D.K.M.COLLEGE FOR WOMEN (AUTONOMOUS), VELLORE

eRESOURCES

Digital Learning

E CONTENT TITLE : BIO ORGANIC CHEMISTRY

DEPARTMENT **: BIO-CHEMISTRY**

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UNIT 1

CARBOHYDRATE

INTRODUCTION

Carbohydrates are important macromolecules that consist of carbon, hydrogen, and oxygen. They are organic compounds organized in the form of aldehydes or ketones with multiple hydroxyl groups coming off the carbon chain.

They are also known as "Saccharides" which is a derivation of the Greek word "Sakcharon" meaning sugar.

The definition of carbohydrates in chemistry is as follows:

"Optically active polyhydroxy aldehydes or polyhydroxy ketones or substances which give these on hydrolysis are termed as carbohydrates".

Carbohydrates are the most abundant organic compounds in living organisms and account for one of the four major biomolecular classes including proteins, lipids, and nucleic acids.

Some of the most common carbohydrates that we come across in our daily lives are in form of sugars. These sugars can be in form of Glucose, Sucrose, Fructose, Cellulose, Maltose etc.

The general formula for carbohydrate is $C_x (H_2O)_y$.

Carbohydrates exist in a variety of isomer forms. Those that differ in arrangements of atoms are known as constitutional isomers, such as glyceradehyde and dihydroxyacetone. Stereoisomers have the same attachments of the atoms, but different in spatial arrangements, which can be further separated into two types: diastereoisomers and enantiomers. Diastereoisomers are the molecules that are not mirror images of each other and enantiomers exists as nonsuperimposable mirror images. The fact that

monosacharides can possess up to three different asymmetric carbon atoms contributes significantly to the wide variety of isomer formations.

- Carbohydrate are among the most widely distributed compounds in both plant and animal kingdoms.

- Plants can build up carbohydrates from carbon dioxide by photosynthesis.

- Many plants and animals contain large quantities of carbohydrates as reserve food material.

- Carbohydrates are also important structural compounds.

- Functional groups present include hydroxyl groups

Classification of Carbohydrates:

The main classification of carbohydrate is done on the basis of hydrolysis. This classification is as follow:

1. **Monosaccharides:** These are the simplest form of carbohydrate that cannot be hydrolyzed any further. They have the general formula of $(CH_2O)n$. Some common examples are glucose, Ribose etc.
   Carbohydrates exist in a variety of isomer forms. Those that differ in arrangements of atoms are known as constitutional isomers, such as glyceradehyde and dihydroxyacetone. Stereoisomers have the same attachments of the atoms, but different in spatial arrangements, which can be further separated into two types: diastereoisomers and enantiomers. Diastereoisomers are the molecules that are not mirror images of each other and enantiomers exists as nonsuperimposable mirror images. The fact that monosacharides can possess up to three different asymmetric carbon atoms contributes significantly to the wide variety of isomer formations.

2. **Oligosaccharides**: Carbohydrates that on hydrolysis yield two to ten smaller units or monosaccharides are oligosaccharides. They are a large catEx ory and further divides into various subcatEx ories.

- **Disaccharides:** A further classification of oligosaccharides, these give two units of the same or different monosaccharides on hydrolysis. For example, sucrose on hydrolysis gives one molecule of glucose and fructose each. Whereas maltose on hydrolysis gives two molecules of only glucose,

- **Trisaccharides:** Carbohydrates that on hydrolysis gives three molecules of monosaccharides, whether same or different. An example is Raffinose.

- **Tetrasaccharides:** And as the name suggests this carbohydrate on hydrolysis give four molecules of monosaccharides. Stachyose is an example.

3. **Polysaccharides:** The final catEx ory of carbohydrates. These give a large number of monosaccharides when they undergo hydrolysis, These carbohydrates are not sweet in taste and are also known as non-sugars. Some common examples are starch, glycogen etc.

Function of Carbohydrates

Carbohydrates play a variety of extensive roles in all forms of life:

* Serve as energy stores, fuels, and metabolic intermediates.
* Formation of the structural framework of RNA and DNA (ribonucleic acid and deoxyribonucleic acid).

* Are structural elements in the cell walls of bacteria (peptidoglycan or murein), plants (cellulose) and animals (chitin).

* They are linked to many proteins and lipids. Such linked carbohydrates are important in cell-cell communication and in interactions between cells and other elements in the cellular environment.

* As "food" for energy supply (starch, glycogen, dextrans) and production of fats.

Monosaccharides

Monosaccharides $(C-H_2O)n$, n > 3, are the simplest form of carbohydrates. They may be subcatEx orized as aldoses or ketoses, if the molecule contains an aldehyde or ketone functional groups respectively.

Aldoses have a carbonyl group in the form of an aldehyde on the end of the carbon chain and ketoses have a ketone group somewhere along the sugar backbone. The simplest ketose is dihydroxyacetone (has no chiral centers) while the simplest aldose is glyceraldehyde, which can be found as either the L or D enantiomer.

Monosaccharides may be further classified based on the number of carbon atoms as triose (n=3), tetrose (n=4), pentose (n=5), hexose (n=6), heptose (n=7), etc. For example, glucose is known as a aldohexose (a six carbon aldose) and ribose is considered an aldopentose (a five carbon aldose).

Sugars have the ability to form cyclic compounds, typically five or six member heterocyclic rings.

In addition, sugars can be reducing or non-reducing depending on whether a sugar has a carbonyl moiety in its linear form. This means that the sugar in cyclic form is either in hemiacetal or hemiketal form.

Monosaccharides aid in the development of nucleic acids. They are often represented by a Fischer Projection. L and D represent the absolute configuration of the asymmetric carbon farthest away from the ketone or aldehyde group on the monosaccharide. Biological carbohydrate is D sugar mostly. Note that the D and L designations are not correlated with the D and L designations for specific rotation (dextro and levorotary molecules).

In addition, each carbon atom bonded to a hydroxyl group except for the first and last ones is a stereocenter of R or S, potentially forming many isomers for the same chemical formula of saccharide. In cyclic form, sugars can be described using Haworth projections.

Monosaccharides are classified as α or β anomers in their cyclic forms where the carbon atom of the carbonyl oxygen is called the anomeric carbon atom. Because the carbonyl carbon is sp2 hybridized in the open chain form, when the sugar cyclizes, the carbonyl carbon forms a new chiral center; the α anomer of glucose has the hydroxyl group attached to anomeric carbon in a trans position relative to heteroatom oxygen.

The β anomer occurs when the hydroxyl group is on the same side or in cis position as the heteroatom oxygen. Both anomers are in equilibrium and are constantly changing between the ring and straight-chain conformations.

Anomeric carbon is defined as hemiacetal or hemiketal carbon for which results in different forms of stereoisomer for the saccharide. The anomeric position is easily oxidized and can form glycosidic linkages readily.

Hemiacetals and hemiketals are formed under acidic conditions when an aldehyde or ketone reacts with an alcohol, respectively. The acidic condition protonates the carbonyl carbon on the aldehyde or ketone, followed by a nucleophilic attack of the hydroxyl group on the alcohol on the carbonyl carbon (electrophile).

In monosaccharides, their physical structure can exist either as an open chain or a cyclized ring. The ring structure is more energetically stable and is more common in the case of glucose, fructose and ribose.

There are two possible ring formations for these sugars, known as pyranose and furanose formations. Pyranose rings are formed from aldoses (aldehyde sugars) and furanose rings are formed from ketoses (ketone sugars).

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Pyranoses are 6 membered rings that resemble the structure of a pyran, whereas furanoses are 5 membered rings that resemble a furan. In hexoses, for example, the C-5 hydroxyl group acts as the alcohol (nucleophile) that attacks the C-1 aldehyde carbonyl carbon (electrophile). This results in an intramolecular hemiacetal at the C-1 position.

To form a intramolecular hemiaketal, the C-5 hydroxyl group of a ketohexose attacks the C-2 keto group to form the 5 membered ring, furanose.

It is possible for pentoses and hexoses to cyclize. In fact, many 5- or 6- carbon sugars, such as glucose, ribose, and fructose, exist predominantly as cyclic molecules.

The carbonyl group in ketoses and aldoses reacts with an alcohol group within the same sugar molecule. This intramolecular reaction produces either an intramolecular hemiacetal or hemiketal. If the resulting cyclic sugar is a 5-membered ring, it is a furanose. If the resulting cyclic sugar is a 6-membered ring, it is a pyranose. Only the 5-membered and the 6-membered ring can be formed because they are the only types of ring formations that are energetically favored.
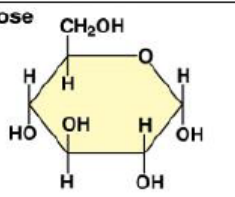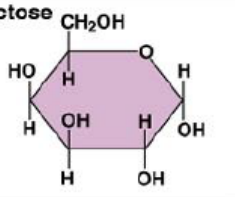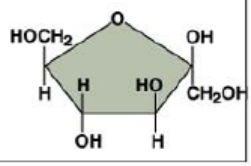
It is important to note that when sugar undergoes a cyclic transformation, an anomeric carbon is formed in the process.
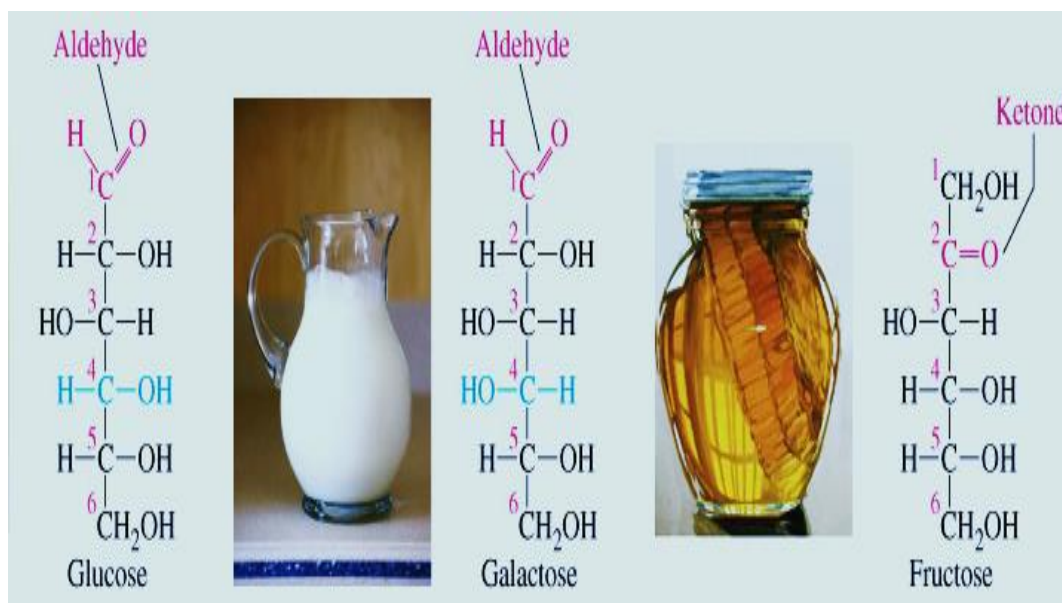
An anomeric carbon is the hemiacetal or hemiketal carbon where there can be two different configurations in cyclic sugar. It can either be α or β depending on the relative position of the -CH$_2$OH group and the -OH group on the anomeric carbon. α refers to the two groups being on opposite sides, and β refers to the two group being on the same side.

The cyclic sugar has conformational isomers. When carbon 2 is out of the plane it is called C-2 endo. Also it has chair and boat conformations. Due to steric hindrance in boat form, chair form is more favorable.

Overall the sugar has many stereochemistry such as enantiomers (D and L), Diasteromers, Anomers (alpha and beta) and molecules with same stereochemical configuration but differ in 3D conformation (chair and boat).
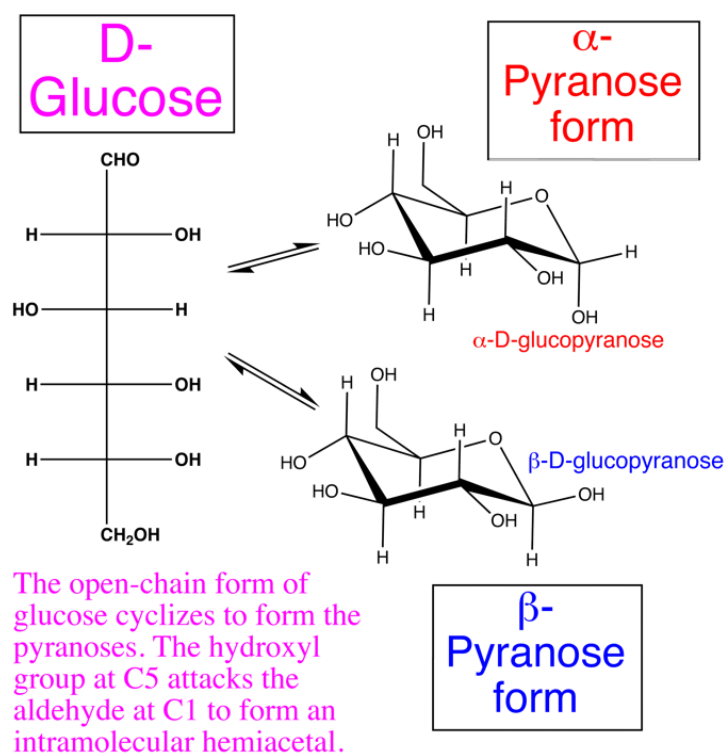
Structure of monosaccharides

Monosaccharides   Hexoses

Glucose
- The essential energy source for all body functions
- Other names: Dextrose and Blood Sugar.
- A component of each disaccharide.

- **Galactose**
  - Seldom occurs freely in nature
  - Binds with glucose to form sugar in milk: lactose.
  - Once absorbed by the body, galactose is converted to glucose to provide energy.

Fructose:
- The sweetest of all sugars
  - (1.5 X sweeter than sucrose)
- Occurs naturally in fruits and honey "the fruit sugar"

D-glucose "dextrose" Blood sugar	D-galactose	D-fructose "Levulose" Fruit sugar

Aldehyde — Glucose, Aldehyde — Galactose, Ketone — Fructose

Structure of monosaccharides can be drawn either as

1. The **Fisher Projection**, which is in straight chain perspective,

The Fischer projection of D-glucose is shown below.

D-Glucose

α-Pyranose form

α-D-glucopyranose

β-D-glucopyranose

β-Pyranose form

The open-chain form of glucose cyclizes to form the pyranoses. The hydroxyl group at C5 attacks the aldehyde at C1 to form an intramolecular hemiacetal.

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2. The **Haworth Projection**, which displays molecules as cyclic or planar with substitutes above or below the ring.

The Haworth projection of α-D-glucopyranose is shown below.

HO
6
5
O
4
OH
1
2
HO
3
OH
OH
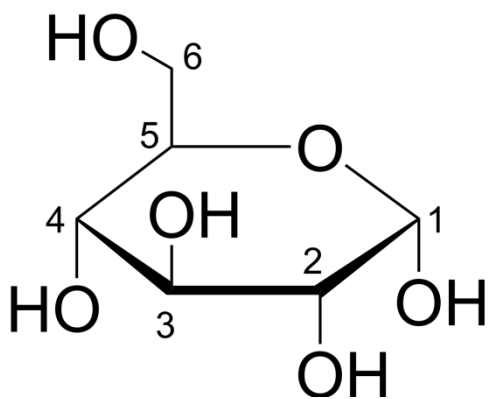
Example of Haworth Projection: Glucose

3. In hetercyclic compounds, five-membered and six-membered rings are favored over others due less steric strain of the cyclic structure. Thus, **furanoses** (pentoses) and **pyranoses** (hexoses) rings are the predominant cyclic structures in monosaccharides.

Straight Chain D Allose

ALLO furanose

ALLO pyranose
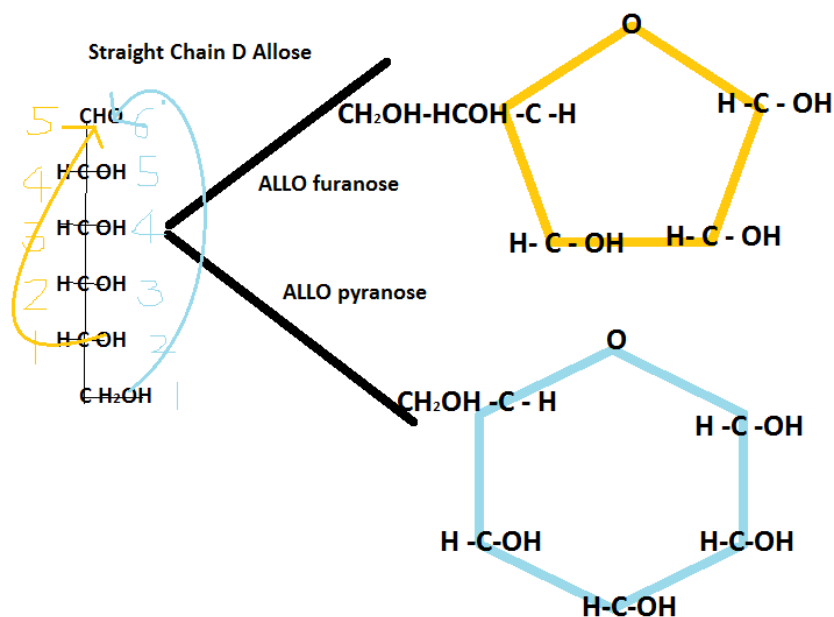
The rings of monosaccarides resemble the molecules furan and pyran, thus the names furanoses and pyranoses.

Example (pyran is left ring and furan is right ring):
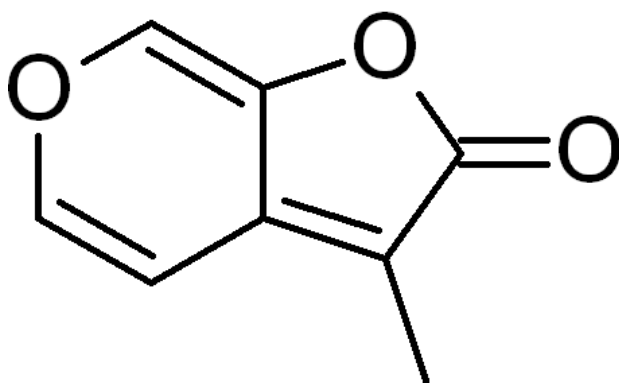
Pyran (left) and Furan (right)

Of straight-chain monosaccharide, aldehyde or ketone group react with other hydroxyl group, for which oxygen is bonded between the two carbon atom, to form a **hemiacetal** or **hemiketal**, which forms a heterocyclic ring; the five-atom furanose and six-atom pyranose forms are in equilibrium with their straight-chain structures. The pyranose ring can take on different

conformations. Either of the chair or boat form. The chair form allows for substituents to be in two positions, either axial or equatorial.

Substituents in the axial position create steric hindrance, thus larger substituents normally are in the equatorial position, due to less crowding. The boat form is disfavored due to steric hindrances. The furanose ring can take on the form of an envelope. It has four nearly coplanar atoms with a puckered fifth atom.

Types of Monosaccharides

Monosaccharides
Classification by Carbon Atoms

	Sugar	Structure formula	Aldoses	Ketoses
1.	Triose	$C_3H_6O_3$	Glyceraldehydes	Dehydroxy acetone
2.	Tetroses	$C_4H_8O_4$	Erythrose, Threose	Erthrulose
3.	Pentoses	$C_5H_{10}O_5$	Xylose Ribose Arabinose	Ribulose
4.	Hexoses	$C_6H_{12}O_6$	Glucose Galactose Mannose	Fructose

Trioses (n=3 where n refers to the number of carbons that make up the skeleton of the sugar) are the smallest forms of monosaccharides: Dihydroxyacetone (a ketose), Glyceraldehyde (an aldose)

Examples of monosaccharides:

- **Glucose** (the most common sugar)-Glucose is Glucose is a polyhydroxyaldehyde. Emil Fischer, a carbohydrate chemist received
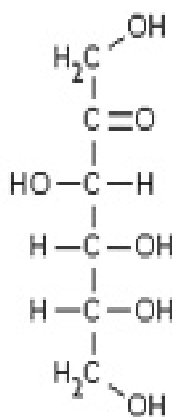
the Nobel Prize for his proof of the structure of glucose.

- **Fructose** (sweetens fruit)- Fructose is a polyhydroxyketone.
- **Galactose** (sugar in milk)-Galactose is the C4 epimer of glucose. Like glucose, galactose mutarotates when it dissolves in water.
- **Ribose** (component of RNA)-The C2 epimer of arabinose, is most stable in its furanose form. Each ribose unit carries a heterocyclic base that provides part of the information needed to specify a particular amino acid in protein synthesis.
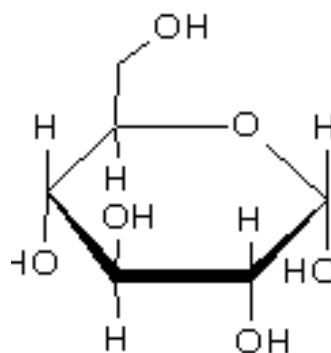
Deoxyribose (component of DNA)-The principal difference between RNA and DNA is the presence of o-2-deoxyribose as the sugar in DNA instead of the o-ribose found in RNA.
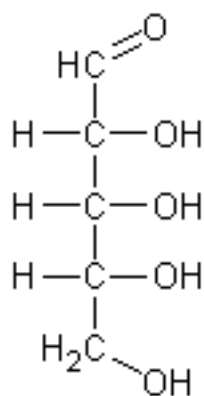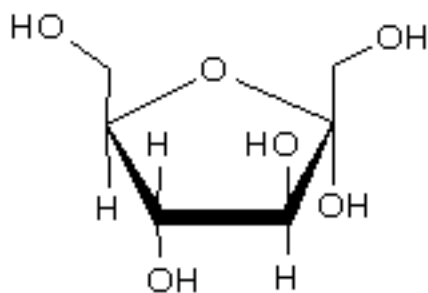
D-Ribose (aldopentose) D-Fructose (ketohexose) α-D-Glucopyranose

α-D-Fructofuranose

Disaccharides

Disaccharides, or chemical formula of $C_n (H_2O)_{n-1}$, n > 5, are namely two

monosaccharides linked by condensation or dehydration synthesis. As a result, a **glycosidic bond** is formed when the -OH of one sugar molecule joins with that of another sugar molecule. The glycosidic bond can be broken by hydrolysis, which is the addition of the water molecule, to form two monosaccharides.

Examples of Disaccharides:

- **Lactose** (sugar in milk) - 1 galactose + 1 glucose

Lactose consists of galactose bonded to glucose by a β-1,4-glycosidic linkage. Lactase is the enzyme in humans that hydrolyzes lactose back to its two monosaccharides.

OCCURRENCE:

Lactose (or) milk sugar occurs in the milk of mammals and is synthesized in the mammary gland.

Human milk contains 6% of lactose and cow's milk contains 4.5% of lactose.

HYDROLYSIS

Lactose on hydrolysis yields equimolar mixture of α-D-glucose and β-D- galactose in the presence of enzymes lactose.

Lactose is present in intestinal juice.

PROPERTIES:

➢ Lactose is less soluble in water and much less sweet than sucrose.

➢ It is dextrorotatory.

Lactose is a reducing sugar because the aldehyde group of C_1 of glucose is free. Since, it is a reducing sugar, it can reduce Fehling's solution , Benedict's reagent and Tollen's reagent.

➢ It can react with phenyl hydrazine to form ozasone.

➢ It undergoes mutarotation.

➢ When lactose is methylated it gives methyl hepta lactoside.

➢ Lactose reacts with acetic anhydride in the presence of sodium acetate to form lactose octa acetate.

➢ When lactose is oxidized with bromine water the free sugar group is oxidized to a carbonyl giving lactoboionic acid.

➢ Hydrolysis of lactobionic acid forms gluconic acid and galactose showing that the sugar group of the glucose unit in lactose is free, were that of the galactose is combined. Lactose is thus a glucose galactoside.

➢ Souring of milk takes place when bacteria found in milk convert lactose into lactic acid.

BIOLOGICAL IMPORTANCE

➢   Lactose present in breast milk is a good source of energy for the new born babies.

➢   Lactose is fermented by coliform bacilli (non-pathogenic) and not by bacillus (pathogenic). This test is used to differentiate pathogenic and non-pathogenic bacteria.

➢   If lactose is not hydrolyzed in the gut due to enzyme deficiency, then it may lead to diarrhoea.

➢ In lactating mother, lactose may appear in urine and that condition is known as lactosuria.

Lactose:

It is formed of β-galactose and α-glucose linked by β-1,4-glucosidic linkage

Contain free anomeric carbon so reducing sugar

It may appear in urine in late pregnancy and during lactation.

β-Galactose   Glucose

α− and β−Lactose
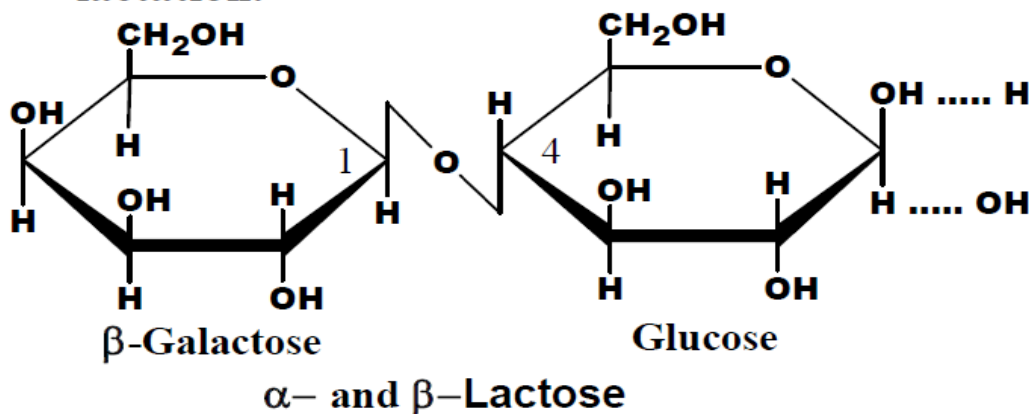
- **Maltose** (malt sugar) - 1 glucose + 1 glucose

Maltose is made from two glucose molecules bonded by a α-1,4-glycosidic linkage. Maltase is the enzyme that hydrolyzes maltose.

Maltose (malt sugar):

It consists of 2 α-glucose units linked by α-1,4-glucosidic linkage,

Contain free anomeric carbon so reducing sugar.

α-Glucose

Glucose

α− and β−Maltose
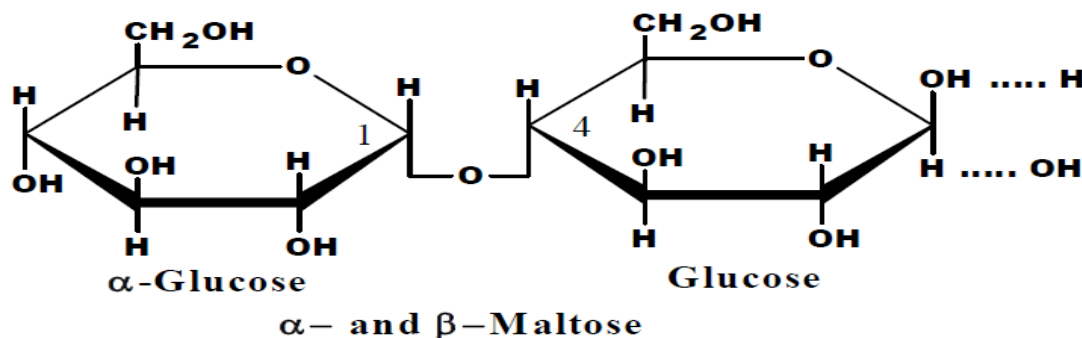
- **Sucrose** (table sugar)- 1 glucose + 1 fructose

Sucrose is formed from a glucose and a fructose. They form a glycosidic linkage between the glucose and β fructose. Since they are linked at their anomeric carbons, sucrose is not a reducing sugar, because either glucose or fructose can be changed to a aldehyde or ketone.

Sucrose

- α D-glucopyranose and β D fructofuranose by α 1- 2 glycosidic bond
- No free aldehyde or keton gp so non reducing sugar
- hydrolysed to glucose and fructose by sucrase (invertase) enzyme.
- * Sucrose is dextrorotatory +66.5.

α-Glucose

β-Fructose
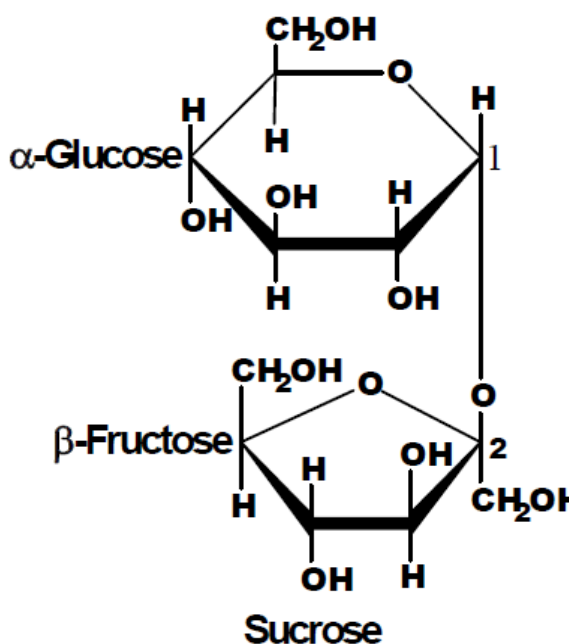
Sucrose

OCCURRENCE

Sucrose is the common sugar of commerce and kitchen and its widely distributed in all photosynthetic plants. It is the chief constituent of sugar cane, beet and maple and is also present in pineapple and carrot.

Sucrose occurs in varying amounts in different plants organs such as fruits, seeds, flowers and root. Nectar of flowers is particularly rich in sucrose.

It is probably the only foodstuff used in the crystalline form.

HYDROLYSIS:

Sucrose on hydrolysis gives equimolar mixture of glucose and fructose in the presence of enzyme called sucrose, which is present in the intestinal juice. Sucrose is hydrolyzed in the intestine and then it is absorbed by our blood.

Sucrose → (Invertase) α-D-glucose+ β-D-fructose

$C_{12}H_{22}O_{11} + H_2O \rightarrow$ (Invertase) $C_6H_{12}O_6 + C_6H_{12}O_6$
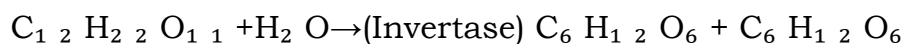
PROPERTIES

Sucrose is a colorless crystalline form, soluble in water and more sweeter than glucose.

- ✓ Sucrose is a non –reducing sugar, it dose not reduce Fehling' s solution, Benedict's reagent and tollen's reagent.

- ✓ It does not react with phenyl hydrazine to form osazone.

- ✓ It does not undergo mutarotation

INVERSION OF SUGAR:

Sucrose is dextrorotatory before hydrolysis but after hydrolysis it is laevorotatory because the laevorotation of fructose is more than the dextrorotation of glucose.

$C_{12}H_{22}O_{11} \rightarrow$ (Invertase) $C_6H_{12}O_6 + C_6H_{12}O_6$

Sucrose   glucose   fructose

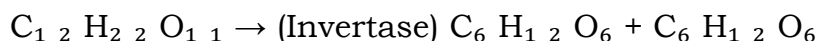This reaction is known as inversion of sugar because the dextrorotatory sugar is converted to laevorotatory due to hydrolysis. The mixture of glucose and fructose is called as invert sugar.

The mixture formed after hydrolysis reduces Fehling's solution, Benedict's reagent and tollen's reagent. Osazone is also formed after hydrolysis. Sucrose on acetylation react with acetic anhydride and gives octa- acetyl sucrose.

On methylation sucrose forms octamethyl sucrose.

Sucrose is fermented by yeast to gives ethyl alcohol and carbon dioxide.

BIOLOGICAL IMPORTANCE:

• Sucrose is a major intermediate product of photosynthesis.

• Sucrose is more sweeter than all the disaccharides and also than glucose.

• Invert sugar is more sweeter than sucrose. So that bakers and candy makers, hydrolyse sucrose before they use as sweeteners.

• Honey contains large proportion of invert sugar, so it is sweeter than sucrose.

When sucrose as heated it loses water and forms a brown substance called barley sugar (or) caramel.

- Sugar like glucose, fructose, galactose, sucrose, maltose, lactose are sweeteners – a part from this there are artificial sweeteners.

- Artificial sweeteners have been developed especially for diabetic patients. So, this artificial sweeteners stimulated the taste buds of the tongue that are stimulated by sugars, but these sweeteners have no nutritional value.

- Example for artificial sweeteners

  1. Saccharin

  2. Sodium cyclamate

  3. Monellin

Glycosidic bond

In general, a **glycosidic bond** is the covalent bond between two monosaccharides to form a disaccharide. It is formed between the hemiacetal group of anomeric carbon of a carbohydrate in acid by a condensation reaction to a hydroxyl group of an organic compound. The condensation reaction causes the formation of one additional water molecule. This results in the formation of an acetal group on the anomeric carbon, which stabilizes the bond.

Glycosidic bonds are notably stable, but they can be broken chemically by strong aqueous acids. There are two types of glycosidic bonds, α and β glycosidic bonds. A substance with a glycosidic bond is called a glycoside.

For saccharides; if they have anomeric carbons, they can form glycosidic bonds to form cyclic structures and not linear forms. The chemical bond is accounted as O-glycosidic bonds (type II linkage), for which, the anomeric carbon is bonded by oxygen to other group.

O-glycosidic bond creates a covalent linkage between a serine or threonine hydroxyl side chain amide and sugar.

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N-glycosidic bond, type I linkage, is a type of carbohydrate-protein covalent linkage between an asparagine side chain amide and a sugar. N-glycosidic bond involves the bonding of the anomeric carbon of a sugar and the nitrogen atom of an amine group.

In the formation of a glycosidic bond, the hemiacetal group of carbohydrates (which contains the anomeric carbon) forms the glycosidic bond in the presence of an acid. Fischer glycosidation is a result from this condensation, which releases a water from the previous reaction.

Though Glycosidic bonds are relatively stable under most conditions, they are easily broken under many aqueous acidic conditions. There are enzymes called **glycoside hydrolases** that also break the bonds, but they are only able to break either the alpha or beta conformation, not both.

The following image shows an example of a reaction that generates glycosidic bonds.
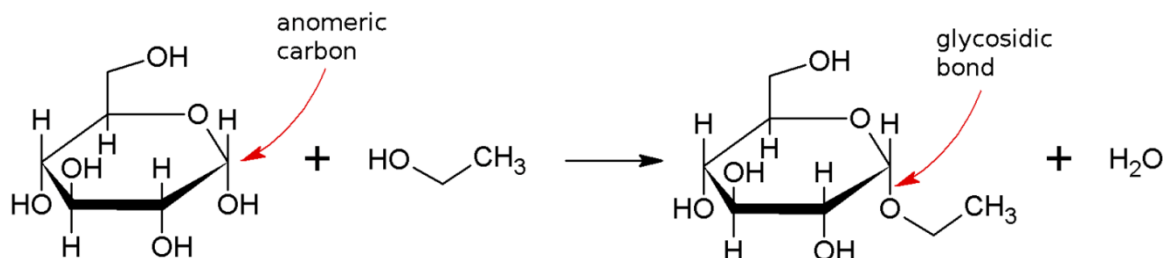
The stereochemistry of the bond is based on the position of the stereocenter furthest away from the C1. The alpha conformation of the glycosidic bond creates an axial conformation, whereas the beta conformation develops an equatorial conformation.

Oligosaccharides

Oligosaccharides are polysaccharides with a smaller number of sugar molecules; disaccharides bonded to simpler polysaccharides, for which, n=3 or 4 simple sugar units. Since almost all the secreted and membrane-

associated proteins of eukaryotic cells are glycosylated, Oligosaccharides are covalently attached to proteins by either N-glycosidic or O-glycosidic bonds.

Oligosaccharides

These are *compound sugars* that yield 2 to 10 molecules of the same or different monosaccharides on hydrolysis. Accordingly, an oligosaccharide yielding 2 molecules of monosaccharide on hydrolysis is designated as a disaccharide, and the one yielding 3 molecules of monosaccharide as a trisaccharide and so on.

Disaccharides – Sucrose, Lactose, Maltose, Cellobiose, Trehalose, Gentiobiose, Melibiose
Trisaccharides – Rhamninose, Gentianose, Raffinose (= Melitose), Rabinose, Melezitose
Tetrasaccharides – Stachyose, Scorodose
Pentasaccharide – Verbascose
The molecular composition of the 3 legume oligosaccharides (viz., raffinose, stachyose and verbascose) is shown below :
α-Galactose (1–6) α-Glucose (1–2) β-Fructose Raffinose
α-Galactose (1–6) α-Galactose (1–6) α-Glucose (1–2) β-Fructose Stachyose
α-Galactose (1–6) α-Galactose (1–6) α-Galactose (1–6) α-Glucose (1–2) β-Fructose Verbascose

$$Maltose + H_2O \xrightarrow{H^+ \text{ or maltase}} glucose + glucose$$

$$Lactose + H_2O \xrightarrow{H^+ \text{ or lactase}} glucose + galactose$$

There are two main types of Oligosaccharides:

• **N-linked Oligosaccharide**: In N-linked oligosaccharides, GlcNAc is variably β-linked to the amide N of an Asn residue in the sequence Asn-X-Thr or Asn-X-Ser, where X is amino acid except possibly Pro or Asp. There is enormous diversity among the oligosaccharides of N-linked glycoproteins. In some glycoproteins, processing if brief, leaving "high-mannose" oligosaccharides. In some other glycoproteins, extensive processing generates large oligosaccharides containing several kinds of sugar residues. N linked oligosaccharides have a common pentasaccharide core which contains 3 mannose and 2 N acetylglucosamine residue.

Proteins containing N-linked oligosaccharides typically are glycosylated and then processed as elucidated, in large part:

1. An oligosacchride containing 9 mannose residues, 3 glucose residues, and 2 GlcNAc residued is attached to the Asn of a growing polypeptide chain that is synthesized by a ribosome associated with the endoplasmic reticulum.

2. Some of the sugars are removed during the process, which bEx ins in the lumen of the endoplasmic reticulum and continues in the Golgi apparatus.

3. Additional monosaccharide residues, including GlcNAc, galactose, fucose, and sialic acid are added by the action of specific glycosyl-transferases in the Golgi apparatus.
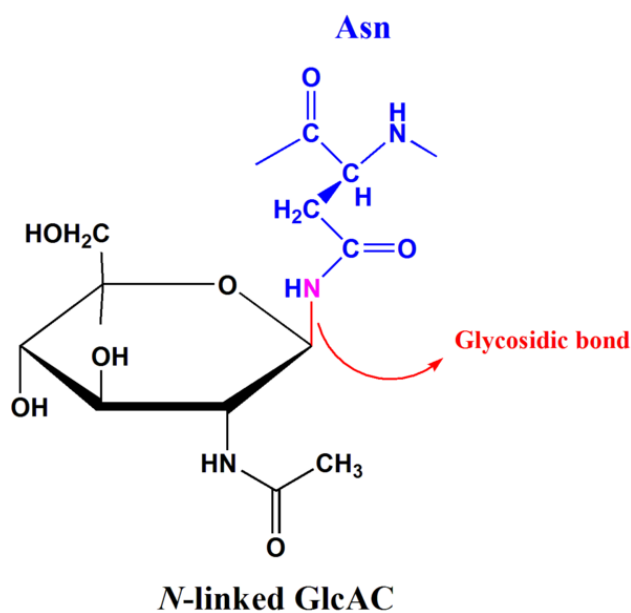
N-linked GlcAC

N-linked Glycosidic Bond

O-linked Oligosaccharide: The most common O-glycosidic attachment involves the disaccharide core β-galactosyl-(1->3)-α-N-acetylgalactosamine linked to the OH group of either Ser or Thr. O-linked Oligosaccharides are synthesized in the Golgi apparatus by the serial addition of monnosaccharide units to a completed polypeptide chain. Synthesis starts

with the transfer of GalNAc to a Ser and Thr residue on the polypeptide. N-linked oligosaccharides are transferred to an Asn in a specific amino acid sequence, but O-glycosulated Ser and Thr residues are not members of any common sequence. Instead, the locations of glycosylation sites are specified only by the secondary or tertiary structure of the polypeptide. O-glycosylation continues with stepwise addition of sugars by the corresponding glycosyltransferases.

Polysaccharides

Polysaccharides (also called Glycans), the chemical formula is written as $Cn(H_2O)n-1$, for which $200 < n < 2500$, or $(C_6H_{10}O_5)n$, $40<n<3000$, are polymers or multiple units of monosaccharides.

They are constructed by glycosidic bonds and make ideal storage products due to their structure and solubility.

Polysaccharides can be further classified into homo-polysaccharides and hetero-polysaccharides based on the composition of individual monosaccharides.

In addition, branched and unbranched polysaccharides are formed by glycosidic bonds on different Carbons of the monosaccharides.

Homo-polysaccharides are composed of one type of monosaccharides. They can be branched or unbranched.

Hetero-polysaccharides can be composed of two or more types of monosaccharides in straight or branched chains. Example of Homopolysaccharides: 1) some are storage of energy (starch, glycogen, and dextrans). 2) Others have structural roles (cellulose, chitin).

Examples of polysaccharides:

- **Cellulose**

Cellulose [$(C_6H_{10}O_5)n$] is a homo-polysaccharide that serves a significant structural role in plant cell walls. Cotton fibers and the wood of trees and bushes, for instance, are largely composed of cellulose.

Glucose residues in cellulose are linked by β-1,4-glycosidic bonds in a linear fashion. Intermolecular and intramolecular hydrogen bonds allow the more hydrophobic part of cellulose to stack, leading to complete insolubility of cellulose in normal aqueous solution.
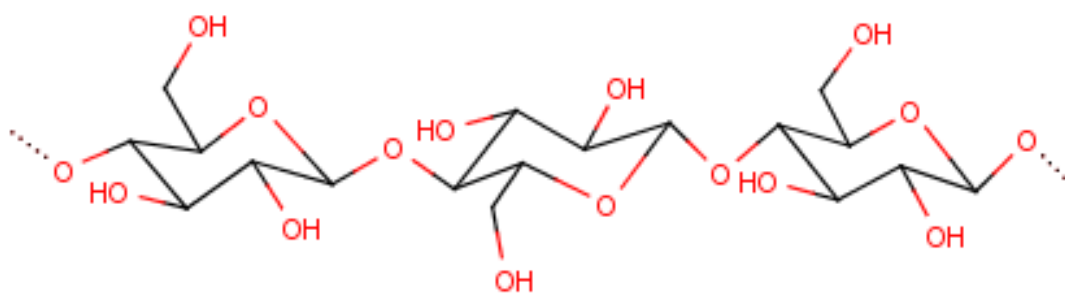
Cellulose (beta-1,4 linkages)

- **Starch**

25
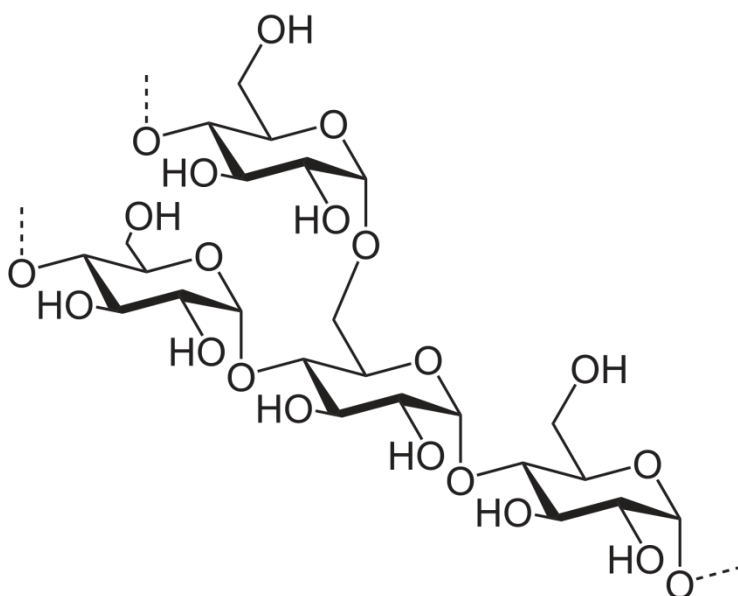
Starch is a polymer of glucose, and is constructed by glycopyranose units that are bonded by α-glycosidic bonds.

Starch is a homo-polysaccharide that serves as a major source of energy storage in plants and also nourishment to most animals, including humans.
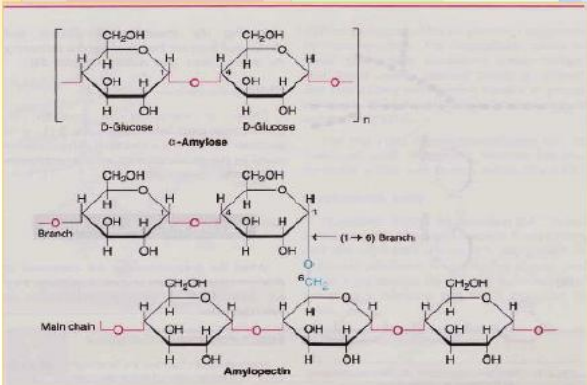
OCCURRENCE:

It is the most important reserve food material of the higher plants and is found in cereals, lEx umes, potatoes and other vEx etables.

1. Amylose:

is in the form of straight chain linked together with α- 1-4, linkages indicating 300 – 5,500 glucose units per molecules, molecular wt range from 105 to 106. Generally it is water soluble and gives blue colour with iodine. Amylopectins: It contain beside straight chain several branched chains, which are arranged in α—1-4 and β-1-6 linkage units, one molecule of amylopectin contains 50,000 to 5,00,000 glucose molecules, molecular wt. range from 107 to 108, it is insoluble in water and gives purple colour with iodine.

Fig. 8–1. Structure of amylose (= A-fraction)

Fig. 8–2. Structure of amylopectin (= B-fraction)
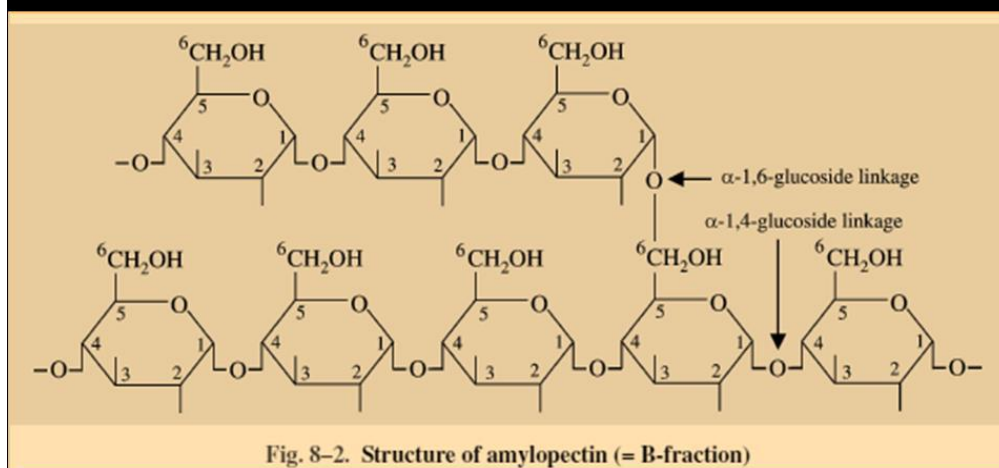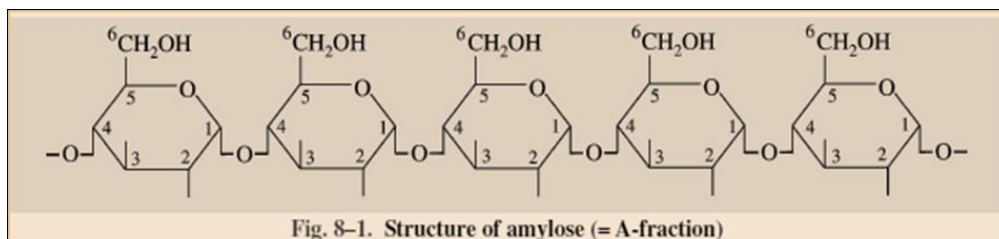
2. AMYLOPCETIN:

- It is insoluble in water.

- It differs from amylose is that they are larger molecules. It constituents about 80%

- They have molecular weight as 20,000 or more.

- This molecule are highly branched.

- They have both α(1,4) and α(1,6) glycosidic linkage. It gives reddish violet colour with iodine.

HYDROLYSIS:

Starch can be hydrolyzed by both acid as well as enzymes. It is hydrolyzed to glucose in which the intermediate product is maltose. Starch on partial hydrolysis, dextrin is formed which is used in infants food preparations, since it is easily digestible.

Starch →(acid hydrolysis) glucose.

Starch →(enzymatic hydrolysis /amylase) glucose.

Starch → (Incomplete hydrolysis) Dextrin.

Amylose has linear complex. So, it occupies more area and it forms starch iodine complex. Since it is able to accommodate more iodine molecules starch gives blue colour with iodine.

PROPERTIES:

➢ It is a white soft amorphous powder and lacks sweetness.

➢ It is insoluble in water, alcohol and either at ordinary temperature.

➢ Starch is a non- reducing sugar, it reduces Fehling's, Benedict's reagent only after hydrolysis.

➢ A compound which yields only glucose molecules on hydrolysis is called a glucosan. Hence, starch is a glucosan.

➢ Partial hydrolysis of starch gives dextrin, it posses adhesive property and it is used as adhesive of paper product.

BIOLOGICAL SIGNIFICANCE:

➢ Starch is used as a food more than half of the carbohydrates ingested by human is starch.

➢ It is used in coating and sizing papers in order to improve the writing qualities.

➢ It is used in the manufacture of glucose. Dextrin is used for feeding infants, since it is easily digestible.

➢ It is a major storage from carbohydrate in plants, synthesis and storage occurs in the chloroplast.

➢ It is used in the manufacture of starch nitrate, which is used as an explosive.

The branching chains of Amylopectin are α-1, 6-linkages. The branching in Amylopectin is less than in glycogen.

These branching occur about once for every thirty α-1,4-linkages. The polymer of glucose is not soluble in water.

CELLULOSE

OCCURRENCE:

It is a homopolysaccharide found in the cell wall of plants.

It is also found in a few micro- organisms and lower organisms.

It is also found in cotton (90-94%), woods (45-50%) and jute (60-65%)

It forms the major part of vEx etables.

STRUCTURE:

FIGURE 13.6 The polysaccharide cellulose is composed of glucose units connected by β-1,4-glycosidic bonds.
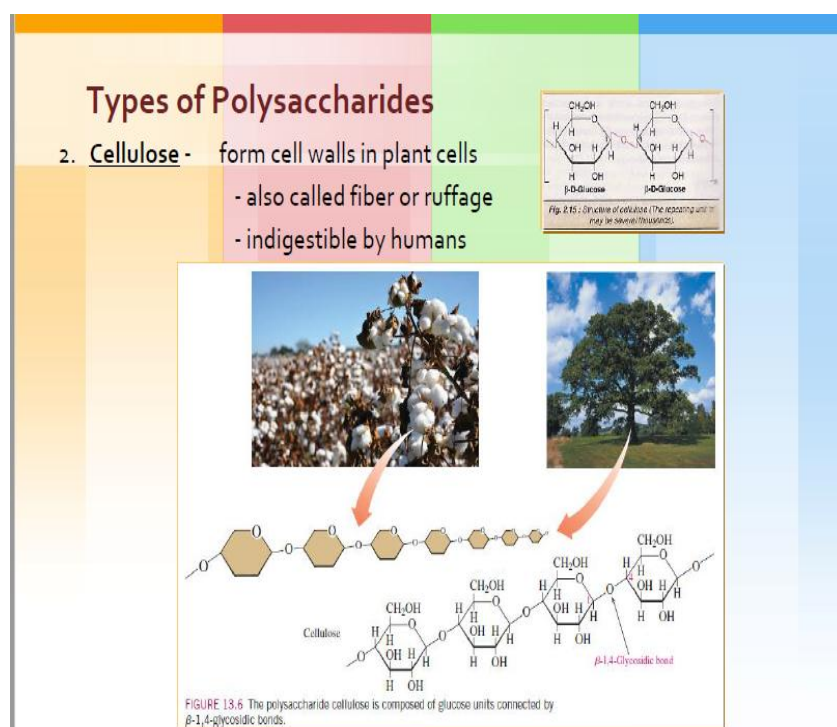
Cellulose is a linear polymer of β-D-glucose units connected through β(1,4) glycosidic bonds.

It, thus resembles the structure of amylose kept that the glucose units are linked together by (1,4)β linkage.

Cellulose is rEx arded as an anhydride of β-D- glucose units.

The groups involved are glycosidic OH on C_1 of glucose and alcoholic OH on C_4 Of glucose.

HYDROLYSIS:

Cellulose on complete hydrolysis gives β-D- glucose by the enzyme cellulose.

Cellulose→(cellulose) β-D-glucose

On partial hydrolysis cellulose gives a disaccharides called cellobiose.

Cellulose →(partial hydrolysis) cellobiose.

PROPERTIES:

➢ Cellulose is insoluble in water and it lacks sweetness.

➢ It gives no colour with iodine.

➢ It is not fermented by yeast.

➢ Cellulose is a non–reducing sugar, it does not reduce Fehling's, Benedict's solution. It does not react with phenyl hydrazine to form osazone.

BIOLOGICAL IMPORTANCE:

➢ In humans cellulose is not digested, because cellulose digesting enzyme is absent.

➢ But in ruminants (cattle, sheep, goat) cellulose digesting enzyme is present and cellulose forms the bulk of faecal matter.

➢ Cellulose reacts with acetic anhydride in the presence of acetic acid and a small amount of concentrated sulpuric acid to give cellulose acetate.

➢ Cellulose→(acetic anhydride, acetic acid and $H_2 SO_4$) Cellulose acetate

➢ Cellulose acetate is used in photographic film and for packing materials.

➢ Cellulose reacts with concentrated nitric acid in the presence of sulpuric acid to form cellulose nitrate or nitro cellulose.

➢ Cellulose →(HNO_3 / $H_2 SO_4$) Cellulose nitrate or Nitro cellulose.

➢ Cellulose nitrate is used in the manufacture of explosives.

➢ The OH groups in cellulose can be replaced by ONO_2 group. When all the OH groups are replaced by ONO, group the product obtained is known as gun cotton, gun cotton looks like a ordinary cotton but is highly explosive.

➢ When only some of the OH group is replaced by ONO_2 group then the product obtained is known as pyroxylin is used in the manufacture of plastics.

➢ When cellulose is dissolved in NaOH and treated with (CS_2) carbon disulphide then artificial silk called Rayon is formed.

➢ Cellulose +NaOH+ CS_2 → Rayon (artificial silk)

➢ Cellulose is a useful substance which is synthesized by plants.

➢ If cellulose is not sufficient in the diet then it may lead to constipation, (difficulty in excretion of faces).

- **Glycogen**

Glycogen, also known as "animal starch", is the storage of glucose as a source of energy to animal cells.
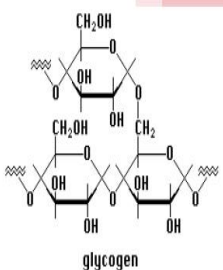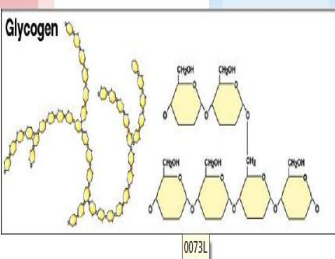
Its structure is similar to that of the amylopectin and has even more branches.

Glycogen is made up of chains of glucopyranose units connected by α-1,4-glycosidic bonds and the branching of the polymer is due to α-1,6-glycosidic bonds.

These branchings occur once for every ten 1,4-linkages.

Glycogen is primarily produced in the liver and muscles cells.
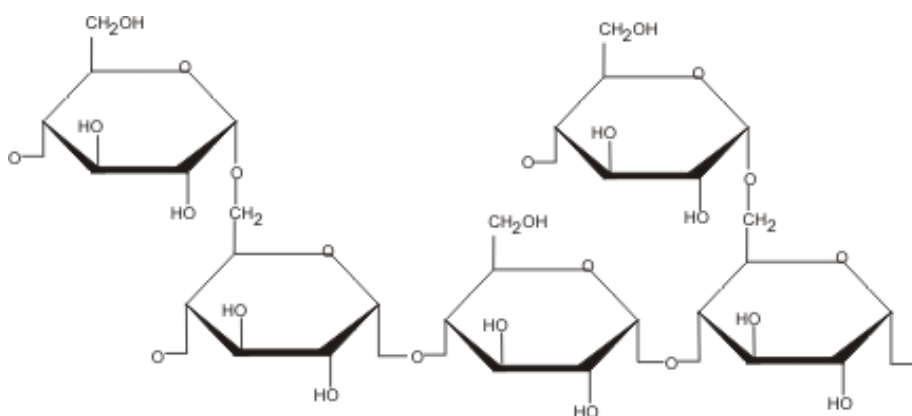
CHITIN

Chitin is a homopolysaccharide made from repeating units of a derivative of glucose, N-Acetylglucosamine.

These units are connected linearly through β-1,4-linkages.

Chitin is a very important structural component making up the cell walls of fungi, the exoskeletons of arthropods and insects, and other such components.

Its structure and linkages are similar to that of cellulose, except that the hydroxyl group on the 2' carbon of glucose is replaced by an acetylamine group.
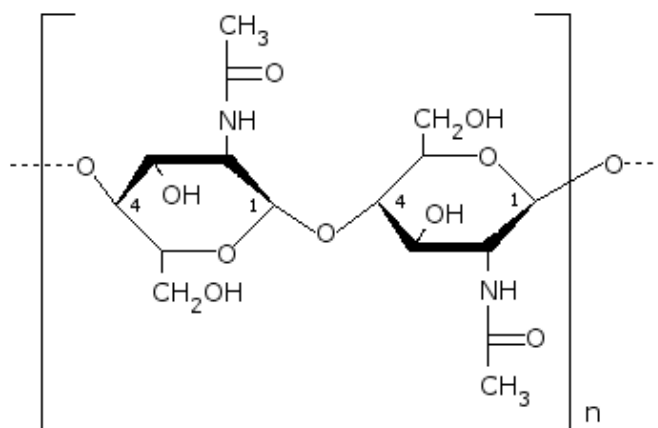
Structure of Chitin

STRUCTURAL DIFFERENCES

The linkage by α-1,4-glycosidic bonds in starch and glycogen give them a very different structure than cellulose(linked by β-1,4-glycosidic bonds).

The α-1,4-glycosidic linkage results in a hollow helical structure that is more suitable for energy storage, whereas the β-1-4-glycosidic bonds

allows cellulose to form linear chains that are stabilized by hydrogen-bonding with adjacent chains to form tensile fibers.

TYPES OF SUGARS

Sugars may also be classified as either **reducing** or **nonreducing** sugars.

REDUCING SUGARS

Reducing sugars have access to their open chain form.

Reducing sugars are basically sugars with an aldehyde group (in their open form) or a **hemiacetal** group (in their ring form) at the anomeric carbon that is ready to oxidize.
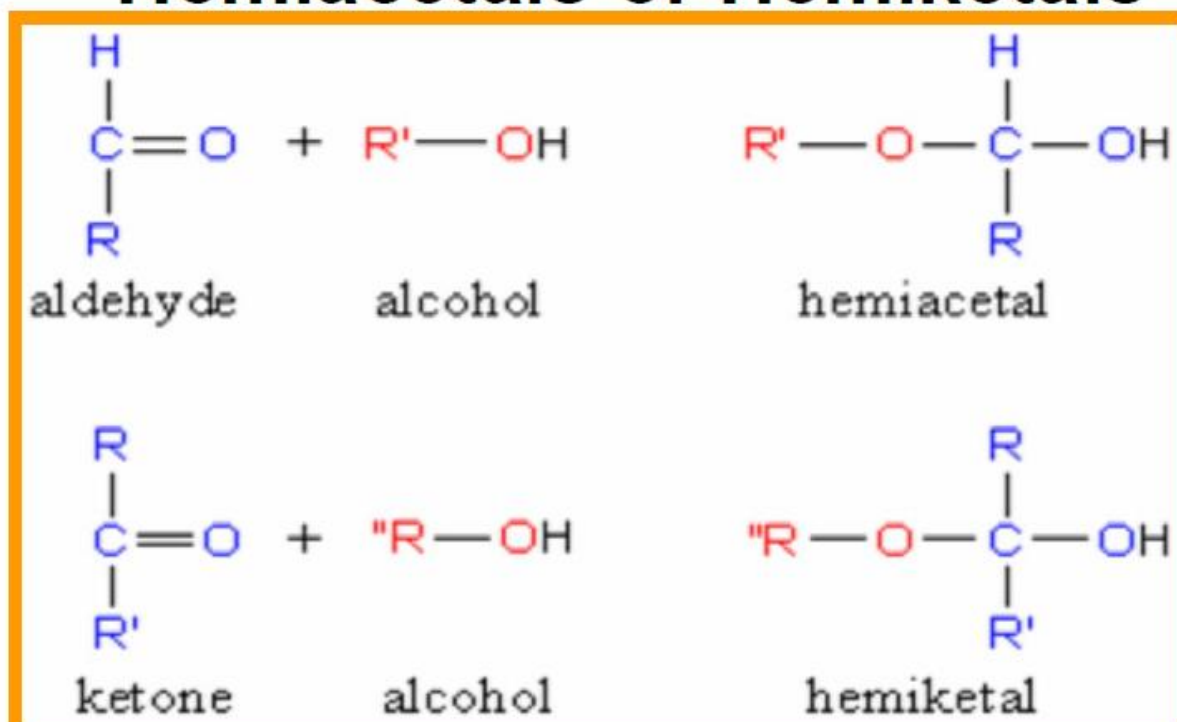
In other words, reducing sugars allow for chain formation and elongation.

The presence of reducing sugars can be tested using Fehling's solution, a solution of Cu^{2+} ions that readily oxidizes free aldehydes or ketones.

A similar test can be done with Benedict's reagent, which also contains the critical Cu^{2+} ion. A characteristic of a reducing sugars is the β-glycosidic bond, where the β anomeric carbon is involved in the linkage. Examples of reducing sugars are glucose, maltose, and lactose.

Reducing sugars can form aldehyde or ketone groups under basic conditions as the carboxyl group is reduced to carbonyl group of aldehyde or ketone group and occurs when the anomeric carbon is not bonded to hemiacetal or hemiketal hydroxyl group.It has only been mentioned that aldoses can form reducing sugars.

What about ketoses? Mechanistically, when ketoses isomerize to their ring form, acetals are formed. So no, ketoses do not form reducing sugars. However, ketoses can tautomerize to aldoses, where a hemiacetal can then be formed upon ring closure.
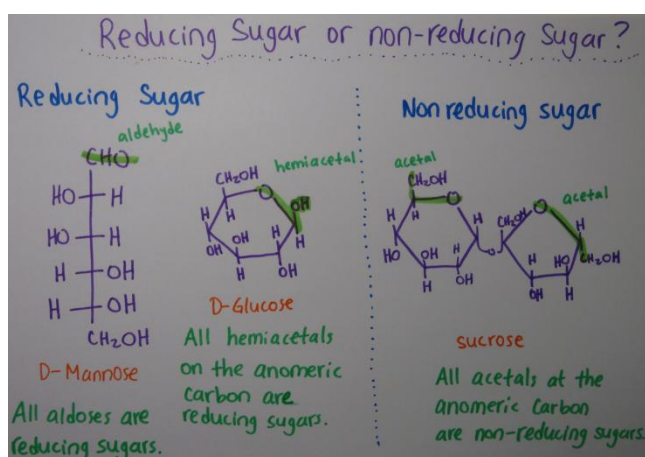
Carbohydrates with free carbonyl groups or in hemiacetal form give positive
tests to Benedict's and Fehling's reagents
without having been hydrolyzed are referred as **'reducing'** sugars ;
others (*i.e.*, the acetal types) are then **'non-reducing'** sugars

Reducing sugar	*Nonreducing sugar*
1. Carbohydrates with a free aldehyde (at C-1) or a free ketone (at C-2) group.	1. Aldehyde or ketone group is not free but instead utilized in bond formation.
2. They are in hemiacetal or hemiketal form.	2. They are in acetal or ketal form.
3. Do exhibit mutarotation.	3. Do not exhiblit mutarotation.
4. Do form osazones with phenyl hydrazine.	4. Do not form osazones.
5. Do form oximes with hydroxylamine.	5. Do not form oximes.
Examples – Glucose, Fructose, Lactose, Maltose, Cellobiose	Examples – Sucrose, Glycogen, Inulin
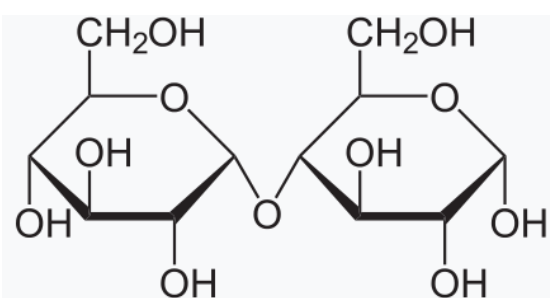
Maltose is a reducing sugar, with a free aldehyde group

Nonreducing sugars

Nonreducing sugars do not have access to free aldehyde or ketone such as glycosides. These type of sugar is basically an **acetal** (in its ring form) at the anomeric carbon. Since the anomeric carbon is fixed in a glycosidic linkage, the sugar chain cannot form or be elongated.

A non-reducing sugar can also be identified by an α-glycosidic bond in which an α anomeric carbon is involved in the linkage. An example of a nonreducing sugar is sucrose.

PROPERTIES OF CHO

Steriochemistry

Optical isomers (= enantiomers) differ from each other in the disposition of the various atoms or groups of atoms in space around the asymmetric carbon atom. These are, in fact, the mirror image of each other. These may also be likened to left- and right-handed gloves.

One form in which H atom at carbon 2 is projected to the left side and OH group to the right is designated as D-form and the other form where H atom is projected to the right side and OH group to the left is called as L-form (note the use of small capital letters D and L)

For example, the glyceraldehyde has only one asymmetric carbon atom (numbered as 2) and it can, therefore, exist in 2 isomeric forms :

D-glyceraldehyde   L-glyceraldehyde
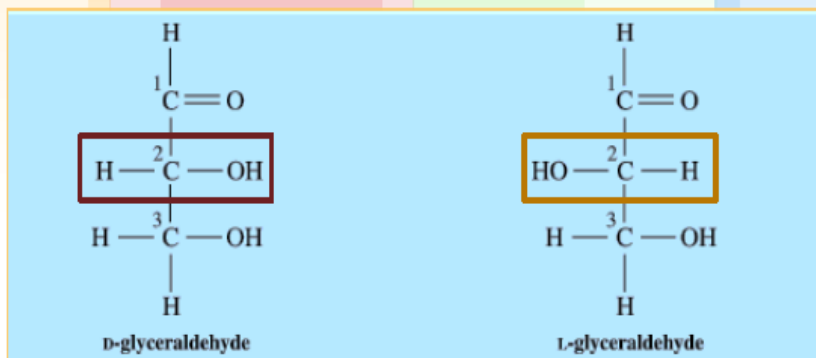
Properties of monosaccharides

1. *Mutarotation* : when a monosaccharide is dissolved in water, the optical rotatory power of the solution gradually changes until it reaches a constant value.   For ex : when D-glucose is dissolved in water, a specific rotation of +112.2° is obtained, but this slowly changes , so that at 24h the value has become +52.7°. This gradual change in specific rotation is known as mutarotation. This phenomenon is shown by number of pentoses, hexoses and reducing disaccharides.

2. *Glucoside formation* : when D-glucose solution is treated with methanol and HCl, two compounds are formed, these are α – and β-D- glucosides. Thus, formed glucosides are not reducing sugar and also doesnot show phenomenon of mutarotation

3. *Reducing power* : Sugars having free or potentially free aldehyde or ketone group have an ability to reduce the cupric copper to cuprous

   Reducing sugar + 2 Cu^{++} →   oxidized + $2Cu^+$

               (cupric)   sugar   (cuprous)

4. *Oxidation / Reduction:* The alcoholic OH, aldehyde (COH) or keto (C=O) group are oxidized to carboxyl group with certain oxidizing agents. The oxidation may be brought under mild or with vigorous oxidizing condition

   i. <u>With mild oxidant like BrH_2O</u> : In this group only aldehyde is oxidized to produce gluconic acid (monocarbonic). Ketoses do not respond to this reaction.

   ii. With strong Oxidizing agent like Conc HNO_3 : Both aldehyde or ketone groups are oxidized to yield dicarboxylic acids

   iii. <u>Oxidation with metal hydroxides:</u> Metal hydroxides like Cu(OH)2,

   Ag OH oxidize free aldehyde or ketone group of mutarotating sugar and reduce themselves to lower oxides of free metals

   Reduction: The aldehyde or ketone group present can be reduced to its respective alcohol with sodium amalgum.

   For ex: Fructose and glucose give the hexahydric alcohol i.e. <u>Sorbitol and Mannitol</u>

Dehydration : The monosaccharides when treated with Conc H_2SO_4, it get dehydrated to from

        5 – hydroxyl – methyl furfural derivative

Methylation or Esterification : The glucosidic and alcoholic OH group of mono saccharides and reducing disaccharides react with acetylating agent like acetic anhydride in pyridine to from acetate derivatives called esters.

ENOLIZATION:

With dilute alkalis, glucose, fructose and mannose are inter convertible in week alkaline solution such as $Ca(OH)_2$ and $Ba(OH)_2$ at low temperature.

This reaction is known as Labry de bruyn- Alberdavenstein transformation.

This reaction proposed in 1890 is as special significance as a similar reaction is support to take place in the human body. The mechanism as this reaction involves enolization, the migration of a proton from a carbon atom onto the oxygen of and adjacent carbonyl group resulting in the formation of an unsaturated alcohol called enolization.

The interconvert ion between glucose, fructose and mannose may in fat be visualized as occurring through a common enoldial form.

MUTAROTATION

When a monosaccharide is dissolved in water, the optical rotatory power of the solution gradually changes until it reaches a constant value.

Ex . A freshly prepared aqueous solution of α-D- glucose has a specific rotation of $(+112.2^0)$. Which this solution is allow to stand the rotation falls to (52.7^0) and remains constant at this value.

The final stage can be attained more quickly by heating the solution or adding some catalyst like acid or alkaline.

The general change is called mutarotation (or) birotation.

If β-D-glucose is dissolved it, optical rotation is (+18.7). This optical rotation gradually increases until it reaches the constant value of (+52.7)

α-D-glucose$\rightarrow 52.7^0 \leftarrow$β-D-glucose

$(+112.2^0)$ $(+18.7^0)$
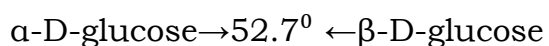
All reducing sugar undergoes mutarotation.

39

MECHANISM OF MUTAROTATION:

At equilibrium open chain structure and structure interconvert. Usually it predominant structure is ring form.

In glucose solution it is assumed that approximately 2/3 of the sugar exits as the form at equilibrium. When the asymmetry is lost each molecules spends some time in open form.

Open chain form passes back to the ring form.

It is possible to form any anomer.

Thus, mutarotation appears due to the changes in α and β form (via) the straight chain structure.

When equilibrium is attained there is αβ mixture and this the constant rotation of $+52.7^0$ is obtained.

CONCEPT MAP
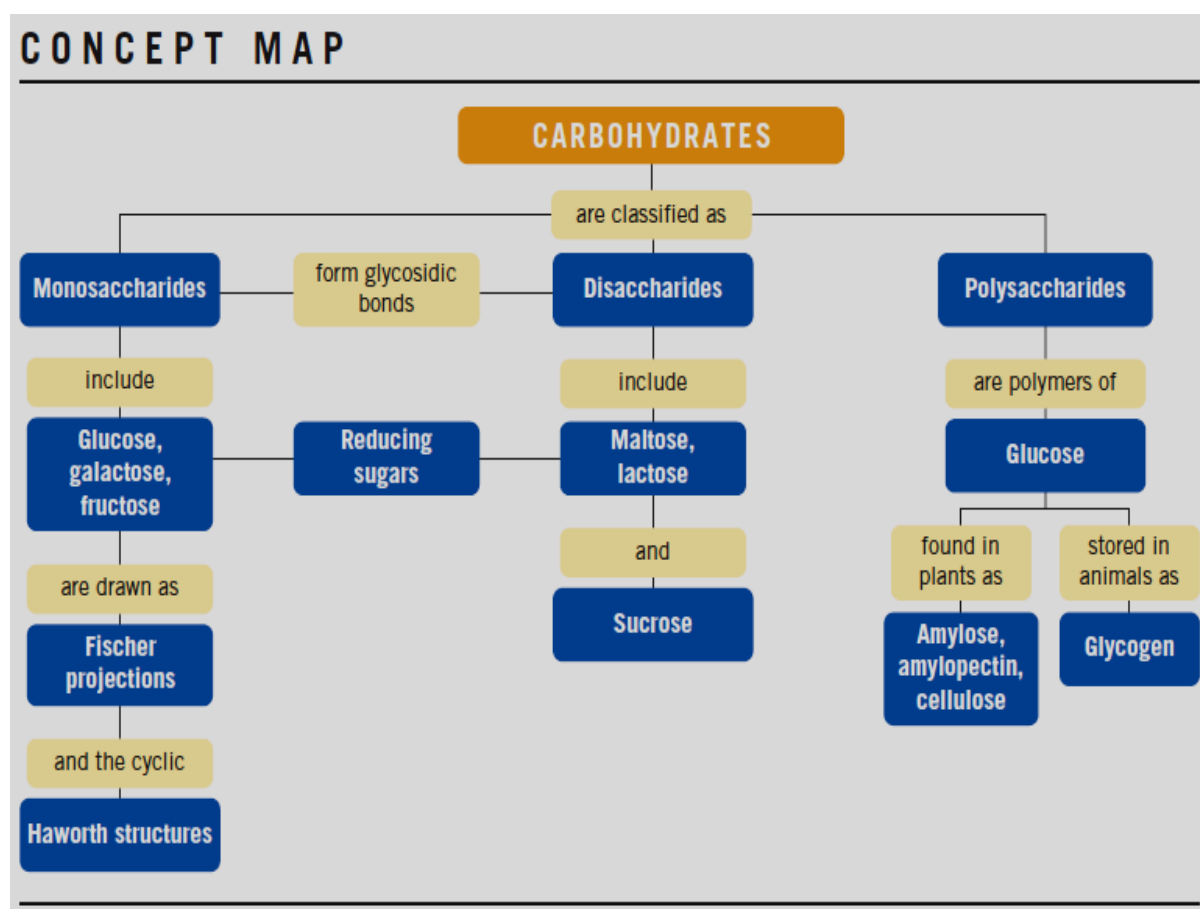
UNIT 2

AMINO ACIDS

INTRODUCTION

- Amino acids are the simplest units of a protein molecule and they form the building blocks of protein structure.

- Proteins are made up of 20 standard amino acids.

Amino acids are organic acids formed of C,H,O,N and in some S.

- They are usually classified into α,β,γ, etc. according to the relative positions of the 2 functional groups.

- But, on hydrolysis , proteins gives exclusively the α- amino acids i.e amino group present on the α-carbon atom.

Since the α- amino acids constitute the protein molecules, the term amino acid is generally used instead of the α- amino acid in reference to proteins.

The general structure of an amino acids is

$$NH_2$$

$$|$$

$$R—C—COOH$$

$$|$$

$$H$$
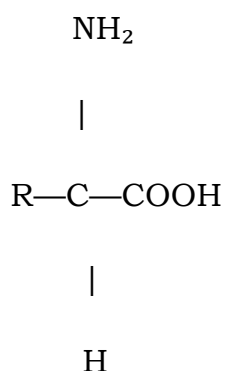
- Amino acid has an amino group (-NH_2), carboxylic group (-COOH) and a hydrogen atom.

- R is called as side chain, it may be hydrogen, aliphatic, aromatic or heterocyclic group. Thus the side chain varies from one amino acid to the other.

L- amino acids are more common than D-forms and most of the naturally occurring amino acids are L-forms.

- The amino acids present in our body is L-α amino acids.

DEFINITION

→ Amino acids are a group of organic compounds containing two functional groups –amino and carboxyl.

→ The amino group (- NH_2) is basic while the carboxyl group (-COOH) is acidic in nature.

20 AMINO ACIDS STRUCTURES

CLASSIFICATION OF AMINO ACIDS

1. Classification based on the composition of the side chain (or) R group

Based on the composition of the side chain amino acids are classified into 9 groups.

1. Aliphatic amino acids

These contain aliphatic group in the side chain.

e.g. Glycine, Alanine, Valine, Leucine and Isoleucine.

2. Hydroxy amino acids:

These contain a hydroxyl group in the side chain.

e.g. Serine and Threonine

3. Sulphur – containing amino acids:

These contain a sulphur atom in their side chain.

e.g. Cysteine and Methionine.

4. Acidic amino acids:

These have carboxyl group in their side chain.

e.g. Aspartic acid and Glutamic acid.

5. Amino acid amides:

These are derivatives of acidic amino acids in which one if the carboxyl group has been transformed into an amide group ($-CO.NH_2$).

e.g. Asparagine and glutamine.

6. Basic amino acids:

These posses an amino group in the side chain.

e.g. Lysine, Arginine and Histidine.

7. Heterocyclic amino acids:

These posses a heterocyclic ring in their structure.

e.g. tryptophan, histidine and proline

8. Aromatic amino acids:

These have a benzene ring in their side chain.

e.g. phenylalanine, tyrosine and tryptophan

9. Imino acids:

These have an imino group ($-NH_2^+$) in their side chain instead of amino group (NH_3^+).

e.g. proline and 4-hydroxy proline.

↗ **2.Classification based on acidic and basic property**

Based on the number of acidic and basic group amino acids are classified into 3 groups.

1. Neutral amino acids:

These amino acids have one amino group and one carboxyl group in their structure. These are mono amino mono- carboxylic acids.

e.g. glycine, alanine, serine, threonine, valine,leucine and isoleucine.

2. Basic amino acids:

These contain one acidic (or) carboxyl and 2 Amino groups in their structure. They are Diamino mono carboxylic acids.

e.g. arginine and lysine.

3.Acidic amino acids:

These amino acids contain 2 carboxyl group and one amino group.

They are mono amino dicarboxylic acids. e.g. aspartic acid, glutamic acid.

3. Classification based on the polarity of the side chain (or) R- group

The polarity reflects the functional role of amino acids in protein structure.

1**. Non- polar amino acids**:

The R group of these amino acids are hydrophobic (water hating) in nature. They have no charge on their R group.

POLAR AMINO ACIDS

(i)   **Polar amino acids with no charge on R-group:**

The R group of these amino acids are hydrophilic (water loving) because they contain functional groups that from hydrogen bonds with water. These amino acids as such carry no charge on them.

e.g. Serine, threonine, tyrosine, cysteine, asparagine and glutamine.

(ii) Polar amino acids with positive R group:

These are diamino mono carboxylic acids. Their side chains contain an extra amino group which imparts basic property to them.

e. g. lysine and arginine

(iii) Polar amino acids with nEx ative R group:

These are mono amino dicarboxylic acids and their side chain contain an extra carboxyl group which gives acidic property to them.

e.g. Aspartic acid and glutamic acid.

4. **Classification based on production inside the body/Nutritional requirement**

1. Essential amino acids:

The amino acids cannot be synthesized inside the body and therefore need to be supplied through the diet. There are 8 essential amino acids, valine, Isoleucine, Leucine, Methionine, phenylalaine, Threonine, Tryptophan.

They are also called indispensable amino acids.

2. Non-essential amino acids:

These amino acids can be synthesized by the body and hence need be consumed in the diet. They are also called dispensable amino acids. They are 10 in numbers.

e.g. Glysine, Alanine, Serine, Cysteine, Aspartate, Glutamate, Asparagine, Glutamine, Tyrosine and Proline.

3. Semi- essential amino acids:

These are partially synthesized by the body but not at the rate to meet the requirement of the body. e.g. Arginine and Histidine

4. **Classification based on their metabolic fate of amino acids**

The carbon skeleton of amino acids can serve as a precursor for the synthesis of glucose or fat or both.

1. Glycogenic amino acids:

These amino acids can serve as precursor for the formation of glucose or glycogen. e.g. Alanine, Aspartate, Glycine, Methionine.

2. Ketogenic amino acids:

These amino acids can serve as a precursors for the synthesis of fat.

e.g. Leucine and Lysine

3. Glycogenic and Ketogenic amino acids:

These are the precursors (Starting material) for the synthesis of glucose as well as fat.   e.g. Isoleucine, Tyrosine, Phenylalanine and Tryptophan.

PROPERTIES OF AMINO AICIDS

PHYSICAL PROPERTIES

1. Amino acids are white crystalline substances.

2. **SOLUBILITY:** Most of the amino acids are usually soluble in water and insoluble in organic solvents.

3.TASTE: Amino acids are usually either sweet, tasteless or bitter.

For example: Glycine, Alanine, Valine, Proline, Hydroxyproline, Serine, Tryptophan, and Histidine are sweet.

→ Leucine is tasteless

→ Isoleucine and Arginine are bitter

→ The sodium salt of glutamic acid, sodium glutamate is valuable as a flavoring agent for certain sauces and foods, since it enhances the flavor.

4. MELTING POINT: Amino acids have high melting point usually more than 200·C.

5.OPTICAL PROPERTIES: All amino acids except glycine are optically active because they contain asymmetric carbon atoms and 3 sides should have different functional group.

6.Aromatic amino acids such as Tryptophan, Phenylalalnine and tyrosine absorb ultraviolet (UV) light.

7. AMPHOTERIC NATURE:

Amino acids contain both acidic (-COOH) and basic (-NH$_2$) groups.

They can donate or accept a proton, hence amino acids are rEx arded as ampholytes. Amino acids react with both acids and bases. Hence, they are amphoteric in nature.

Thus, in acid solution the coo⁻ ion acquires a proton and the amino acid becomes an ammonium salt of the acid.

In alkaline solution, the NH$_3$ $^+$ ion loose proton and the amino acid becomes the anion of a salt.

These reactions are of reversible nature and depend on the PH change when an acid or base is added.

8. ISOELECTRIC POINT:

The PH at which a molecule carries is known as its iso electric point PI.

Since the amino acid carries not net charge at PI ,they are immobile in an electric field.

9. ISOELECTRIC PH:

It is denoted as PI. Isoelectric PH is defind as the PH at which a molecule exists as a Zwitter ion or dipolar ion and carries no net charge. Thus the molecule is electrically neutral.

10. ZWITTER ION:

Dipolar ion (or) inner salt. The name zwitter derived from the german word which means hybrid.

Zwitter ion is a hybrid molecule containing positive and nEx ative ionic groups.

Titration Curve

Definition

Titrations are often recorded on graphs called **titration curves**, which generally contain the volume of the titrant as the independent variable and the pH of the solution as the dependent variable (because it changes depending on the composition of the two solutions.

- Titration curves are produced by monitoring the pH of given volume of a sample solution after successive addition of acid or alkali

- The curves are usually plots of pH against the volume of titrant added or more correctly against the number of equivalents added per mole of the sample

Titration of acetic acid

- At the starting point the acid form predominates (CH3COOH).

- As strong base is added (e.g. NaOH), the acid is converted to its conjugate base.

- At the mid point of the titration, where pH=pK, the concentrations of the acid and the conjugate base are equal.

At the end point(equivalence point), the conjugate base predominates, and the total amount of OH added is equivalent to the amount of acid that was present in the starting point.

pH

$[CH_3COOH]=[CH_3COO^-]$

CH_3COO^-

pK=4.8

CH_3COOH

Equivalents of NaOH added
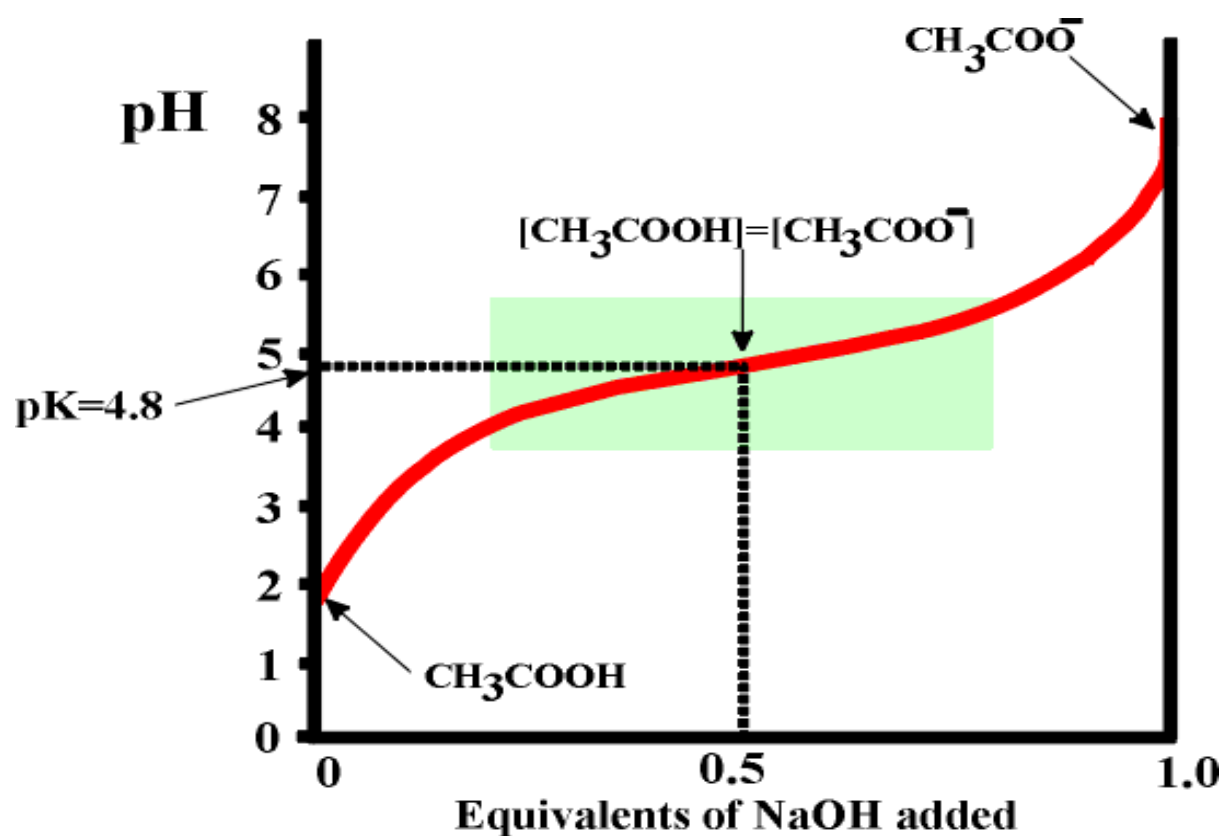
UNIT 3

PROTEIN

INTRODUTION

The term protein is derived from the greek word 'Protos' which means 'of first importance'. Berzelius in 1838, referred proteins as complex organic compounds with a high molecular weight formed of carbon, hydrogen, oxygen and nitrogen and may also contain sulfur, phosphorous and non-protein organic groups and metal ions. Proteins occupy central position in the architecture and functioning of living matter. All proteins are macromolecules, because of their very high molecular weight. Amino acids are the building blocks of proteins. Proteins are the main constituents of our body such as muscles, skin, hair and nails. Proteins carry all vital life processes

All proteins contain carbon, hydrogen, oxygen and nitrogen. Some contain sulphur and some phosphorous.

ELEMENTAL COMPOSITION OF PROTEINS

CARBON – 51%, HYDROGEN – 7%,

OXYGEN – 20%, NITROGEN – 16%,

SULPHUR – 1%, PHOSPHOROUS - 2%

The molecular weight of proteins varies from about 12,000 daltons to several million.

Cells contain a very large number of proteins. The number may vary from 1000-2000 in the simplest bacteria to as many as 1,00,000 different proteins in human cells. Proteins serve as building blocks for celluar and organic structure. Proteins from about 15% of the total body weight of an average adult.

SOURCES

Proteins are obtained both from animal and plant sources.

Animal sources → milk, Ex g, meat, fish, liver

Plant sources → pulses, nuts, cereals.

DEFINTION

Proteins are defined as complex nitrogenous substances found in the protoplasm of all animal and plant cells.

Proteins are biopolymers containing large number of amino acids joined to each other by peptide bonds.

CLASSIFICATION

Proteins can be classified in several ways based on their shape, size, solubility, function and composition.

I CLASSIFICATION BASED ON THE SHAPE AND SIZE OF PROTEINS

On the basis of shape and size proteins are classified into 2 types.

1. Fibrous proteins (or) Fibrillar proteins

2. Globular proteins (or) Corpuscular proteins.

FIBROUS PROTEINS

When the axial ratio length, width of a protein molecule is more than 10, it is called fibrous proteins. Fibrous proteins are unbranched and they appear as long ribbons or fibres in shape.

They are mostly condensation products of neutral amino acids.

They are insoluble in water, acids and alkalies. They long linear protein chains are held together by intermolecular hydrogen bonds. They are highly resistant to digestion by proteolytic enzymes.

Ex. Keratin of hair, collagen of tendons, fibroin of silk, elastin of connective tissue.

GLOBULAR PROTEINS

When the axial ratio length; width of a protein molecule is less than10, it is called as globular protein. They are spherical or ovoid in shape.

They are highly branched and cross-linked condensation products of basic or acidic amino acids. They are soluble in water, acids and alkalies.

They are folded into three- dimensional structures; the peptide chain is stabilized by intermolecular hydrogen bonds.

As a result of various physical interactions, the hydrophobic amino acids are oriented towards the interior of the protein structure, whereas hydrophilic amino acids are oriented towards the exterior surface.

Ex : Enzymes, antibodies, haemoglobin, myoglobin.

II CLASSIFICATION BASED ON COMPOSITION AND SOLUBILITY

Proteins are classified into 3 main groups based on their physical properties like solubility and composition.

1. SIMPLE

2. CONJUGATED

3. DERIVED

SIMPLE PROTEINS

These are the proteins which on hydrolysis yield only amino acids.

They are further classified based on their solubilities and heat coagulabilities. Mostly simple proteins are globular type expect scleroproteins which are fibrous in albumins. Albumins are soluble in water. They are coagulated by heat.

They are precipitated by full saturation with ammonium sulphate. They are deficient in glycine.

Ex : Lactalbumin in milk, Ovalbumin in Ex g, Plasma albumin, Serum albumin in blood.

GLOBULINS

Globulins are insoluble in water but soluble in dilute neutral salt solutions.They are coagulated by heat. They are precipitated by half saturation with ammonium sulphate. Globulins generally contain glycine.

Ex : Ova globulin in Ex g white, Lacto globulin of milk plasma globulin in blood.

GLUTELINS

Glutelins are insoluble in water but soluble in dilute acids and alkalies. They are coagulated by heat. Glutelins are plant proteins. Glutelins are rich in glutamic acid.

Ex : Glutenin from wheat, glutelin from corn and oryzenin from rice.

PROLAMINES

They are insoluble in water but soluble in 70-80% alcohol. They are also called as alcohol soluble proteins. These are not coagulated by heat. Prolamines are isolated from plant seeds. They are rich in proline but but poor in lysine. Ex ; Zein of corn, Gliadin of wheat, Hordein of oat.

SCLEOPROTEINS OR ALBLUMINOIDS

The albluminoids are the least soluble of all proteins.

These are insoluble in water, dilute acids, alkalies. These proteins occur in animals and they are commonly known as "animal skeletal proteins"

Ex ; Collagen of bones, Cartilage and tendons, Keratin of hair, elastin of connective tissue.

HISTONES

They are soluble in water, acids and insoluble in ammonia. They are not coagulated by heat. Histones are basic proteins, rich in arginine and histidine. Ex ; Globin of haemoglobin, nucleo histones of nuclei.

PROTAMINES

They are the simplest of the proteins and contain about 8 amino acids.

They are soluble in water, acids, alkalies. They are not coagulated by heat. They are more basic than histones. They are rich in arginine and do not contain cysteine, tryptophan and tyrosine. Ex ; Salmine from salmon sperm, Protamines are principally found in Ex g cells.

CONJUGATED PROTEINS

These are proteins in which simple proteins are combined with a non protein group called prosthetic group. On hydrolysis, they yield amino acids as well as the prosthetic group. Conjugated proteins→($H_2 O$) α-amino acids+ prosthetic group.

Based on the nature of the prosthetic group they further classified as following.

NUCLEO PROTEINS

The prosthetic group is a nucleic acid. The nucleic acids are attached with basic proteins. Ex ; Protamines or Histones.

The nucleoproteins are found in all cells, in the cytoplasm as well as in the nucleus.

(i) Deoxyribonucleoproteins:

It contains DNA as their prosthetic group and found in nuclei, mitochondira and chloroplast.

(ii) Ribonucleoproteins:

It contains RNA as their prosthetic group and are found in nuclei and ribosome granules.

GLYCOPROTEINS AND MUCOPROTEINS

These are the proteins containing carbohydrates as prosthetic group.

Glycoproteins contain small amounts of carbohydrates i.e, less than 4 % . Ex ; Ex g albumins , certain serum globulins and serum albumins.

Mucoproteins or mucoids are combined with mucopolysaccharides such as hyaluronic acid and chondroitin sulphate.   Mucoproteins contain higher amount of carbohydrates more than 4%.

Ex : Ovo mucoid from Ex gwhite, mucin from saliva.

LIPOPROTEINS

The prosthetic group is lipids such as fatty acids, cholesterol and Phosphlipids.   Ex ; Lipoproteins of blood

PHOSPHOPROTEINS

The prosthetic group is phosphoric acid which is attached to the hydroxyl group of protein by an ester linkage.

Ex ; Casein of milk, Vitellin of Ex g.

METALOPROTEINS

These are the proteins linked with various metals such as Fe, Cu , Zn.

Ex ; Ferritin contain Fe, Carbonic anhydrase contain Zn,

Cerruloplasmin contain Cu.

CHROMOPROTEINS

- These are the proteins that contain coloured substance as their prosthetic group. Ex. Haemoglobin, Chlorophyll, Cytochrome.

DERIVED PROTEIN

These are protein formed from simple and conjugated proteins by the action of physical agents such as heat or chemical agents.

Derived proteins are further classified in to 2 groups.

1. Primary derived proteins.

2. Secondary derived proteins.

PROTEANS

- ➢ These are insoluble products formed by the action of acids, enzymes or water. Ex ; Myosan from Myosin, Fbrin from fibrinogen.

METAPROTEINS

- ➢ Metaproteins are produced by further action of acid or alkali on proteins.

- ➢ These are insoluble in water.   Ex ; acid and alkali metaproteins

COAGULATED PROTEINS

The coagulated proteins are insoluble products formed by the action of heat or alcohol on proteins. Ex ; Cooked Ex g albumin, cooked meat.

SENCONDARY DERIVED PROTEINS

These are the derivatives of proteins in which the hydrolysis has take place. The molecules are smaller than that of original proteins. These are of 3 types

1. **PROTEOSES**

These are hydrolysis products of proteins.

Ex ; Albuminoses from Albumin.

Primary proteoses which are precipitated by half saturation with ammonium sulphate. Secondary proteoses which are precipitated by full saturation with ammmonium sulphate.

2. **PEPTONS**

Peptons are hydrolytic products of proteoses.

3. **POLYPEPTIDES**

These are combination of two are or more amino acids.

III CLASSIFICAION BASED ON BIOLOGICAL FUNCTIONS

Based on the functions proteins are classified into following groups.

ENZYMES

Except ribozy me all the enzymes are proteins.

Enzymes act as biological catalyst that catalyse various reactions

Ex ; amylase, catalase, urease

STRUCTURAL PROTEINS

Some proteins function as biological structural materials. They are needed for strengthening and protecting biological structure.

Ex ;keratin of hair and nails,collagen

TRANSPORT OR CARRIER PROTEINS

Certain proteins are involved in the transport of many essential biological factors to various parts of the organisms.

Ex ; cerruloplasmin transports copper in blood, hameoglobin carries O_2 .

HORMONAL PROTEINS

Hormones are the rEx ulatory substances of the body. Some hormones are protein in nature .   Ex ; Insulin, growth hormone

CONTRACTILE PROTEINS

Some proteins function in the contractile system.   Ex ; Actin,myosin

NUTRIENT OR STORAGE PROTE

Milk protein casein stores amino acids. Ferritin stores iron.

TOXIC PROTEIN

Some proteins act as toxic substances. Ex ; Abrin, ricin which are poisonous plant proteins

GENETIC PROTEINS

They are associated with genetic material.   Ex ; Nucleohistones.

DEFENSE PROTEIN

Some proteins products outer body from various antigens.   Ex ; Antibodies

IV NUTRITIONAL CLASSIFICATION OF PROTEINS

The nutritive values of proteins are determined by the composition of essential amino acids. From the nutritional point of view, proteins are classified into 3 catEx ories.

1. COMPLETE PROTEINS

These proteins have all the 10 essential amino acids in the required proportion by the human body to promote good growth.

Ex ; Ex g albumin, milk casein.

2. PARTIALLY INCOMPLETE PROTEINS

These proteins are partially lacking one or more essential amino acids and hence can promote moderate growth.   Ex ; Wheat and rice proteins

3. INCOMPLETE PROTEINS

These proteins completely lack one or more essential amino acids. Hence they do not promote growth at all.   Ex ; Gelatin, Zein

S.No	Class of proteins	Function	Examples
1	Enzymic proteins	Biological catalysts	Urease, amylase, catalase, cytochrome C, alcohol dehydrogenase
2	Structural proteins	Protecting biological structures	Collagen, elastin, keratin and fibroin
3	Defense proteins	Defend against other organisms	Antibodies, fibrinogen, thrombim
4	Toxic proteins	Hydrolyze enzymes	Snake venom, ricin
5	Motile proteins	Function in the contractile system	Actin, myosin, tubulin

PHYSICAL PROPERTIES

1. COLOUR AN TASTE

Proteins are colourless and tasteless expect serinc which is sweet.

2. ODOR

Pure proteins are odorless. When heated, they turn brown and char give off odor of burning feathers or hair.

3. MOLECULAR WEIGHT

Proteins generally have larger molecular weight. The proteins vary in their molecular weights, which, in turn, is dependent on the number of amino acids residues.

Each amino acids on an average contributes to a molecular weight of about 110. Ex ; Insulin -51 amino acids- molecular weight-5,700.

4. SHAPE AND SIZE

Proteins range in shape from simplest crystalloid spherical structures to long fibrillar structures. Based on shape, they are of 2 types

(i) GOBULAR PROTEINS

These are spherical in shape and occur mainly in plants, especially in seeds and in leaf cells. These are bundles formed by folding and crumbling of proteins chains.

Ex ; pepsin, insulin, ribonuclease etc.

(ii) FIBRILLAR PROTEINS

These are thread-like or ellipsoidal in shape and occur generally in animal muscles. Ex ; Fibrinogen, myosin, collagen

Each protein molecule is characterized for its specific size.

Haemoglobin has diameter of $65A^0$. Collagen is one of the longest protein with length of $3000A^0$

5. COLLIODAL NATURE

Because of their giant size, the proteins exhibit many colloidal properties.

6. ISOELECTRIC POINT

Like amino acids, the proteins are amphoteric i.e they acts as acids and alkalies both. At physiological PH, proteins exit as charged molecules.

At isoelectric PH(pi) the proteins exit as zwitter ions or dipolar ions. They are electrically neutral.

7. OPTICAL ACTIVITY

Proteins are levorotatory form.

8. SOLUBILITY

Solubility of proteins is influenced by PH. Solubility is lowest at isoelectric point and increases acidity or alkalinity. This is because as when the protein molecules exist either cations or anions, repulsive from between the ions are high.

Since the molecules possess excess charges of the same sign. Thus they will be more soluble than in the isoelectric point.

SALTING IN

The solubility of many proteins is increased in the presence of small concentrations of various neutral salts. This is referred to as salting in of protein. The phenomenon of "salting in" of proteins is caused by forces of attraction between salt and proteins at low salt concentration, leading to increased solubility.

Globulins are sparingly soluble in water but their solubility is greatly increased by the addition of neutral salts like NaCl.

SALTING OUT

As the concentration of the neutral salt is increased, the solubility increases to maximum and then starts decreasing and finally the protein is precipitated. This is referred to as salting out.

The phenomenon of salting out of protein is caused due to the salt at high concentration. The salting out of proteins is an effective method of purification.

Proteins are precipitated from aqueous solution by high concentration of neutral salts.

The salts commonly used for this purpose are Na_2SO_4, magnesium salts and phosphates.

DENATURATION

The phenomenon of disorganization of native protein structure is known as denaturation. Denaturation results in the loss of secondary, tertiary and quaternary structure of proteins.

This involves a change in physical, chemical and biological properties of protein molecules.

AGENTS OF DENATRATION

Denaturation may be bright about by a variety of agents, both physical and chemical.

1. PHYSICAL AGENTS

The physical agents include mechanical action like vigorous shaking, heat treatment, cooling and freezing operations, rubbing, high hydrostatic pressures, ultra violet rays X- rays etc.

2. CHEMICAL AGENTS

Acids, alkalies, organic solvents (acetone, alcohol), salts of heavy metals (pb, Hg), urea, aromatic anions (salicylates), anionic detergents (like sodium dodecyl sulphate).

CHARACTERISTICS OF DENATURATION

The following changes take place in denaturation.

1. PHYSICAL CHANGES

Solubility decreases, viscosity increases, therefore rate of diffusion decreases. The native helical structure is lost.

The primary structure of a protein with peptide linkages remains intact ie, peptide bonds are not hydrolysed. Decrease in size and shape of protein. Denatured protein connot be crystallized since crystallization depends upon a high dEx ree of organization of a molecule.

2. CHEMICAL CHANGES

The structure of the peptide chains of some proteins are held in coiled from by 3 types of cross linkages namely, hydrogen bonds, disulphide and salt linkages. Denaturation causes splitting of one or more of these linkages resulting in and of peptide chains in turn produce certain chemical changes.

As a result of the unfolding process, chemical groups which are essential for activity of the protein molecules loose their activity.

Examples of such chemical groups are

→ The sulphydryl (SH) group of cystine

→ The disulphate groups of cystine

→ The phenolic groups of tyrosine.

BIOLOGICAL CHANGES

The protein loses its biological activity.

Hormonal and enzymatic activities are destroyed after denaturation.

Denaturation protein is more easily digested. This is increased exposure of peptide bonds to enzymes. Cooking causes protein denatuation and therefore, cooked food (protein) is more easily digested.

If the denaturation is severe, the protein molecules, becomes insoluble and precipitation occurs.

REVERSIBLE DENATURATION

Reversal of denaturation is generally called renaturation or refolding.

Ex . Of reversible or controlled denaturation

Ribonuclease is heated with urea and mercaptoethanol.

During this, breakage of disulphate bond and uncoiling of the polypeptide chain occurs. The denaturation protein on slow reoxidation without the urea is again changed into the original tertiary structure.

IRREVERSIBLE DENATURATION

Denaturation is generally irreversible. In this type of uncontrolled denaturation, the proteins cannot be brought back to their original state.

Irreversible denaturation results in coagulation. The term " coagulum" refers to a semisoild viscous precipitate of protein.

Ex . Boiling of an Ex g during which tertiary structure of the protein is destroyed.

RENATURATION OF PROTEINS

The reversal of denaturation is called renaturation or refolding.

If the denaturation is effected by heat, renaturation may be carried out by very slow cooling. The process of this type of renaturation is called as anneling.

Ex : If trypsin is exposed to a temperature of 80-90^0 C ,it denatures and when this solution is cooled at 37^0 C , the solubility and the activity of this protein enzyme is rEx ained.

SIGNIFICANCE

Denaturaton property of proteins help in the clinical laboratory. The protein free substances of blood such as glucose, uric acid and drugs are analysed by precipitating the proteins of blood by the addition of certain acids.

PEPTIDE BOND

The amino acid units are linked together through the carboxyl and amino acid groups to produce the primary structure of the protein chain.

The bond between 2 adjacent amino acids is a special type of amide bond , know as the peptide bond and the chain, thus formed is called a peptide chain, When the amino group of an amino acids combines with the carboxyl group of another amino acid, a peptide bond is formed.

If a peptide bond is made up of 2 amino acids→ Dipeptide, 3 amino acids→ tripeptide.

If a peptide bond is made up of less than 10 amino acids, it is called as polypeptide.

These peptide bonds are rather strong and serve as the cementing material between the individual amino acids.

STRUCTURE OF PROTEINS

Proteins are the polymers of L-α- amino acids.

Proteins structure can be classified into 4 levels of organization.

1. Primary structure

2. Secondary structure

3. Tertiary structure

4. Quaternary structure

1. PRIMARY STRUCTURE

Primary structure of a protein refers to the number and sequence of amino acids in the polypeptide chains. The amino acids are linked by peptide bonds. The free NH^+_3 group of the terminal amino acid is called N-terminal and the free COO^- terminal end.

The primary structure of a protein is largely responsible for its function. Any change in the sequence is abnormal and may affect the function and properties of protein.

When 2 amino acids are joined together by one peptide bond, then it is called as dipeptide. When 3 amino acids are joined, they from a tripeptide. Less than 10 amino acids from oligo peptide, more than 10 amino acids from a polypeptide. The amino acids in a polypeptide chain are numbered from the N- terminal end.

In primary structure the proteins are linear and unbranched.
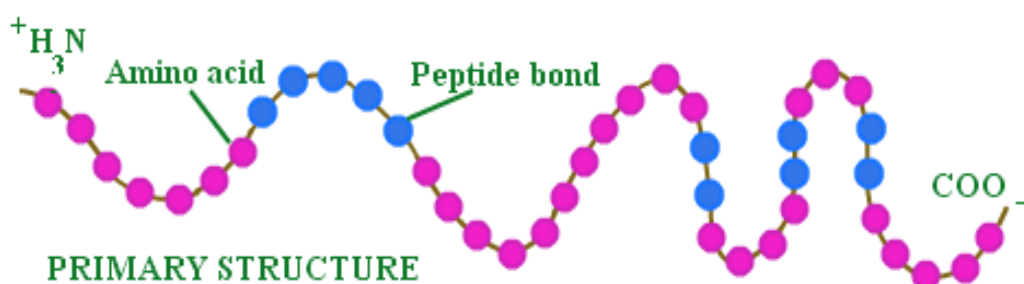
Many structure proteins, especially those which form fibres, are of linear, unfolded type.

Silk fibroin is composed of mainly glycine, alanine and serine.

SECONDARY STRUCTURE

- It refers to the coiling or folding of a polypeptide chain that gives the protein its 3-D shape.
- Based on the nature of hydrogen bonding (whether intramolecular or and intermolecular), Pauling and Corey (1951) identified two rEx ular types of secondary structure in proteins.
    1. **Alpha (α) helix**
    2. **Beta (β) pleated sheet (Fig. 3)**.

1. <u>**Alpha (α) helix :**</u>
- Pauling and Corey found that a polypeptide chain with planar peptide bonds would form a right handed helical structure by simple twist about the α-carbon-to-nitrogen and the α-carbon-to-carboxyl carbon bonds.
- They called this helical structure as α-helix. The helix is so named because of the mobility of α-carbon atoms. Collagen is one example of a protein that has triple helix structure.

2. <u>**β-pleated sheet:**</u>
- The formation of β-pleated sheets depends on intermolecular hydrogen bonding, although intramolecular hydrogen bonds are also present.
- B-sheet structures are quite common in nature and are favoured by the presence of glycine and alanine. Silk and synthetic fibres consist of β-structures.
- There are 2 types of β-pleated sheet structures. If the N-terminal ends of all the participating polypeptide chains lie on the same edge of the sheet, with all C-terminal ends on the opposite edge, the structure is known as parallel β-pleated sheet.
- If the direction of the chains alternates so that alternating chains have their N-terminal ends on the same side of the sheet, while their C-

terminal ends lie on the opposite edge, the structure is known as the antiparallel β-pleated sheet.

- Silk fibroin is one example of a protein that has the antiparallel pleated sheet structure.
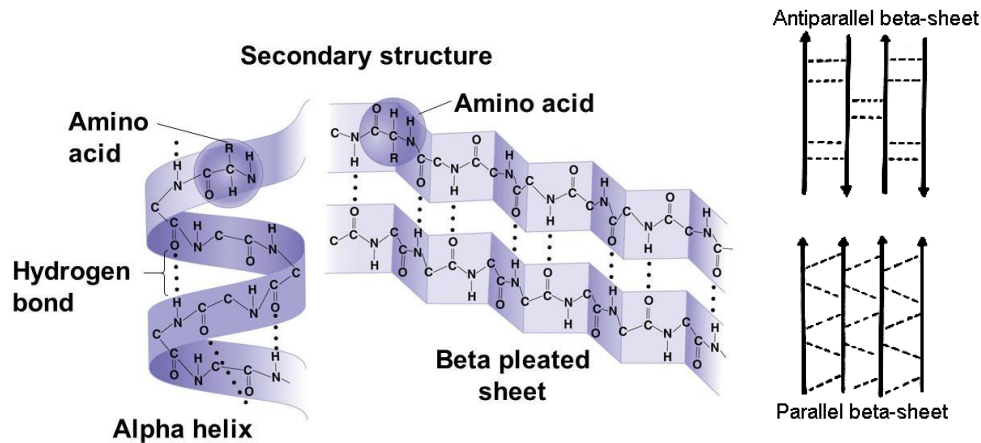
Secondary structure of proteins

TERTIARY STRUCTURE

- The polypeptide chain with secondary structure may be further folded and twisted producing structural coils of different sizes and shapes to form a compact three dimensional globular molecular structure called the tertiary structure.
- Hydrophobic side chains present in the interior of protein structure and hydrophilic side chains are found on the outside of protein structure.
- Tertiary structure of protein is stabilized by covalent and non covalent forces.
- They are 1. **Hydrogen bonds 2. Salt bonds 3. Hydrophobic interaction** (Fig. 4).
- **Hydrogen bonds:** These are the major forces that hold and maintain the protein structure. Hydrogen bond formed between peptide bonds in the same chain or between peptide bond of different amino acids.

- If the hydrogen bonds form between peptide bonds in the same chain, helical structures such as the α-helix, develop.
- If the hydrogen bonds are formed between peptide bonds in different chains, extended structures such as the β-configuration, develop.
- **Salt bonds or ionic bonds** link oppositely charged chain groups.
- A **disulphide bond (SH) is a covalent bond** that contributes to the stability of the 3-dimensional shapes of the protein molecules and prevents them from becoming denatured in the various extracellular environments.
- E.g. Myoglobin, cytochrome C, Ribonuclease, Chymotrypsin, lysozyme and carboxypeptidase.
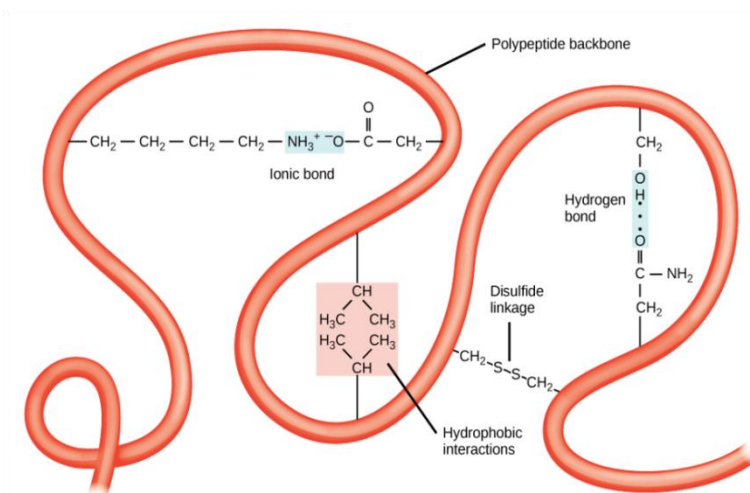
Tertiary structure of proteins

Bonds involved in stabilizing tertiary structure

1. Hydrogen bonds

2. Hydrophobic interactions

3. Ionic or electrostatic interactions

4. Disulphide bonds

5. Vanderwaal's forces

QUATERNARY STRUCTURE

Quaternary structure refers to the structure of a protein macromolecule formed by interactions between multiple polypeptide chains. Each polypeptide is referred to as a subunit. Hemoglobin is an example of a protein with quaternary structure. Hemoglobin, found in blood, is an iron containing protein that binds oxygen molecules. It contains 4 subunits: two alpha subunits and 2 beta subunits (Fig. 5).
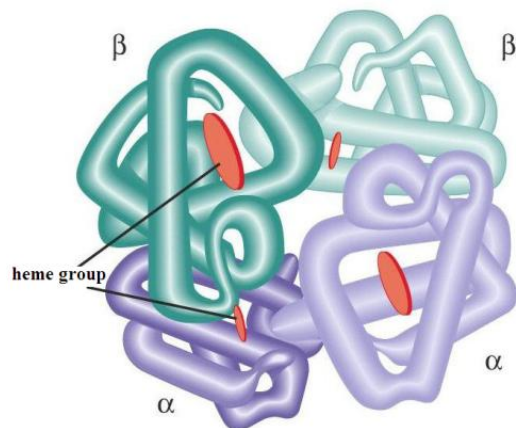
Quaternary Protein Sructue: Three-dimensional assembly of subunits

.

Quaternary structure of proteins

The quartarnary structure is of 2 types

1. Homogenous quarternary structure

2. Heterogenous quarternary structure.

Based on the nature of the polypeptide chain

1. Homogenous quarternary structure

In this type, the polypeptide chains are identical.

Ex ; LDH (lactate dehydrogenase)

2. Heterogenous quarternary structure

In this type the polypeptide chains are non-identical.

Ex - haemoglobin.

Forces stabilizing the quarternary structure

1. Hydrogen bond
2. Hydrophobic interactions
3. Ionic bond
4. Disulphide bond
5. Vanderwaal's forces.

UNIT 4

LIPIDS

What are Lipids?

➢ These organic compounds are soluble only in non-polar solvents and insoluble in water because water is polar molecules. In the human body, these molecules can be synthesized in the liver and are generally found in the oil, butter, whole milk, cheese, fried foods, and also in some red meats.

INTRODUCTION

Lipids- The word lipid derived from a greek word Lipos-fat.

Lipids are a heterogeneous group of compounds which are sparingly soluble in water, but soluble in non-Polar solvents (fat solvents) such as acetone, ether, chloroform and benzene.

The lipids are of great biochemical importance because of their role as the chief storage form of energy and because of their role in cellular structures.

They are commercially important in the form of soaps, detergents, greases and the various oils of the paints industry. Chemically, they are esters of fatty acids or are capable of forming esters.

They all contain carbon, hydrogen and oxygen and some have also phosphorous and nitrogen.

DEFINITION

Lipids are organic substances relatively insoluble in water, soluble in organic solvents (alcohol, ether etc.) actually or potentially related to fatty acids and utilized by the living cells.

OCCURRENCE

Lipids are widely distributed throughout the plant and animal kingdom.

- In plants, they occur in the seeds, nuts and fruits.

- In animals, they are stored in adipose tissues, bone marrow and nervous tissues.

  Food sources rich in fat are

  ANIMAL SOURCES: Milk, Ex g, meat, liver and fish oils

  PLANT SOURCES: Nuts, seed and oils.

FUNCTIONS OF LIPIDS

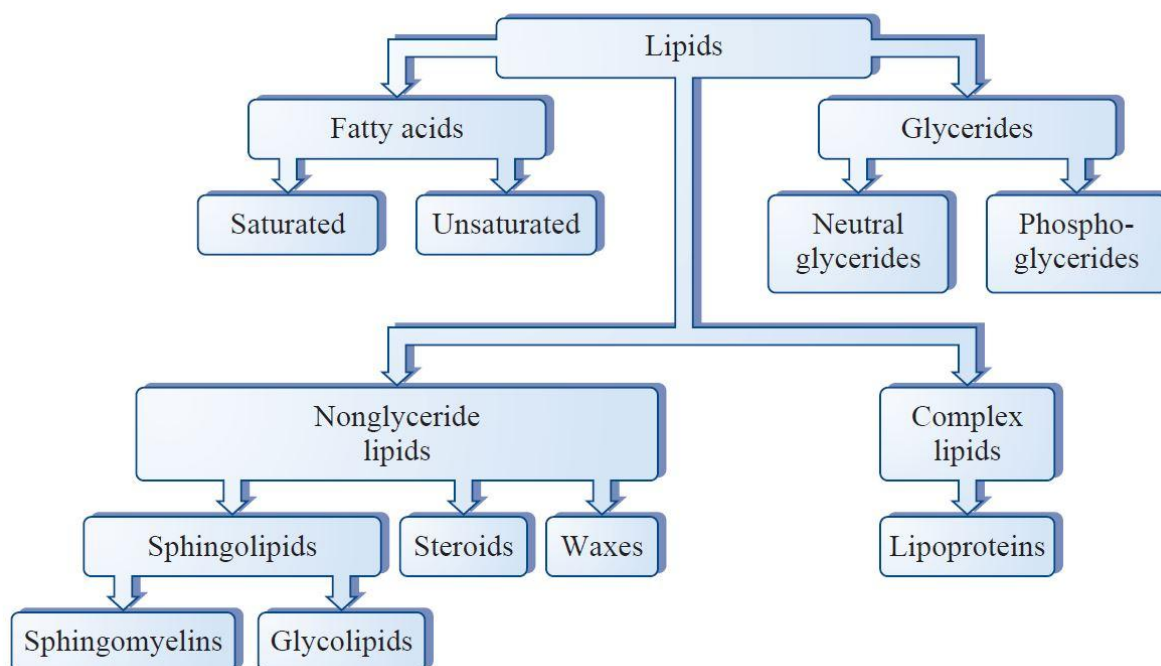They are the concentrated fuel reserve of the body (triacylglycerol).

Lipids are the constituents of membrane structure and rEx ulate the membrane permeability (phospholipids and cholesterol).

They serve as a source of fat soluble vitamins (A, D , E and K)

Lipids are important as cellular metabolic rEx ulators (steroid hormones and prostaglandins).

CLASSIFICATION

Bloor has proposed the following classification of lipids based on their chemical composition. Used in the year 1943.

```
                                    Lipids
               ┌──────────────────────┬┼──────────────────────┐
          Fatty acids                 │                   Glycerides
          ┌─────┴─────┐               │               ┌────────┴────────┐
      Saturated   Unsaturated         │           Neutral        Phospho-
                                       │           glycerides     glycerides
           ┌───────────────────────┬──┴────────────────────┐
      Nonglyceride                                      Complex
        lipids                                           lipids
    ┌──────┬──────┐                                        │
Sphingolipids  Steroids  Waxes                        Lipoproteins
  ┌────┴────┐
Sphingomyelins  Glycolipids
```

1. SIMPLE LIPIDS (OR) HOMOLIPIDS

There are esters of fatty acids with various alcohols.

a. Fats and oils

There are esters of fatty acid with glycerol.

A fat is solid at room temperature, an oil is lipid at room temperature.

b. Waxes:

These are esters of fatty acids with high molecular weight monohydric alcohols.

2. COMPLEX (OR) COMPOUND LIPIDS (OR) HETEROLIPIDS

These are esters of fatty acids with alcohol and posses additional group.

a. Phospholipids

These are compounds containing in addition to fatty acids and glycerol, a phosphoric acids, nitrogen bases and other substituents.

(i) GLYCEROPHOSPHOLIPID:

In this type glycerol is the alcohol.   Ex : Lecithin, caphalin.

(ii) SPINGOPHOSPHOLIPID:

Spingosine is the alcohol in this group. Ex ; spingomyclin.

b. GLYCOLIPIDS:

They are lipids containing carbohydrates and nitrogen, but no phosphoric acid and glycerol. Alcohol is sphingosine, hence they are also called as glycosphingolipid.

c. SULPHOLIPIDS:

They are lipids containing sulphate groups.

d. LIPOPROTEINS:

These are lipids attached to proteins. They are present in plasma and tissues.

3. DERIVED LIPIDS

These are the substances derived from simple and compound lipids by hydrolysis. These include glycerol and other alcohols, fatty acids, mono and diacylglycerols, lipid soluble vitamins, steroid hormones, hydrocarbons and ketone bodies.

4. MISCELLANEOUS LIPIDS

These include a large number of compounds possessing the characteristics of lipids. Ex . Carotenoids.

FUNCTIONS OF LIPIDS

Functions of lipids

In the human body, **triglycerides** are mostly stored in fat cells, called **adipocytes**, which form **adipose tissue**. Adipose tissue is primarily used as an **energy store**, but also helps to **protect** and **insulate** the body. Lipids have a variety of functions in the cell.

Energy storage – Triglyceride breakdown yields more energy than the breakdown of carbohydrates because the carbons are all bonded to hydrogens (and they, therefore, have a higher proportion of hydrogens relative to oxygens). This means they are electron-rich and can contribute to the production of **acetyl-CoA**, which is an important co-enzyme in aerobic respiration.

Biological membranes – As previously discussed, cell membranes are principally composed of a **phospholipid bilayer**. Phospholipids are another type of lipid, created when a phosphate group replaces one of the three fatty acid chains. Phospholipids have a **hydrophobic** part and a **hydrophilic part**. The fatty acid chains remain hydrophobic, forming the tail of the molecule, but the addition of the phosphate group to the head makes this part of the molecule hydrophilic, meaning a bilayer forms.

Hormone production – many hormones are lipid-derived, and they usually belong to a class of hormones called **steroid hormones**. These hormones are usually derived from cholesterol and are often structurally similar to cholesterol. Steroid hormones are important signalling molecules that can enter the cell directly through the cell membrane and influence gene expression and signalling pathways. Examples include cortisol and testosterone

➢ The lipids are the important constituent of diet.

➢ They also serve as an insulating material.

➤ Many hormones such as steroid are lipids.

➤ It is a bad conductor of heat.

➤ It maintains the body temperature.

➤ Myelin sheath of nerve fibres contains lipids which are electrical insulators.

➤ Waxes generally form protective covering in plants and animals.

➤ Fatty tissues around many vital organs protect against mechanical injury.
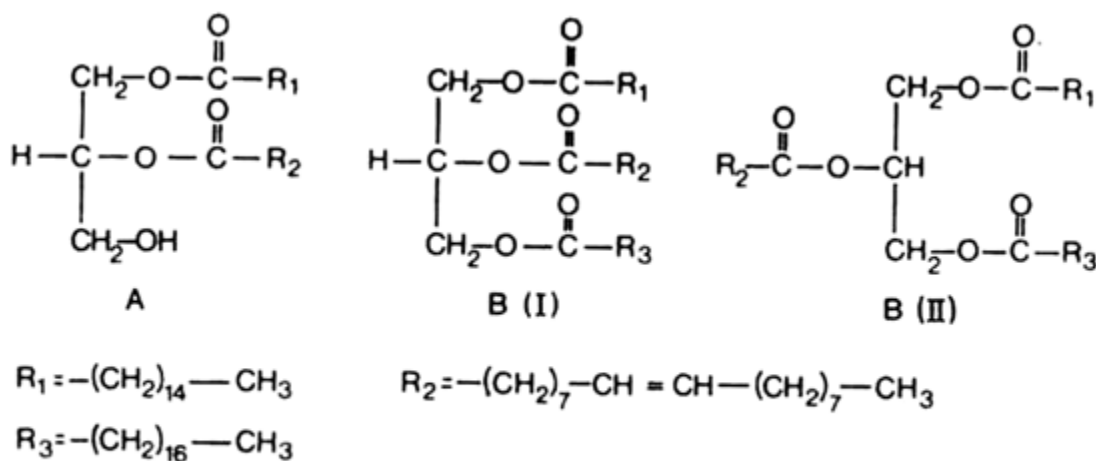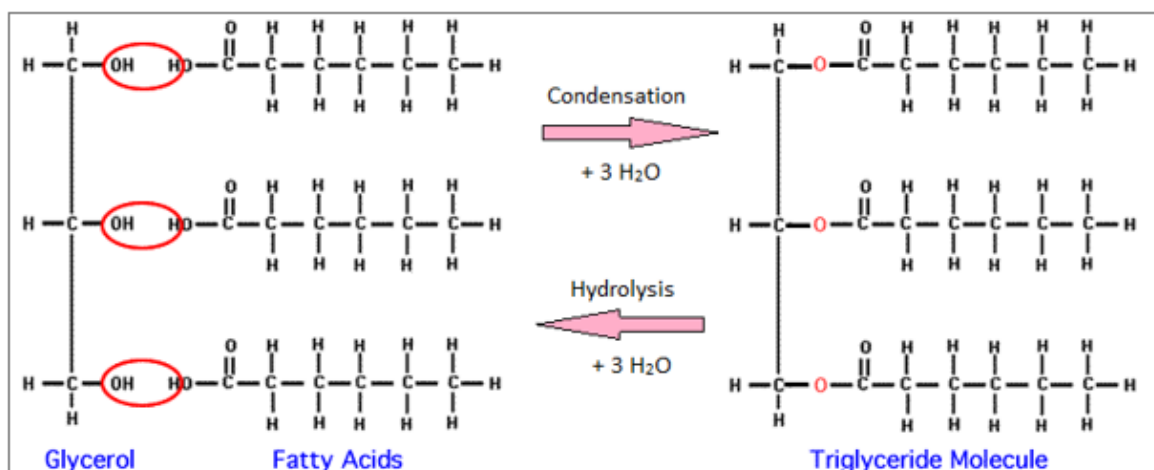
SIMPLE LIPIDS

FATS AND OILS (triglycerides)

$$R_1 = -(CH_2)_{14} - CH_3 \qquad R_2 = -(CH_2)_7 - CH = CH - (CH_2)_7 - CH_3$$
$$R_3 = -(CH_2)_{16} - CH_3$$

FIG. 5-2. — Structures of di- and triglycerides.

Glycerol   Fatty Acids   Triglyceride Molecule

OCCURRENCE:

The most abundant form of the simple lipids are the fats and oils which make up over 90% of the lipid adipose tissue in animals.

In plants, they are particularly abundant in nuts and seeds, where they represent reserve food material.

The triglycerides are the most abundant lipids in nature.

It is also called as neutral fat.

CHEMISTRY:

A fat molecule contains 3 moles of fatty acids which may be similar or dissimilar. Most of the naturally occurring triglycerides are mixed triglycerides i.e they contain 2 or 3 different fatty acid units in the molecule.

Ex . Oleodipalmitin, oleopalmitostearin

Symmetrical triglycerides, in which all the 3 fatty acid units are the same, occur very rare. They are mostly synthesized in the laboratory

Ex . Tristearin, triotein.

Fats and oils are generally differentiated by their melting ranges.

Oils are lipids at room temperature; fats are solids (or) semisolid.

Fats contain larger proportion of saturated fatty acids (animal fats) with high melting point. Oils contain larger proportion of unsaturated fatty acids (plant fat) with low melting point.

BIOLOGICAL FUNCTIONS

Triglycerides are the most abundant group of lipids that primarily function as fuel reserves of animals.

WAXES

OCCURRENCE:

Waxes are present in plants and animals.

CHEMISTERY:

These are esters of fatty acids with alcohol than glycerol.

In some waxes, the alcohol component may be cholesterol. Such waxes are found in blood. Ex. Cholesteryl palmitate i.e esters of cholesterol and palmitic acid.

COMMON WAXES

Sperm whale wax, bees wax, carnauba wax. Waxes are insoluble in water, but soluble in fat solvents.

They are not easily hydrolyzed like fats or digested by lipases. Therefore, they are of no value nutritionally.

BIOLOGICAL FUNCTIONS

➢ The most important physiological function of waxes is a protective agent on the surfaces of animals and plants.

➢   Waxes are found on the surfaces of feathers and hair with form the excessive loss of moisture. Hence desert plants like palm and cactus can live for long periods without rain.

➢   Waxy coating protects the plant from becoming infected with fungi and bacteria which causes disease.

➢ The waxes serve as water barrier in insects, birds and furred animals.

PHOSPHOLIPIDS

OCCURRENCE

Phospholipids are the most abundant the complex lipids.

It is found in every living cell and makes up as much as 70% of the complex lipid contents of the tissues. These substances are known as phospholipids. There are 2 classes of phospholipids

1. GLYCEROPHOSPHOLIPIDS

Phosphoglycerides, they contain glycerol as the alcohol. Glycerophospholipids are the major lipids that occur in biological membranes. Ex ; phosphatidic acid lecithin cephalin , phosphatidylinoitol, phosphatidylserine.

2. SPHINGOMYELINS

Sphingosine is an amino alcohol present in sphingomyelins (sphingophospholipids). They do not contain glycerol.

Sphingosine is attached by an amide linkage to fatty acids to produce ceramide. Sphingomyelins are important constituents of myelin and are found in good quantity in brain and nervous system.

BIOLOGICAL FUNCTIONS

- In association with proteins, phospholipids form the structure compounds of membranes and rEx ulate membrane permeability.

- Phospholipids are essential for the synthesis of different lipoproteins and thus participate in the transport of lipids.

- Accumulation of fat in liver (fatty liver) can be prevented by phospholipids, hence they are rEx arded as lipotropic factors.

Arachidonic acid, an unsaturated fatty acid liberated from phospholipids serves as a precursor for the synthesis of eicosanoids (prostaglandins, thrombaxane).

STEROIDS

Steroids are the compounds containing a cyclic nucleus namely cyclopentanoperhydrophenanthrene (CPPP). If the steroid is having more than one or more hydroxyl groups, it is commonly known as sterol.

Ex . Cholesterol

FIG. 5-7. — Structures of various steroids.
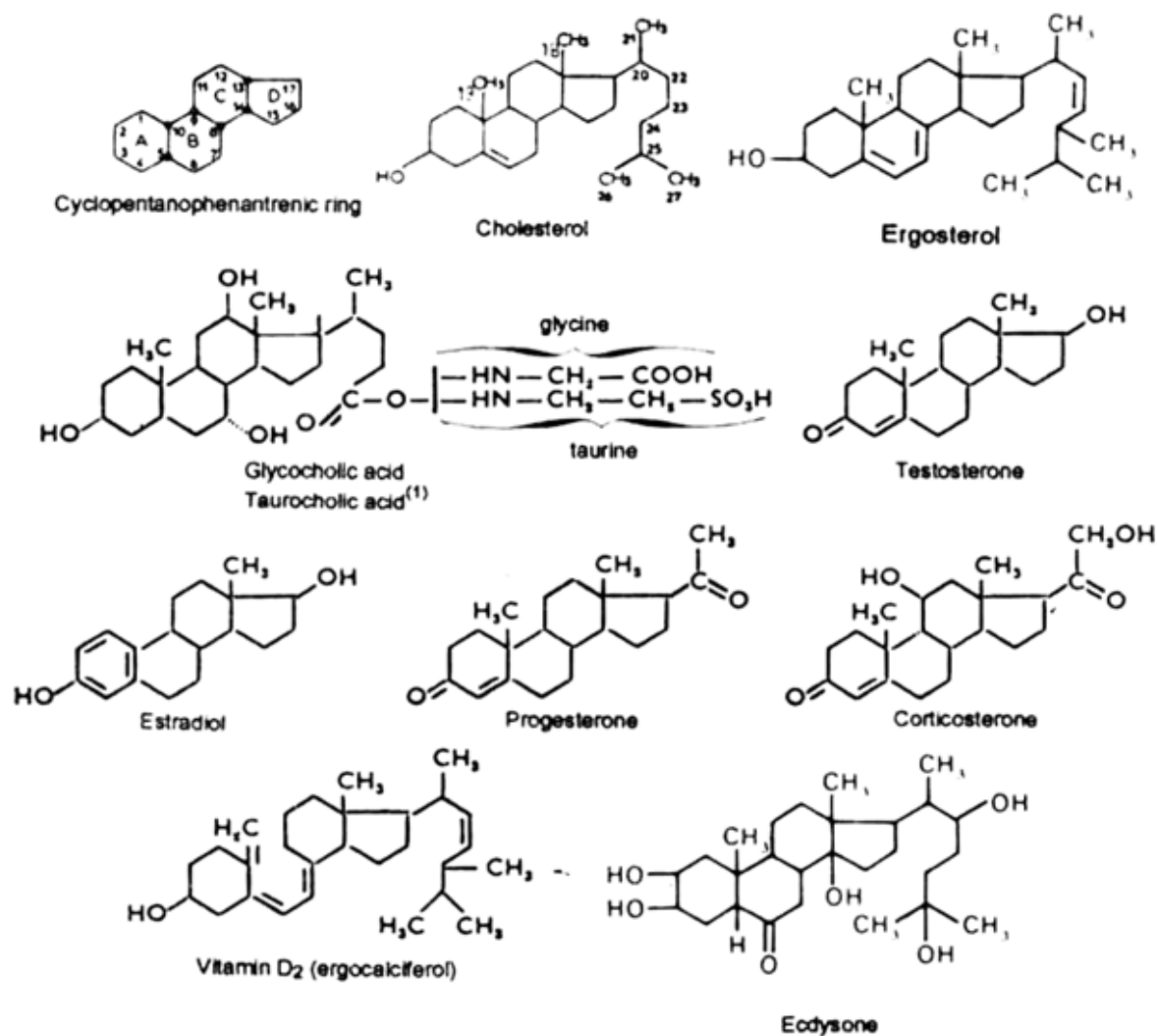
(1) The structures of glycodeoxycholic and taurodeoxycholic acids are identical except for the fact that in position 7 there is a H atom instead of a OH group.

CHOLESTEROL

OCCURRENCE

It is the principal sterol of higher animals and is especially abundant in nerve tissues and in gallstones.

CHEMISTRY:

Molecular formula – $C_{27}H_{45}OH$

BIOLOGICAL FUNCTIONS

Cholesterol functions as an insulating cover for the transmission of electrical impulse in the nervous tissue. It performs several other role in membrane structure and function, in the synthesis of bile acids, hormones and vitamin D

FATTY ACIDS

They are carboxylic acids with hydro carbons side chain. They are the simplest form of lipids.

SATURATED FATTYACIDS

These are the fatty acids containing only single bonds. General molecular formula $CnH_{2n+1}COOH$. They are found in both plants and animals. They contain even number of carbon atoms. Generally presence of saturated fatty acid makes the fat solid

Ex ; Acetic acid CH_3COOH (VinEx ar)

Butyric acid $CH_3(CH_2)_2COOH$ (Butter)

Arachidonic acid $CH_3(CH_2)_{18}COOH$ (Peanut oil)

Stearic acid $CH_3(CH_2)_{16}COOH$ (Animal and vEx etables fat)

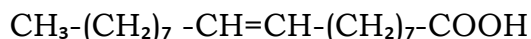Palmitic acid $CH_3(CH_2)_{14}COOH$(Animal and vEx etable fats)

UNSATURATED FATTY ACIDS

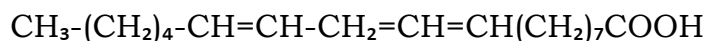These are fatty acids containing one or more double bonds.

General molecular formula $CnH_{2n-1}COOH$.

Fatty acids with one double bond are known as monounsaturated and those with 2 or more double bonds are collectively known as polyunsaturated fatty acids (PUFA). Unsaturated fatty acids are present in oil.

Ex . Oleic acid(1=)

$CH_3-(CH_2)_7 -CH=CH-(CH_2)_7-COOH$

   Linleic acid (2=)

$CH_3-(CH_2)_4-CH=CH-CH_2=CH=CH(CH_2)_7COOH$

   Linolenic acid (3=)

$CH_3CH_2CH=CHCH_2CH=CHCH_2CH= CH(CH_2)_7COOH$

ESSENTIAL FATTY ACID (EFA)

The fatty acid that cannot be synthesized by the body and therefore, should be supplied in the diet are know as essential fatty acids (EFA).

Chemically, they are polyunsaturated fatty acids, namely linoleic acid , linolecic acid, arachidonic acid.

PHYSICAL PROPERTIES

Lipids are insoluble in water and soluble in organic solvents like benzene, ether and chloroform. They are thus hydrophobic in nature. They are oily and greasy in nature.

EMULSIFICATION

It is the process by which the lipid mass is converted   into a number of small lipid droplets.

The fats may be emulsified by shaking either with water or with emulsifying agents like soaps, gum, proteins etc.

The agent that is used in emulsification is called emulsifying agent.

This process is accomplished by the bile juice secreted from liver.

The carboxyl group of fats and the hydrocarbons group will tend to aggrEx ate with the emulsifier leaving the water molecule as aqueous solution.

CHEMICAL PROPORTIES

SAPONIFICATION (OR) SOAP FORMATION

The hydrolysis of fats by alkali is called saponification. This reaction results in the formation of glycerol and salts of fatty acids called soap.

Fat → Glycerol+ salts of fatty acids (soap).   (alkali)

The soaps are of 2 types

(i) **HARD SOAP:**

These are the common bar soap are the Na salts of fatty acids.

(ii) **SOFT SOAP:**

These are the potassium salts of fatty acids which are semisolid or paste in nature.

RANCIDITY

The process of auto oxidation taking place in edible oils and fats is called rancidity. The mechanism taking place in this is called rancification.

The chemical changes taking place in rancification is called rancidity. It is of 2 types

(i) HYDROLYTIC RANCIDITY

It takes place in butter and fats, when butter is stored for a long time rancidity is caused by the growth of micro organisms which secret enzymes like lipases. These split the fats into glycerol and free fatty acids. This causes an unpalatable odour and taste to the fat.

It can be prevented by refrigeration.

(ii) OXIDATIVE RANCIDITY

It takes place mainly in the oils containing unsaturated fatty acids. The oxidation takes place slowly and results in the formation of short chain fatty acids and aldehydes which give a rancid taste and odour to the fats. It is due to the reaction called "antioxidant".

It takes place mainly in animal fat than in vEx etable fats, because the animal fat does not contain any antioxidant.   Ex . Tocopheral (or) vitamin E.

ACID NUMBER

It is the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in 1 gram of fat.   It tells about the quantity of free fatty acid present in a fat.

SAPONIFICATION NUMBER

It is the number of milligrams of potassium hydroxide required to saponify 1 gram of fat. It provides information of the average chain length of fatty acids in fat.

IODINE NUMBER

It is the number of grams of iodine absorbed by 100 gm of fat. It gives a measure of the dEx ree of unsaturation of fatty acid.   Oils like soybean, corn and cottonseed have higher iodine number. It does not give any information on the number of double bonds.

REICHERT MEISSL NUMBER

It is the number of millitres of 0.1 N KOH required to neutalize the soluble, volatile fatty acids derived from 5g of fat. It gives a measure on the

quantity of short chain fatty acids. The reichert meissl number of coconut and palm oil range from 5-8 and for butter from 17-35.

This makes possible detection of any foreign fats.

BILE ACID

The liver secretes a clear, golden yellow, viscous fluid known as bile.

It is stored in the gall bladder and is mainly useful for digestive system. Bile consists of inorganic (chiefly HCO^-_3, Cl^-, Na^+, K^+ etc) ions as well as organic compounds.

Among organic compounds the main constituents are bile acids, bile pigments, lipids, fatty acids and cholesterol. The bile acids are present as the sodium salt of amide with glycine or taurine.

Ex . Sodium glyocholate (glycine+ cholic acid) and taurocholate (taurine + cholic acid)

Bile acids are the hydroxy derivatives of either cholanic or allocholanic acid and dehydration followed by reduction of the bile acids gives the latter (cholanic (or) allocholanic acid). The most abundant bile acids in human bile are cholic acid (25- 60 % of the total bile acids), deoxy cholic acid (5- 25 %) and chenodeoxy cholic acid (30- 50%) .

Bile acids are formed by oxidation of cholesterol in the liver.

BILE SALTS

Since bile contains significant quantities of sodium and potassium and the ph is alkaline, it is assumed that the bile acids and their conjugates are actually in salt form, hence the term bile salts bile salts synthesized in the liver are excreted in bile.

FUNCTIONS

Bile salts the digestion promoting constituents of bile. They are surface active agents, i.e they lower surface tension and thus can emulsify fats. They also activate lipases.

FUNCTIONS OF BILE ACIDS

They facilitate the digestion of fats by emulsifying them and thereby increasing the surface area of the material for pancreatic enzymes. They also activate the enzyme cholesterol esterase and pancreatic lipase.

They help in the absorption of cholesterol fat soluble vitamins (A, D, E,K) , farming water soluble complexes.

They also keep cholesterol in solution, if the ratio between bile acids and cholesterol is precipitated and forms gall stones in liver , and gallbladder.

The bile acid in the bile entering the intestine are rapidly absorbed into the blood, taken back by the liver and reutilized. This is called enter hepatic circulation of bile salts. Unabsorbed bile acids are attacked by bacteria and decomposed into various products which are excreted in faeces.

UNIT 5

NUCLEIC ACID

INTRODUCTION

Nucleic acid, naturally occurring chemical compound that is capable of being broken down to yield phosphoric acid, sugars, and a mixture of organic bases (purines and pyrimidines). Nucleic acids are the main information-carrying molecules of the cell, and, by directing the process of protein synthesis, they determine the inherited characteristics of every living thing. The two main classes of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is the master blueprint for life and constitutes the genetic material in all free-living organisms and most viruses. RNA is the genetic material of certain viruses, but it is also found in all living cells, where it plays an important role in certain processes such as the making of proteins.

Nucleic acid were first reported by Miescher in 1871.

They have high molecular weight.
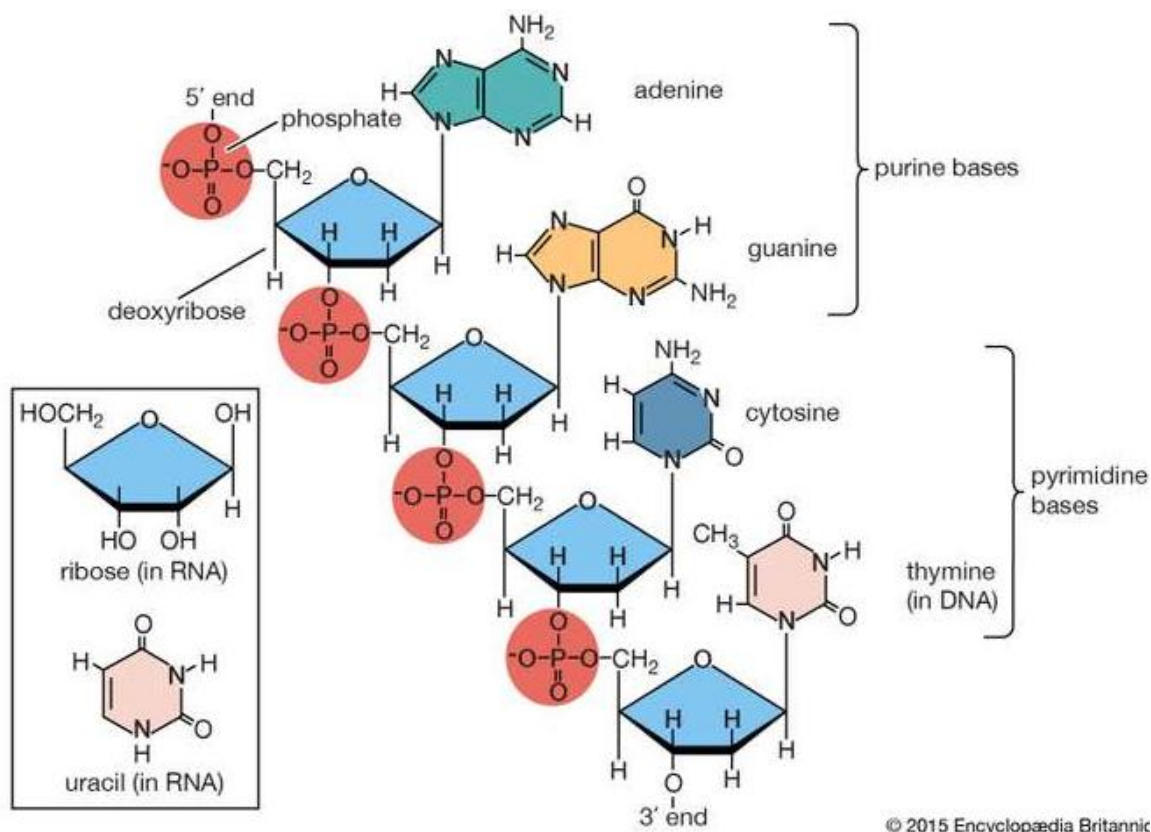
Elements constituting nucleic acid are C,H,O,N and P.

Nucleic acids are colourless complex compounds which are made up of 3 units.

→Nitrogenous bases (purine (or) pyrimidine)

→ Sugar

→Phosphoric acid

There are 2 types of nucleic acids

   1. Ribo nucleic acid (RNA)

   2. Deoxy Ribo nucleic acid (DNA)

DEFINITION

Nucleic acids are defined as polynucleotide in which repeating units of are ribonucleotides (or) deoxyribonucleotides.

FUNCTION OF NUCLEIC ACIDS

➢ RNA directs protein synthesis (translation).

DNA acts as a genetic material, transfer information from parents to their offspring.

NUCLEOSIDE

A nucleoside is a chemical combination of a pentose sugar and a pyrimidine or a purine base.

Nitrogenous base + pentose sugar→ Nucleoside + H_2O

Nucleoside containing Ribo sugar are called as ribonucleosides.

Ex . Adenosine→ Adenine + Ribose

Nucleoside containing Deoxy ribose suger are called as deoxyribonucleoside.

Ex . Deoxyadenosine → Adenine + Deoxy ribose.

NUCLEOTIDES

They are phosphoesters of nucleosides.

Nucleotides are composed of 3 units

Phosphate group + pentose sugar + nitrogenous bases.

Nucleoside+ phosphoric acid→ Nucleotide + H_2O

Ex . Adenylic acid →Adenosine+ phosphoric acid.

POLY NUCLEOTIDES

They are composed of many mononucletide units.

Ex . Nucleic acids composed of nucleotides.

DNA- is a polymer Deoxyribonucleotides.

RNA- is a polymer of Ribonucleotides.

They are linked by phosphodiester bond.

$3' \rightarrow 5'$ phosphodiester bond forms the "backbone" of polynucleotides such as RNA and DNA.
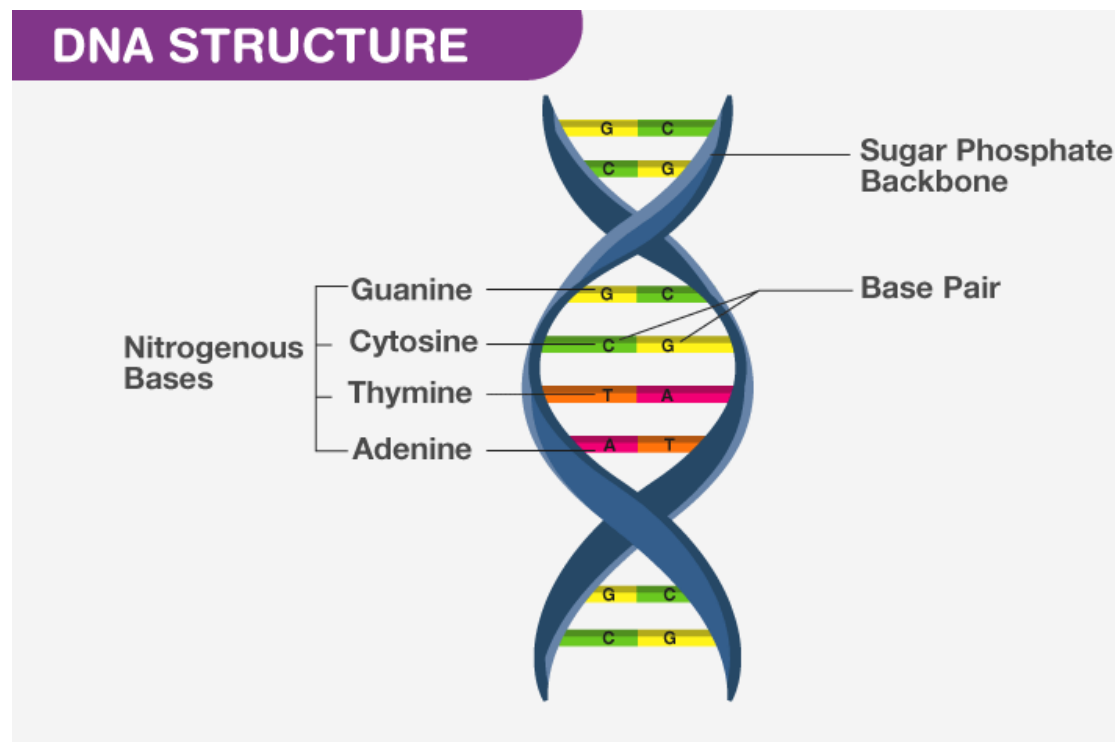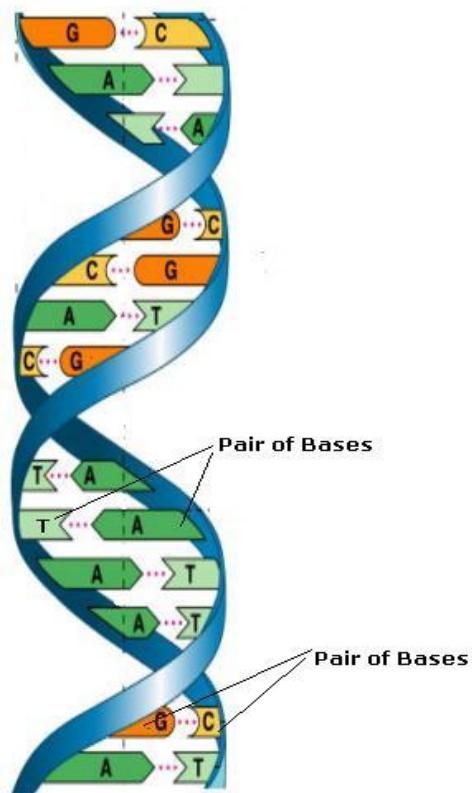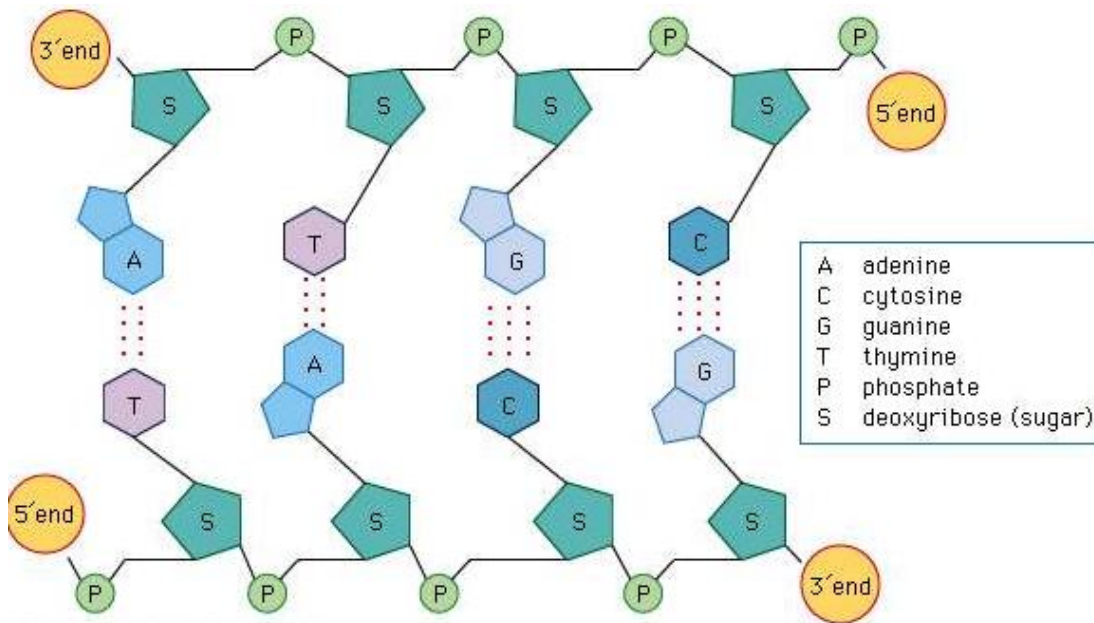
DNA

INTRODUCTION

➢ DNA- Deoxyribonucleic acid

➢ DNA is polymer of deoxyribonucleotides.

➢ Double helical model of DNA was proposed by J.D.Watson and F.H.C.Crick in 1953.

DNA is present in cells of the all plants, animals, prokaryotes and in number of viruses.

STRUCTURE OF DNA

> ➤ The DNA is right handed helix.

> ➤ It consists of 2 polydeoxyribonucleotide chains (strands) twisted around each other on a common axis.

> ➤ The 2 stands are antiparallel in direction i.e, one strand is 3'→5' while another strand is 5'→3' direction.

> ➤ The width of a double helix is 20s (=2.0 nm)

> ➤ Each turn (pitch) of the helix is 34 A°(=3.4 nm)

> ➤ There are 10 nucleotides per turn

The back bone of DNA is phosphodiester bond between sugar and phosphate.

> ➤ The 2 strands are held together by hydrogen bonds formed by complementary base pairs.

i.e. Adenine always pair with thymine by 2 hydrogen bonds

$$A = T$$

Guanine always pair with cytosine by 3H bonds

94

G Ɛ C

Uracil is absent in DNA

ERWIN CHARGAFF'S RULE

Sum of purinnes = Sum of purimidines

A + G

------- = 1

T + C

The ratio of adenine to thymine is one. The ratio of guanine to cytosine is also one.

Chemical structure

In 1953 James D. Watson and Francis H.C. Crick proposed a three-dimensional structure for DNA based on low-resolution X-ray crystallographic data and on Erwin Chargaff's observation that, in naturally occurring DNA, the amount of T equals the amount of A and the amount of G equals the amount of C. Watson and Crick, who shared a Nobel Prize in 1962 for their efforts, postulated that two strands of polynucleotides coil around each other, forming a double helix.

The two strands, though identical, run in opposite directions as determined by the orientation of the 5′ to 3′ phosphodiester bond.

The sugar-phosphate chains run along the outside of the helix, and the bases lie on the inside, where they are linked to complementary bases on the other strand through hydrogen bonds.
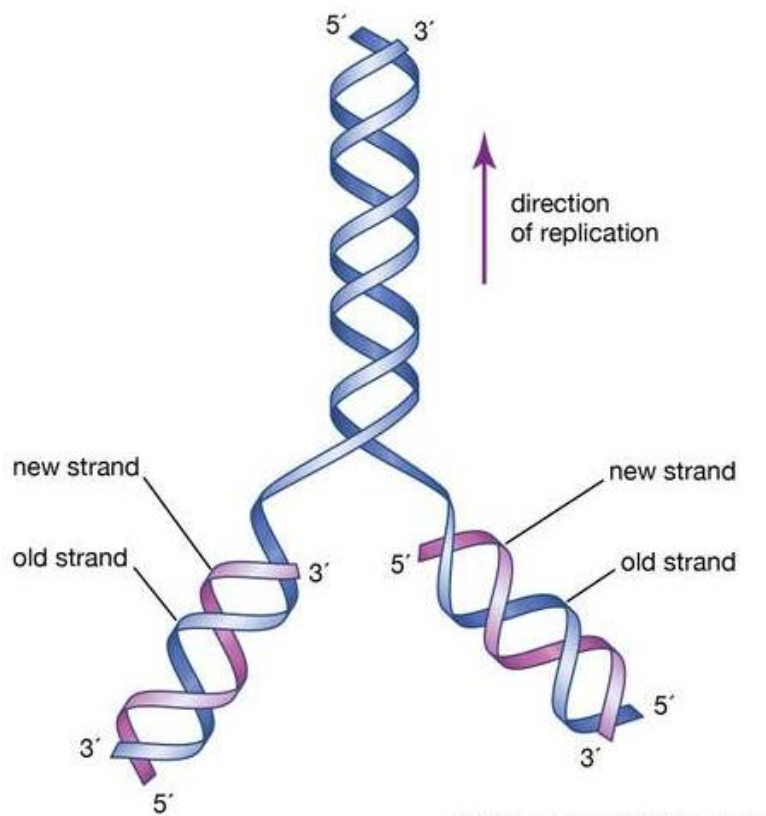
Biological structures

Naturally occurring DNA molecules can be circular or linear. The genomes of single-celled bacteria and archaea (the prokaryotes), as well as the genomes of mitochondria and chloroplasts (certain functional structures within the cell), are circular molecules.

In addition, some bacteria and archaea have smaller circular DNA molecules called plasmids that typically contain only a few genes. Many plasmids are readily transmitted from one cell to another. For a typical bacterium, the genome that encodes all of the genes of the organism is a single contiguous circular molecule that contains a half million to five million base pairs.

The genomes of most eukaryotes and some prokaryotes contain linear DNA molecules called chromosomes.

Human DNA, for example, consists of 23 pairs of linear chromosomes containing three billion base pairs.

In all cells, DNA does not exist free in solution but rather as a protein-coated complex called chromatin.

In prokaryotes, the loose coat of proteins on the DNA helps to shield the nEx ative charge of the phosphodiester backbone. Chromatin also contains proteins that control gene expression and determine the characteristic shapes of chromosomes.

In eukaryotes, a section of DNA between 140 and 200 base pairs long winds around a discrete set of eight positively charged proteins called a histone, forming a spherical structure called the nucleosome.

Additional histones are wrapped by successive sections of DNA, forming a series of nucleosomes like beads on a string.

Transcription and replication of DNA is more complicated in eukaryotes because the nucleosome complexes have to be at least partially disassembled for the processes to proceed effectively.
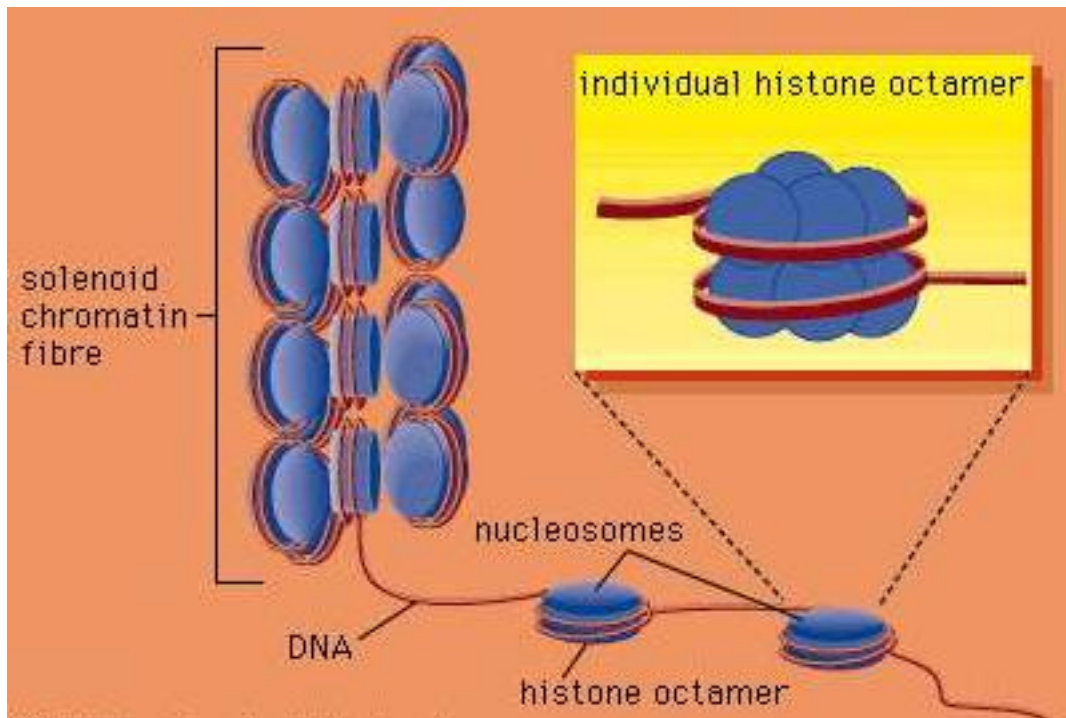
Figure 4-DNA wrapped around clusters of histone proteins to form nucleosomes

Most prokaryote viruses contain linear genomes that typically are much shorter and contain only the genes necessary for viral propagation.

Bacterial viruses called bacteriophages (or phages) may contain both linear and circular forms of DNA. For instance, the genome of bacteriophage λ (lambda), which infects the bacterium Escherichia coli, contains 48,502 base pairs and can exist as a linear molecule packaged in a protein coat.

The DNA of phage λ can also exist in a circular form (as described in the section Site-specific recombination) that is able to intEx rate into the circular genome of the host bacterial cell.

Both circular and linear genomes are found among eukaryotic viruses, but they more commonly use RNA as the genetic material.

Types of DNA

Three major forms of DNA are double stranded and connected by interactions between complementary base pairs. These are terms A-form, B-form, and Z-form DNA.

B-form DNA

The information from the base composition of DNA, the knowledge of dinucleotide structure, and the insight that the X- ray crystallography suggested a helical periodicity were combined by Watson and Crick in 1953 in their proposed model for a double helical structure for DNA. They proposed two strands of DNA-each in a right- hand helix -- wound around the same axis. The two strands are held together by H- bonding between the bases (in anti conformation) as shown in Figure.7
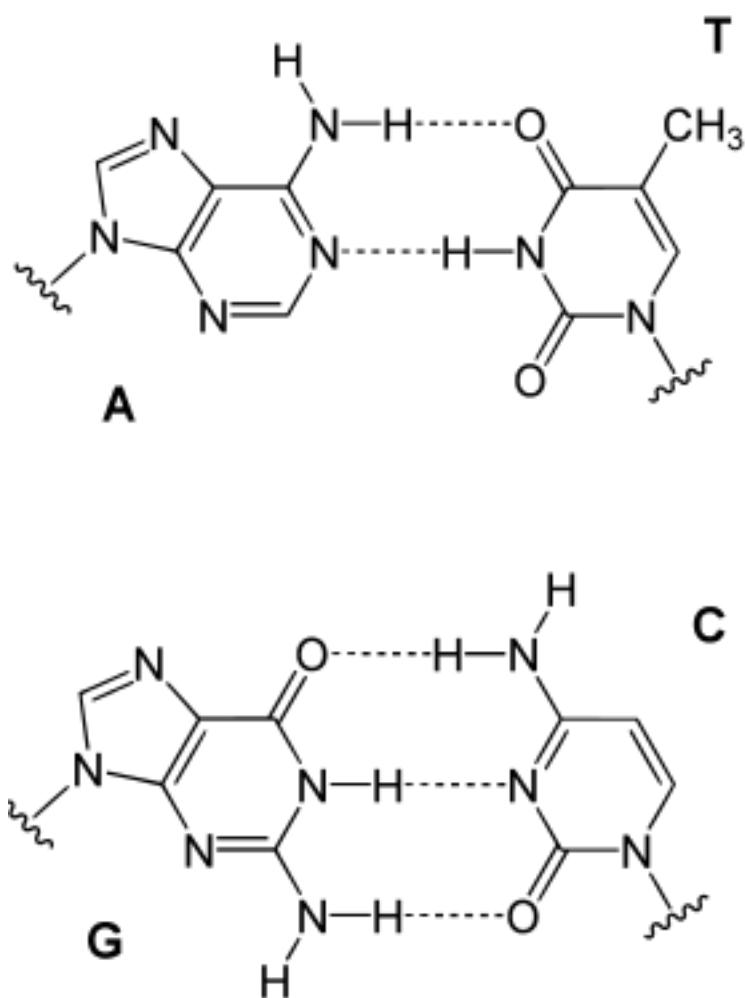
Figure 7-Hydrogen bond between bases (Top-An A:T base pair and Bottom a G:C base pair).

Bases fit in the double helical model if pyrimidine on one strand is always paired with purine on the other. From Chargaff's rules, the two strands will pair A with T and G with C. Two H- bonds can form between A and T, and three can form between G and C. This third H-bond in the G:C base pair is between the additional exocyclic amino group on G and the C2 keto group on C. The pyrimidine C2 keto group is not involved in hydrogen bonding in the A:T base pair.

These are the complementary base pairs. The base- pairing scheme immediately suggests a way to replicate and copy the genetic information.

(a)

0.34 nm

3.4 nm

2 nm

(b)

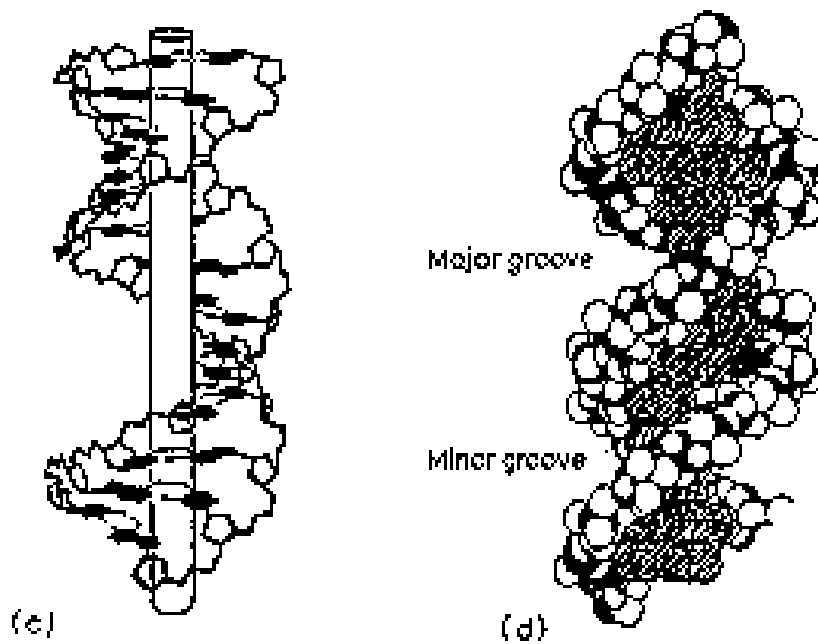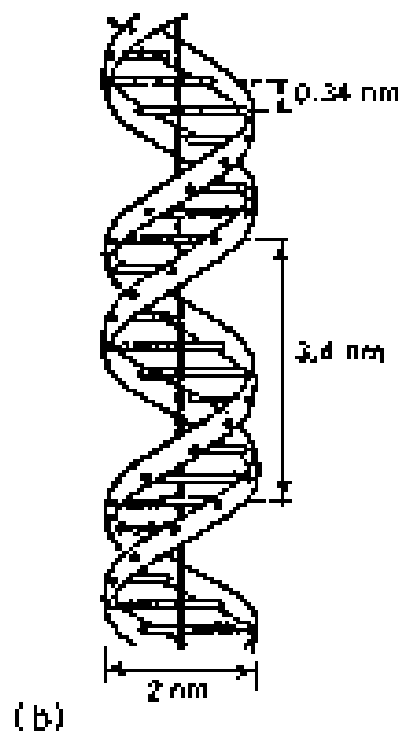(c)

Major groove

Minor groove

(d)
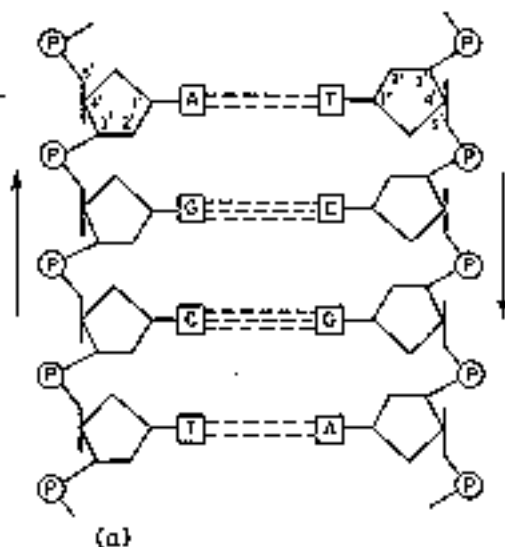
Figure 8 explains about the Antiparallel (a), plectonemically coiled (b, c, d) DNA strands. The arrows in a are pointed 3' to 5', but they illustrate the antiparallel nature of the duplex. The two strands of the duplex are antiparallel and plectonemically coiled.

The nucleotides arrayed in a 5' to 3' orientation on one strand align with complementary nucleotides in the 3' to 5' orientation of the opposite strand.

The two strands are not in a simple side- by- side arrangement, which would be called a paranemic joint. Rather the two strands are coiled around the same helical axis and are intertwined with themselves (which is referred to as a plectonemic coil). One consequence of this intertwining is that the two strands cannot be separated without the DNA rotating, one turn of the DNA for every "untwisting" of the two strands.
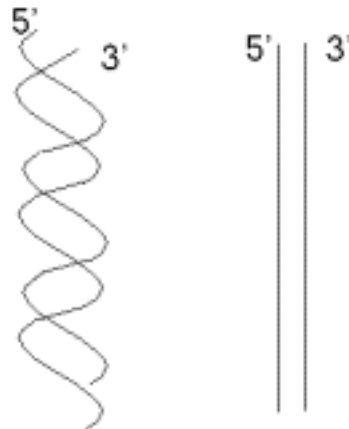
In a plectonemic coil, the two strands wrap around each other.
In a paranemic joint, the two strands align side-by-side.

Figure 9-Duplex DNA has the two strands wrapped around each other in a plectonemic coil (left), not a paranemic duplex (right)

Dimensions of B-form (the most common) of DNA

- 0.34 nm between bp, 3.4 nm per turn, about 10 bp per turn

- 1.9 nm (about 2.0 nm or 20 Angstroms) in diameter

Major and minor groove

The major groove is wider than the minor groove in DNA (Figure 8.d), and many sequence specific proteins interact in the major groove. The N7 and C6 groups of purines and the C4 and C5 groups of pyrimidines face into the major groove, thus they can make specific contacts with amino acids in DNA-binding proteins.

Thus specific amino acids serve as H- bond donors and acceptors to form H-bonds with specific nucleotides in the DNA.

H- bond donors and acceptors are also in the minor groove, and indeed some proteins bind specifically in the minor groove. Base pairs stack, with some rotation between them.

A-form nucleic acids and Z-DNA

Three different forms of duplex nucleic acid have been described. The most common form, present in most DNA at neutral pH and physiological salt concentrations, is B-form. That is the classic, right-handed double helical structure we have been discussing. A thicker right-handed duplex with a shorter distance between the base pairs has been described for RNA-DNA duplexes and RNA-RNA duplexes. This is called A-form nucleic acid.

A third form of duplex DNA has a strikingly different, left-handed helical structure. This Z DNA is formed by stretches of alternating purines and pyrimidines, e.g. GCGCGC, especially in nEx atively supercoiled DNA.

A small amount of the DNA in a cell exists in the Z form. It has been tantalizing to propose that this different structure is involved in some way in rEx ulation of some cellular function, such as transcription or rEx ulation, but conclusive evidence for or against this proposal is not available yet.

Differences between A-form and B-form nucleic acid

The major difference between A-form and B-form nucleic acid is in the conformation of the deoxyribose sugar ring. It is in the C2' endoconformation for B-form, whereas it is in the C3' endoconformation in A-form. As shown in Figure 10, if you consider the plane defined by the C4'-O-C1' atoms of the deoxyribose, in the C2' endoconformation, the C2' atom is above the plane, whereas the C3' atom is above the plane in the C3' endoconformation. The latter conformation brings the 5' and 3' hydroxyls (both esterified to the phosphates linking to the next nucleotides) closer together than is seen in the C2' endoconfromation. Thus the distance between adjacent nucleotides is reduced by about 1 Angstrom in A-form relative to B-form nucleic acid (Figure 10).
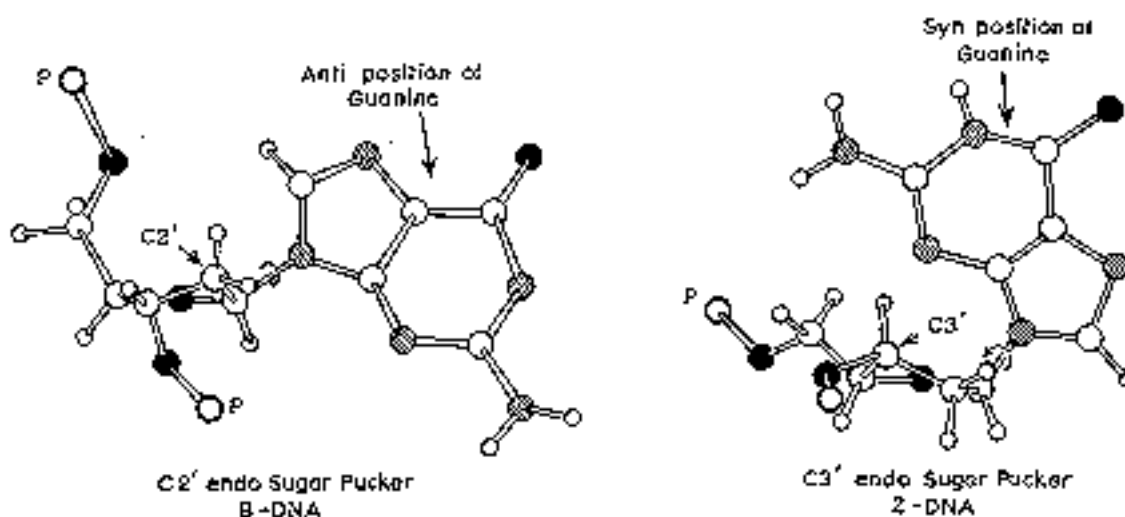
Figure 10-Syn and anti conformations of the base relative to the sugar in nucleotides.

A second major difference between A-form and B-form nucleic acid is the placement of base-pairs within the duplex. In B-form, the base-pairs are almost centered over the helical axis (Figure 10), but in A-form, they are displaced away from the central axis and closer to the major groove. The result is a ribbon-like helix with a more open cylindrical core in A-form.

Z-form DNA

Z-DNA is a radically different duplex structure, with the two strands coiling in left-handed helices and a pronounced zig-zag (hence the name) pattern in the phosphodiester backbone.

As previously mentioned, Z-DNA can form when the DNA is in an alternating purine-pyrimidine sequence such as GCGCGC, and indeed the G and C nucleotides are in different conformations, leading to the zig-zag pattern. The big difference is at the G nucleotide. It has the sugar in the C3' endoconformation (like A-form nucleic acid, and in contrast to B-form DNA) and the guanine base is in the synconformation. This places the guanine back over the sugar ring, in contrast to the usual anticonformation seen in A- and B-form nucleic acid. Note that having the base in the anticonformation places it in the position where it can readily form H-bonds with the complementary base on the opposite strand. The duplex in Z-DNA has to accommodate the distortion of this G nucleotide in the synconformation. The cytosine in the adjacent nucleotide of Z-DNA is in the "normal" C2' endo, anticonformation.

Even classic B-DNA is not completely uniform in its structure. X-ray diffraction analysis of crystals of duplex oligonucleotides shows that a given sequence will adopt a distinctive structure. These variations in B-DNA may differ in the propeller twist (between bases within a pair) to optimize base stacking, or in the 3 ways that 2 successive base pairs can move relative to each other: twist, roll, or slide.

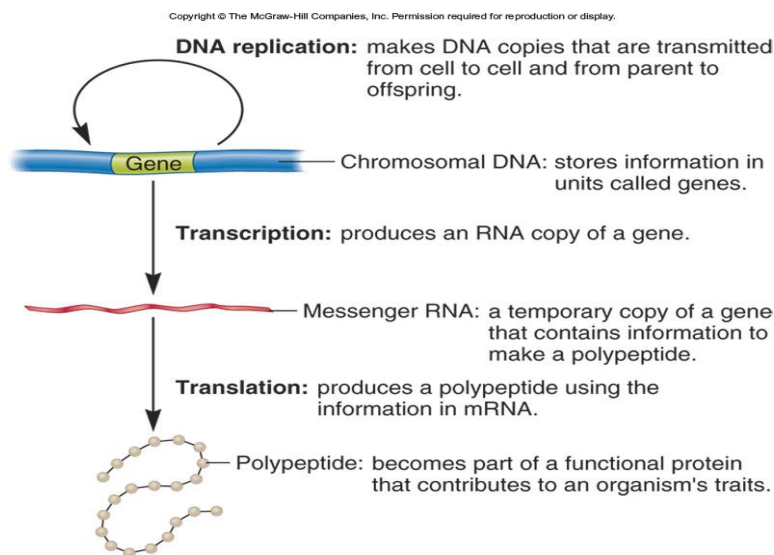**Comparisons of B-form, A-form and Z- DNA**

	B-Form	**A-Form**	**Z-Form**
Helix sense	Right Handed	Right Handed	Left Handed
Base pairs per turn	10	11	12
Vertical rise per bp	3.4 Å	2.56 Å	19 Å

Rotation per bp	+36°	+33°	- 30°
Helical diameter	19 Å	19 Å	19 Å

BIOLOGICAL FUNCTIONS

➢ DNA is a genetic material.

➢ It transfers genetic information from one generation to the next generation.

➢ Synthesis of DNA is known as replication.

➢ DNA→ (Replication) DNA →(Transcription) RNA →(Translation) Protein.

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DNA replication: makes DNA copies that are transmitted from cell to cell and from parent to offspring.

Chromosomal DNA: stores information in units called genes.

Transcription: produces an RNA copy of a gene.

Messenger RNA: a temporary copy of a gene that contains information to make a polypeptide.

Translation: produces a polypeptide using the information in mRNA.

Polypeptide: becomes part of a functional protein that contributes to an organism's traits.

RNA

➢ RNA → Ribonucleic acid

➢ RNA is a polymer of ribonucleotides held together by 3'- 5' phosphodiester bridges.

➢ Ribose sugar is present in RNA, thynine is absent, instead of thymine it is having uracil.

➢ RNA is usually single stranded polynucleotide.

TYPES OF RNA

There are 3 types of RNA

1. Messenger RNA (mRNA) : 5-10%

2. Transfer RNA (tRNA) : 10-20%

3. Ribosomal RNA (rRNA) : 50-80%

m RNA

OCCURRENCE:

➢ m-RNA forms about 3-5% of the total cellular RNA.

➢ In eukaryotes m-RNA is synthesized in the nucleus.

In the cytoplasm mRNA's are deposited on the ribosomes. In ribosomes mRNA acts as a template for protein synthesis.

CHEMISTRY

➢ The genetic information carried by M-RNA is called as genetic code. The genetic code is formed of several codons.

➢ Each codon is the sequence of 3 nitrogenous bases which codes for 1 amino acid.

➢ M-RNA carries message in the form of triplet codons.

TYPES OF m-RNA

1. MONOCISTRONIC mRNA

If the MRNA carries codes for the synthesis of a single protein molecule. Then it is called monocistronic.

Ex . Eukaryotic MRNA's are monocistronic

2. POLYCISTRONIC mRNA

If the MRNA carries codes for more than one kind of protein then it is called as polycistronic M-RNA.

STRUCTURE OF m-RNA

Among the RNA's MRNA is the longest. It is single stranded polynucleotide chain containing 500-1500 nucleotides. At 5' end cap is present which is methylated by T- methyl guanine. This cap helps the mRNA to bind with ribosome's.

The cap is followed by a non-coding rEx ion- 1. It is not contain any message for protein. Hence it cannot translate protein.

Structure of mRNA
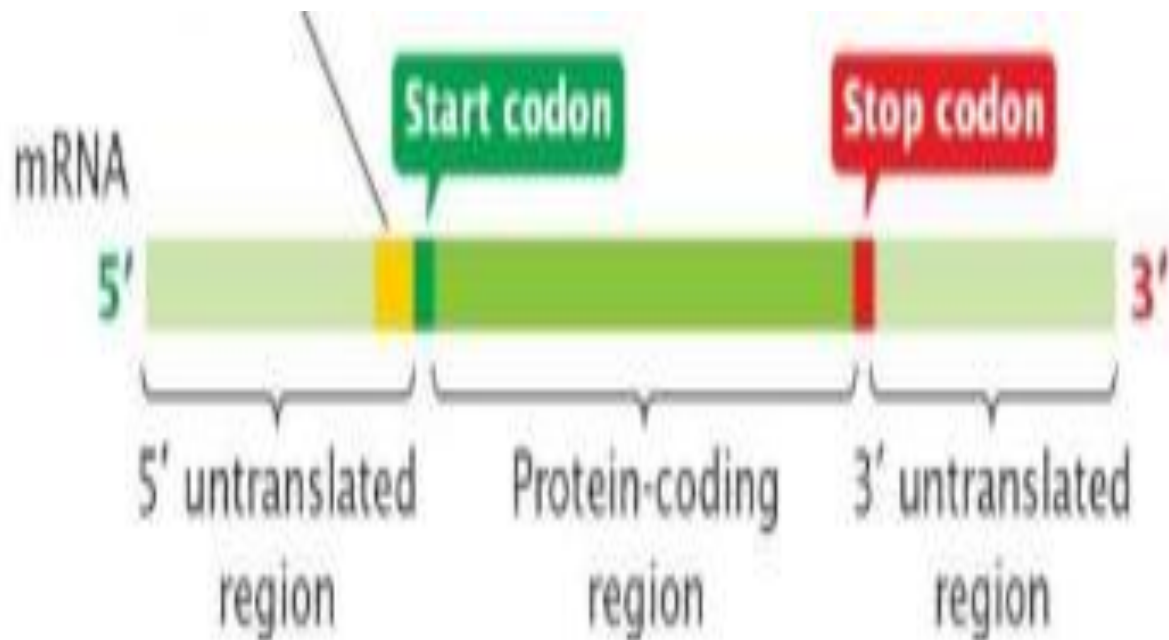
Next to the non-coding rEx ion, initiation codon which is AUG. The initiating codon is followed by a coding rEx ion which contains the code for protein.

Next to the coding rEx ion is the termination codon which may be UAA (OR) UGA. Next to this the non-coding rEx ion –II

At 3' end of mRNA is polyadenylate sequence, this contains about 200-250 adenylate residues of nucleotides.

FUNCTION

m-RNA carries genetic information from the DNA in the nucleus to the ribosomes in the cytoplasm for protein synthesis.

r RNA

OCCURRENCE

➢ r RNA is about 80% of total cellular RNA.

➢ r RNA is persent in ribosomes hence it is called as ribosomal RNA.

CHEMISTRY

➢ r RNA is composed of RNA and protein.

➢ There are 2 subunits present in ribosomes larger and smaller subunit.

There are different types of RNA

16s r RNA

23s r RNA = **PROKARYOTIC RNA**
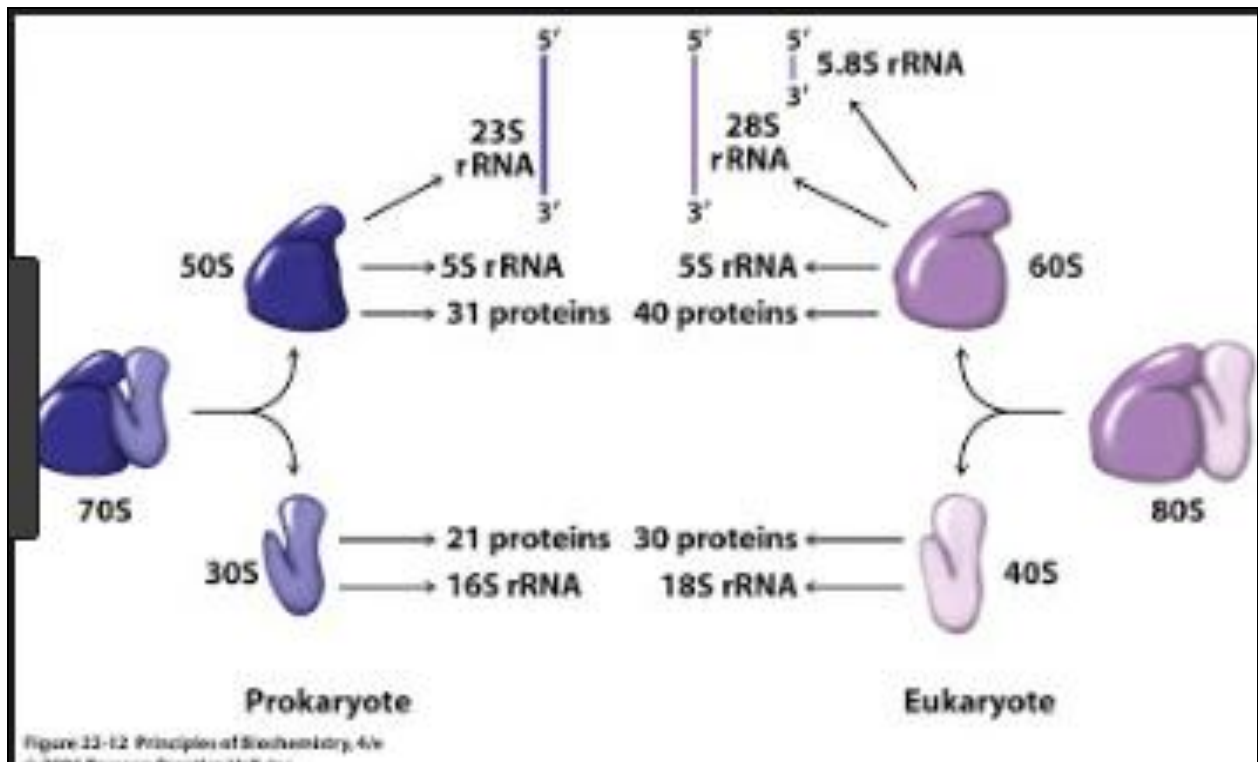
55s r RNA

28s r RNA

18s r RNA = **EUKARYOTIC RNA**

5.8s r RNA

S- Svedberg

Prokaryotic r RNAs bind with prokaryotic ribosome and eukaryotic r RNAs bind with eukaryotic ribosome.


Figure 11-12 Principles of Biochemistry, 4/e

STRUCTURE

r RNA is formed of single strand but in some rEx ions the single strand is twisted upon itself to from a double helix.

In the helical rEx ions most of the bases are complementary, they are joined by H_2 bonds. In the unfolded rEx ions the base pairs are not complementary.

FUNCTION

They play significant role in the binding of M RNA to ribosome's and protein synthesis.

rRNA structure
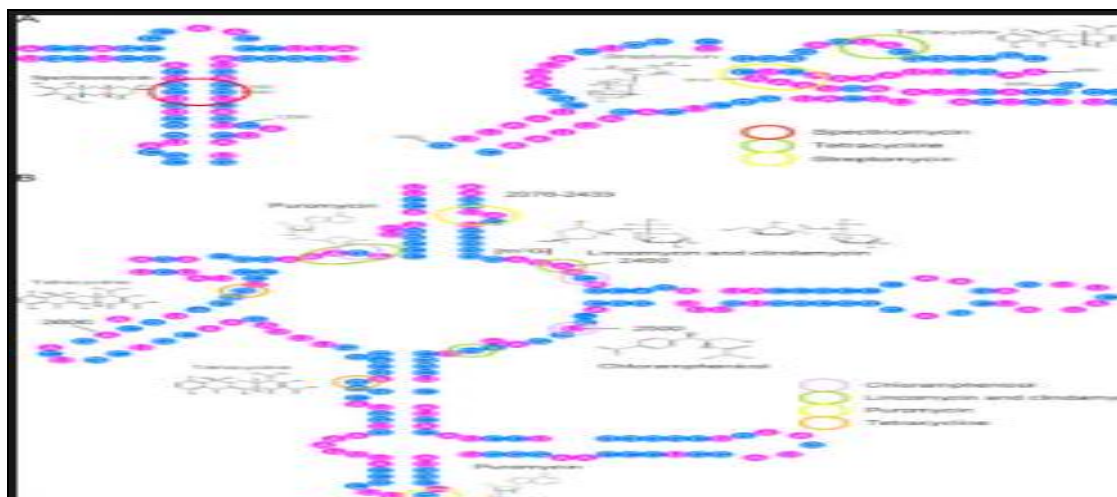
t- RNA TRANSFER RNA

OCCURRENCE:

- t-RNA is about 10-15% of the total cellular RNA.

- t-RNA is also called as soluble RNA because it is so small and remains in the supernatent during centrifugation.

CHEMISTRY:

- The sedimentation co-efficient of T-RNA is 3.8s.

- Molecular weight of t-RNA is about 22,000-37,000 daltons.

- t-RNA is also called as adaptor RNA because serves as an adaptor for the incoming amino acid.

STRUCTURE

t-RNA is a single polynucleotide chain which is folded on itself to attain the shape of clover leaf.

t-RNA has 5 arms

1. Acceptor arm

2. D-arm

3. Anticodon arm

4. Variable arm

5. Tψc (pseudouridine) arm

1. ACCEPTOR ARM

In the acceptor arm, the stem does not end with the loop. In the 3' end CCA bases are present to this bases amino acids are attached to from amino acyl t-RNA.

5' end terminates with C or G base.

2. D-ARM

➢ This arm is also called as dihydrouidine loop (or) D- loop (DHU) loop.

➢ About 3-4 bases are present in the stem and 7-11 bases in the loop.

3.ANTICODON ARM

➢ The arm that is opposite to the acceptor arm is called as anticodon arm.

➢ The stem has 5 paired bases and about 7 unpaired bases are present in the loop.

3 of the 7 unpaired bases determine the pairing of t-RNA with specific codon of M-RNA.

4. VARIABLE ARM

- ➢ In the variable arm the stem may or may not be formed.

- ➢ Variable arm (or) mini arm has a loop with 4-5 bases.

5. TψC ARM

- ➢ The arm that is opposite to D- arm is TψC arm.

- ➢ The loop rEx ion contains 9- unpaired bases.

- ➢ The stem contains 3- Paired bases. This is the arm in which the ribosomes are recognised.

- ➢ T-RNA is named according to the amino acid to which it gets attached.

Ex . T-RNA carry alanine is called t-RNA alanine.

Structure of t RNA

tRNA^{Ala}
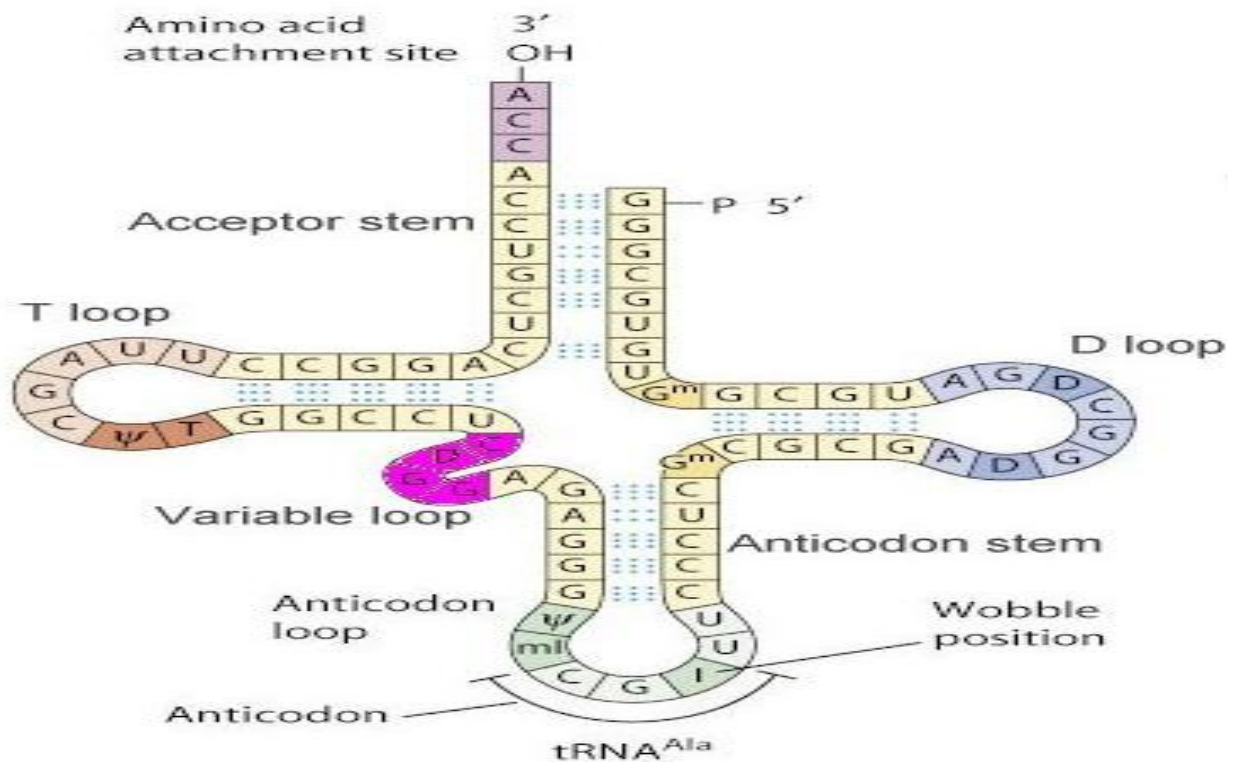
The unusual bases that are found in t-RNA are

INOSINE(I)

PSEUDOURIDINE(ψ)

DIHYDROURIDINE(DHU)

FUNCTIONS

t-RNA transfers the activated amino acids from the amino acid pool in the cytoplasm to the ribosomes for protein synthesis.

DNA vs. *RNA*

Cytosine C

Guanine G

Adenine A

Uracil U
replaces Thymine in RNA

Nitrogenous
Bases

RNA
Ribonucleic acid

AUCG's

Base pair

Sugar
phosphate
backbone

ATCG's

DNA
Deoxyribonucleic acid

C Cytosine

G Guanine

A Adenine

T Thymine

Nitrogenous
Bases

115