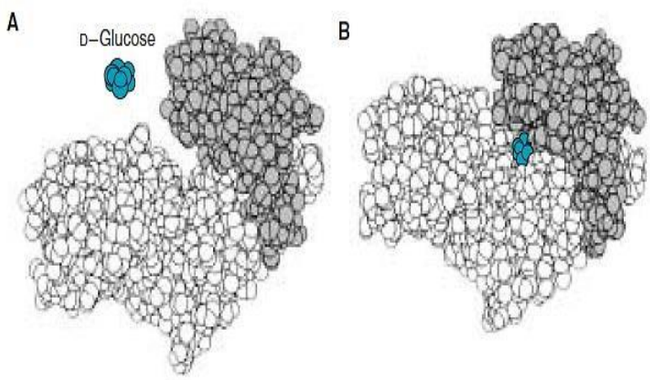
Mechanism of Enzyme Action

Enzymes bind and chemically transform other molecules— they catalyze reactions.

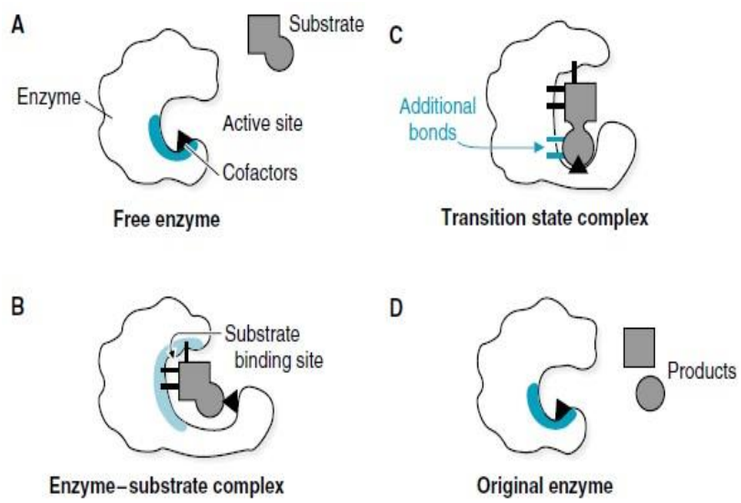
- The molecules acted upon by enzymes are called reaction substrates rather than ligands.

-
- Enzymes are highly effective catalysts, commonly enhancing reaction rates by a factor of 10^5 to 10^{17}.
- The distinguishing feature of an enzyme-catalyzed reaction is that it takes place within the confines of a pocket on the enzyme called the **active site.**
- The surface of the active site is lined with **amino acid** residues with side chains that complement and bind the substrate and catalyze its chemical transformation.

  Often, the active site encloses a substrate, sequestering it completely from solution.
- The enzyme substrate complex, whose existence is central to the action of enzymes.



- Enzyme-catalyzed reactions have three basic steps:
- binding of substrate: E+S↔ES
- conversion of bound substrate to bound product: ES ↔ EP • release of product : EP ↔ E+P



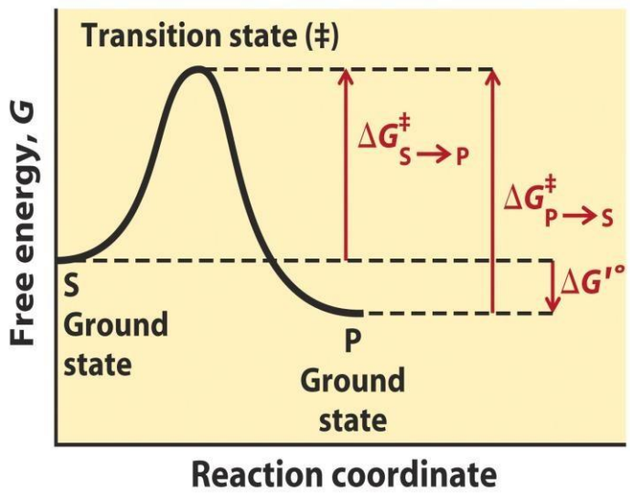
-
- To understand catalysis, we must first appreciate the important distinction between reaction equilibria and reaction rates.

197 Enzymes- Mechanism of enzyme action (continued..)

Reaction Equilibria

- The function of a catalyst is to increase the rate of a reaction.
- Catalysts *do not affect reaction equilibria*
- Any reaction, such as S ↔ P, can be described by a reaction coordinate diagram
- The free energy of the system is plotted against the progress of the reaction S → P.

- A diagram of this kind is a description of the energy changes during the reaction.
- The horizontal axis (reaction coordinate) reflects the progressive chemical changes (e.g., bond breakage or formation) as S is converted to P.



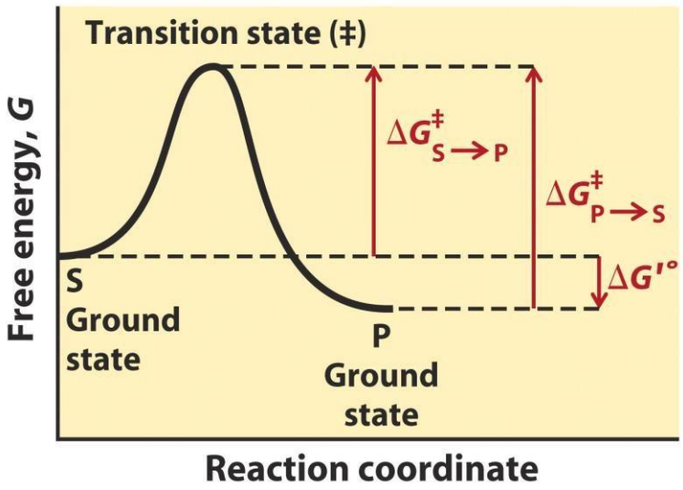
- **Reaction coordinate diagram for a chemical reaction.**
- The free energy of the system is plotted against the progress of the reaction S→P. A diagram of this kind is a description of the energy changes during the reaction, and the horizontal axis (reaction coordinate) reflects the progressive chemical changes (e.g., bond breakage or formation) as S is converted to P. The activation energies, *G*‡, for the S→P and P→S reactions are indicated. *G* is the overall standard free-energy change in the direction S→P.
- **Activation energies, *G‡,***
- The activation energies, *G‡, for* the
- **S → P and P → S** reactions are indicated.
- *G'º is the overall standard* free-energy change in the direction **S → P**
- **Free-energy change ∆*G***
- When a reacting system is not at equilibrium, the tendency to move toward equilibrium represents a driving force
- the magnitude of which can be expressed as the free-energy change for the reaction, ∆*G*.
- **Standard free-energy change, ∆*G*º**
- Under standard conditions (298 K = 25 C)
- when reactants and products are initially present at 1 M concentrations or
- for gases, at partial pressures of 101.3 (kPa), or 1 atm
- the force driving the system toward equilibrium is defined as the standard free-energy change, ∆*G*º
- However, because the conditions in the body systems are different from standard conditions
- energy in biological systems is described in terms of free energy, *G'º.*

198 Enzymes-Mechanism of action (continued...)

Ground State &Transition State

- The starting point for either the forward or the reverse reaction is called the ground state

- The equilibrium between S and P reflects the difference in the free energies of their ground states.
- The free energy of the ground state of P is **lower** than that of S
- So *G'º for the* reaction is negative and the equilibrium favors P.
- The position and *direction* of equilibrium are *not affected by* any catalyst.



- **Reaction coordinate diagram for a chemical reaction.**
- The free energy of the system is plotted against the progress of the reaction
- S→P. A diagram of this kind is a description of the energy changes during the reaction, and the horizontal axis (reaction coordinate) reflects the progressive chemical changes (e.g., bond breakage or formation) as S is converted to P. The activation energies, *G*‡, for the S→P and P→S reactions are indicated. *G* is the overall standard free-energy change in the direction S→P.But there is an **energy barrier** between S and P**:** The energy required for alignment of reacting groups formation of transient unstable charges bond rearrangements and other transformations
- This is illustrated by the energy "hill"
- To undergo reaction, the molecules must overcome this barrier and therefore must be raised to a higher energy level
- This is called the **transition state.**
- It is simply a fleeting molecular moment in which events such as bond breakage, bond formation, and charge development have proceeded to the precise point at which decay to either substrate or product is equally likely.
- The difference between the energy levels of the ground state and the transition state is the **activation energy, *G*‡.**
- At the top of the energy hill is a point at which decay to the S or P state is equally probable (it is downhill either way)
- A substance that modifies the transition state to lower the activation energy is termed a catalyst; a biological catalyst is termed an enzyme.

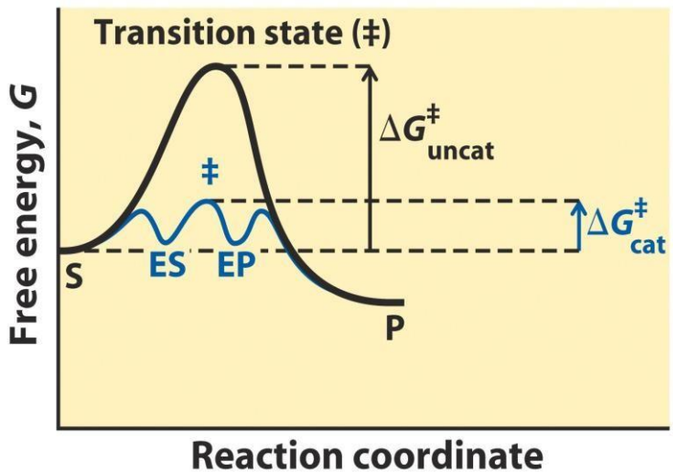
199 Enzymes-Mechanism of Action- Activation Energy (continued...)

Mechanism of Enzyme Action (Contd.)

Activation energy $\Delta G^{\ddagger}$.

- The difference between the energy levels of the ground state and the transition state is the activation energy $\Delta G^{\ddagger}$.

- The rate of a reaction reflects this activation energy: a higher activation energy corresponds to a slower reaction.
- Reaction rates can be increased by raising the temperature, thereby increasing the number of molecules with sufficient energy to overcome the energy barrier.
- Alternatively, the activation energy can be lowered by adding a catalyst.
- Catalysts enhance reaction rates by lowering activation energies.



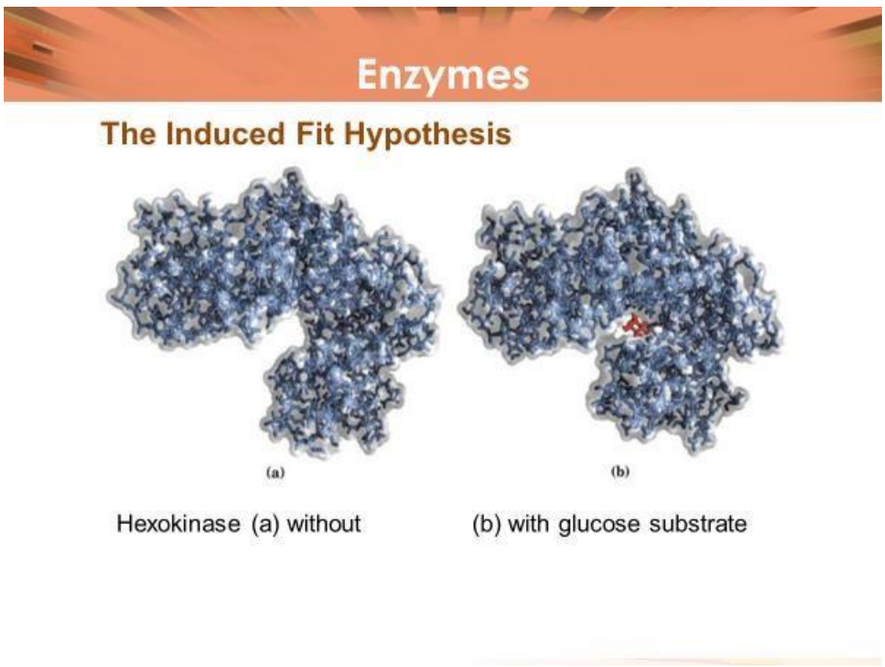
- **Reaction coordinate diagram comparing enzyme catalyzed**
- **and uncatalyzed reactions.** In the reaction S→P, the ES
- and EP intermediates occupy minima in the energy progress curve of
- the enzyme-catalyzed reaction. The terms *G*‡
- uncat and *G*‡ cat correspond to the activation energy for the uncatalyzed •

  reaction and the overall activation energy for the catalyzed reaction, respectively.
- The activation energy is lower when the enzyme catalyzes the reaction.
- The role of enzymes is to accelerate the inter-conversion of S and P.
- i.e ***enzymes lower the energy of activation, Δ G‡, of a reaction.***
- The enzyme is not used up in the process, and the equilibrium point is unaffected.
- However, the reaction reaches equilibrium much faster when the appropriate enzyme is present,
- because the rate of the reaction is increased.

200 Enzymes- induced fit hypothesis

The Induced Fit Hypothesis

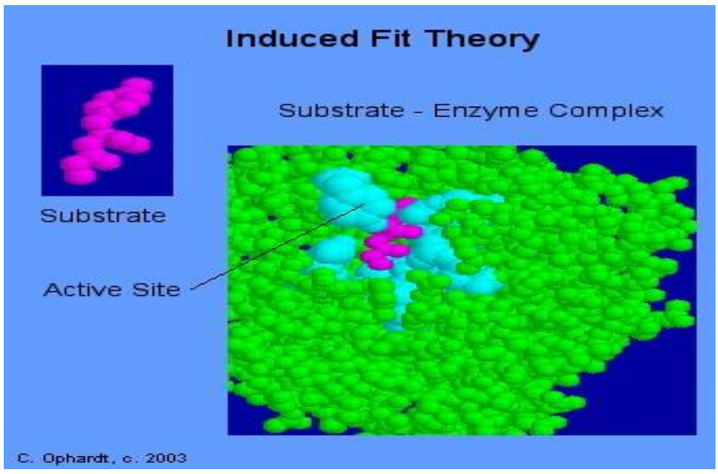
- Some proteins can change their shape (conformation)
- When a substrate combines with an enzyme, it induces a change in the enzyme's conformation
- This change in conformation when the substrate binds is induced by multiple weak interactions with the substrate.
- There may also be rearrangements of covalent bonds during an enzyme-catalyzed reaction.
- This conformational change is referred to as induced fit.
- The Induced Fit Hypothesis



- Chemical reactions of many types take place between substrates and enzyme's functional groups (specific amino acid side chains, metal ions, and coenzymes
- Induced fit serves to bring specific functional groups on the enzyme into the proper position to catalyze the reaction.

Induced Fit Theory

- Enzyme is not rigid, changes shape with substrate.



- The active site is also moulded into a precise conformation
- Making the chemical environment suitable for the reaction

201 Enzymes- co factors, coenzymes

Cofactors, Coenzymes and Prosthetic groups

- Some enzymes require no chemical groups for activity other than their amino acid residues.
- Whereas some enzymes require molecules other than proteins for enzymic activity.
- If the non-protein moiety is a metal ion such as Zn^{2+} or Fe^{2+}, it is called a cofactor.
- If it is a complex organic molecule or metallo-organic compound it is termed a coenzyme.

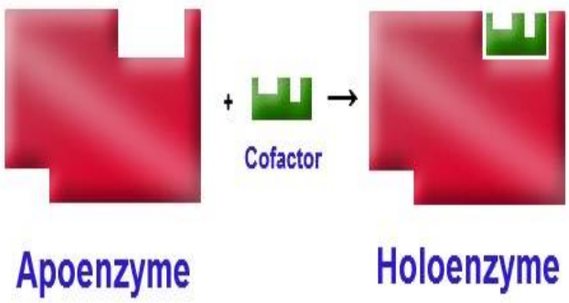
Some Coenzymes That Serve as Transient Carriers

Coenzyme	*Examples of chemical groups transferred*
Biocytin	CO_2
Coenzyme A	Acyl groups
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups
Flavin adenine dinucleotide	Electrons
Lipoate	Electrons and acyl groups
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)

Some Inorganic Elements That Serve as Cofactors for Enzymes

Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^+	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase

- A coenzyme or metal ion that is very tightly or even covalently bound to the enzyme protein is called a prosthetic group.
- The term holoenzyme refers to the active enzyme with its non-protein component,
- whereas the enzyme without its non-protein moiety is termed an apoenzyme (apoprotein) and is inactive.

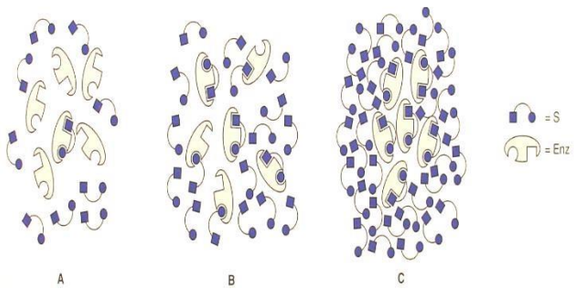


- Coenzymes serve as recyclable shuttles that transport many substrates from one point within the cell to another.
- The function of these shuttles is twofold.
- First, they stabilize species
- such as hydrogen atoms (FADH) or hydride ions (NADH)
- that are too reactive to persist for any significant time in the presence of the water or organic molecules that permeate cells.
- Second, they serve as an adaptor or handle that facilitates the
- recognition and binding of small chemical groups, such as acetate (coenzyme A) or glucose (UDP), by their target enzymes

202 Enzymes-Reaction rates and order

Reaction Velocity (v)

- The rate or velocity of a reaction (v) is the number of substrate molecules converted to product per unit time;
- Velocity is usually expressed as **µmol of product** formed **per minute**.
- The rate of an enzyme-catalyzed reaction increases with substrate concentration until a maximal velocity (Vmax) is reached- reflecting the **saturation** with substrate of **all available binding sites are occupied** on the enzyme molecules present.



(Vmax) is reached- reflecting the **saturation** with substrate of **all available binding sites are occupied** on the enzyme molecules present.

- An equilibrium such as S ↔ P is described by an **equilibrium constant, Keq**, or simply **K.**
- Under the standard conditions used to compare biochemical processes, an equilibrium constant is denoted ***K´eq*** (or ***K´).***
- The rate of any reaction is determined by the concentration of the reactant (or reactants) and by a **rate constant,** usually denoted by **k.**
- For the uni molecular reaction S → P, the rate (or velocity) of the reaction, V—representing the amount of S that reacts per unit time—is expressed by a **rate equation:** V = k[S].
- In this reaction, the rate depends only on the concentration of S.
- This is called a first-order reaction.
- The factor k is a proportionality constant that reflects the probability of reaction under a given set of conditions (pH, temperature, and so forth).
- If a reaction rate depends on the concentration of two different compounds.
- or if the reaction is between two molecules of the same compound, the reaction is second order.
- The rate equation then becomes
- **$V = k[S_1][S_2]$**

203 Enzyme-Factor affecting enzyme activity

- Enzymes can be isolated from cells, and their properties studied in a test tube (that is, in vitro).

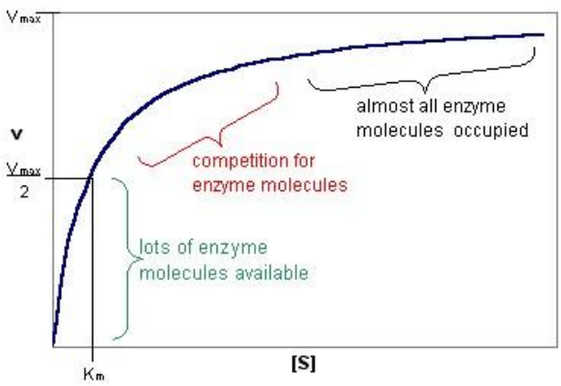
Factors Affecting Enzymatic Activity

- **Different enzymes show different responses to changes in;**
- **substrate concentration**
- **temperature, and**

- **pH.**
- Enzymic responses to these factors give us valuable clues as to how enzymes function in living cells (that is, in vivo)

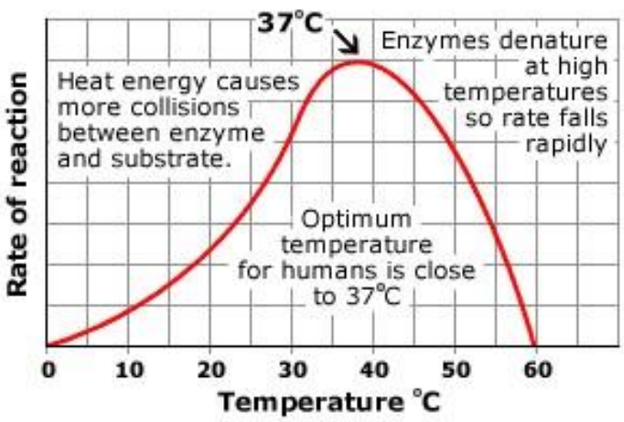
Substrate concentration

- The rate of an enzyme-catalyzed reaction increases with substrate concentration until maximal velocity (Vmax) is reached
- The leveling off of the reaction rate at high substrate concentrations
- reflects the saturation with substrate
- of all available binding sites on the enzyme molecules present.



Temperature

- The reaction velocity increases with temperature until a peak velocity is reached
- This increase is the result of the increased number of molecules having sufficient energy to pass over the energy barrier and form the products.



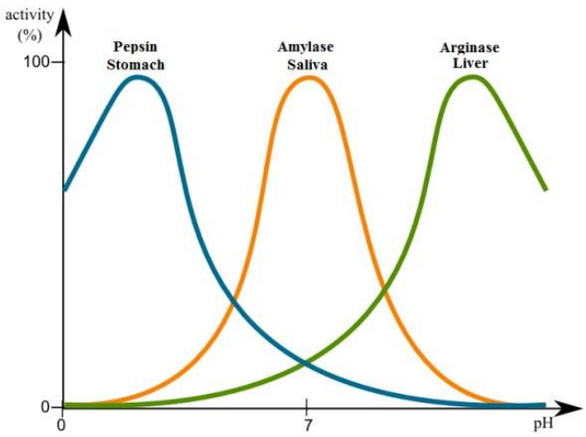
- Further elevation
- of the temperature results in a decrease in reaction velocity as a
- result of temperature-induced denaturation of the enzyme

204 Enzymes-Factors (continued..)

Effect of pH

- The pH optimum varies for different enzymes:
- The pH at which maximal enzyme activity is achieved is different for different enzymes, and often reflects the [H+] at which the enzyme functions in the body.
- For example, pepsin, a digestive enzyme in the stomach, is maximally active at pH 2.

- Whereas other enzymes, designed to work at neutral pH, are denatured by such an acidic environment
- Another examples is that there are two types of phosphatases in the body.
- The one that acts in the alkaline pH is called alkaline phosphatase and
- the other which acts at acidic pH is known as acid phosphatase.



- Effect of pH on the ionization of the active site:
- The concentration of H+ affects reaction velocity in several ways.
- First, the catalytic process usually requires that the enzyme and substrate have specific chemical groups in either an ionized or un-ionized state in order to interact.
- For example, catalytic activity may require that an amino group of the enzyme be in the protonated form (–NH3 +).
- At alkaline pH, this group is deprotonated, and the rate of the reaction, therefore, declines.
- Extremes of pH can also lead to denaturation of the enzyme.

205 Enzymes-Enzyme kinetics

Enzyme Kinetics

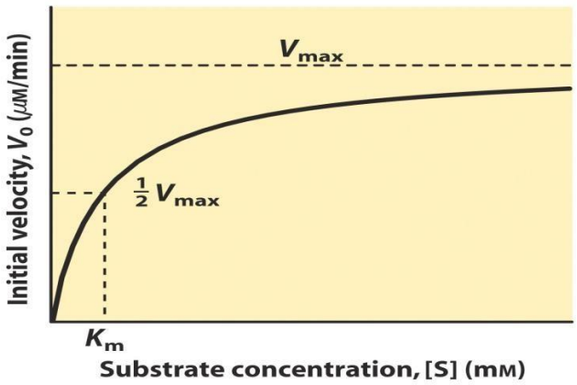
Substrate Concentration Affects the Rate of Enzyme-Catalyzed Reactions

E + S ↔ ES ↔ EP

↔ E + P

- A key factor affecting the rate of a reaction catalyzed by an enzyme is the concentration of substrate, [S].
- However, studying the effects of substrate concentration is complicated because substrate is converted to product
- and because of reversibility of reactions, e.g. conversion of product back to substrate.
- One simplifying approach in kinetics experiments is to measure the **initial rate (or initial velocity), designated V_0,** when [S] is much greater than the concentration of enzyme,
  [E].
- The velocity (v) of a reaction is the rate of product formation
- Whereas V_0 is the initial velocity and is measured as soon as the reactants and enzymes are mixed.

- At the start of a reaction, [S] is in large excess of [P].
- Thus the initial velocity of the reaction will be dependent on substrate concentration
- At that time, the concentration of product is very small and, therefore, the rate of the back reaction from P to S can be ignored.

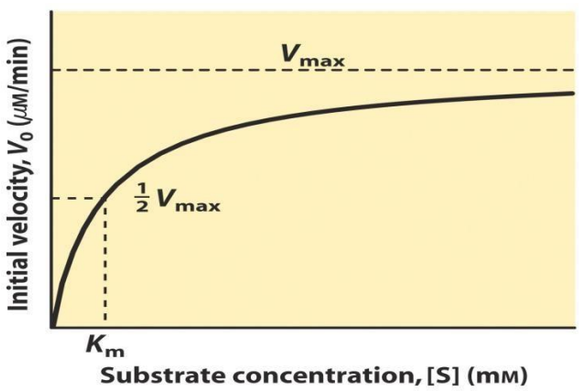


- Increase in substrate concentration increases V_0 i.e.
- initial velocity is increased whenever a fixed concentration of enzyme is mixed with an increased concentration of substrate.

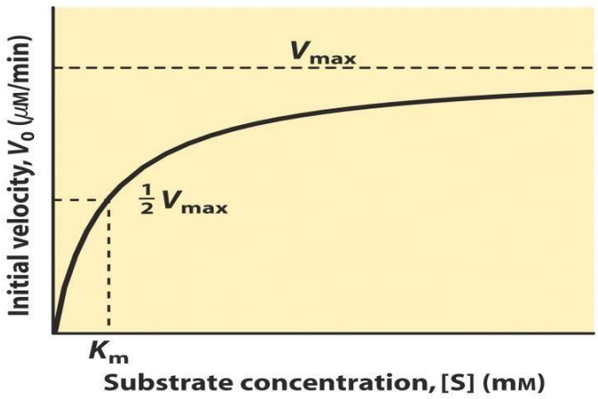
206 Enzymes-Enzyme kinetics (continued..)

Maximal Velocity V_{max}

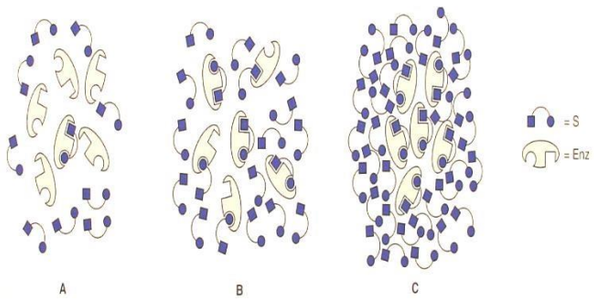
- When initial velocity **(V_0)** is plotted against [S],
- a **hyperbolic curve** results,
- where V_{max} represents the maximum reaction velocity.



- At relatively low concentrations of substrate, V_0 increases almost linearly.
- At higher substrate concentrations, V_0 *increases by smaller and smaller amounts in* response to increases in [S].
- This plateau-like V_0 *region is close to the* **maximal velocity, V_{max}.**
- V_{max} *is extrapolated from the* plot, because V_0 *approaches but never quite reaches* V_{max}.



- At this point in the reaction, if **[S] >> E**, all available enzyme is "saturated" with bound substrate, meaning only the **ES complex** is present.
- This dictates that further increasing the substrate concentration will not result in increased V_0



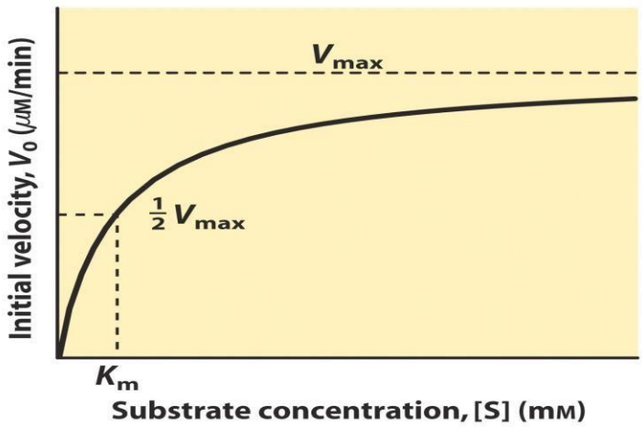
A. Low [S] B. 50% [S] or K_m C. High, saturating [S]

- The leveling off of the reaction rate at high substrate concentrations
- reflects the saturation with substrate
- of all available binding sites on the enzyme molecules present.
- This finite limit of V_{max} is called saturation kinetics.
- Saturation kinetics is a characteristic property of all rate processes dependent on the binding of a ligand to a protein e.g. mThe process by which a carrier protein transfers a solute molecule across the lipid bilayer resembles an enzyme-substrate reaction, and in many ways carriers behave like enzymes. In contrast to ordinary enzyme-substrate reactions, however, the transported solute is not covalently modified by the carrier protein, but instead is delivered unchanged to the other side of the membrane.
- embrane transporter proteins.

207 Enzymes-Enzyme kinetics Km (continued...)

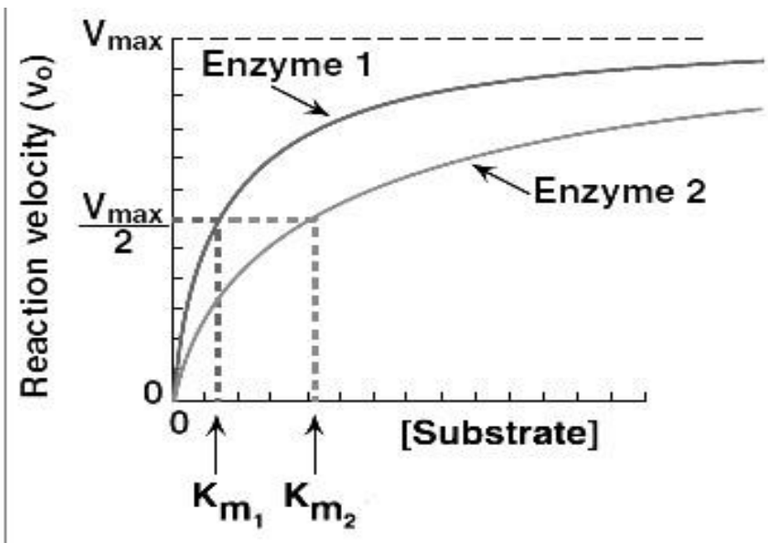
Michaelis constant K_m

- The substrate concentration at which V_0 is half maximal is K_m, the Michaelis constant.



The effect of varying [S], on V_0, when the enzyme concentration is held constant

- K_m reflects the affinity of the enzyme for the substrate.
- Small K_m reflects a high affinity of the enzyme for substrate, because a low concentration of substrate is needed to half-saturate the enzyme i.e. to reach a velocity that is 1⁄2V_{max}. • A numerically large (high) Km reflects a low affinity of enzyme for substrate because
- a high concentration of substrate is needed to half-saturate the enzyme.



a. **Small K_m:** A numerically small (low) K_m reflects a high affinity of the enzyme for substrate, because a low concentration of substrate is needed to half-saturate the enzyme—that is, to reach a velocity that is 1⁄2 V_{max}

b. **b. Large Km:** A numerically large (high) Km reflects a low affinity of enzyme for substrate because a high concentration of substrate is needed to half-saturate the enzyme

c. The velocity of an enzyme is most sensitive to changes in substrate concentration over a concentration range below its Km.

d. At substrate concentrations less than 1⁄10th of the Km, a doubling of substrate concentration nearly doubles the velocity of the reaction

e. At substrate concentrations 10 times the Km, doubling the substrate concentration has little effect on the velocity.

- A comparison between the isozymes of hexokinase illustrates the significance of the K_m.
- Hexokinase catalyses the first step in glucose metabolism in most cells, the transfer of a phosphate from ATP to glucose to form glucose 6-phosphate.

- Hexokinase I, the isozyme in red blood cells has a low K_m for glucose of approximately 0.05 mM- helpful in utilizing blood glucose even when the blood glucose concentration is very low.
- The isozyme of hexokinase, called glucokinase, which is found in the liver has a much higher K_m of approximately 5 to 6 mM- helpful in storing large amounts of "excess" glucose as glycogen or converting it to fat after a carbohydrate meal.

208 Enzymes-Michaelis menten equation

Michaelis-Menten equation, the rate equation

- Leonor Michaelis and Maud Menten in 1913, proposed a simple model that accounts for most of the features of enzyme-catalyzed reactions.
- They postulated that the enzyme first combines reversibly with its substrate to form an enzyme-substrate complex in a relatively fast reversible step: *k1*

E + S ↔ ES

K-1

- The ES complex then breaks down in a slower second step to yield the free enzyme (E) and the reaction product (P):

K2

ES ↔ E + P

K-2

- Early in the reaction, the concentration of the product, **[P], is negligible**, and we make the simplifying assumption that the reverse reaction, P → S (described by *k-2), can be ignored*
- This assumption is not critical but it simplifies our task.
- The overall reaction then reduces to *k1* *K2*
- **E + S ↔ ES → E + P**

K-I

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

E = Enzyme S = Substrate P = Product

ES = Enzyme-Substrate complex k_1 rate

constant for the forward reaction

k_{-1} = rate constant for the breakdown of the ES to substrate k_2

= rate constant for the formation of the products

- Because the slower second reaction must limit the rate of the overall reaction.
- The overall rate must be proportional to the concentration of the species that react in the second step, that is, ES.
- The maximum initial rate of the catalyzed reaction (*Vmax) is observed when virtually all the* enzyme is present as the ES complex and [E] is vanishingly small.

- Under these conditions, the enzyme is “saturated” with its substrate, so that further increases in [S] have no effect on rate.
- This condition exists when [S] is sufficiently high that essentially all the free enzyme has been converted to the ES form.
- After the ES complex breaks down to yield the product P,
- the enzyme is free to catalyze reaction of another molecule of substrate.

209 Enzymes-Michaelis Menten equation (continued..)

Michaelis-Menten equation, the rate equation (Contd.)

$$V_0 = \frac{Vmax\,[S]}{Km + [S]}$$

- Where;
- V_0 = initial reaction velocity
- Vmax = maximal velocity
- Km = Michaelis constant
- = K1+K2 / K-1
- **[S] = substrate concentration**
- This is the **Michaelis-Menten equation**-statement of the quantitative relationship between the;
- initial velocity V_0,
- *the maximum velocity Vmax, and*
- *the initial substrate concentration [S], all related* through the Michaelis constant *Km*
- An important numerical relationship emerges from the Michaelis-Menten equation in the special case;
- when V_0 *is exactly one-half Vmax*

$$V_0 = \frac{Vmax\,[S]}{Km + [S]}$$

$$\frac{Vmax}{2} = \frac{Vmax\,[S]}{Km + [S]}$$

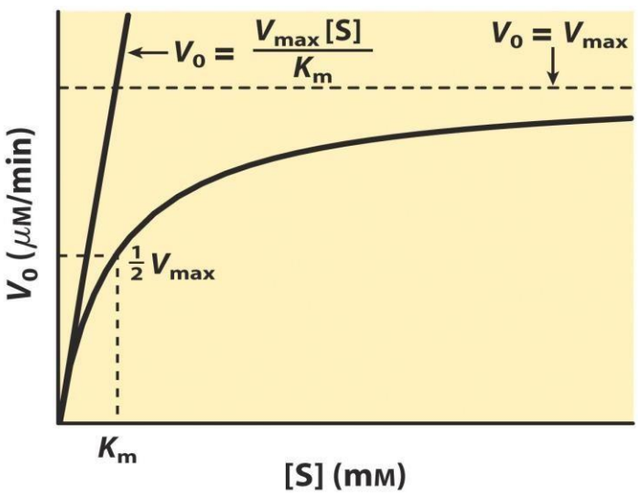
- On dividing by *Vmax, we obtain*
- 1/2 = [S] / Km + [S]
- Solving for *Km, we get*
- *Km + [S] = 2[S], or*
- *Km = [S], when* V_0 = 1/2 Vmax
- This is a very useful, practical definition of *Km: Km is* equivalent to the substrate concentration at which V_0 *is* one-half *Vmax.*
- The equation describes the kinetic behavior of a great many enzymes, and all enzymes that exhibit a hyperbolic dependence of *V0 on [S] are said to follow* ***Michaelis-*** **Menten kinetics.**
- The practical rule that *Km= [S] when V0 =½Vmax holds for all enzymes* that follow Michaelis-Menten kinetics.

- The most important exceptions to Michaelis-Menten kinetics are the regulatory enzymes.

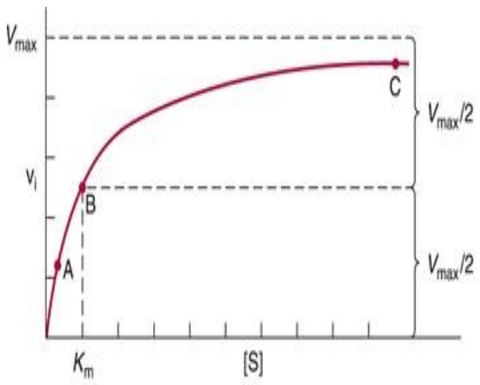
210 Enzymes-Michaelis menten equation at low [S]

The Michaelis-Menton Equation at low [S]

- Interpreting Vmax and Km shows a simple graphical method for obtaining an approximate value for *Km.*
- This graph shows the kinetic parameters that define the limits of the curve at high and low [S].



- At low [S], *Km>>[S]* and the *[S]* term in the denominator of the Michaelis-Menten equation becomes insignificant.
- low [S]; $K_m >> [S]$
- $V_o = \frac{Vmax\,[S]}{K^m + [S]}$
- $V_o = \frac{Vmax\,[S]}{K^m}$
- Since V_{max} and K_m are both constants, their ratio is a constant.
- In other words, when [S] is considerably below K_m,
- V_0 is proportionate to k[S].
- The initial reaction velocity, V_0, therefore is directly proportionate to [S].
- V_0 exhibits a linear dependence on [S]*, as observed here.*
- *(First order Reaction)*



Source: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA: *Harper's Illustrated Biochemistry, 28th Edition*: http://www.accessmedicine.com

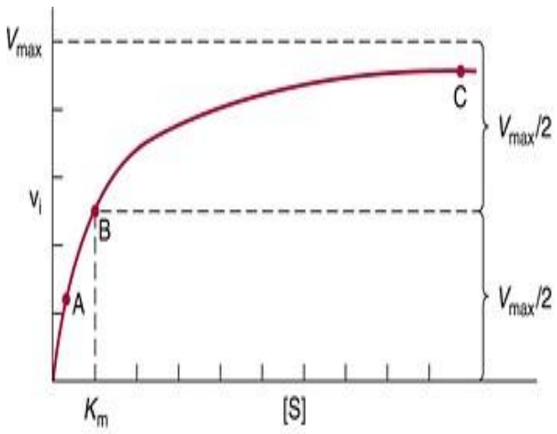
When [S] is much less than K_m (point A in Figure)

- Therefore at concentrations below K_m reaction rate is first order i.e. it is directly proportional to the concentration of the substrate.

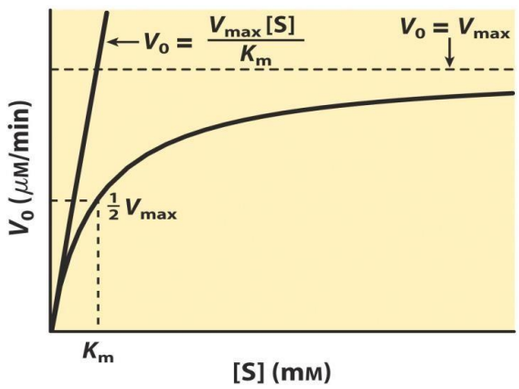
211 Enzymes-Michaelis Menten equation at high S

The Michaelis-Menton Equation at high [S]

- At high [S] [S] >> K^m
- The term K_m + [S] is essentially equal to [S].
- *The Km term in the denominator of the Michaelis*- Menten equation becomes insignificant
- Replacing K_m + [S] with [S] reduces equation
- high [S] [S] >> K^m
- V_0 = Vmax [S]
- K_m + [S]
- Ignoring K_m
- V_0 = Vmax [S]
- [S]
- V_0 = Vmax.
- *This is consistent with the plateau observed at high [S]. (Zero Order Reaction)*



- The rate of reaction is then independent of substrate concentration ***[S]*****,** and is said to be **zero order** with respect to substrate concentration.

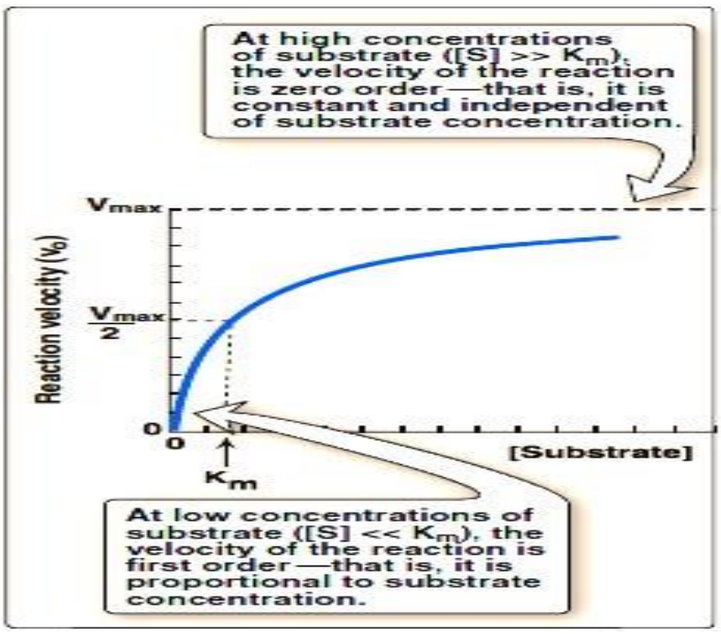


- The Michaelis-Menten equation is therefore consistent with the observed dependence of V_0 on [S], and the shape of the curve is defined by the terms;
- *V_{max}/K_m at low [S] and*
- *V_{max} at high [S].*

212 Enzymes-Order of reaction

Order of Reaction

- When [S] is much less, then the velocity of the reaction is approximately proportional to the substrate concentration.
- The rate of reaction is then said to be first order with respect to substrate.
- When [S] is much greater than Km the velocity is constant and equal to V_{max}.
- The rate of reaction is then independent of substrate concentration, and is said to be zero order with respect to substrate concentration

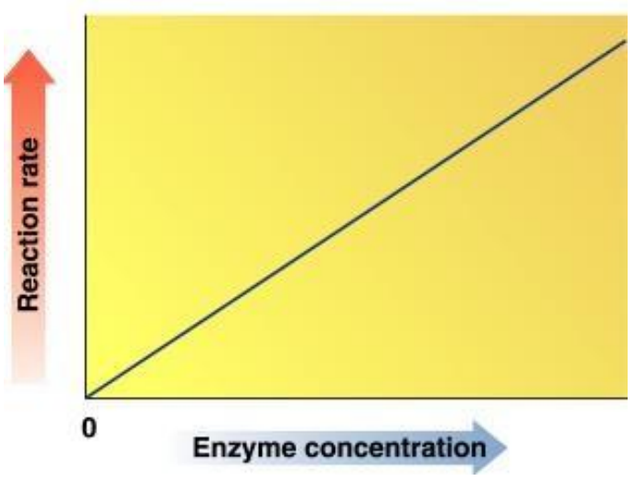


Reaction Orders with Respect to Substrate Concentration

Order	Rate Equation	Comments
Zero	Rate = k	Rate is independent of substrate concentration
First	First rate = k[S]	Rate is proportional to the first power of substrate concentration
Second	Rate = k[S1][S2]	Rate is proportional to the first power of each of two reactants

Relationship of velocity to enzyme concentration

- The rate of the reaction is directly proportional to the enzyme concentration.
- There is a linear relationship between reaction rate and enzyme concentration (at constant substrate concentration)

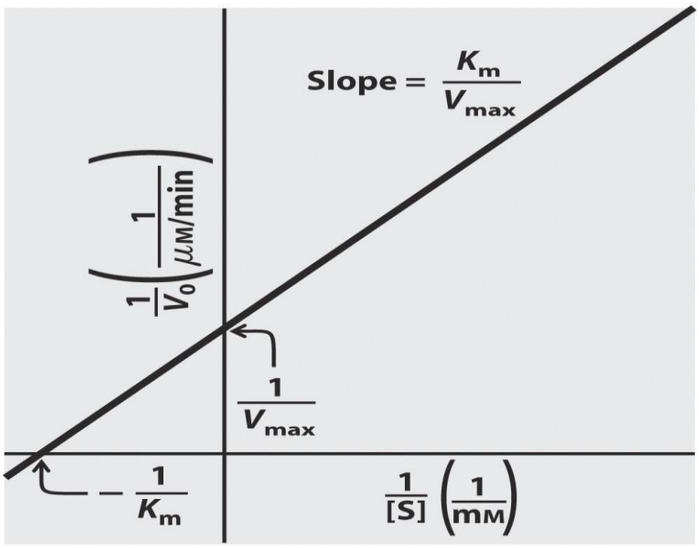


- For example,
- if the enzyme concentration is halved, the initial rate of the reaction (V_0) as well as that of Vmax are reduced to one half that of the original.

213 Enzymes-Line Weaver Burke plot

Lineweaver-Burke plot

- The Michaelis-Menten equation can be algebraically transformed into **LineweaverBurke plot,** a *Double Reciprocal Plot***,** that is useful in the practical determination of K_m and V_{max}.



However, if $1/V_0$ is plotted versus 1/[S], a straight line is obtained. This graph, the LineweaverBurke plot can be used to calculate

K_m and V_{max}, as well as to determine the mechanism of action of

enzyme inhibitors.

$$V_0 = \frac{Vmax\,[S]}{K^m + [S]}$$

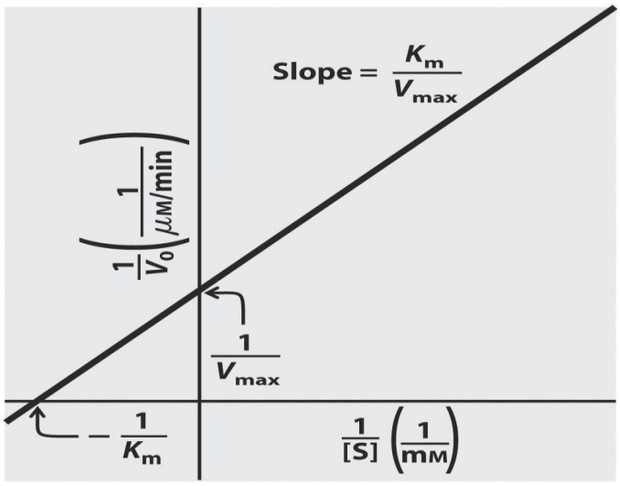
- Lineweaver-Burke transformation is derived simply by taking the reciprocal of both sides of the Michaelis-Menten equation:

$$\frac{1}{V_0} = \frac{K^m + [S]}{Vmax\,[S]}$$

$$\frac{1}{V_o} = \frac{K^m + [S]}{Vmax\,[S]}$$

$$\frac{1}{V_o} = \frac{Km}{Vmax[S]} + \frac{[S]}{Vmax[S]}$$

$$\frac{1}{V_o} = \frac{K^m}{Vmax[S]} + \frac{1}{Vmax}$$



In lineweaver Burke plot y- intercept is $1/V_{max}$ and x-intercept is $1/K_m$.

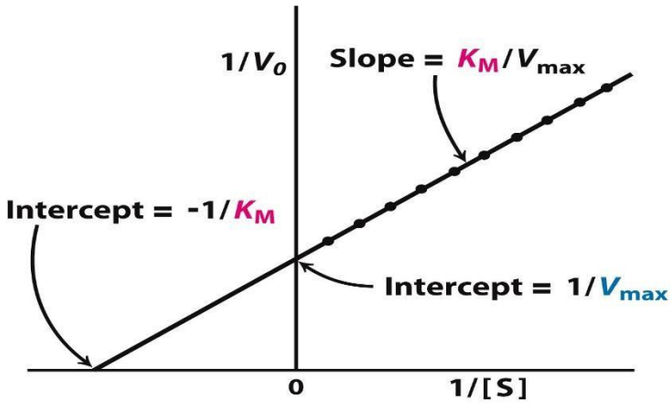


Figure 8.12
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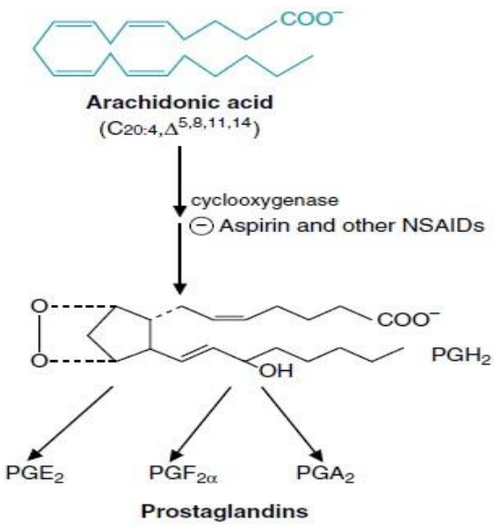
In lineweaver Burke plot y- Axis intercept is $1/V_{max}$ and x- Axis intercept is $-1/K_m$.

- Lineweaver-Burk plot, has the great advantage of allowing a more accurate determination of V_{max},
- *Which can only be approximated from* a simple plot of V_0 *versus [S].*
- The double-reciprocal plot of enzyme reaction rates is very useful in distinguishing between certain types of enzymatic reaction mechanisms and in analyzing enzyme inhibition.

214 Enzymes-Inhibition of Enzyme Activity

INHIBITION OF ENZYME ACTIVITY

- Enzyme inhibitors are molecular agents that interfere with catalysis, slowing or halting enzymatic reactions.
- Any substance that can diminish the velocity of an enzyme-catalyzed reaction is called an inhibitor.
- Enzymes catalyze virtually all cellular processes, so it should not be surprising that enzyme inhibitors are among the most important pharmaceutical agents known.



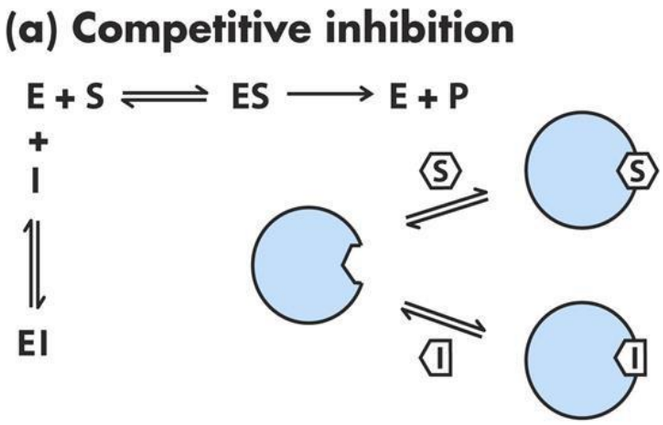
For example, aspirin inhibits the enzyme (cyclooxygenase) that catalyzes the first step in the synthesis of prostaglandins, compounds involved in many processes, including some that produce pain.

- Inhibitors can be classified on the basis of their site of action on the enzyme,
- on whether they chemically modify the enzyme, or on the kinetic parameters they influence.
- Two broad classes of enzyme inhibitors:
- **1. Reversible**
- **2. Irreversible**.
- In general, irreversible inhibitors bind to enzymes through covalent bonds.
- Reversible inhibitors typically bind to enzymes through noncovalent bonds,
- The two most commonly encountered types of reversible inhibition are;
- competitive and
- noncompetitive.
- Competitive inhibitors resemble the substrate and compete for binding to the active site of the enzyme.
- Noncompetitive inhibitors do not bind at the active site. They bind either free enzyme at a site other than active site or the ES complex.

215 Enzymes-Competitive Inhibition

Competitive Inhibition

- This type of inhibition occurs when the inhibitor binds reversibly to the same site that the substrate would normally occupy i.e. active site and, therefore, competes with the substrate for that site.
- Competitive inhibitors bind to the enzyme's active site.

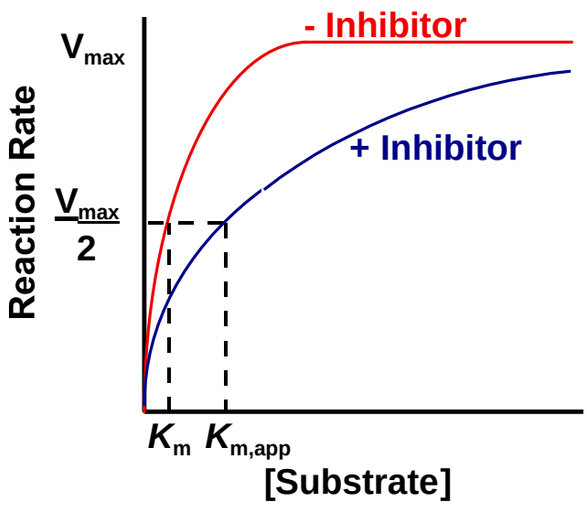


While the inhibitor (I) occupies the active site it prevents binding of the substrate to the enzyme. Many competitive inhibitors are compounds that resemble the substrate and combine with the enzyme to form an EI complex, but without leading to catalysis.

- Reversible inhibitors bind to enzymes through non covalent bonds.
- Dilution of the enzyme-inhibitor complex results in dissociation of the reversibly bound inhibitor, and recovery of enzyme activity.

1. Effect on Vmax

- The effect of a competitive inhibitor is reversed by increasing [S].
- At a sufficiently high substrate concentration, the reaction velocity reaches the V_{max} as observed in the absence of inhibitor.



Vmax,app = Vmax *K*m,app > *K*m

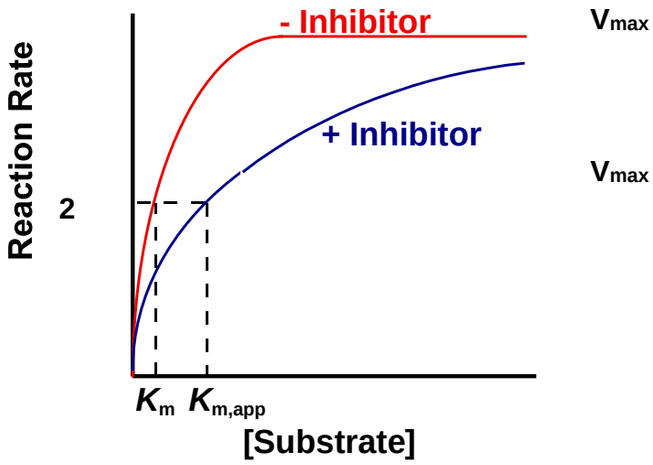
Even if high conc. of substrate is required V_{max} is achieved. ***K*m,app becomes greater than km**

- When [S] far exceeds [I], the probability that an inhibitor molecule will bind to the enzyme is minimized and the reaction exhibits a normal V_{max}.
- Therefore, a competitive inhibitor does not decrease V_{max}

216 Enzymes-Competitive inhibition (continue...)

2. Effect on Km

- The [S] at which $V_0 = 1/2\ V_{max}$, the apparent Km, increases in the presence of inhibitor.
- A competitive inhibitor increases the apparent K_m (αK_m) for a given substrate.



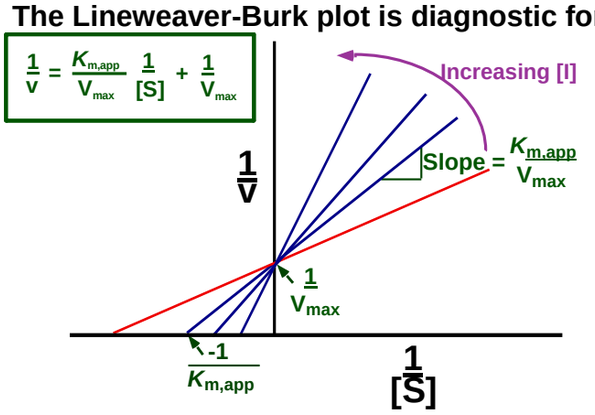
Vmax,app = Vmax *K*m,app > *K*m

Increase in the apparent K_m (α*K_m*) for a given substrate means that, more substrate is now needed to achieve 1/2V_{max}.

3. **Effect on Lineweaver-Burk plot**

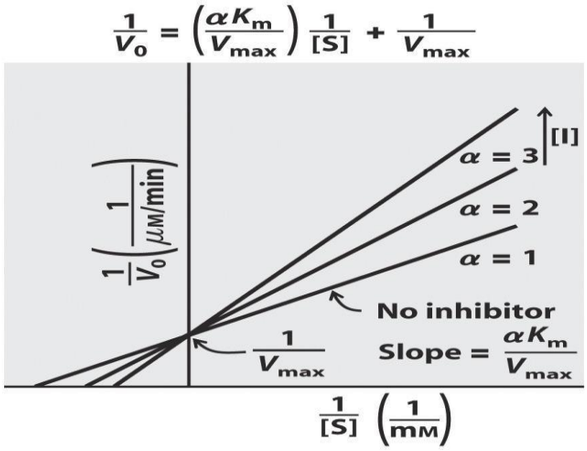
- Competitive inhibition shows a characteristic Lineweaver-Burke plot
- the plots of the inhibited and uninhibited reactions intersect at a single point on the y-axis at 1/V_{max}.
- V_{max} is unchanged
- However, the inhibited and uninhibited reactions show; different x axis intercepts
- Therefore indicating that the apparent K_m is increased in the presence of the competitive inhibitor because -1/K_m moves closer to zero from a negative value.

The Lineweaver-Burk plot is diagnostic for competitive inhibition



Increasing [I] results in a family of lines with a common intercept on the 1/V_0 *axis but with* different slopes. Because the intercept on the 1/V_0 *axis equals* 1/V_{max}.

Competitive inhibition.



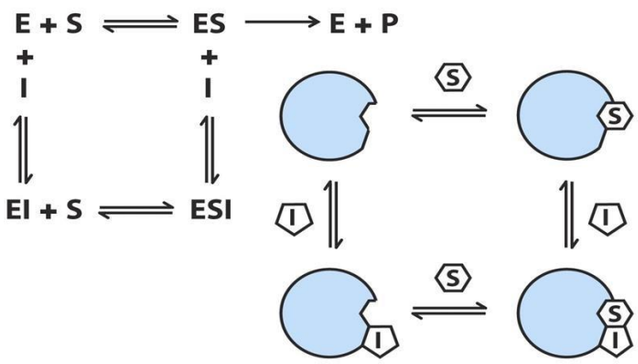
- We know that V_{max} *is* unchanged by the presence of a competitive inhibitor.
- This is because more substrate means more formation of ES complex vs EI complex.

217 Enzymes-Non competitive inhibition

Noncompetitive Inhibition

- Inhibitors bind enzymes at sites distinct from the substrate-binding site and
- generally bear little or no structural resemblance to the substrate.

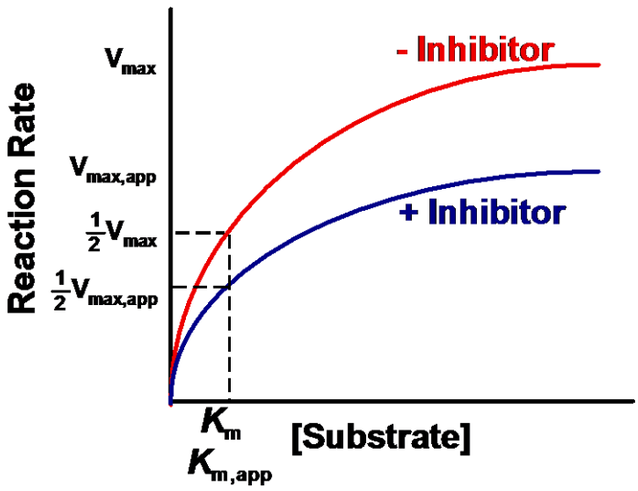
Noncompetitive inhibitors bind at a separate site, but may bind to either E or ES.



- Noncompetitive inhibition cannot be overcome by increasing the concentration of substrate.

1. Effect on V_{max}

- the apparent V_{max} changes, because the inhibitor is capable of preventing catalysis regardless of whether the substrate is bound to the enzyme.
- Noncompetitive inhibition cannot be overcome by increasing the concentration of substrate.
- Thus, noncompetitive inhibitors decrease the V_{max} of the reaction.
- V_{max} decreases in noncompetitive inhibition.
- V_{max} decreases in noncompetitive inhibition.



V_{max} decreases in noncompetitive inhibition.

- The noncompetitive inhibitor, in effect, lowers the concentration of the active enzyme and therefore decreases the V_{max} of the enzyme.

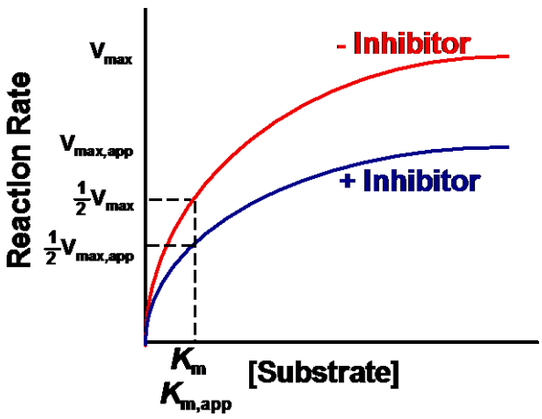
218 Enzymes-Non competitive inhibition (continued...)

Noncompetitive Inhibition

(Contd.)

2. Effect on Km

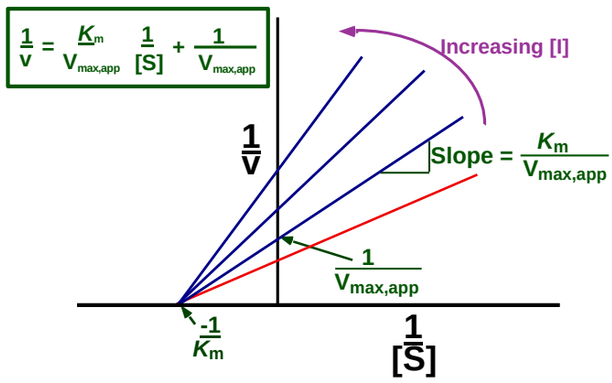
- Noncompetitive inhibitors do not interfere with the binding of substrate to enzyme.
- Thus, the enzyme shows the same K_m in the presence or absence of the noncompetitive inhibitor.



V_{max} changes but the [S] required to achieve ½ V_{max} i.e. K_m remains the same

3. Effect on Lineweaver-Burk plot

- Noncompetitive inhibition shows a characteristic Lineweaver-Burke plot
- the plots of the inhibited and uninhibited reactions intersect at a single point on the x-axis at K_m
- K_m is unchanged
- However, the inhibited and uninhibited reactions show; different y axis intercepts
- indicating the deccrease in V_{max} in the presence of the competitive inhibitor.



- While certain inhibitors exhibit characteristics of a mixture of competitive and noncompetitive inhibition, the evaluation of these inhibitors exceeds the scope of our level of study.

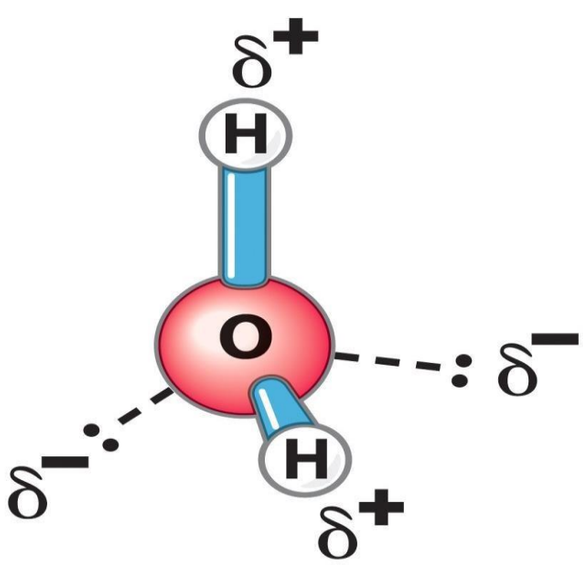
219 Water, PH and Buffers

Water

- Water is the most abundant substance in living systems, making up 70% or more of the weight of most organisms
- **The hydrogen bonding between water molecules and the slight tendency of water to ionize are of crucial importance to the structure and function of biomolecules**

Hydrogen Bonding gives Water its Unusual Properties

- Water has a higher
- melting point,
- boiling point, and
- heat of vaporization than most other common solvents
- Each hydrogen atom of a water molecule shares an electron pair with the central oxygen atom
- The geometry of the molecule is dictated by the shapes of the outer electron orbitals of the oxygen atom

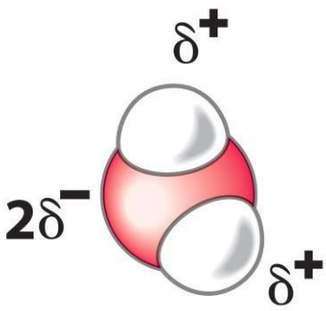


These orbitals describe a rough tetrahedron, with a hydrogen atom at each of two corners and unshared electron pairs at the other two corners.

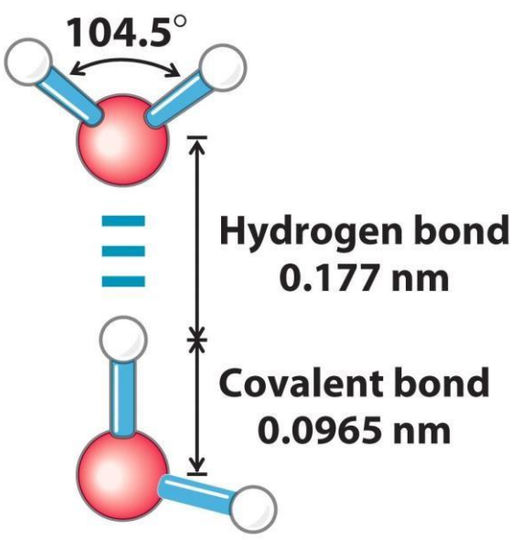
The oxygen nucleus attracts electrons more strongly than does the hydrogen nucleus. That is, oxygen is more electronegative.

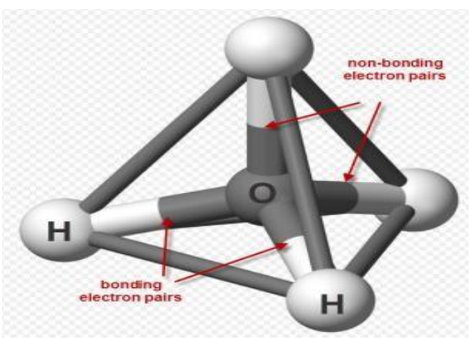
This means that the shared electrons are more often in the vicinity of the oxygen atom than of the hydrogen

- The result of this unequal electron sharing is two electric dipoles in the water molecule
- Each hydrogen bears a partial positive charge (δ+), and
- the oxygen atom bears a partial negative charge equal to the sum of the two partial positives (2δ-)

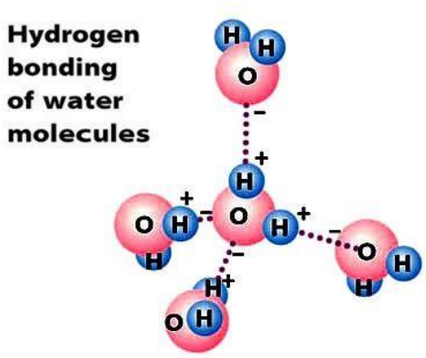


- As a result, there is an electrostatic attraction between the oxygen atom of one water molecule and the hydrogen of another, called a hydrogen bond





- The nearly tetrahedral arrangement of the orbitals about the oxygen atom allows each water molecule to form hydrogen bonds with as many as four neighboring water molecules



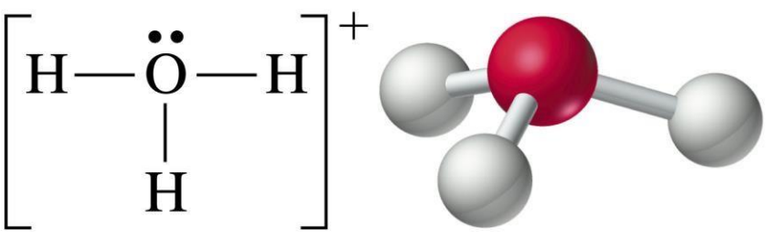
Each water molecule can form two hydrogen bonds involving their hydrogen atoms plus two further hydrogen bonds utilizing the hydrogen atoms attached to neighboring water molecules.

These four hydrogen bonds optimally arrange themselves tetrahedrally around each water molecule

220 Water, PH and buffer systems-ionization of water

Ionization of Water

- Although many of the solvent properties of water can be explained in terms of the uncharged H_2O molecule, the small degree of ionization of water into hydrogen ions (H^+) and hydroxide ions (OH^-) must also be taken into account
- Pure Water ls Slightly lonized
- Water molecules have a slight tendency to undergo reversible ionization to yield a hydrogen ion (a proton) and a hydroxide ion, giving the equilibrium reaction
- $H_2O \leftrightarrow H^+ + OH^-$
- Hydrogen ions formed in water are immediately hydrated to hydronium ions ($H3O^+$)
- The ionization of water can be measured by its electrical conductivity
- Pure water carries electrical current as $H3O^+$ migrates toward the cathode and OH^- toward the anode



- The lonization Of Water ls Expressed By an Equilibrium Constant
- The degree of ionization of water at equilibrium is small
- At 25^0C only about two out of every 10^9 molecules in pure water are ionized at any instant
- The equilibrium constant for the reversible ionization of water is

$$\mathbf{Keq} = \frac{[H^+][OH^-]}{[H_2O]}$$

$$\mathbf{K_{eq}} = \frac{[H^+][OH^-]}{[H_2O]}$$

- Since 1 mole (mol) of water weighs 18 g,
- 1 liter (L) (1000 g) of water contains
- 1000 ÷ 18 = 55.56 mol
- Pure water thus is 55.56 molar(M)
- Accordingly, we can substitute 55.5 M in the equilibrium constant expression to yield
- $Keq = [H^+][OH^-]/55.5$
- $Keq = [H^+][OH^-]/55.5$
- On rearranging, this becomes (Keq) (55.5)= $[H^+][OH^-]= Kw$
- Where Kw designates the product (55.5M)(Keq), the ion product of water at 25^0C

- The value for Keq, determined by electrical conductivity measurements of pure water, is

  1.8×10^{-16} M at 25^0C

- Kw= (Keq)(55.5)= $[H^+][OH^-]$
- Kw = $(1.8 \times 10^{-16}M)$ (55.5M)= $[H^+][OH^-]$
- Kw = 1.0×10^{-14} M^2= $[H^+][OH^-]$
- Kw= $[H^+][OH^-]$= $[H^+]^2$ = $[OH^-]^2$
- Solving for$[H^+]$ gives:

$[H^+]= \sqrt{Kw} = \sqrt{10^{-14}}$ M^2

$[H^+]= 10^{-7}$ M

- Thus the product $[H^+][OH^-]$ in aqueous solutions at 25^0C always equals 1×10^{-14} M^2
- When there are exactly equal concentrations of H^+ and OH^- , as in pure water, the solution is said to be at neutral pH
- As the ion product of water is constant, whenever $[H^+]$ is greater than 1×10^{-7} M, $[OH^-]$ must be less than 1×10^{-7} M, and vice versa

221 Water, PH and Buffer systems-working examples

WORKING EXAMPLES

- From the ion product of water we can calculate $[H^+]$
- if we know $[OH^-]$, and vice versa
- **What is the concentration of H^+ in a solution of 0.1 M NaOH?**

Solution:

Kw= $[H^+][OH^-]$

- **With $[OH^-]$ = 0.1 M, solving for $[H^+]$ gives**
- **$[H^+] = K_w/[OH^-]$**

$= 1 \times 10^{-14}M^2/ 0.1M$

$= 10^{-14}M^2/0.1M$

$= 10^{-13}M$

- **What is the concentration of OH^- in a solution with an H^+ concentration of 1.3×10^4M?**
- **Solution:**
- Kw= $[H^+][OH^-]$
- With [H+] = 1.3×10^{-4}M, solving for $[OH^-]$ gives
- $[OH^-] = Kw/[H^+]$
- = $1 \times 10^{-14}M^2/ 1.3 \times 10^{-4}M$
- = **7.7×10^{-11} The pH Scale**
- Designates the H^+ and OH^- Concentrations
- The **pH** of a solution is defined as the logarithm to the base 10 of the reciprocal of the $[H^+]$, i. e, the negative logarithm of the $[H^+]$

- pH = log 1/[H^+]
- = -log[H^+]
- The pH of water at 25°C, in which H^+ and OH^- ions are present in equal numbers, is 7.0
- pH = -log [1 x 10^{-7}] = 7
- The symbol p denotes "negative logarithm of"
- For each pH unit less than 7.0, the [H^+] is increased tenfold;
- for each pH unit above 7.0, it is decreased tenfold

TABLE 2–6 The pH Scale

$[H^+]$ (M)	pH	$[OH^-]$ (M)	pOH*
10^0 (1)	0	10^{-14}	14
10^{-1}	1	10^{-13}	13
10^{-2}	2	10^{-12}	12
10^{-3}	3	10^{-11}	11
10^{-4}	4	10^{-10}	10
10^{-5}	5	10^{-9}	9
10^{-6}	6	10^{-8}	8
10^{-7}	7	10^{-7}	7
10^{-8}	8	10^{-6}	6
10^{-9}	9	10^{-5}	5
10^{-10}	10	10^{-4}	4
10^{-11}	11	10^{-3}	3
10^{-12}	12	10^{-2}	2
10^{-13}	13	10^{-1}	1
10^{-14}	14	10^0 (1)	0

*The expression pOH is sometimes used to describe the basicity, or OH^- concentration, of a solution; pOH is defined by the expression pOH = −log [OH^-], which is analogous to the expression for pH. Note that in all cases, pH + pOH = 14.

TABLE 2–6 The pH Scale

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10^{-4}	4	10^{-10}	10
10^{-5}	5	10^{-9}	9
10^{-6}	6	10^{-8}	8
10^{-7}	7	10^{-7}	7
10^{-8}	8	10^{-6}	6
10^{-9}	9	10^{-5}	5
10^{-10}	10	10^{-4}	4
10^{-11}	11	10^{-3}	3
10^{-12}	12	10^{-2}	2
10^{-13}	13	10^{-1}	1
10^{-14}	14	10^0 (1)	0

*The expression pOH is sometimes used to describe the basicity, or OH^- concentration, of a solution; pOH is defined by the expression pOH = −log [OH^-], which is analogous to the expression for pH. Note that in all cases, pH + pOH = 14.

- **What will be the pH of 0.1 M HCl?**
- Assuming that being a strong acid HCl is completely dissociated, it's 0.1 M solution will contain 0.1 or 10^{-1} grams H^+ per litre
- pH= - log [H^+]
- pH = - log [10^{-1}]
- = - [- 1]

- = **1**

222 Water, PH and buffer systems-weak acids and bases

Weak Acids and Bases

- Each acid has a characteristic tendency to ionize in an aqueous solution
- The stronger the acid, the greater its tendency ionize i.e. to lose its proton
- This tendency is measured by an acid dissociation constant
- **Weak Acids and Bases have Characteristic Acid Dissociation Constants**
- **HCl, H_2SO_4, and HNO_3, commonly called strong acids, are fully ionized in aqueous solutions**
- **The strong bases NaOH and KOH are also completely ionized**
- Of more interest is the behavior of weak acids and bases-those not completely ionized when dissolved in water
- These are ubiquitous in biological systems and play important roles in metabolism and its regulation
- Acids may be defined as proton donors and bases as proton acceptors
- A proton donor and its corresponding proton acceptor make up a conjugate acidbase pair
- Acetic acid (CH3COOH), a proton donor, and the acetate anion ($CH3COO^-$), the corresponding proton acceptor, constitute a conjugate acid-base pair, related by the reversible reaction:
- $CH3COOH \leftrightarrow CH3COO^- + H+$
- The tendency of any acid (HA) to lose a proton and form its conjugate base (A-) is defined by the acid dissociation constant (Ka) for the reversible reaction
- $HA \leftrightarrow H^+ + A^-$ **;**
- Ka = [H+][A-]/[HA]
- Stronger acids, have larger dissociation constants (Ka) i.e they ionize completely
- Weaker acids, have smaller dissociation constants (Ka) i.e. they ionize only partially.

pKa

- analogous to pH, pKa is defined by the equation
- **pKa = log 1/Ka**

  = -logKa
- The stronger the tendency to dissociate a proton, the stronger is the acid and the lower its pKa

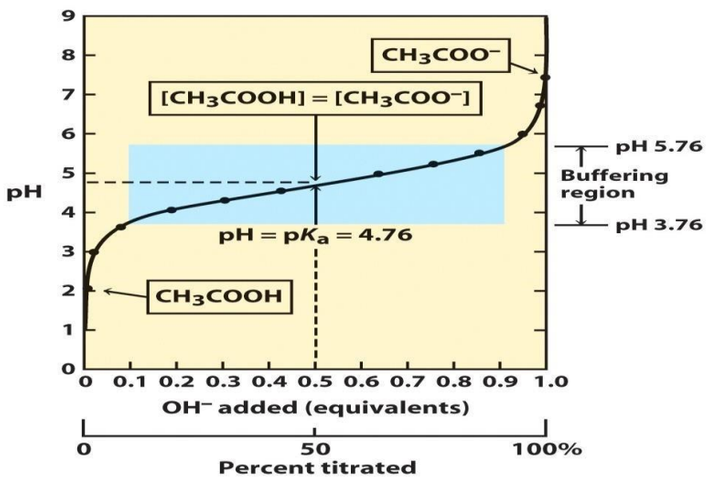
223 Water, PH and buffer systems-working with pKa

Working with pKa

- Titration is used to determine the amount of an acid in a given solution
- A measured volume of the acid is titrated with a solution of a strong base, usually sodium hydroxide (NaOH), of known concentration
- Consider the titration of a 0.1M solution of acetic acid with 0.1M NaOH at 25^0C
- Two reversible equilibria are involved in the process (here, for simplicity, acetic acid is denoted HAc) o H_2O □ $H^+ + OH$

  o HAc □ $H^+ + Ac^-$

- The equilibria must simultaneously conform to their characteristic equilibrium constants, which are, respectively,
- Kw = [H+][OH-] o = 1 x 10^{-14} M2 • Ka = [H+][Ac-]/[HAc] o = 1.74 x 10^{-5} M
- At the beginning of the titration, the acetic acid is only slightly ionized
- As NaOH is gradually added, OH^- combines with the free H^+ in the solution to form H_2O, • As free H+ is removed, HAc dissociates further to satisfy its equilibrium constant



The net result as the titration proceeds is that more and more HAc ionizes, forming Ac-, as the NaOH is added

At the midpoint of the titration, at which exactly 0.5 equivalent of NaOH has been added, onehalf of the original acetic acid has undergone dissociation, so that the concentration of the proton donor, [HAc], now equals that of the proton acceptor, [Ac-]

- At the midpoint a very important relationship holds:
- The pH of the equimolar solution of acetic acid and acetate is exactly equal to the pKa of acetic acid (pKa = 4.76)
- At pKa of a weak acid, half of the acid is in dissociated form
- whereas other half is un-dissociated.
- Increasing the pH will result in an increased dissociation and vice versa.

224 Water, PH and buffer systems-HH equation

The Henderson-Hassebalch (HH) Equation

- The HH equation relates pH, pka, and buffer concentration
- This equation is simply a useful way of restating the expression for the ionization constant of an acid
- For the ionization of a weak acid HA, the HH equation can be derived as follows:
- Ka = [H+][A-]/[HA]
- First solve for $[H^+]$:

  $[H^+] = ka[HA]/[A^-]$
- Then take the negative logarithm of both sides:

  $-\log[H^+] = -\log ka - \log[HA]/[A^-]$
- Substitute pH for -log [H+] and pKa for -log Ka pH = pka – log[HA]/[A-]
- Now invert -log [HA]/[A-],

pH = pka + log[A-]/[HA]

- This equation shows why the pKa of a weak acid is equal to the pH of the solution at the midpoint of its titration
- At that point, [HA]= [A-]
- pH = pka + log[A-]/[HA]
- pH = pka + log 1
- pH = pKa + 0
- pH = pka
- **Calculate the pKa of lactic acid, given that when the concentration of lactic acid is 0.01M and the concentration of lactate is 0.087 M, the pH is 4.80**
- pH= pKa + log [lactate]/[lactic acid]
- **pKa** = pH- log [lactate]/[lactic acid]

  = 4.80 - log 0.087/0.01

  = 4.80 - log 8.7

  = 4.80 - 0.94

  = **3.9**
- **Calculate the ratio of the concentrations of acetate and acetic acid required in a buffer system of pH 5.30 (pKa = 4.76)**
- pH= pKa + log [acetate]/[acetic acid] • log [acetate]/[acetic acid]= pH- pKa

  = 5.30-4.76

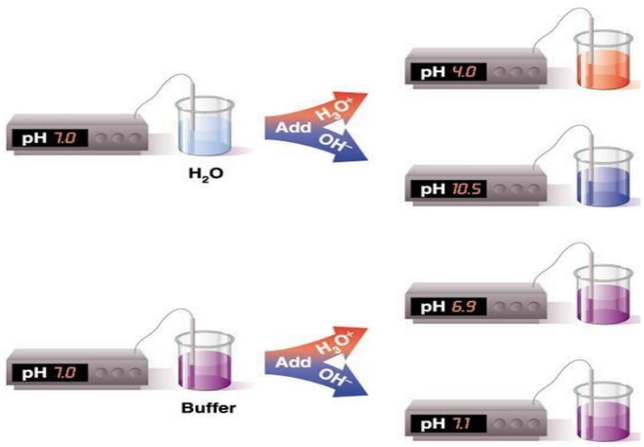
  = 0.54
- [acetate]/[acetic acid] = antilog 0.54

  = **3.5**
- In summary,
- when [HA] = [A^-] ; pH=pKa
- when [HA] > [A^-] ; pH<pKa
- when [HA] < [A^-] ; pH>pKa

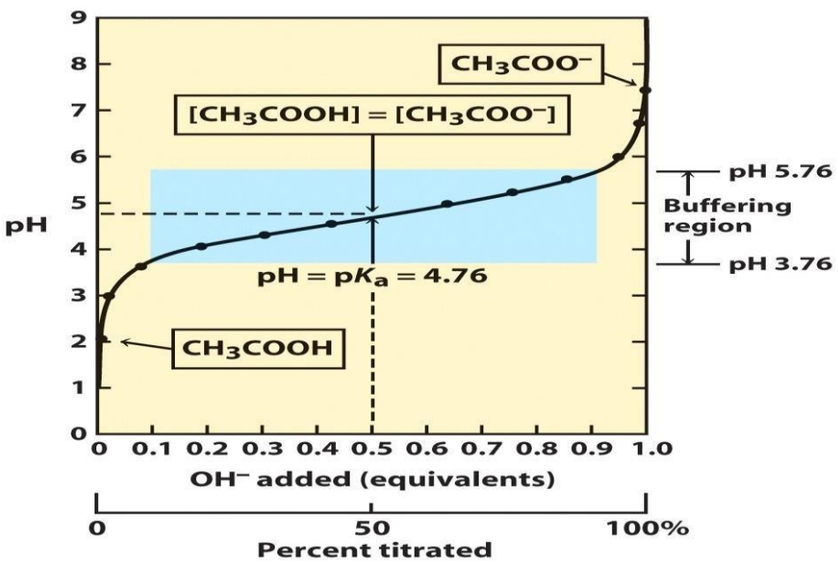
225 Water, PH and buffer systems-buffer solutions

Buffer Solutions

- Buffers are aqueous systems that tend to resist changes in pH when small amounts of acid (H^+) or base (OH^-) are added
- A buffer system consists of a weak acid (the proton donor) and its conjugate base (the proton acceptor)



- As an example, a mixture of ;acetic acid and acetate ion, is a buffer system,



- If more H^+ is added to this solution, it simply shifts the equilibrium to the left, absorbing H^+, so the $[H^+]$ remains unchanged.
- If H^+ is removed (e.g. by adding OH-) then the equilibrium shifts to the right, releasing H^+ to keep the pH constant
- Notice that the titration curve of acetic acid has a relatively flat zone

extending about 1 pH unit on either side of its midpoint pH of 4.76.

- In this zone, a given amount of H+ or OH- added to the system has much less effect on pH than the same amount added outside the zone
- This relatively flat zone is the buffering region of the acetic acid-acetate buffer pair
- At the midpoint of the buffering region, where the concentration of the proton donor (acetic acid) exactly equals that of the proton acceptor (acetate), the buffering power is maximal;
- The pH at this point in the titration curve of acetic acid is equal to its pKa
- The pH of the acetate buffer system does change slightly when a small amount of H^+ or OH^- is added, but this pH change is very small
- compared with the pH change that would result if the same amount of H+ or OH- were added to pure water.
- Each conjugate acid-base pair has a characteristic pH zone in which it is an effective buffer
- For example, the $H_2PO_4^-/HPO_4^{2-}$ pair has a pKa of 6.86 and thus can serve as an effective buffer system between approximately pH 5.9 and pH 7.9
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