KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University Established Under Section 3 of UGC Act 1956)

Pollachi Main Road, Eachanari Post, Coimbatore – 641 021. INDIA

Phone: 0422-6471113-5, 6453777; Fax No: 0422-2980022-3

Email: info@karpagam.com; Web: www.kahedu.edu.in

19MBU103 **BIOCHEMISTRY I**

Instruction Hours / week:L: 4 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

COURSE OBJECTIVES

To provide the basics of biochemistry and its applications.

It serves as good research techniques and the ability to combine and analyzeinformation.

COURSEOUTCOME (CO'S)

A candidate able to understand structures and functions of enzymes, proteins, carbohydrates, fats and nucleic acids and its functions.

Unit I- Carbohydrates

Monosaccharides- families, stereo isomerism, epimers, Mutarotation and anomers. Forms of glucose and fructose, Fischer and Haworth projection.Sugar derivatives.Disaccharides-occurrence, concept of reducing and non-reducing sugars and Haworth projections.Polysaccharides-storage and structural polysaccharides.

Unit II- Lipids

Classification and functions of lipids.Storage lipids- structure and function of fatty acids.Triacylglycerols.Saponification. Structural lipids- structure, functions and properties of phosphoglycerides and sphingolipids.

Unit III- Proteins

Classification and functions of proteins and amino acids, Structure of amino acids and concept of zwitterion. Ninhydrinreaction.Natural modifications of amino acids in proteins. Non protein amino acids, Primary and Secondary structure of proteins- alpha helix, beta pleated sheet. Tertiary and quaternary structures of proteins.Humanhaemoglobin structure.

Unit IV- Enzymes

Structure and classification of enzymes, specificity of enzymes.Michaelis-Menten equation, Km, Vmax, isoenzymes.Allosteric enzyme and its mechanism.Multienzyme complex.Enzyme inhibition.

Unit V- Nucleic Acids

Nucleic Acids-Purines & Pyrimidinesnucleotides, RNA, & DNA base pairing schemes, types of RNA: mRNA, rRNA, tRNA, Secondary structure of DNA, Watson and Crick model.

SUGGESTED READINGS

1. Campbell, M.K. (2012) Biochemistry, 7th edition. Published by Cengage Learning.
2. Campbell, P.N., and Smith, A.D., (2011) Biochemistry Illustrated, 4th edition. Published by Churchill Livingstone.
3. Tymoczko, J.L., Berg, J.M., and Stryer, L. (2012) Biochemistry: A short course, 2ndedition. W.H.Freeman.
4. Berg, J.M., Tymoczko, J.L., and Stryer, L. (2011) Biochemistry, W.H.Freeman and Company. Nelson, D.L and Cox, M.M. (2008) Lehninger Principles of Biochemistry, 5th edition. W.H. Freeman andCompany.
5. Willey, M.J., Sherwood, L.M., &Woolverton, C. J. (2013) Prescott, Harley and Klein's Microbiology. 9th edition. McGrawHill.

Lesson Plan	2019 - 2022 Batch

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Coimbatore – 641 021.

LECTURE PLAN

DEPARTMENT OF BIOCHEMISTRY

STAFF NAME: Dr. S. RUBILA

SUBJECT NAME: BIOCHEMISTRY-I SUBJECT CODE:19MBU103

SEMESTER: I CLASS: I B.Sc., (MICROBIOLOGY)

S.No	Lecture Duration Period	Topics to be Covered	Support Material/Page No.
UNIT-I			
1	1	Monosaccharides-families	R1: 235-236
2	2	Stereo isomerism, epimers, Mutarotation and anomers	R1: 237-238
3	1	Forms of glucose and fructose, Fischer and Haworth projection	R1:239
4	1	Sugar derivatives	R1: 240
5	1	Disaacharides-occurrence, concept of reducing and non reducing sugars and Haworth projection	R1: 239, 243-249
6	1	Storage Polysaccharides	R1:244-247
7	1	Structural polysaccharides	R1: 247-252
8	1	Revision will be given	
Total No of hours Planned for Unit I: 09			
UNIT-II			

1	1	Classification of lipids and Functions of lipids	R1: 357-363
2	1	Storage of lipids	R1: 343-346
3	1	Structure and function of fatty acids	R1: 357-363, 344-346
4	1	Triacylglycerols and Saponification	R1: 346
5	2	Structural lipids-structure, functions and properties of phosphoglycerides	R1: 350-352
6	2	Structure, function and properties of sphingolipids	R1: 349-354
7	1	Revision will be given	
Total No of hours Planned for Unit II :09			
UNIT-III			
1	2	Classification and functions of proteins and aminoacids	R1: 74-76
2	1	Structure of aminoacids and concepts of zwitterion. Ninhydrin reaction	R1: 72-79
3	1	Natural modification of aminoacids in proteins and Non protein amino acids	R1: 114-116
4	1	Oligopeptides: Structure and functions of glutathione, insulin and aspartame	R1:876-878,439,559
5	1	Primary and Secondary structure of proteins- alpha, beta pleated sheet	R1: 113-123
6	1	Tertiary and quaternary structures of proteins	R1:113-123
7	1	Human haemoglobin structure	R1:154-158
8	1	Revision will be given on the possible questions	
Total No of Hours Planned for Unit III : 09			
Unit-IV			
1	2	Structure and classification of enzymes	R1:183-185
2	2	Specificity of enzymes, MM equation, K_m, V_{max} and isoenzyme	R1:186-194,197-201
3	1	Allosteric enzyme and its mechanism	R1: 220-223

4	2	Multienzyme complex	R1:223-225
5	1	Enzyme inhibition	R1:223-225
6	1	Revision will be given	
		Total No of hours planned for Unit IV - 09	
		UNIT-V	
1	2	Nucleic acids -Purines and Pyrimidines nucleotides	R1: 273-278
2	1	RNA & DNA base pairing schemes	R1
3	2	Types of RNA: mRNA, rRNA, tRNA, aminoacyl tRNA synthetase	R1:1008-1020
4	2	Secondary structure of DNA, Watson and Crick model	R1:279-280
5	1	Denaturation of DNA-enol tautomerism and consequences	R1:95-96
6	1	Revision will be given	
Total No of Hours Planned for Unit V: 09			
Previous year End Semester Question papers			
1	1	Revision will be given in the previous year end Semester Question papers	-
2	1	Revision will be given in the previous year end Semester Question papers	-
3	1	Revision will be given in the previous year end Semester Question papers	-
Total No of Hours Planned for discussion: 03			
Total Planned Hours	**48**		

References Books:

R1: David L Nelson and Michael M Cox (2008) Lehninger Principles of Biochemistry, 5th edition. W.H. Freeman and Company.

R2: Berg, J.M., Tymoczko, J.L., and Stryer, L. (2011) Biochemistry, W.H. Freeman and company.

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DEPARTMENT OF MICROBIOLOGY

Subject	**:**	**Biochemistry-I**	**Semester**	**:**	**I**
Subject code	**:**	**19MBU103**	**Class**	**:**	**I B.Sc Microbiology**

UNIT-I: COURSE MATERIAL

Unit-I

Monosaccharides-families, stereo isomerism, epimers, mutarotation and anomers. Forms of glucose and fructose, Haworth projection. Sugar derivatives. Disaccharides-occurrence, concept of reducing and non-reducing sugars and Haworth projections. Polysaccharides-storage and structural polysaccharides.

Suggest Readings

1. Nelson, D.L and Cox, M.M. (2008). Lehninger Principles of Biochemistry, 5th edition. W.H. Freeman and Company.

Carbohydrates

A carbohydrate is a biological molecule consisting of carbon (C), hydrogen (H) and oxygen (O) atoms, usually with a hydrogen–oxygen atom ratio of 2:1 (as in water); in other words, with the empirical formula $C_m(H_2O)_n$ (where *m* could be different from *n*). Carbohydrates are hydrates of carbon; technically they are polyhydroxy aldehydes and ketones. Carbohydrates are also known as saccharides, the word saccharide comes from Greek word sakkron which means sugar.

Functions of Carbohydrate

All animals derive the major portion of their food calories from the different types of Carbohydrates in their diets. Most of the energy for the metabolic activities of the cell in all organisms is derived from the oxidation of Carbohydrate. Important functions of Carbohydrate are that of storing food, acting as a framework in body, performs are listed below.

Carbohydrate functions as Bio Fuel

Carbohydrate functions as an energy source of the body and acts as Bio fuel. Step wise details for the process of production of energy are discussed below.

- Polysaccharides such as starch and glycogen are first hydrolyzed by enzymes to Glucose.
- Glucose is the transported from one cell to another by blood in case of animals and cell sap in case of plants.
- Glucose is then oxidized to produce carbon dioxide and water.
- Energy is released in this process which is used for functioning of the cells.

Carbohydrate functions as Primary Source of Energy

The process of production of energy by carbohydrates is described in above steps. Now it is important to note, that fats and proteins can also be burned to provide energy but carbohydrate functions as primary source of energy. Fats are only burned if there is non availability of carbohydrates. When fat is burned in absence of carbohydrates, toxic compounds like called ketone bodies are produced. Accumulation of these ketone bodies over long period causes a condition called Ketosis. In this condition blood becomes unable to carry oxygen properly and this can be fatal. Thus, one of important function of carbohydrate is help burn fat properly.

Carbohydrate functions as storage food

Different forms of Carbohydrate are stored in living organism as storage food.

- Polysaccharide starch acts as storage food for plants.
- Glycogen stored in liver and muscles acts as storage food for animals.
- Insulin acts as storage food of dahlias, onion and garlic.

Thus carbohydrate performs the function of storing food.

Carbohydrate functions as framework in body

Different Carbohydrates especially Polysaccharides act as framework in living organism.

- Cellulose forms cell wall of plant cell along with hemicelluloses and Pectin
- Chitin forms cell wall of fungal cell and exoskeleton of arthropods
- Peptidoglycan forms cell wall of bacteria and cyanobacteria.

Thus carbohydrates function as contributing material to the cellular structure.

Carbohydrate functions as Anticoagulant

Heparin is a polysaccharide (carbohydrate) which acts as anticoagulant and prevents intravascular clotting.

Carbohydrate functions as Antigen

Many antigens are glycoprotein (which contains oligosaccharide) in nature and give immunological properties to the blood.

Carbohydrate functions as Hormone

Many Hormones like FSH (Follicular Stimulating Hormone which takes part in ovulation in females) and LH (Leteinizing Hormone) are glycoprotein and help in reproductive processes.

Carbohydrates provide raw material for industry

Carbohydrates are an important component of many industries like textile, paper, lacquers and breweries.

Other Functions

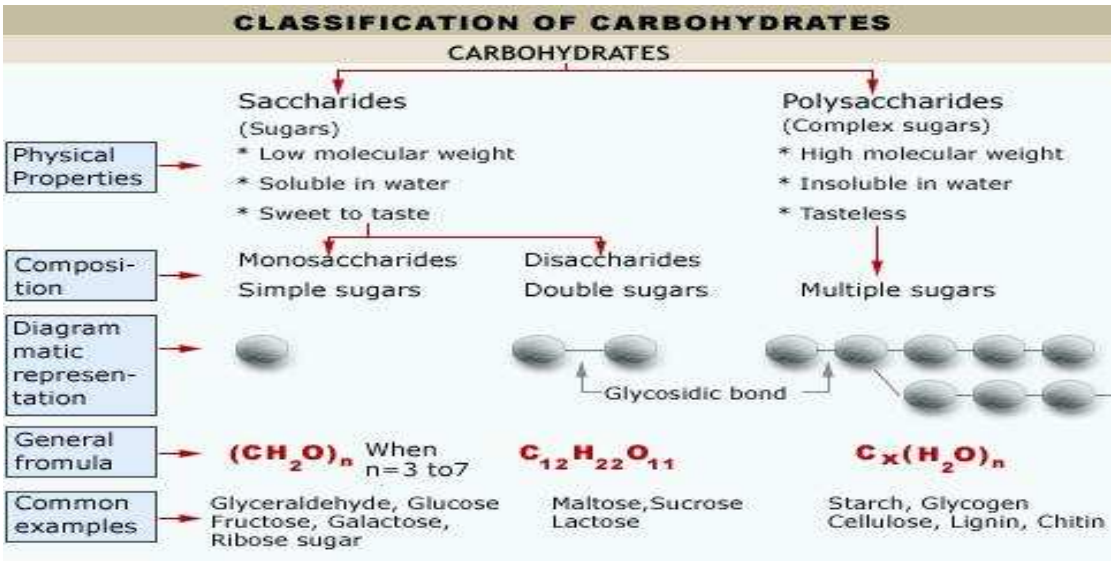
Agar is polysaccharide used in culture media, laxative and food.

Cellulose acts as roughage of food. It stimulates peristalsis movement and secretion of digestive enzymes.

Hyaluronic acid found in between joints acts as synovial fluid and provides frictionless movement.

Classes of carbohydrates

Carbohydrates are classified into three groups



Monosaccharides (From Greek, *mono*=one; *sakchron*=sugar)

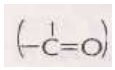
The following table shows the classification of monosaccharides based on the number of their carbon atoms, their general structure, and examples for each.

- They have the general formula $Cn(H_2O)_n$, and they cannot be further hydrolyzed.
- The monosaccharides are divided into different categories, based on the functional group and the number of carbon atoms.

Monosaccharides (empirical formula)	*Aldose*	*Ketose*
Trioses ($C_3H_6O_3$)	Glyceraldehyde	Dihydroxyacetone
Tetroses ($C_4H_8O_4$)	Erythrose	Erythrulose
Pentoses ($C_5H_{10}O_5$)	Ribose	Ribulose
Hexoses ($C_6H_{12}O_6$)	Glucose	Fructose
Heptoses ($C_7H_{14}O_7$)	Glucoheptose	Sedoheptulose

Classification of monosaccharide with selected examples

Aldoses: When the functional group in monosaccharides in aldehyde  they are known as aldoses e.g. glyceraldehydes, glucose.

Ketoses: When the functional group is a keto  group, they are referred to as ketoses

e.g. dihydroxyacetone, fructose.

- Based on the number of carbon atoms, the monosaccharides are regarded as trioses (3C), tetroses (4C), pentoses (5C), hexoses (6C) and heptoses (7C).
- These terms along with functional groups are used while naming monosaccharides.
- For instance, glucose is an aldohexose while fructose is a ketohexose.
- The common monosaccharides and disaccharides of biological importance are given.

Monosaccharides	*Occurrence*	*Biochemical importance*
Trioses		
Glyceraldehyde	Found in cells as phosphate	Glyceraldehyde 3-phosphate is an intermediate in glycolysis
Dihydroxyacetone	Found in cells as phosphate	Its 1-phosphate is an intermediate in glycolysis
Tetroses		
D-Erythrose	Widespread	Its 4-phosphate is an intermediate in carbohydrate metabolism
Pentoses		
D-Ribose	Widespread as a constituent of RNA and nucleotides	For the structure of RNA and nucleotide coenzymes (ATP, NAD^+, $NADP^+$)
D-Deoxyribose	As a constituent of DNA	For the structure of DNA
D-Ribulose	Produced during metabolism	It is an important metabolite in hexose monophosphate shunt
D-Xylose	As a constituent of glycoproteins and gums	Involved in the function of glycoproteins
L-Xylulose	As an intermediate in uronic acid pathway	Excreted in urine in essential pentosuria
D-Lyxose	Heart muscle	As a constituent of lyxoflavin of heart muscle
Hexoses		
D-Glucose	As a constituent of polysaccharides (starch, glycogen, cellulose) and disaccharides (maltose, lactose, sucrose). Also found in fruits	The 'sugar fuel' of life; excreted in urine in diabetes. Structural unit of cellulose in plants
D-Galactose	As a constituent of lactose (milk sugar)	Converted to glucose, failure leads to galactosemia
D-Mannose	Found in plant polysaccharides and animal glycoproteins	For the structure of polysaccharides
D-Fructose	Fruits and honey, as a constituent of sucrose and inulin	Its phosphates are intermediates of glycolysis
Heptoses		
D-Sedoheptulose	Found in plants	Its 7-phosphate is an intermediate in hexose monophosphate shunt, and in photosynthesis
Disaccharides	***Occurrence***	***Biochemical importance***
Sucrose	As a constituent of cane sugar and beet sugar, pineapple	Most commonly used table sugar supplying calories
Lactose	Milk sugar	Exclusive carbohydrate source to breast fed infants. Lactase deficiency (lactose intolerance) leads to diarrhea and flatulence
Maltose	Product of starch hydrolysis, occurs in germinating seeds	An important intermediate in the digestion of starch

Stereoisomerism of monosaccharides:

All the monosaccharides except dihydroxyacetone contain one or more asymmetric (chiral) carbon atoms and thus occur in optically active isomeric forms. The simplest aldose, glyceraldehyde, contains one chiral center (the middle carbon atom) and therefore has two different optical isomers, or **enantiomers**

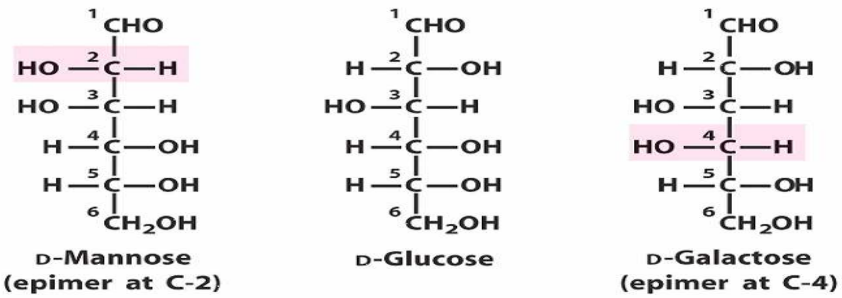
- Carbon 2 of glyceradehyde is a chiral center.
- There are thus 3 steroisomers of glyceraldehydes: D-glyceraldehyde and L-glyceraldehyde.

- By convention, sugars are written with the most oxidized carbon (i.e. aldehyde or ketone) at the top.
- The chiral center farthest from the most oxidized carbon determines if it is D or L.
- If the hydroxyl points to the left, then it is the L configuration if to the right then it is D.
- In general, only the D isomers are used biologically, but there are many exceptions to this generalization.
- Sugars can be conveniently written as Fischer projections to indicate stereochemistry.
- The most oxidized carbon is placed at the top and each carbon between it and the last carbon is a cross from which are appended the hydrogen and hydroxyl group.
- It makes a difference if the hydroxyl group is written to the or lift.
- It is important to recognize that a Fischer projection indicates the stereochemistry of each chiral center.
- One must imagine that the groups to the left and right (-H and –OH) are coming out the plane towards the viewer, while the substituent's above and below are out of the plane directed away from the viewer.
- In general, a molecule with *n* chiral centers can have 2^n stereoisomers. Glyceraldehyde has $2^1 = 2$; the aldohexoses, with four chiral centers, have $2^4 = 16$ stereoisomers.

Epimers

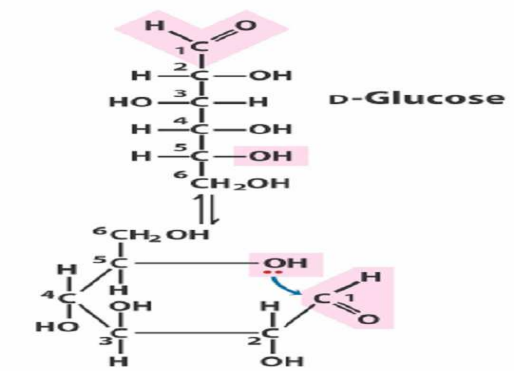
Carbohydrates that differ only in their stereochemistry at one position are called Epimers.

- Eg. Glucose and mannose (C-2)
- Glucose and galactose (C-4)



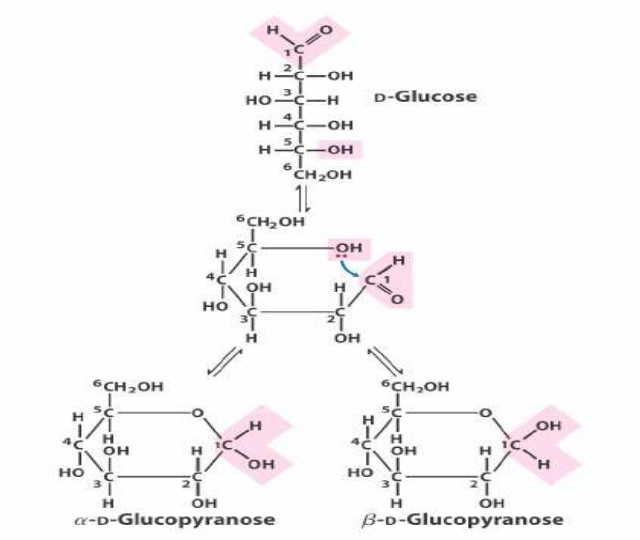
Mutarotation and anomers:

- In aqueous solution, D-glucose exists in one of 2 forms: α-D-glucose and ß-D-glucose.
- This is because Aldehydes can react with alcohols to form a hemiacetal.
- In this case, the hydroxyl oxygen attacking the molecule it is an intermolecular reaction, which results in formation of a ring.
- Rings with 6 members are the most stable, but 5-membered rings are possible.
- The oxygen that attacked the carbonyl carbon will be a member of the ring.
- The carbonyl oxygen is converted to a hydroxyl group in the process.
- The stereochemistry of this hydroxyl group is determined by the position of the carbonyl during the attack; it can be one of 2 possible configurations: α or ß.
- Six-member rings resemble pyran and are referred to as pyranosides.
- Five – member rings resemble furan and are referred to as furanosides.



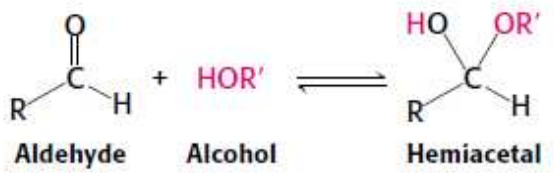
Anomers

- Isomeric forms of monosaccharides that differ only in their configuration about the hemiacetal or hemiketal carbon atom are called **anomers**. The hemiacetal (or carbonyl) carbon atom is called the **anomeric carbon**. The _ and _ anomers of D-glucose interconvert in aqueous solution by a process called **mutarotation**
- The aldehyde or ketone carbon is referred to as the anomeric carbon, as this is the chiral center that differs between 2 Anomers.
- For D-sugars the anomer has the hydroxyl group down in the Haworth projection and on the same side as the ring oxygen in the Fisher projection.

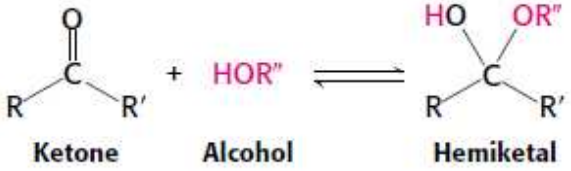


Haworth Projections

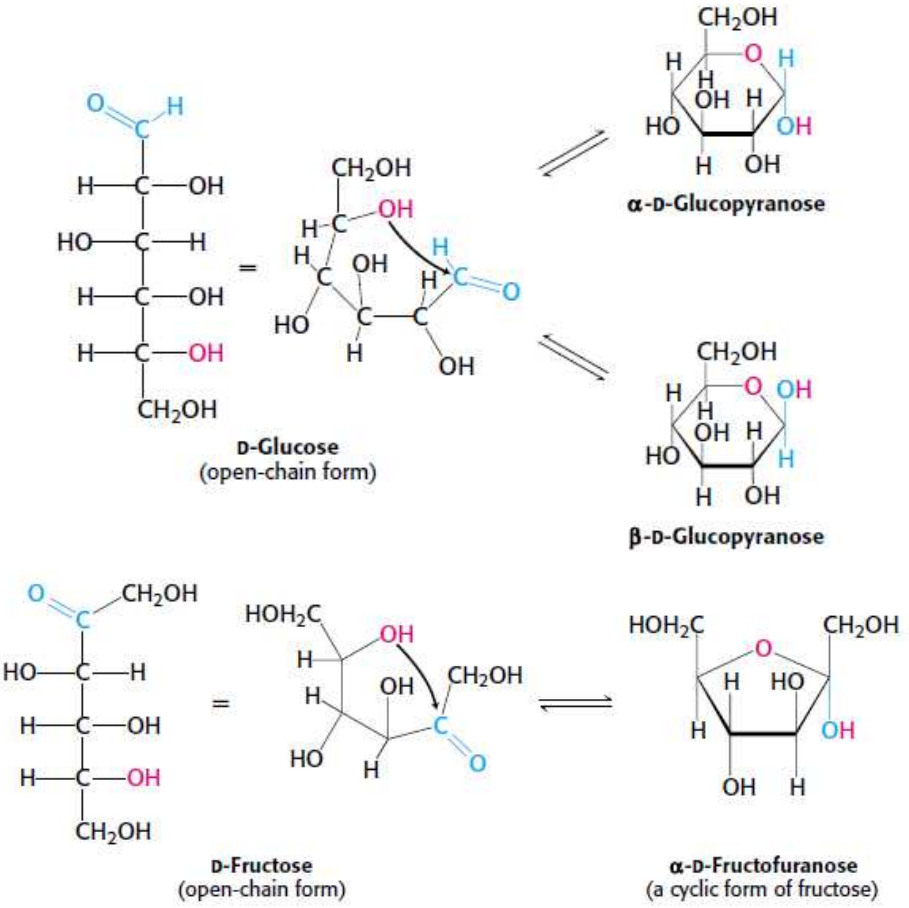
The predominant forms of ribose, glucose, fructose, and many other sugars in solution are not open chains. Rather, the open-chain forms of these sugars cyclize into rings. In general, an aldehyde can react with an alcohol to form a hemiacetal.



For an aldohexose such as glucose, the C-1 aldehyde in the open-chain form of glucose reacts with the C-5 hydroxyl group to form an intramolecular hemiacetal. The resulting cyclic hemiacetal, a six-membered ring, is called pyranose because of its similarity to pyran. Similarly, a ketone can react with an alcohol to form a hemiketal.



The C-2 keto group in the open-chain form of a ketohexose, such as fructose, can form an intramolecular hemiketal by reacting with either the C-6 hydroxyl group to form a six-membered cyclic hemiketal or the C-5 hydroxyl group to form a five-membered cyclic hemiketal. The five-membered ring is called a furanose because of its similarity to furan.



The depictions of glucopyranose and fructofuranose shown below are Haworth projections. In such projections, the carbon atoms in the ring are not explicitly shown. The approximate plane of the ring is perpendicular to the plane of the paper, with the heavy line on the ring projecting toward the reader. Like Fischer projections, Haworth projections allow easy depiction of the stereochemistry of sugars.

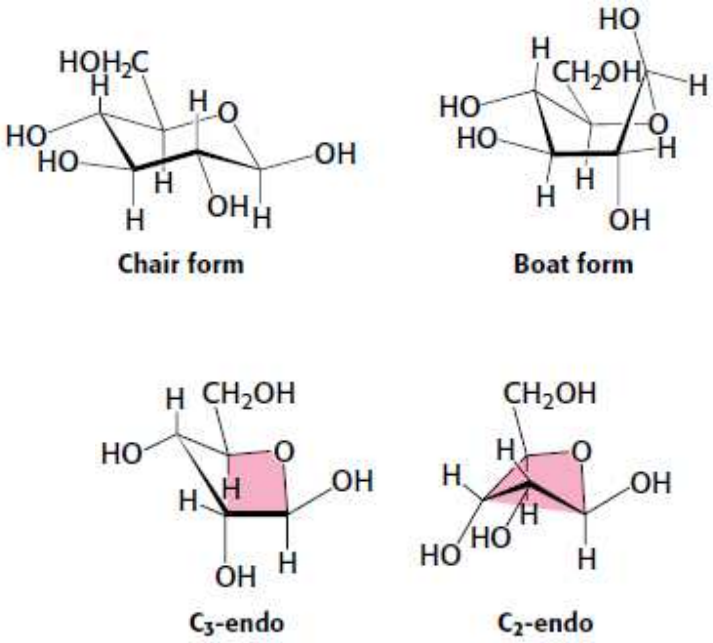
An additional asymmetric center is created when a cyclic hemiacetal is formed. In glucose, C-1, the carbonyl carbon atom in the open-chain form, becomes an asymmetric center. Thus, two ring structures can be formed: α-D-glucopyranose and β-D-glucopyranose. For D sugars drawn as Haworth projections, the designation α means that the hydroxyl group attached to C-1 is below the plane of the ring; β means that it is above the plane of the ring. The C-1 carbon atom is called the anomeric carbon atom, and α and β forms are called anomers. An equilibrium mixture of glucose contains approximately one-third α anomer, two-thirds β anomer, and <1% of the open-chain form.

The same nomenclature applies to the furanose ring form of fructose, except that α and β refer to the hydroxyl groups attached to C-2, the anomeric carbon atom. Fructose forms both pyranose and furanose rings. The pyranose form predominates in fructose free in solution, and the furanose form predominates in many fructose derivatives. Pentoses such as D-ribose and 2-deoxy-D-ribose form furanose rings, as we have seen in the structure of these units in RNA and DNA.

Chair and boat forms of glucose

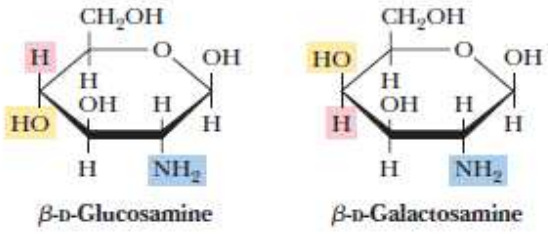
The six-membered pyranose ring is not planar, because of the tetrahedral geometry of its saturated carbon atoms. Instead, pyranose rings adopt two classes of conformations, termed chair and boat because of the resemblance to these objects. In the chair form, the substituents on the ring carbon atoms have two orientations: axial and equatorial. Axial bonds are nearly perpendicular to the average plane of the ring, whereas equatorial bonds are nearly parallel to this plane. Axial substituents sterically hinder each other if they emerge on the same side of the ring (e.g., 1,3-diaxial groups). In contrast, equatorial substituents are less crowded. The chair form of β-D-glucopyranose predominates because all axial positions are occupied by hydrogen atoms. The bulkier –OH and –CH2OH groups emerge at the less-hindered periphery. The boat form of glucose is disfavored because it is quite sterically hindered.

Furanose rings, like pyranose rings, are not planar. They can be puckered so that four atoms are nearly coplanar and the fifth is about 0.5 Å away from this plane. This conformation is called an envelope form because the structure resembles an opened envelope with the back flap raised. In the ribose moiety of most biomolecules, either C-2 or C-3 is out of the plane on the same side as C-5. These conformations are called C2- endo and C3-endo, respectively.



Sugar Derivatives

Amino sugars, including D-glucosamine and D-galactosamine, contain an amino group (instead of a hydroxyl group) at the C-2 position. They are found in many oligosaccharides and polysaccharides, including chitin, a polysaccharide in the exoskeletons of crustaceans and insects.



Glucosamine

Glucosamine ($C_6H_{13}NO_5$) is an amino sugar and a prominent precursor in the biochemical synthesis of glycosylated proteins and lipids. Glucosamine is part of the structure of the polysaccharides chitosan and chitin, which compose the exoskeletons of crustaceans and other arthropods, as well as the cell walls of fungi and many higher organisms. Glucosamine is one of the most abundant monosaccharides. It is produced commercially by the hydrolysis of crustacean exoskeletons or, less commonly, by fermentation of a grain such as corn or wheat.

Glucosamine is naturally present in the shells of shellfish, animal bones, bone marrow, and fungi. D-Glucosamine is made naturally in the form of glucosamine-6-phosphate, and is the biochemical precursor of all nitrogen-containing sugars.[30]Specifically in humans, glucosamine-6-phosphate is synthesized from fructose 6-phosphate and glutamine by glutamine fructose-6-phosphate transaminase as the first step of the hexosamine biosynthesis pathway. The end-product of this pathway is uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), which is then used for making glycosaminoglycans,proteoglycans, and glycolipids.

Galactosamine

Galactosamine is a hexosamine derived from galactose with the molecular formula $C_6H_{13}NO_5$. This amino sugar is a constituent of some glycoprotein hormones such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Other sugar constituents of FSH and LH include glucosamine, galactose and glucose. Galactosamine is a hepatotoxic, or liver-damaging, agent that is sometimes used in animal models of liver failure.

Disaccharides

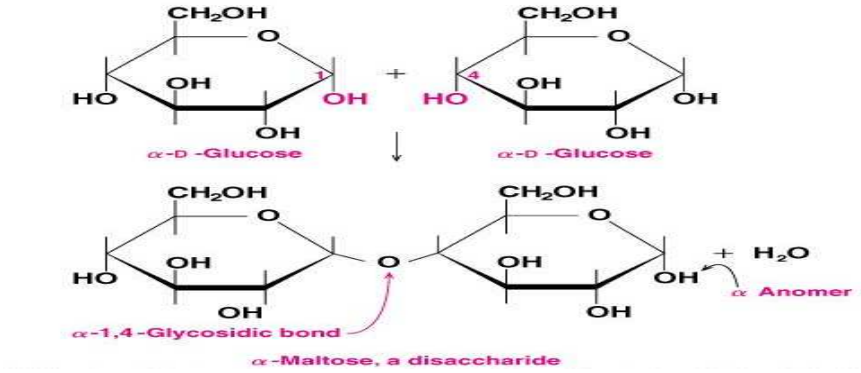
- A disaccharide is formed when a hydroxyl group on one monosaccharide reacts with the anomeric carbon of another monosaccharide to form a glycosidic bond.
- Each disaccharide has a specific glycosidic linkage (depending on which hydroxyl reacts with which anomer).
- The three most common disaccharides are **maltose, lactose and sucrose.**
- When hydrolyzed using acid or an enzyme, the following monosaccharide are produced.
- The disaccharides are of two types

  1. Reducing disaccharides with free aldehyde or keto group e.g. maltose, lactose.
  2. Non-reducing disaccharides with no free aldehyde or keto group e.g. sucrose,

Maltose

Occurrence: Not occur in our body, but present in germinating cereals and malt; It is the breakdown product of starch

Structure: Maltose (malt sugar or corn sugar) is composed of two glucose molecules are joined through α-1,4 glycosidic linkage



Properties

- Because one of the glucose molecules is a hemiacetal (having a free aldehyde group) it can undergo mutarotation (Gradual change in specific rotation; Glucose if freshly prepared have sp rotation of +112°, but on standing gives a rotation of + 52.).
- It exist in α and β forms
- Since it is having a free aldehyde group, it reduce compounds and and so maltose is a reducing sugar.
- Reduce Fehling and Benedicts solution but not Barfoeds solution
- Forms osazone with phenyl hydrazine
- Maltose can be fermented by yeast to produce ethanol.
- Maltose is also used in cereals, candies and malted milk.

  Hydrolysis: Hydrolyzed by maltase present in alimentary canal; two glucose molecules are released upon hydrolysis

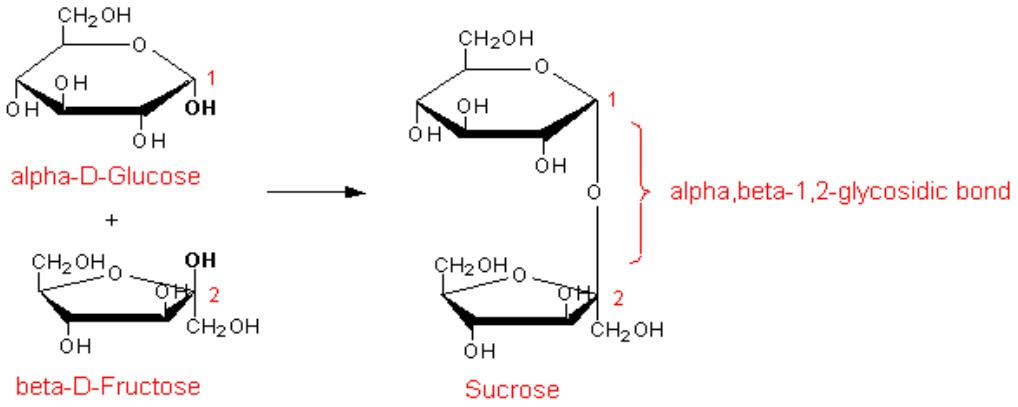
Sucrose

Occurrence

It is the sweetest of all the sugars; does not exist in our body, occur in cane sugar, pineapple, carrot root, sweet potato and honey. Sucrose is the most abundant disaccharide and is commercially produced from sugar cane and sugar beets.

Structure

Sucrose (table sugar) consists of one glucose molecule and one fructose molecule linked by an α,β-1,2-glycosidic bond.



- It is not having a free aldehyde or ketone group, so don't have mutarotation; does not exist in α and β forms. **Because the glycosidic bond in sucrose involves both** anomeric carbons, neither monosaccharide can undergo mutorotation, and so sucrose is not a reducing sugar.

Properties

- White crystalline solid powder; sparingly soluble in water
- The specific rotation of fructose is 66.5, but upon hydrolysis it is changed to -19.5. This because the hydrolyzed product, fructose, which is having more levorotary than the glucose. This reaction is called inversion and the sugar is called invert sugar.
- it does not reduce Fehling , Benedicts s and Barfoeds solution
- it cannot from crystals with phenyl hydrazine
- **Hydrolysis:**
- Hydrolyzed by sucrose present in alimentary canal; one glucose and one fructose molecules are released upon hydrolysis

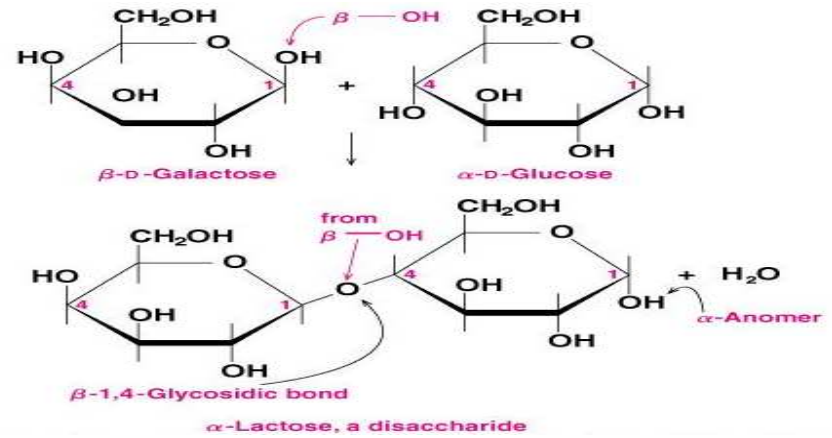
Lactose

Occurrence

- Present in human milk (9.8%) produced by mammary gland of human beings; It comes from milk products (about 4-5% of cow's milk).; also occur in urine during pregnancy.

Structure

- **Lactose** (milk sugar) consists of one glucose molecule and one galactose molecule linked by a β-1,4 glycosidic bond.



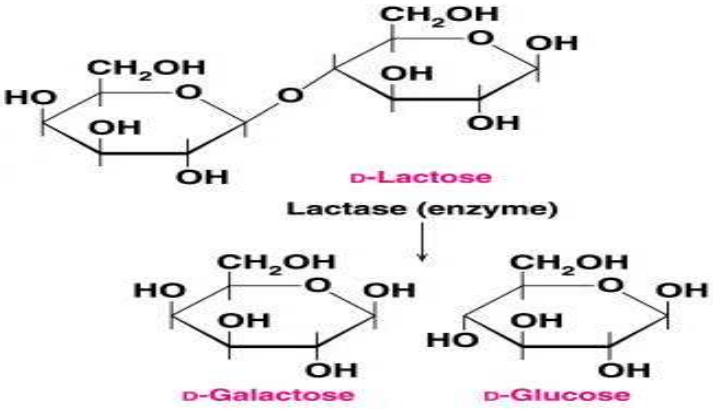
- Because the glucose is a hemiacetal, it can undergo mutorotation, and it is having a free aldehyde group, which reduce compounds and so lactose is a reducing sugar.

Properties

- White crystalline solid powder; sparingly soluble in water
- The specific rotation is + 55.2·
- Exist in α and β forms
- Reduce Fehling and Benedicts solution but not Barfoeds solution
- Forms osazone with phenyl hydrazine

Hydrolysis of Lactose

Hydrolyzed by lactase present in alimentary canal; one glucose and one galactose molecules are released upon hydrolysis.



- Some people don't produce enough lactase, the enzyme that hydrolyzes lactose, and so can't digest lactose.
- Many adults become lactose intolerant, and develop abdominal cramps, nausea and diarrhea.
- Lactase can be added to milk products (or taken as a supplement) to combat this problem

Polysaccharides

A **polysaccharide** is a polymer consisting of hundreds to thousands of monosaccharide joined together by glycosidic linkages.

They are further classified into

Homopolysaccharides

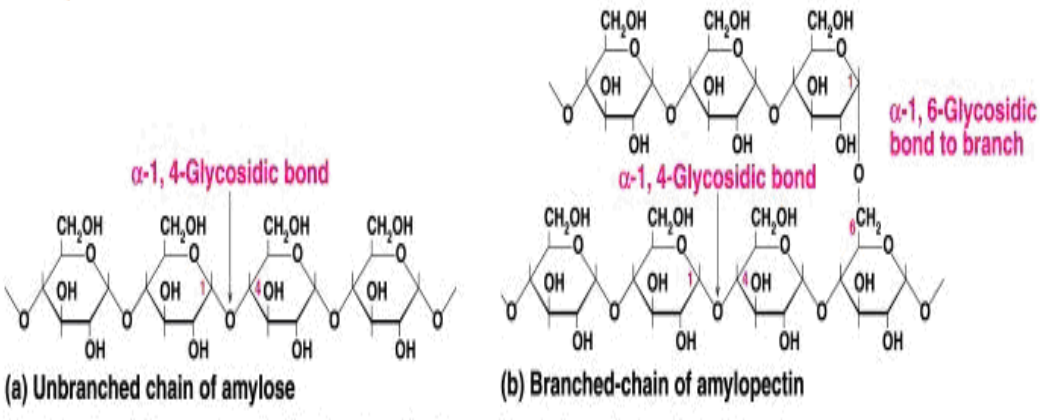
1. Storage polysaccharides: Eg-Starch (Plant); glycogen (animal)
2. Structural polysaccharides: Eg-Cellulose (Plant); Chitin (animal)

Heteropolysaccharides

1. Glycoproteins
2. Glycosaminoglycans

- Heparin/Heparin sulfate
- Chondroitin sulfate
- Keratin sulfate
- Hyaluronic acid

- Three biologically important polysaccharides are **starch, glycogen** and **cellulose** all three are polymers of D-glucose, but they differ in the type of glycosidic bond and/or the amount of branching
- Starch and glycogen are used for storage of carbohydrates
- Starch is found in plants and glycogen in animals
- The polymers take up less room than would the individual glucose molecules, so are more efficient for storage
- Cellulose is a structural material used in formation of cell walls in plants Plant Starch (Amylose and Amylopectin)



Storage polysaccharides

Starch

- Half of the carbohydrate ingested by human is starch.
- It is the source of carbohydrates and fundamental source of energy.
- Starch is the carbohydrate reserve of plants which is the most important dietary source for higher animals, including man.

Occurrence: It is the storage form of carbohydrate in plants ; It is present in cereals, potato, and legumes, root, tubers, tubers, vegetables etc fruits. It is found as granules in cytoplasm of chloroplast

Structure

- Starch is a homopolymer composed of D-glucose units held by a-glycosidic bonds.
- It is known as glucosan or glucan.

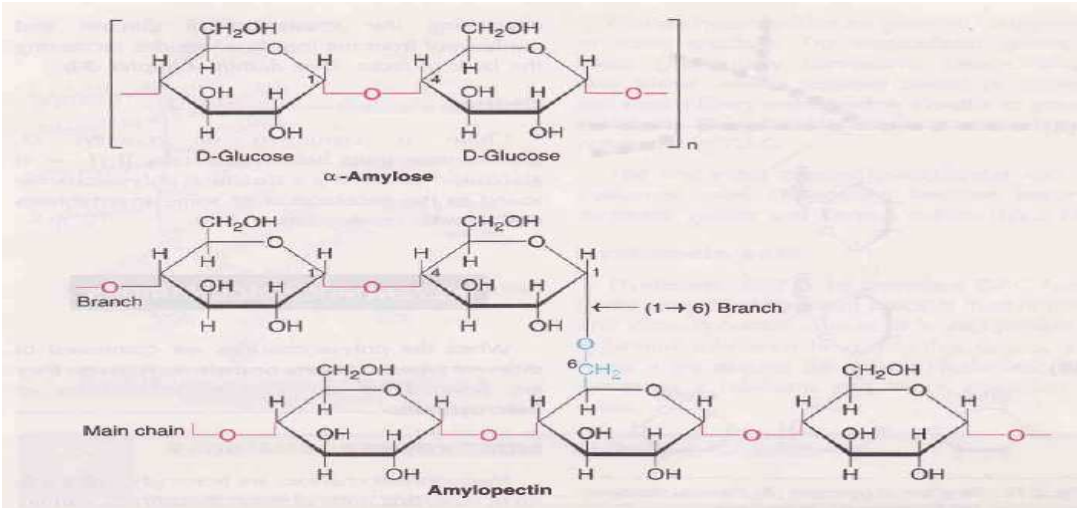
- Starch consists of two polysaccharide components-water soluble amylose (15-20%) and a water insoluble amylopectin (80-85%).

Amylose (α- amylose)

It is a long unbranched polysaccharide; made of α- D glucose joined by α (1 → 4) glycosidic linkage. In starch it constitute about 15-20%. It is in the helical form and 6 glucose unit per turn. It have nearly 300-400 glucose units; molecular weight is 1000-50,000. It form blue color with iodine.

Amylopectin (β-amylose)

Amylopectin on the other hand, is a branched polysaccharide atleast 80 branch with an interval of 24-30 glucose units(20-30 glucose units per branch).It is made of α- D glucose joined by α 1,4 glycosidic linkage and the branch is established with α 1,6 glycosidic linkage(α (1 → 6) glycosidic bonds at the branching points and α (1 → 4) linkages everywhere). In starch it constitute about80-85%. It have nearly 300-5500 glucose units; molecular weight is 5,00,000. It form blue colour with iodine.



Structure of starch (α-amylose and amylopectin)

Properties of starch

White , soft powder, tasteless; insoluble in water; specific rotation is+196.

Hydrolysis

Starch is a glucosan, because it yields only glucose molecule on hydrolysis; with water it form hydrated micelle

- Starches are hydrolyzed by amylase (pancreatic or salivary) to liberate dextrins, and finally maltose and glucose units.
- Amylase acts specifically on a (1 → 4) glycosidic bonds.

α- amylase

Amylose ------→ Maltose + glucose

α- amylase attacks the α 1,4 glycosidic linkage. It is present in saliva

α- amylase/ β- amylase

Amylo pectin ----------→ Maltose + glucose

α- amylase attacks the α 1,4 glycosidic linkage. It is present in saliva. α 1,6 glycosidic linkage is attacked by as α 1,6 glucosidase

Starch with mineral acid gives glucose. This glucose reacts with iodine and give gradual change in colour ie., -blue----------purple----------red----------none

Starch on partial hydrolysis yield dextrin which gives stiffness to cloths

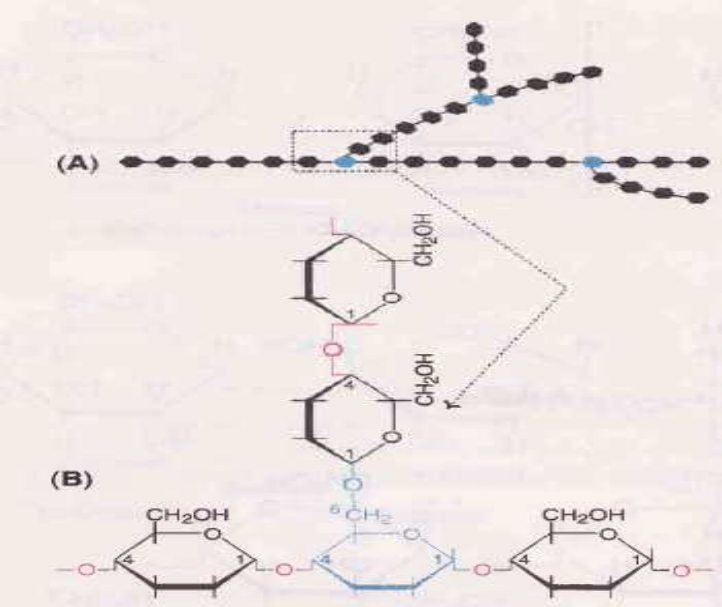
Glycogen

Glycogen is the carbohydrate reserve in animals, hence often referred to as animal starch. It is the reserve carbohydrate found in liver and muscle of animal and human beings

It is present in high concentration in liver, followed by muscle, brain etc. Liver have more glycogen (7% of its weight) than muscle. Glycogen is also found in plants that do not possess chlorophyll (e.g. yeast, fungi).

Structure

- The structure of glycogen is similar to that of amylopectin with more number of branches. It is a branched polymer of carbohydrate ; made of α-D glucose; Glucose is the repeating unit in glycogen joined together by α (1 → 4) glycosidic bonds, and α (1 → 6) glycosidic bonds at branching points, the branching is established by α 1,6 glycosidic linkage.
- The molecular weight (up to 1 x 10^8) and the number of glucose units (up to 5000-25,000) vary in glycogen depending on the source from which glycogen is obtained.



Structure of glycogen (A) General structure (B) Enlarged at a branch point

Properties

White, tasteless powder; readily soluble in water; Non reducing; give red color with iodine

Hydrolysis

On complete hydrolysis, glycogen yields glucose and maltose

Dextrin

This is formed by the partial (incomplete) hydrolysis of starch by salivary amylase; and also by dilute mineral acid and heat.

Inulin

It is a fructosan; made of repeating units of fructose. It is found in roots and tubers of dahlia and dandelions; it mainly used in assessing the kidney function.

Structural Polysaccharides

Structural polysaccharides are the polysaccharides that are found to form the structure of an organism.

Eg. Cellulose - in plants

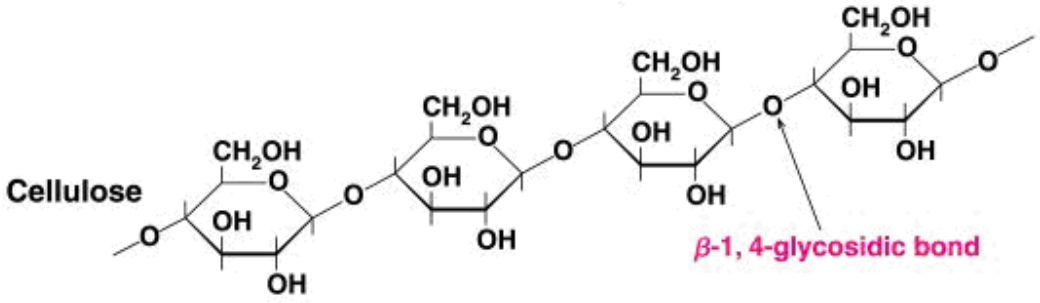
Chitin - found in outer skeleton of insects and crabs

Lignin - wood

Cellulose

It is the most abundant of all biomolecule in biosphere. 50% carbon in vegetation is contributed by cellulose. In plant, it is the main constituent of supporting tissue. It is not present in animal.

- Cellulose is a polymer made with repeated glucose units bonded together by *beta*-linkages.
- The structural components of plants are formed primarily from cellulose.
- Wood is largely cellulose and lignin, while paper and cotton are nearly pure cellulose.



Properties

- Cellulose is insoluble in water. It does not change color when mixed with iodine. On hydrolysis, it yields glucose. It is the most abundant carbohydrate in nature.
- Fibrous, tough, white solid; insoluble in ordinary solvents and water; give no color with iodine.

Hydrolysis

- Humans and many other animals lack an enzyme to break the *beta*-linkages, so they do not digest cellulose.
- Certain animals such as termites can digest cellulose, because bacteria possessing the enzyme are present in their gut.
- It is not acted upon by amylase in human intestine, so doesn't have any nutritive value. It adds bulk to the intestinal constituents and stimulates the peristaltic movement of bowel so it aids in relieving constipation.
- On complete hydrolysis by cellulase enzyme it yields α-D glucose. This enzyme is mainly present in termites, which are able to digest the wood.

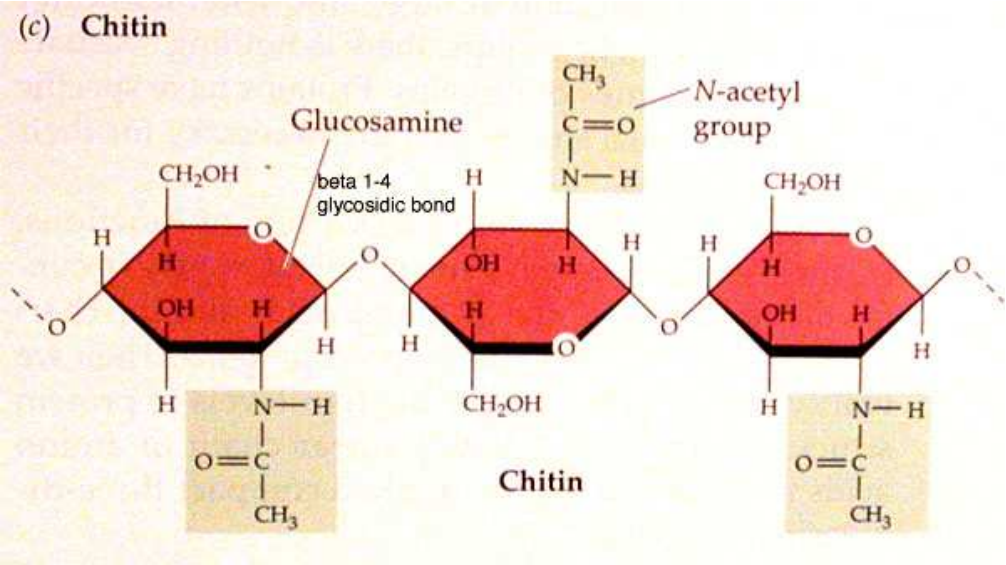
- It is also hydrolyzed by acids such as sulfuric acid, nitric acid and sodium hydroxide.

Chitin

Chitin is a polysaccharide found in the outer skeleton of insects, crabs, shrimps, and lobsters and in the internal structures of other invertebrates.

Structure

It is a long-chain polymer of a *N*-acetylglucosamine, a derivative of glucose, Joined through ß(1-4) linked units of the amino sugar N-acetyl-glucosamine.



Properties

In its unmodified form, chitin is translucent, pliable, resilient, and quite tough. but in most invertebrates it occurs largely as a component of composite materials

Application

Chitin is the main source of production of chitosan, which is used in a number of applications, such as a flocculating agent, a wound healing agent, a sizing and strengthening agent for paper, and a delivery

All Possible Questions

1. Define epimers and anomers?
2. Differences between reducing and nonreducing sugar? Give example?
3. Give an account of polysaccharides?
4. Write short notes on D and L sugars?
5. What are carbohydrates?
6. Differences between glucose and fructose?
7. List out the different categories of carbohydrates?

Detailed Question

1. Explain the detail of mutarotation?
2. How carbohydrate can be classified? Explain with example?
3. Classification of polysaccharides?
4. Differences between amylose and amylopectin?
5. Functions of carbohydrates?
6. Explain in detail about the families of monosaccharides?

7. Write a detailed note on structural polysaccharides?

8. Describe the Haworth projection formulae and chair and boat forms of glucose.

9. Explain in detail about the storage polysaccharides.

10. Describe the forms of glucose and fructose.

11. Explain detail about the mutarotation?

Questions	Option A	Option B	Option C	Option D	Answers
Two monosaccharides are joined by	Peptide bond	Phosphodiester bond	Glycosidic bond	Hydrogen bond	**Glycosidic bond**
All the following are storage polysaccharides except	Starch	Cellulose	Dextran	Glycogen	**Dextran**
The glycosidic linkage between glucose molecule in maltose is	β 1 – 4	α 1 – 2	α 1 – 4	β 1 – 2	**α 1 – 4**
The glycosidic linkage between two glucose molecules in isomaltose is	α 1 – 4	β 1 – 4	α 1 – 6	β 1 – 6	**α 1 – 6**
Which of the following sugar give a positive result with Seliwanoff test	Sucrose	Glucose	Galactose	Mannose	**Sucrose**
Maltose is a disaccharide of_____	Glucose and galactose	Glucose and glucose	Glucose and lactose	Fructose and lactose	**Glucose and glucose**
Glycosidic bond in sucrose is _____	α 1 – 4	β 1 – 4	α 1 – 2	β 1 – 2	**β 1 – 2**
Majority of the monosaccharides found in the human body are of …	L-type	D-type	DL-types	None of the above	**D-type**
Example of Epimers is	Glucose &	Glucose & Ribose	Mannose & Glucose	a & c	**a & c**
The end product of hydrolysis of “Starch” by amylase is …	Soluble starch	Glucose	Dextrins	Maltose	**Glucose**
Cellulose fibers resemble with the protein structure in the form of	ß-sheets	α-helices	ß-turns	None of these	**ß-sheets**
Hydrolysis of lactose yields	galactose and fructose	galactose and glucose	glucose and fructose	fructose and galactose	**galactose and glucose**

Boat and chair conformations are found	in pyranose sugars	in any sugar without axial -OH groups	in any sugar without equatorial -OH groups	only in D-glucopyranose	**in pyranose sugars**
Storage polysaccharide made by animals is	amylopect	glycogen	cellulose	collagen	**glycogen**
The glycosaminoglycan which does not contain uronic acid is	Dermatan	Chondroiti	Keratan sul	Heparan sulphate	**Keratan sulphate**
Keratan sulphate is found in abundance in	Heart mus	Liver	Adrenal cor	Cornea	**Cornea**
Repeating units of hyaluronic acid are	N-acetyl glucosamine and D-glucuronic acid	N-acetyl galactosamine and D-glucuronic acid	N-acetyl glucosamine and galactose	N-acetyl galactosamine and L-iduronic acid	**N-acetyl glucosamine and D-glucuronic acid**
The approximate number of branches in amylopectin is	10	20	40	80	**80**
In amylopectin the intervals of glucose units of each branch is	10–20	24–30	30–40	40–50	**24–30**
The general formula for polysaccharide is	(C6H10O5)n	(C6H12O5)n	(C6H10O6)n	(C6H10O6)n	**(C6H10O5)n**
α-D-glucose and β -D-glucose are	Stereoisomers	Epimers	Anomers	Keto-aldo pairs	**Anomers**
The general formula of monosaccharides is	CnH2nOn	C2nH2On	CnH2O2n	CnH2nO2n	**CnH2nOn**
The aldose sugar is	Glycerose	Ribulose	Erythrulose	Dihydoxyacetone	**Glycerose**
A triose sugar is	Glycerose	Ribose	Erythrose	Fructose	**Glycerose**
A pentose sugar is	Dihydroxyacetone	Ribulose	Erythrose	Glucose	**Ribulose**

The pentose sugar present mainly in the heart muscle is	Lyxose	Ribose	Arabinose	Xylose	**Lyxose**
Polysaccharides are	Polymers	Acids	Proteins	Oils	**Polymers**
The number of isomers of glucose is	2	4	8	16	**16**
Two sugars which differ from one another only in configuration around a single carbon atom are termed	Epimers	Anomers	Optical isomers	Stereois omers	**Epimers**
Isomers differing as a result of variations in configuration of the —OH and —H on carbon atoms 2, 3 and 4 of glucose are known as	Epimers	Anomers	Optical isomers	Steroiso mers	**Epimers**
The most important epimer of glucose is	Galactose	Fructose	Arabinose	Xylose	**Galactose**
α-D-glucose + 112.0 → + 52.50 ← + 19.0 β-D-glucose for glucose above represents	Optical isomeris m	Mutarota tion	Epimerisat ion	D and L isomeris m	**Mutarotation**
Compounds having the same structural formula but differing in spatial configuration are known as	Stereoiso mers	Anomers	Optical isomers	Epimers	**Epimers**
In glucose the orientation of the —H and —OH groups around the carbon atom 5 adjacent to the terminal primary alcohol carbon determines	D or L series	Dextro or levorotat ory	α and β anomers	Epimers	**D or L series**
The sugar found in milk is	Galactose	Glucose	Fructose	Lactose	**Lactose**
Invert sugar is	Lactose	Sucrose	Hydrolytic products of sucrose	Fructose	**Hydrolytic products of sucrose**

Sucrose consists of	Glucose + glucose	Glucose + fructose	Glucose + galactose	Glucose + mannose	**Glucose + fructose**
The monosaccharide units are linked by 1 → 4 glycosidic linkage in	Maltose	Sucrose	Cellulose	Cellobiose	**Maltose**
Which of the following is a non-reducing sugar?	Isomaltose	Maltose	Lactose	Trehalose	**Trehalose**
Which of the following is a reducing sugar?	Sucrose	Trehalose	Isomaltose	Agar	**Isomaltose**
A dissaccharide formed by 1,1-glycosidic linkage between their monosaccharide units is	Lactose	Maltose	Trehalose	Sucrose	**Trehalose**
A polysacchharide which is often called animal starch is	Glycogen	Starch	Inulin	Dextrin	**Glycogen**
The homopolysaccharide used for intravenous infusion as plasma substitute is	Agar	Inulin	Pectin	Starch	**Agar**
The polysaccharide used in assessing the glomerular fittration rate GFR) is	Glycogen	Agar	Inulin	Hyaluronic acid	**Inulin**
The constituent unit of inulin is	Glucose	Fructose	Mannose	Galactose	**Fructose**
The polysaccharide found in the exoskeleton of invertebrates is	Pectin	Chitin	Cellulose	Chondroitin sulphate	**Chitin**
Which of the following is a heteroglycan?	Dextrins	Agar	Inulin	Chitin	**Agar**
A positive Benedict's test is not given by	Sucrose	Lactose	Maltose	Glucose	**Sucrose**
Starch is a	Polysaccharide	Monosaccharide	Disaccharide	None of these	**Polysaccharide**
A positive Seliwanoff's test is obtained with	Glucose	Fructose	Lactose	Maltose	**Fructose**
Osazones are not formed with the	Glucose	Fructose	Sucrose	Lactose	**Sucrose**
The most abundant carbohydrate found in nature is	Starch	Glycogen	Cellulose	Chitin	**Cellulose**
The total Glucose in the body is _____ gms.	10–15	20–30	40–50	60–80	**20–30**

Whcih of the following features are common to monosaccharides?	Contain asymmetric centres	Are of 2 types – aldoses and ketoses	Tend to exist as ring structures in solution	Include glucose, galactose and raffinose	**Tend to exist as ring structures in solution**
The following examples are important heteropolysaccharides except	Amylopectin	Heparin	Peptidoglycan	Hyaluronic acid	**Amylopectin**
Glucosamine is an important constituent of	Homopolysaccharide	Heteropolysaccharide	Mucopolysaccharide	Dextran	**Mucopolysaccharide**
Glycogen is present in all body tissues except	Liver	Brain	Kidney	Stomach	**Brain**
Iodine test is positive for starch, dextrin and	Mucoproteins	Agar	Glycogen	Cellulose	**Glycogen**
The distinguishing test between monosaccharides and dissaccharide is	Bial's test	Seliwanoff's test	Barfoed's test	Hydrolysis test	**Barfoed's test**
Cane sugar is known as	Galactose	Sucrose	Fructose	Maltose	**Sucrose**

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DEPARTMENT OF MICROBIOLOGY

Subject	**:**	**Biochemistry**	**Semester**	**:**	**I**
Subject code	**:**	**19MBU103**	**Class**	**:**	**I B.Sc Microbiology**

UNIT-II: COURSE MATERIAL

Unit-II

Classification and functions of lipids, storage lipids-structure and function of fatty acids. Triacylglycerols. Saponification. Structural lipids-structure, functions and properties of phosphoglycerides and sphingolipids.

Suggest Readings

1. Nelson, D.L and Cox, M.M. (2008). Lehninger Principles of Biochemistry, 5th edition. W.H. Freeman and Company.

Lipids

Definition

- The lipids are heterogeneous group of compounds related to fatty acids.
- They constitute a broad group of naturally occurring molecules that include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids, and others.

Biological significance

The main biological functions of lipids include

- Fat serve as an efficient sources of energy storage,
- Serve as insulating material
- Helps in blood clotting
- Serve as structural components of cell membranes, and as important signaling molecules.
- Lipoproteins and glycolipids are important for maintaining cellular integrity.

Classification of Lipids

- They are broadly classified into simple lipids, complex lipids, derived lipids and miscellaneous lipids based on their chemical composition.

Simple lipids

Esters of fatty acids with alcohols. These are mainly of two types

Fats and oils (triacylglycerols)

- These are esters of fatty acids with glycerol.
- The difference between fat and oil is only physical.
- Thus, oil is a liquid while fat is a solid at room temperature.

Waxes

- Esters of fatty acids (usually long chain) with alcohols other than glycerol.
- These alcohols may be aliphatic or alicyclic.
- Cetyl alcohol is most commonly found in waxes.

Complex (or compound) lipids

- These are esters of fatty acids with alcohols containing additional groups such as phosphate, nitrogenous base, carbohydrate, protein etc.

- They are further divided as follows

Phospholipids

- They contain phosphoric acid and frequently a nitrogenous base.
- This is in addition to alcohol and fatty acids.

(i) Glycerophospholipids: These phospholipids contain glycerol as the alcohol e.g., lecithin, cephalin.

(ii) Sphingophospholipids: Sphingosine is the alcohol in this group of Phospholipids
e.g., sphingomyelin.

Glycolipids

- These lipids contain a fatty acid, carbohydrate and nitrogenous base.
- The alcohol is sphingosine; hence they are also called as glycosphingolipids.
- Glycerol and phosphate are absent e.g., cerebrosides, gangliosides.

Lipoproteins

- Macromolecular complexes of lipids with proteins.

Other complex lipids

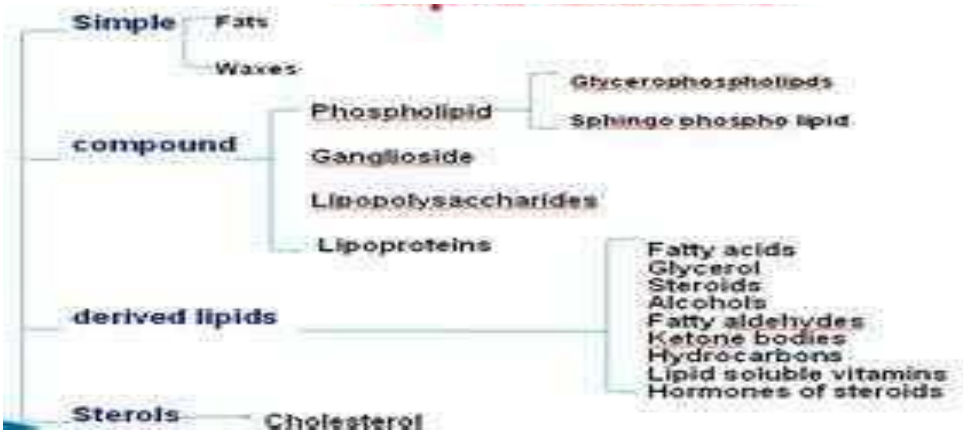
- Sulfolipids, amino lipids and lipopolysaccharides are among the other complex lipids.

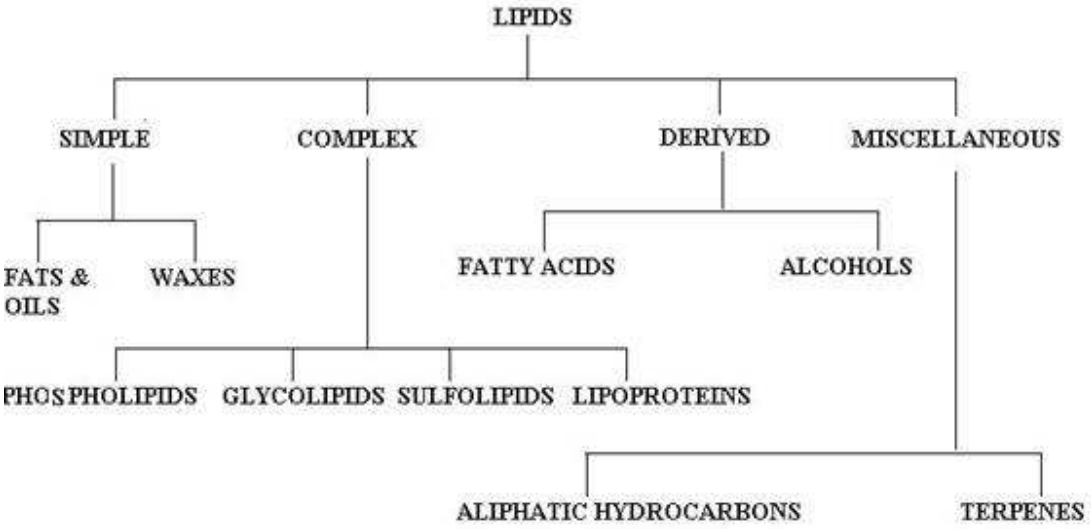
Derived lipids

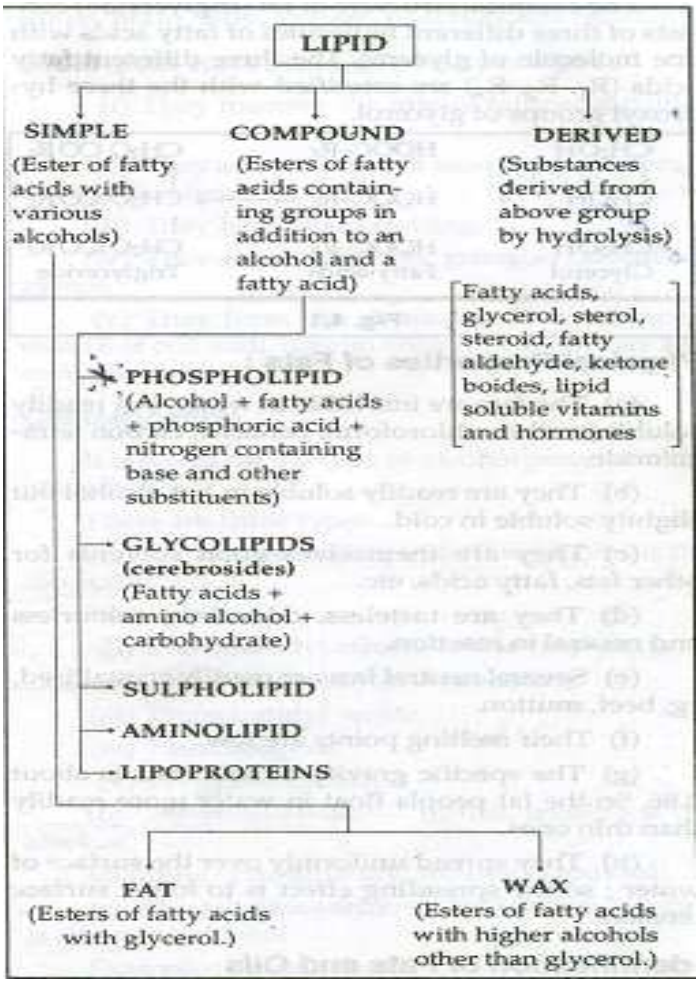
- These are the derivatives obtained on the hydrolysis of group 1 and group 2 lipids which possess the characteristics of lipids.
- These include glycerol and other alcohols, fatty acids, mono- and diacylglycerols, lipid (fat) soluble vitamins, steroid hormones, hydrocarbons and ketone bodies.

Miscellaneous lipids

- These include a large number of compounds possessing the characteristics of lipids e.g., carotenoids, squalene, hydrocarbons such as pentacosane (in bees wax), terpenes etc.


Simple
Fats
Waxes
compound
Phospholipid
Glycerophospholipids
Sphingo phospho lipid
Ganglioside
Lipopolysaccharides
Lipoproteins
derived lipids
Fatty acids
Glycerol
Steroids
Alcohols
Fatty aldehydes
Ketone bodies
Hydrocarbons
Lipid soluble vitamins
Hormones of steroids
Sterols
Cholesterol


LIPIDS
SIMPLE
COMPLEX
DERIVED
MISCELLANEOUS
FATS & OILS
WAXES
FATTY ACIDS
ALCOHOLS
PHOSPHOLIPIDS
GLYCOLIPIDS
SULFOLIPIDS
LIPOPROTEINS
ALIPHATIC HYDROCARBONS
TERPENES

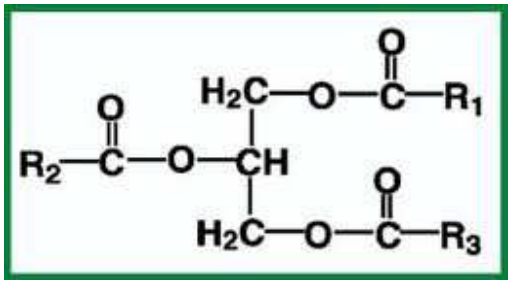


Simple Lipids

Fats

- Fat, any substance of plant or animal origin that is nonvolatile, insoluble in water, and oily or greasy to the touch. They are esters of fatty acids with glycerol.
- They are found in nature in large quantities. Fats are usually solid at ordinary temperatures, such as 25 °C (77 °F), but they begin to liquefy at somewhat higher temperatures.
- Chemically, fats are identical to animal and vegetable oils, consisting primarily of glycerides, which are esters formed by the reaction of three molecules of fatty acids with one molecule of glycerol.

  Eg-Triacylglycerol



- They are the best reserve of food material in the human body.
- They act as insulator for the loss of body heat.
- They act as a padding material for protecting internal organs.
- The chemical structure of fat (triglyceride) consists of three different molecules of fatty acids with one molecule of glycerol. The three different fatty acids (R_1, R_2, R_3) are esterified with the three hydroxyl groups of glycerol.

Physical and Chemical Properties

- Fats (and oils) may be divided into animal and vegetable fats according to source. Further, they may be classified according to their degree of unsaturation as measured by their ability to absorb iodine at the double bonds.
- This degree of unsaturation determines to a large extent the ultimate use of the fat.
- Liquid fats (i.e., vegetable and marine oils) have the highest degree of unsaturation, while solid fats (vegetable and animal fats) are highly saturated.
- Solid vegetable fats melting between 20 and 35 °C (68 and 95 °F) are found mainly in the kernels and seeds of tropical fruits.
- They have relatively low iodine values and consist of glycerides containing high percentages of such saturated acids as lauric, myristic, and palmitic.
- Fats are practically insoluble in water and, with the exception of castor oil, are insoluble in cold alcohol and only sparingly soluble in hot alcohol.
- They are soluble in ether, carbon disulfide, chloroform, carbon tetrachloride, petroleum benzin, and benzene. Fats have no distinct melting points or solidifying points because they are such complex mixtures of glycerides, each of which has a different melting point.

- Glycerides, further, have several polymorphic forms with different melting or transition points.
- Fats can be heated to between 200 and 250 °C (392 and 482 °F) without undergoing significant changes provided contact with air or oxygen is avoided.
- Above 300 °C (572 °F), fats may decompose, with the formation of acrolein (the decomposition product of glycerol), which imparts the characteristic pungent odour of burning fat.
- Hydrocarbons also may be formed at high temperatures.
- Fats are hydrolyzed readily.
- This property is used extensively in the manufacture of soaps and in the preparation of fatty acids for industrial applications.

Physical Properties of Fats

- The fats are insoluble in water, but readily soluble in ether, chloroform, benzene, carbon tetrachloride.
- They are readily soluble in hot alcohol but slightly soluble in cold.
- They are themselves good solvents for other fats, fatty acids, etc.
- They are tasteless, odourless, colorless and neutral in reaction,
- Several neutral fats are readily crystallized, eg, beef, mutton
- Their melting points are low.
- The specific gravity of solid fats is about 0.86. So the fat people float in water more readily than thin ones.
- They spread uniformly over the surface of water; so the spreading effect is to lower surface tension.

Chemical properties of fats

Hydrolysis

1. Hydrolysis of triacylglycerol takes place by lipases producing fatty acids and glycerol.
2. Phospholipases attack the ester linkage of phospholipids.

Saponification

- Boiling with an alcoholic solution of strong metallic alkali hydrolyzes triglycerides into glycerol and fatty acids are called saponification.
- The products are glycerol and the alkali salts of the fatty acids which are called soaps.
- Fats, phospholipids, glycolipids and waxes are called saponifiable lipid.
- Steroids, polyisoprenoids and higher alcohols are grouped as unsaponifiable lipids because they cannot give rise to soap.

Saponification number

- The number of milligrams of KOH is required to saponify 1 gram of fat or oil.
- The amount of alkali needed to saponify a given quantity of fat will be depended upon the number of -{:OOH group present. It is inversely proportional to the average molecular weight of the fatty acids in the fat i.e. the fats containing short chain fatty acids will have more -{:OOH groups per gram than long chain fatty acids and this will take up more alkali and hence will have higher saponification number.

Example: Butter containing a larger proportion of short chain fatty acids such as butyric and caproic acids, has relatively high saponification number 220 to 230.

Acid number

- The number of milligrams of KOH is required to neutralize the free fatty acids of 1 gram of fat.
- Significance: The acid number indicates the degree of rancidity of the given fat.

Iodine number

- This is the amount (in grams) of iodine absorbed by 100 grams of fat.
- This is the measure of the degree of unsaturation of a fat.
- 3. Significance: If the fat contains higher number of unsaturated fatty acids, it becomes essential for the protection of heart disease. These unsaturated fatty acids being combined with the cholesterol are oxidized in the liver producing bile acids, bile salts, Vitamin D, gonadotrophin hormones. They prevent atherosclerosis.

Acetyl number

- The number of milligrams of KOH required to neutralize the acetic acid obtained by saponification of 1 gram of fat after it has been acetylated.
- This is a measure of the number of hydroxy acid groups in the fat.

Polenske number

- The number of milliliters of 0.l (N) KOH required to neutralize the insoluble fatty acids from 5 grams of fat.

Reichert-Miessl number

- This is the same as the Polenske number except that the soluble fatty acids are measured by titration of the distillate obtained by steam distillation of the saponification mixture.
- Significance: It measures the amount of volatile soluble fatty acids.

Halogenation

- Chlorine, bromine and iodine atoms may be added to the double bonds of unsaturated fatty acids containing fats.

Rancidity

- Nearly all natural fats are oxidized when ex- posed to air, light, moisture, particularly, if warm, it develops an unpleasant odour and taste. The enzyme lipase which in the presence of moisture and temperature bring about hydrolysis rapidly.
- This happens so due to the formation of peroxides at the double bonds of unsaturated fatty acids.
- Vitamin E is an important natural antioxidant and prevents development of rancidity.

Soaps

- Soaps are metallic salts of fatty acids.
- Soaps are formed by adding alkalis to fatty acids.
- Soaps of unsaturated fatty acids are softer and more water soluble than those of saturated fatty acids.
- Potassium soap of an acid is more water soluble and softer than the sodium soap, calcium and magnesium soaps are far less soluble.

Compound lipids

Phospholipids

These are complex or compound lipids containing phosphoric acid, in addition to fatty acids, nitrogenous base and alcohol. Based on the type of alcohol present in the phospholipid they are classified into three types.

Glycerophosphatides - In this, glycerol is the alcohol group.

Example

- Phosphatidyl ethanolamine (cephalin).
- Phosphatidyl choline (Lecithin).
- Phosphatidyl serine.
- Plasmalogens.
- Phosphatidic acid.

Phosphoinositides - In this, inositol is the, alcohol.

Example: Phosphatidyl inositol (Lipositol).

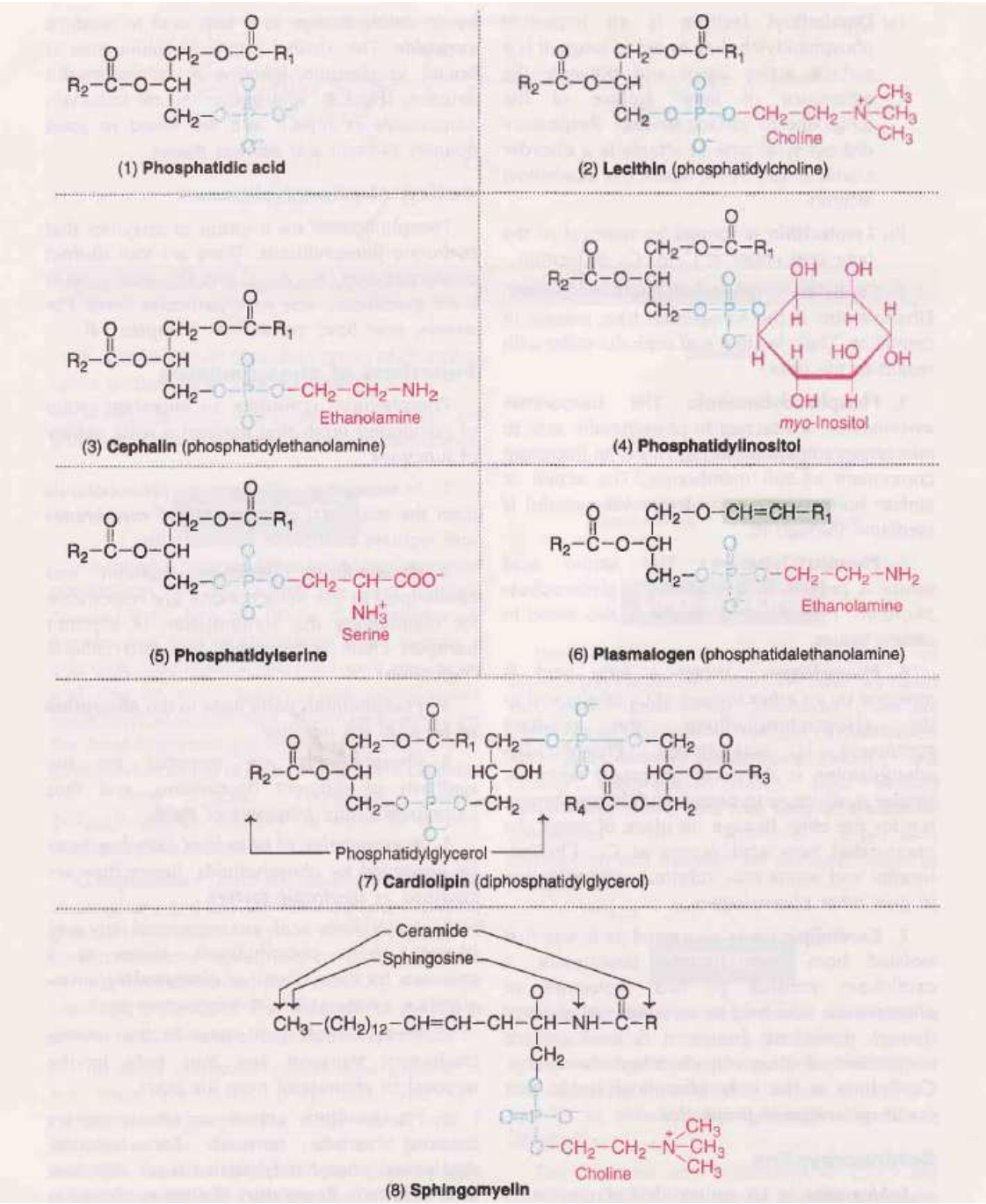
Phosphosphingosides - In this, sphingosine is an amino alcohol. .

Example: Sphingomyelin, ceramide.

Structure

- **Phosphatidic acid:** This is the simplest phospholipid. It does not occur in good concentration in the tissues.
- **Lecithins (phosphatidylcholine):** These are the most abundant group of phospholipids in the cell membranes.
- **Cephalins (phosphatidylethanolamine):** Ethanolamine is the nitrogenous base present in cephalins, thus lecithin and cephalin differ with regard to the base.
- **Phosphatidylinositol:** The steroisomer myo-inositol is attached to phosphatidic acid to give Phosphatidylinositol.
- **Phosphatidylserine:** The amino acid serine is present in this group of glycerophospholipids. Phosphatidylthreoninise also found in certain tissues.
- **Plasmalogens:** When a fatty acid is attached by an ether linkage at C1 of glycerol in the glycerophospholipids, the resultant compound is plasmalogen.

- **Cardiolipin**: It is so named as it was first isolated from heart muscle. Structurally, a cardiolipin consists of two molecules of phosphatidic acid held by an additional glycerol through phosphate groups.



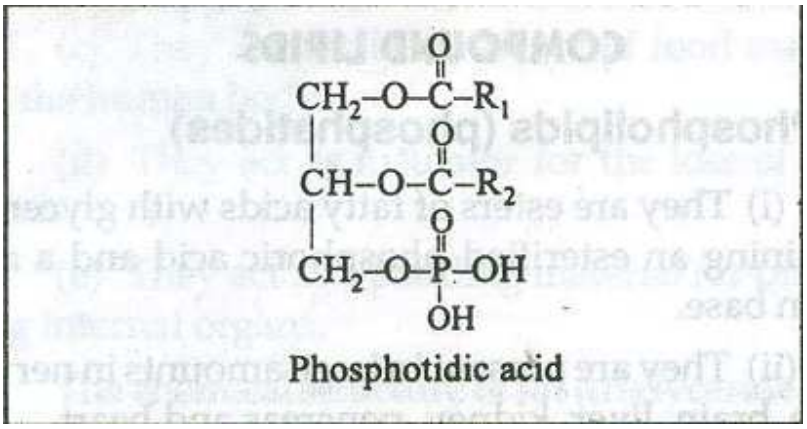
Structure of phospholipids

Phosphatidic acid and phosphat idyl glycerols

Phosphatidic acid is important as an intermediate in the synthesis of triacylglycerols and phospholipids.

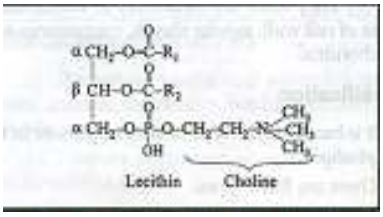
Cardiolipin

- It is formed from phosphatidyl glycerol.
- Chemically, it is diphosphatidyl glycerol.
- It is found in inner membrane of mitochondria and bacterial wall.



Lecithins (Phosphatidylcholine)

The lecithins contain glycerol and fatty acids, phosphoric acid and choline (nitrogenous base). Lecithins generally contain a saturated fatty acid at a position and an unsaturated fatty acid at f} position. They can exist in a or f} forms.



Physical Properties

- Lecithins are waxy, white substances but become brown soon when exposed to air.
- They are soluble in ordinary fat solvents except acetone.
- They decompose when heated.
- They constitute valuable agents for the emulsifications of fats and oils.

Chemical Properties of Lecithin

- When aqueous solution of lecithins is shaken with ~S04' choline is split off, forming phosphatidic acid.'
- When lecithins are boiled with alkalis or mineral acids, not only choline is split off; phosphatidic acid is further hydrolyzed to glycerophosphoric acid and 2 molecules of fatty acids.

Lecithin H_2SO4-+ Phosphatidic acid + cholin.

Phosphatidic acid -+ Glycerophosphoric acid + fatty acids (2 mol)'

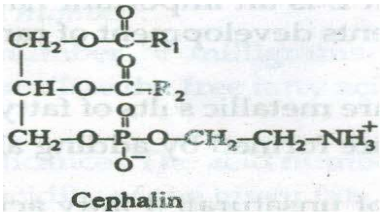
Physiological Functions of Lecithin

- It facilitates the combinations with proteins to from lipoproteins of plasma and cells.
- Acetylcholine formed from choline has an important role in the transmission of nervous impulses across synapses.
- Choline is the most important lipotropic agent as it can prevent formation of fatty liver.
- Lecithin lowers the surface tension of lung alveoli. Dipalmityllecithin is a major constituent of "lung surfactant" which prevents the adherence *ql* the inner surface of the alveoli of the lungs (preventing the collapse of the alveoli) by its surface tension lowering effect. The absence of this in the alveolar membrane of some premature infants causes the respiratory distress syndrome in them.
- It lowers the surface tension of water molecule and helps in the emulsification of fat.

Difference of Lecithin and Cephalin

Cadmium chloride compound of Cephalin is soluble but cadmium chloride compound of lecithin is insoluble.

Cephalins (Phosphatidyl ethanplamine)

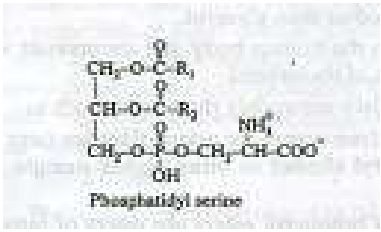


Cephalin

They always occur in the tissues in association with lecithins and are very similar in

properties. The only difference is the nitrogenous base.

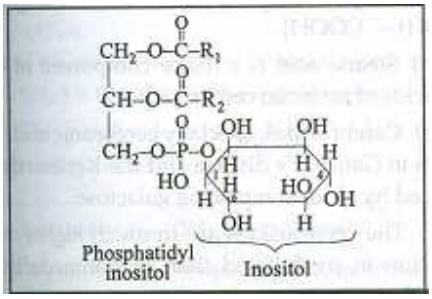
Phosphatidyl Serine



A cephaline like phospholipid is found in tissues.

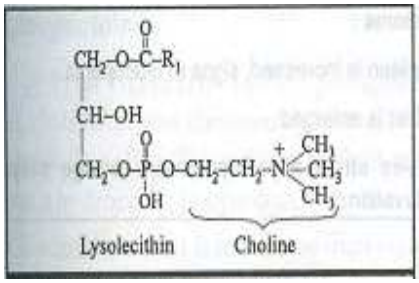
Phosphatidyl inositol (Lipositol or Phosphoinasitides)

Phosphatidyl~ inositol Inositol



- It acts as second messenger in Ca ++ de- pendent hormone action.
- Some signals must provide communication between the hormone receptor on the plasma membrane and intracellular Ca ++ reservoirs.
- They are more acidic than the other phospholipids.

Lysophospholipids:



- These are phosphoacylglycerols containing only one acyl radical in ex position eg, Lysolecithin.

Formation

(a) By the action of phospholipase A_2.

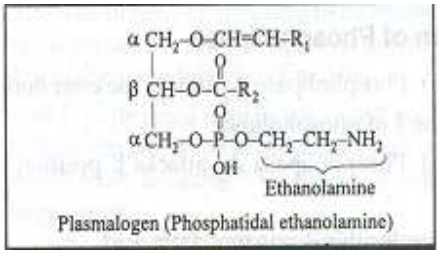
(b) By interaction of lecithin and cholesterol in presence of the enzyme lecithin

cholesterol acyl transferase, so lysolecithin and cholesterol ester are formed

Lecithin + cholesterol .t LCAT

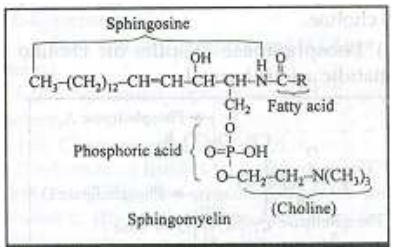
Lysolecithin + cholesterol ester.

Plasmalogens



- These are the contents of brain and muscle.
- Structurally, these resemble lecithins and cephalins but give a positive reaction when tested for aldehydes with Schiff's reagent (fuchsin sulfurous acid) after pretreatment of the phospholipid with mercuric chloride. Plasmalogen (phosphatidal ethanolamine)
- They possess an ether link in ex position instead of ester link. The alkyl radical is an unsaturated alcohol.

Sphingomyelins



- These are found in large quantities in brain and nerve tissue.
- The concentrations of these phospholipids are increased in Niemann-Pick disease in the liver and spleen.
- These contain sphingosine (18 carbon) (amino alcohol) fatty acid, phosphoric acid and choline. No glycerol is present.
- In sphingosine molecule -N~ group.· binds a fatty acid by an amide linkage to produce ceramide. When phosphate group is attached to ceramide it is called ceramide phosphate.

- When choline is split off from sphingomyelin, ceramide phosphate is left.

Clinical Aspect

- In Niemann-.Pick disease excess amount of sphingomyelin are deposited in brain, liver, spleen.
- It is a lipid storage disease (lipidoses) and hereditary. It is caused by the deficiency of enzyme sphingomyelinase.
- The clinical findings are:

(a) Enlarged liver and spleen.

(b) Mental retardation.

(c) Nervous system is affected.

(d) Anemia and leukocvtosis.

Action of Phospholipase

(a) Phospholipase A_1 attacks the ester bond in position **1** of phospholipid.

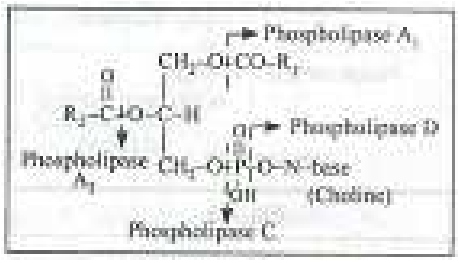
(b) Phospholipase A_2 attacks β position and form

Lysolecithin + one mol. fatty acid.

(c) Phospholipase B (lysophospholipase) attacks lysolecithin and hydrolyzes ester bond in a position and forms glyceryl phosphoryl choline + **1** mol fatty acid.

(d) Phospholipase C hydrolyzes phosphate ester bond and produces α, β diacyl glycerol + phosphoryl choline.

(e) Phospholipase D-splits off choline and phosphatidic acid is formed



Function of Phospholipids

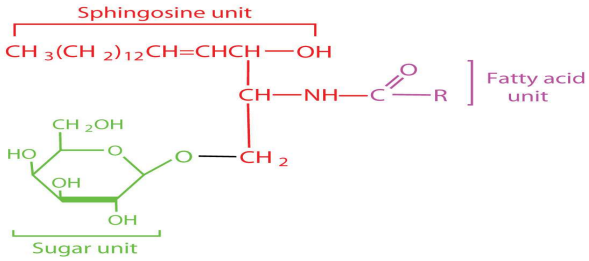
- Phospholipids constitute an important group of compound lipids that perform a wide variety of functions.

- In association with proteins, phospholipids form the structural components of membranes and regulate membrane permeability.
- Phospholipids (lecithin, cephalin and cardiolipin) in the mitochondria are responsible for maintaining the conformation of electron transport chain components, and thus cellular respiration.
- Phospholipids participate in the absorption of fat from the intestine.
- 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 regarded as lipotropic factors.
- Arachidonic acid, an unsaturated fatty acid liberated from phospholipids, serves as a precursor for the synthesis of eicosanoids (prostaglandins, prostacyclinst, hromboxanes etc.,).
- Phospholipids participate in the reverse cholesterol transport and thus help in the removal of cholesterol from the body.
- Phospholipids act as surfactants (agents. lowering surface tension). For instance dipalmitoyl phosphatidylcholinies an important lung surfactant. Respiratory distress syndrome infants are associated with insufficient production of this surfactant.
- Cephalins, an important group of phospholipids participate in blood clotting.
- Phospholipids (phosphatidylinositol) are involved in signal transmission across membranes.

Glycolipids

Glycolipids are lipids with a carbohydrate attached. Their role is to provide energy and also serve as markers for cellular recognition. Eg: One type of glycolipid found in human red blood cells is involved in the ABO blood type antigens.

They contain an amino alcohol (Sphingosine) attached with an amide linkage to fatty acid and glycosidically to a carbohydrate moiety (Sugar, amino sugar, sialic acid).



Classification

They are classified into (i) Cerebrosides, (ii) Gangliosides.

Cerebrosides

- Cerebrosides contain galactose, a high molecular weight fatty acid and sphingosine.

Therefore, they may also be classified as sphingolipids.

- They are the chief constituent of myelin sheath.
- They may be differentiated by the type of fatty acid in the molecule.

These are

- Kerasin-Containing lignoceric acid [CH_3 - (CH_2)22 - COOH].
- Cerebron-Containing a hydroxylignoceric acid (cerebronic acid).
- [CH3-(C~h1 - CH(OH) - COOH].
- Nervon-Containing an unsaturated homologue of lignoceric acid called nervonic acid. [CH_3 - {C~h- CH = CH - (CH_2)13 - COOH].
- Oxynervon-Containing hydroxynervonic acid [CH_3 - (CH_2)7 - CH = CH - (CH_2)12- CH(OH) - COOH].
- Stearic acid is a major component of the fatty acids of rat brain cerebrosides.
- Cerebrosides, specially cerebronic acid, increases in Gaucher's disease and the Kerasin characterized by glucose replacing galactose.
- The cerebrosides are in much higher concentration in medullated than in nonmedullated nerve fibers.

Clinical Aspect

Gaucher's disease

- The cerebroside content of the reticuloendothelial cell (spleen) is very high.
- In cerebroside molecule, the kerasin is characterised by glucose replacing galactose.

- The disease is caused by the deficiency of enzyme glucocerebrosidase.

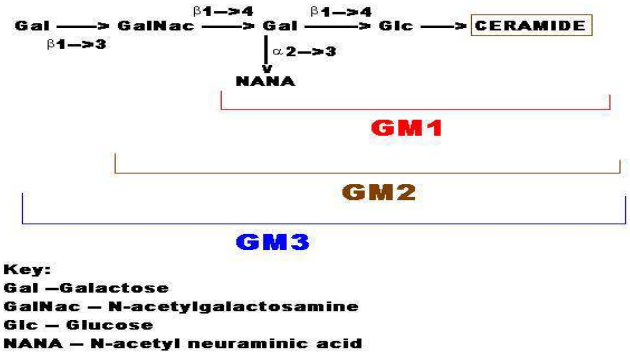
Symptoms

- Spleen is increased, signs of leucopenia.
- Liver is enlarged.
- Eyes show a yellow brown wedge shaped elevation.

Gangliosides

- These are glycolipids occurring in the brain.
- Gangliosides contain ceramide (sphingosine + fatty acids), glucose, galactose, N-acetylgalactosamine and sialic acid.
- Some gangliosides also contain dihydrosphingosine or Gangliosine in place of sphingosine.
- Most of the gangliosides contain a glucose, two molecules of galactose, one N-acetylgalactosamine and upto three molecules of sialic acid.

They are further classified into GM1, GM2, GM3. The following figure depict this.

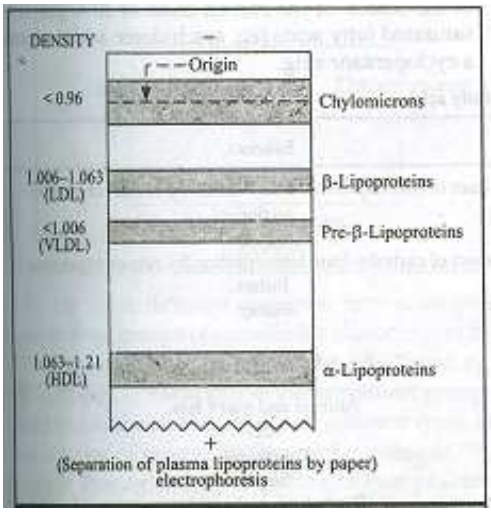


Lipo Proteins

Lipids are transported in blood as large macromolecules called lipoproteins. These are complexes with proteins. Free fatty acids are the exception, mainly binding to albumin.

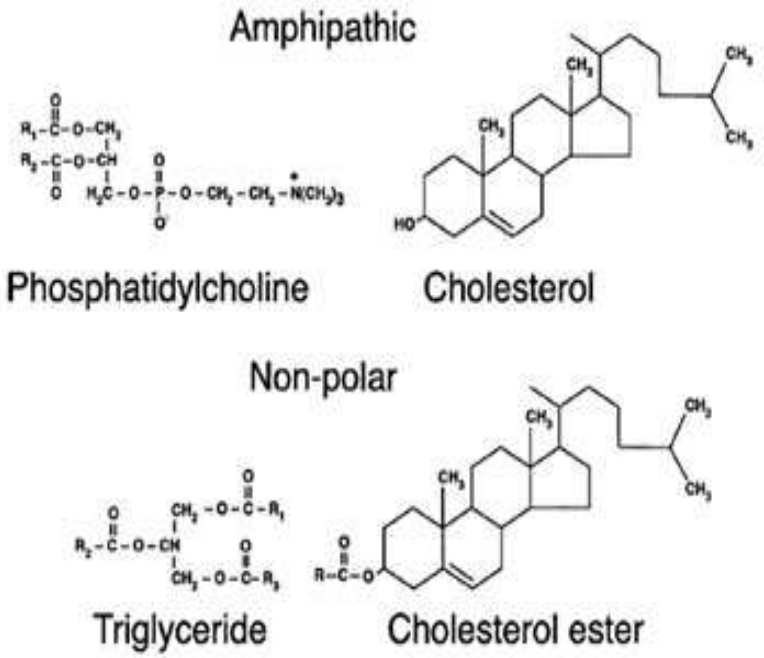
(i) Triacylglycerol (45%), phospholipids (35%), cholesterol and cholesteryl esters (15%), free fatty acids (less than 5%) and also protein combine to form a hydrophilic lipoprotein complex.

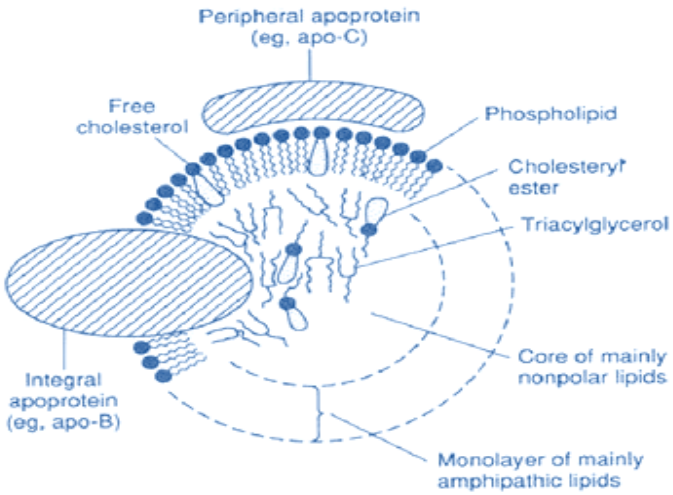
(ii) Since pure fat is less dense than Water, the proportion of lipid to protein in lipoproteins in plasma is separated by ultracentrifugation.



Structure of lipo protein

Hydrophobic lipids, triglycerides and phospholipids are within the lipoprotein core, with the polar portions of phospholipids and the water-soluble alcohol portion of free cholesterol projecting into the aqueous environment, causing solubilization of the lipoprotein.





Types of lipoproteins

Lipoprotein classes can be separated physico chemically, either by electrophoresis which uses surface charge or by ultracentrifugation which uses relative density. Four major groups of lipoproteins have been identified which are important physiologically and in clinical diagnosis in some metabolic disorders of fat metabolism.

(i) chylomicrons,
(ii) Very-low-density lipoprotein (VLDL),
(iii) Intermediate-density lipoprotein (IDL),
(iv) Low-density lipoprotein (LDL)
(v) High-density lipoprotein (HDL).

Predominant lipid is triacylglycerol (50%) and cholesterol (23%). The concentrations of these are increased in atherosclerosis and coronary thrombosis etc.

LDL: Predominant lipid is cholesterol (46%) and phospholipids (23%).Increase in atherosclerosis and coronary thrombosis, etc.

HDL: Predominant lipid is phospholipid (27%) and proteins (45%). The protein moiety lipoprotein is known as an apo protein which constitute nearly 60% of some HDL and 1% of chylomicrons. Many lipoproteins contain more than one type of apoprotein polypeptide.

The larger lipoproteins (such as chylomicrons and VLDL) consist of a lipid core of non-polar triacylglycerol and cholesteryl ester surrounded by more polar phospholipid,

cholesterol and apoproteins.

The table gives the properties of different lipo proteins

Lipoprotein class	Density (g/mL)	Diameter (nm)	Protein % of dry wt	Phospholipid %	Triacylglycerol % of dry wt
HDL	1.063-1.21	5 – 15	33	29	8
LDL	1.019 – 1.063	18 – 28	25	21	4
IDL	1.006-1.019	25 - 50	18	22	31
VLDL	0.95 – 1.006	30 - 80	10	18	50
chylomicrons	< 0.95	100 - 500	1 - 2	7	84

Importance

- To transport and deliver the lipids to tissues.
- To maintain structural integrity of cell surface and sub cellular particles like mitochondria and microsomes.
- The β-lipoprotein fraction increases in severe diabetes mellitus, atherosclerosis etc. Hence determination of the relative concentrations of α and β-lipoproteins and pre-β- lipoproteins are of diagnostic importance.

Aminolipids

Phosphatidyl ethanolamine and serines are aminolipids and sphingomyelins and gangliosides contain substituted amino groups.

Sulpholipids (Sulphatides)

- These have been isolated from brain and other animal tissues.
- These are sulphate derivatives of the a1actosyl residue in cerebrosides.

Derived Lipids

Fatty Acids

- In chemistry, especially biochemistry, a fatty acid is a carboxylic acid with a long aliphatic tail (chain), which is either saturated or unsaturated.

- Most naturally occurring fatty acids have a chain of an even number of carbon atoms, because they are synthesized from 2-carbon units and are straight chain derivatives (from 4 to 28.)
- These are obtained by the hydrolysis of fats. Fatty acids are usually derived from triglycerides or phospholipids.
- When they are not attached to other molecules, they are known as "free" fatty acids.
- The straight chain may be saturated (containing no double bonds) or unsaturated (containing one or more double bonds).
- Carbon atoms of fatty acids are numbered from the carboxyl carbon (carbon No.1). The carbon atom adjacent to the carboxyl carbon (Carbon No. 2) is also known as the α-carbon. Carbon atom No. 3 is the β-carbon and the end methyl carbon is known as the γ-carbon.
- Various conventions are used for indicating the number and position of the double bonds, eg, Δ^9 indicates a double bond between carbon atoms 9 and 10 of the fatty acid.

Functions

- Fatty acids are important sources of fuel because, metabolized, they yield large quantities of ATP.
- Many cell types can use either glucose or fatty acids for this purpose.
- In particular, heart and skeletal muscle prefer fatty acids.
- The brain cannot use fatty acids as a source of fuel; it relies on glucose or ketone bodies

Types

1. Straight chain.
2. Branched chain.
3. Substituted (methyl substituted -cerebronic acid)
4. Cyclic (chaulmoogric acid) used in leprosy.

Straight chain

(a) Saturated {odd (less than 10 carbon atom)} & even (greater than 10 carbon atom)}.

(b) Unsaturated (odd & even). (Straight chain even number fatty acid is common)

Saturated Fatty Acids

General formula for saturated fatty acids is CnH2n+l COOE. Other higher fatty acids occur in waxes. A few branched-chain fatty acids have also been isolated from both plant and animal sources. Prostanoids include Prostaglandins (PC), and thromboxanes (TX).

General characteristics of prostanoid

(a) All are 20 carbon compound.

(b) Trans double bond at 13 position.

(c) -OH group at 15 position.

Saturated Fatty Acids

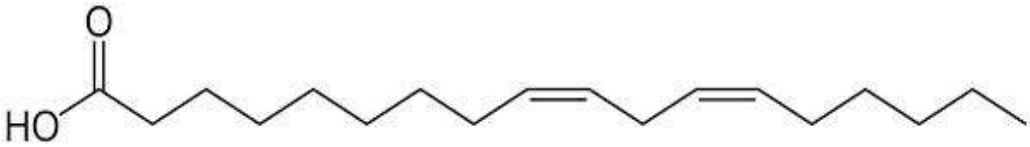
Acid	*Formula*	*Carbon atoms*	*Sources*
Acetic	CH_3COOH	2	Product of carbohydrate fermentation by rumen organisms
Propionic	C_2H_5COOH	3	— do—
Butyric	C_3H_7COOH	4	Butter.
Caproic	$C_5H_{11}COOH$	6	Product of carbohydrate fermentation by rumen organisms
Caprylic	$C_7H_{15}COOH$	8	Butter.
Decanoic (Capric)	$C_9H_{19}COOH$	10	Butter.
Lauric	$C_{11}H_{23}COOH$	12	Coconut oils.
Myristic	$C_{13}H_{27}COOH$	14	Coconut oils.
Palmitic	$C_{15}H_{31}COOH$	16	Animal and plant fats.
Stearic	$C_{17}H_{35}COOH$	18	–do–
Arachidic	C_{19} H_{39} COOH	20	Peanut oil.
Behenic	$C_{21}H_{43}COOH$	22	Seeds.
Lignoceric	$C_{23}H_{47}COOH$	24	Peanut oil, crebrosides.

Unsaturated fatty acids

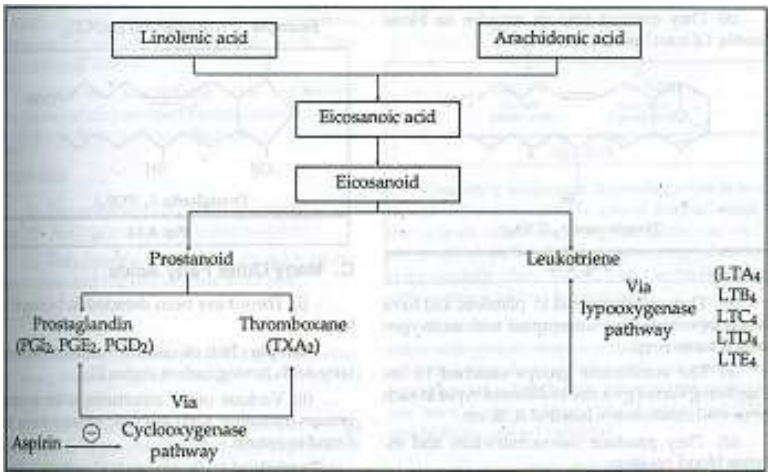
A.General formula C_nH2n-l COOH

Type of acid	*Acid*	*Formula*	*Unsaturation at carbon atoms*	*Number of double bonds*	*Sources*
Mono-unsaturated	Palmitoleic	$C_{15}H_{29}COOH$	Δ^9	1	Near all fats
,,	Oleic	$C_{17}H_{33}COOH$	Δ^9	1	do
Poly-unsaturated	Linoleic	$C_{17}H_{31}COOH$	Δ^9, Δ^{12}	2	Animal and plant fat
,,	Linolenic	$C_{17}H_{29}COOH$	$\Delta^9, \Delta^{12}, \Delta^{15}$	3	do
,,	Arachidonic	$C_{19}H_{31}COOH$	$D^5, \Delta^8, \Delta^{11}, \Delta^{14}$	4	Peanut oil
Eicosanoids Prostanoids & Leukotrienes	Timnodonic	$C_{19}H_{33}COOH$	$\Delta^5, \Delta^8, \Delta^{11}, \Delta^{14}, \Delta^{17}$	5	Fish oils, eg. cod liver oil
,,	Clupanodonic	$C_{12}H_{32}COOH$	$\Delta^7, \Delta^{10}, \Delta^{13}, \Delta^{16}, \Delta^{19}$	5	Fish oils, phospholipids in brain
,,	Cervonic	$C_{21}H_{31}COOH$	$\Delta^4, \Delta^7, \Delta^{10}, \Delta^{13}, \Delta^{16}, \Delta^{19}$	6	Fish oils, phospholipids in brain

- Fatty acids with one double bond are monounsaturated and those with 2 or more double bonds are collectively known as polyunsaturated fatty acids (PUFA).
  Eg: Linoleic acid



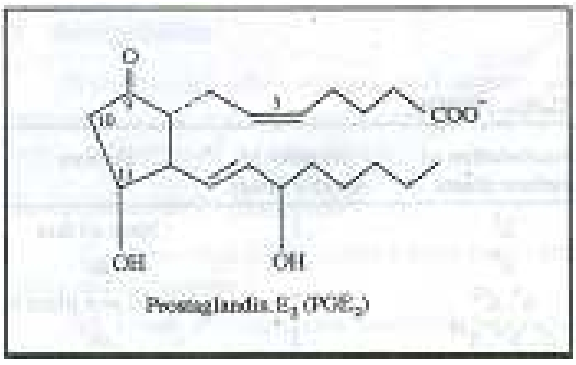
Classification



Three different eicosanoic fatty acids give rise to three groups of eicosanoids characterized by the number of double bonds in the side chains, eg, PG_1, PG_2, PG_3. Variations in the substituent groups attached to the rings give rise to different types in each series of prostaglandins, as for example, "E" type of Prostaglandin has a keto group in position9,whereas the "F" type has a hydroxyl group in this position,

Prostacyclins (PGI)

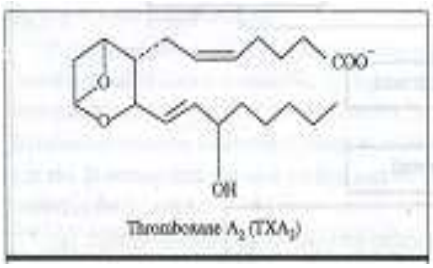
- They are formed in vascular endothelium and continually formed in heart. They are also formed in kidneys.
- They are formed from cyclic endoperoxide PGH_2 by the action of microsomal Prostacyclin synthetase.
- They inhibit platelet aggregation and gastric secretion from the pyloric mucosa.



Prostaglandin E_2 (PGE_2)

- They decrease blood pressure and protect coronary arteries.
- They increase renal blood flow and stimulate renin production.
- They are inhibited by hyperlipemia, vit. E deficiency and radiation.

Thromboxanes

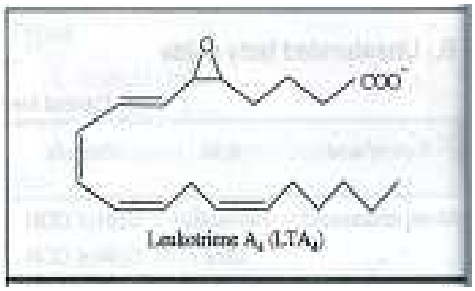


Thromboxane A_2 (TXA_2)

- They contract smooth muscles on blood vessels, GI tract, uterus, bronchioles.
- They are discovered in platelets, and have the cyclopentane ring interrupted with an oxygen atom (Oxane ring).
- The substituent groups attached to the rings being varied give rise to different types in each series of thromboxanes labelled A, B, etc.
- They produce vasoconstriction and in- crease blood pressure.
- They cause release of serotonin and calcium ion (Ca ++) from platelet granules.
- lmidazoles inhibit their synthesis.

Leukotrienes

- They are the third group of eicosanoid derivatives formed via the lipoxygenase pathway rather than cyclization of the fatty acid chain.

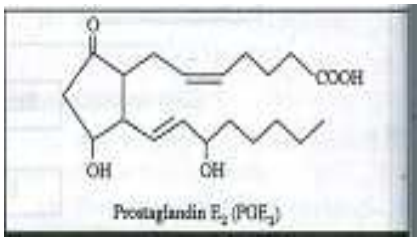
- 

Leukotriene A_4 (LTA_4)

- They are first described in leukocytes.
- They are characterized by the presence of three conjugated double bonds.
- They are stimulators of mucus secretion and are responsible for vasoconstriction of bronchial muscles.
- They are inhibited by prolonged use of aspirin.
- The groups of compounds known as prostaglandins are synthesized from arachidonic acid in the body. They have pharmacologic and biochemical activity.

Prostaglandins (PG)

- They virtually exist in every mammalian tissue and act as local hormones.
- They have important physiologic anc pharmacologic activities.
- They are synthesized in vivo by cyclization of the center of the carbon chain of 2OC polyunsaturated fatty acids (eg, arachidonic acid) to fom a cyclopentane ring.
- **Example:** Prostaglandin E_2(PGE_2)



Prostaglandin E_2 (PGE_2)

Many Other Fatty Acids

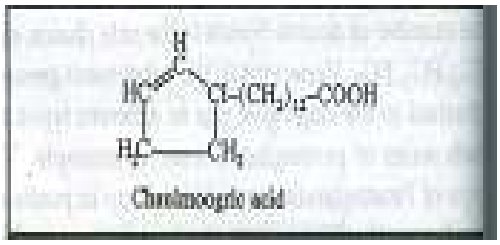
- These have been detected in biologic material.

Example: Fish oil contain 5 and. 6 unsaturated fatty acids having carbon atoms 22.

- Various other structures with hydroxy groups (ricinoleic acid) or cyclic groups have been found in nature. .

Example of cyclic groups is chaulmoogric acid which was used many years ago in the

treatment of leprosy.



Chaulmoogric acid

Essential fatty acids	Chemical name	General formula	No. of double bonds	Structure	Sources
Linoleic	9, 12-Octadecadi-enoic acid	$C_nH_{2n-4}COOH$	$2(\Delta^9,\Delta^{12})$	$CH_3-(CH_2)_4$ $(CH=CH-CH_2)_2$ $(CH_2)_6COOH$	Corn, Peanut, Cotton seed, Soyabean oil.
Linolenic	6,9,12-Octadeca-trienoic acid	$C_nH_{2n-5}COOH$	$3(\Delta^9,\Delta^{12},\Delta^{15})$	$CH_3-(CH_2)_4$ $(CH=CH-CH_2)_3$ $(CH_2)_3COOH$	Found frequently with linoleic acid but particularly in linseed oil.
Arachidonic	5,8,11,14-eicosatetra-enoic acid	$CnH_{2n-7}COOH$	$4(\Delta^5,\Delta^8,\Delta^{11},\Delta^{14})$	$CH_3-(CH_2)_4(CH$ $=CH-CH_2)_4$ $(CH_2)_2COOH$	Found in small quantities with linoleic and linolenic acids but particularly in peanut oil.

Essential Fatty Acids

Burr and Burr (1930) introduced the term "Essential Fatty Acids" (EF A) on the basis that they are essential for the growth and health of young albino rats. These polyunsaturated fatty acids which are not synthesized in the body but are taken from natural sources are called essential fatty acids. They are (mentioned above): linolenic and arachidonic acids are formed from linoleic acids provided linoleic acids are available in the body in sufficient quantities.

Properties

- The essential fatty acids of vegetable oils have low melting points and iodine number.
- They become saturated fatty acids on hydrogenation and the oils become solid fats.

Functions

- The essential fatty acids in high concentration along with the lipids constitute the structural elements of the tissues.
- The lipids of gonads also contain a high concentration of polyunsaturated fatty acids which suggest the importance of reproductive function.

- They effect the prolongation of clotting time and increase the fibrinolytic activity.
- They retard atherosclerosis being esterified and emulsified with cholesterol and are incorporated into lipoproteins for transport to the liver for further oxidation.
- They cure skin lesions.
- The deficiency of these acids in the diet of babies causes eczema.

Isomerim in Unsaturated Fatty Acids

Variations in the locations of the double bond in unsaturated fatty acid chains produce isomers. Oleic acid has 15 different positional isomers.

Geometric isomerism depends on the orientation of radicals around the axis of double bonds. If the radicals which are being considered are on the same side of the bond, the compound is called "cis", if on opposite side, "trans". This can be illustrated with maleic acid and fumaric acid.

There are more geometric isomers in case of acids with greater degree of unsaturation. The un- saturated long chain of fatty acids occurring in nature are nearly all in tbe 'cis' form and the molecules are "bent" at the position of the double bond. Thus, arachidonic acid is D-shaped.

Refined and Hydrogenated Oils

Refined oil: It is prepared in the following manner:

- Free fatty acids are removed by alkali treatment
- Colouring matter is removed by activated carbon.
- Odour is '∼ov"" by superheated steam,

Essential fatty acids, or EFAs, are fatty acids that humans and other animals must ingest because the body requires them for good health but cannot synthesize them. The term "essential fatty acid" refers to fatty acids required for biological processes but does not include the fats that only act as fuel. Only two fatty acids are known to be essential for humans: alpha-linolenic acid (anomega-3 fatty acid) and linoleic acid (an omega-6 fatty acid).

Some other fatty acids are sometimes classified as "conditionally essential," meaning that they can become essential under some developmental or disease conditions;

examples include docosahexaenoic acid (an omega-3 fatty acid) and gamma-linolenic acid(an omega-6 fatty acid).

The essential fatty acids start with the short chain polyunsaturated fatty acids (SC-PUFA):

- ω-3 fatty acids:
- α-Linolenic acid or ALA (18:3n3)
- ω-6 fatty acids:
- Linoleic acid or LA (18:2n-6)

These two fatty acids cannot be synthesized by humans because humans lack the desaturase enzymes required for their production.

They form the starting point for the creation of longer and more desaturated fatty acids, which are also referred to as long-chain polyunsaturated fatty acids (LC-PUFA):

ω-3 fatty acids

eicosapentaenoic acid or EPA (20:5n-3)

docosahexaenoic acid or DHA (22:6n-3)

ω-6 fatty acids

gamma-linolenic acid or GLA (18:3n-6)

dihomo-gamma-linolenic acid or DGLA (20:3n-6)

arachidonic acid or AA (20:4n-6)

ω-9 fatty acids are not essential in humans because they can be synthesized from carbohydrates or other fatty acids.

Functions of Essential Fatty Acids

Essential fatty acids have a ton of benefits in our body. They are

- They help with cellular development and the formation of healthy cell membranes, and they have actually been shown to block tumor formation in animals, as well as block the growth of human breast cancer cells.
- Essential fatty acids assist in the development and function of the brain and nervous system.
- Helps to regulate proper thyroid and adrenal activity.
- They play a role in thinning blood, which can prevent blood clots that lead to heart attacks and stroke.

- They also possess natural anti-inflammatory qualities that can relieve symptoms of both arthritis and other autoimmune system diseases.
- Essential fatty acids regulate blood pressure, immune responses and liver function, as well as help with blood clotting and breaking down cholesterol.
- Diet low in these fatty acids has been shown to create skin problems, including eczema, dandruff, split nails and brittle hair.

Hydrogenated oils

The refined oils are hydrogenated under optimum temperature and pressure with hydrogen in the" presence of nickel catalyst. . Unsaturated fatty acids are converted into saturated fatty' acids.

Hydrogenation

Oleic acid----------Stearic acid

The liquid oil becomes solid fat and the unsaturated fatty acid content decreases. Vanaspati is hydrogenated refined groundnut oil.

Alcohols

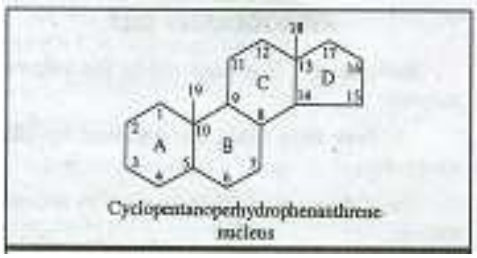
Alcohols found in lipid molecules include glycerol, cholesterol and higher alcohols (cetyl alcohol), usually found in the waxes.

The unsaturated alcohols are important pigments. Phytyl alcohol is a constituent of chlorophyll and lycophyll ($C_{40}H_{56}O_2$); a polyunsaturated dihydroxy alcohol occurs in tomatoes as a purple pigment.

Steroids

The steroids are often found in association with fat. They have a similar cyclic nucleus resembling phenanthrene (rings A, B, C) to which a cyclopentane ring (D) is attached. The parent substance is better designated as cyclopentano-perydrophen anthrene. The positions on the steroid nucleus are numbered as shown in the figure.

Methyl side chains occur typically at positions 10 and 13 (constituting C atoms 19 and 18). A side chain at position 17 is usual (as in cholesterol). *H* the compound has one or more hydroxyl groups and no carbonyl or carboxyl groups, it is a *sterol,* and the name terminates in-OL.



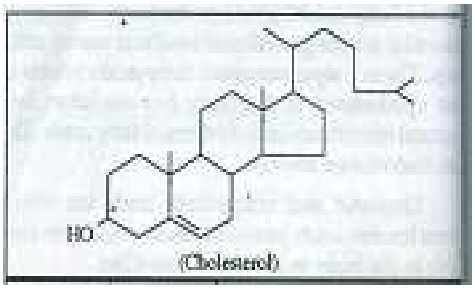
Cyclopentanope Rhydrophenanthrene. nucleus

Steroids may be divided in the following manner:

- Sterols=cholesterol, ergosterol, coprosterol.
- Bile acids-Glycocholic acid and taurocholic acid.
- Sex hormones-Testosterone, Estradiol.
- Vitamin D-Vit. 02 and 03 .
- Adrenocortical hormones-Corticosterone.
- Cardiac glycosides-Stropanthin.
- Saponins-Digitonin.

Cholesterol

It is widely distributed in all cells of the body. It occurs in animal fats but not in plant fats. Its structure is given below. The metabolism of cholesterol is discussed in the chapter of lipid metabolism



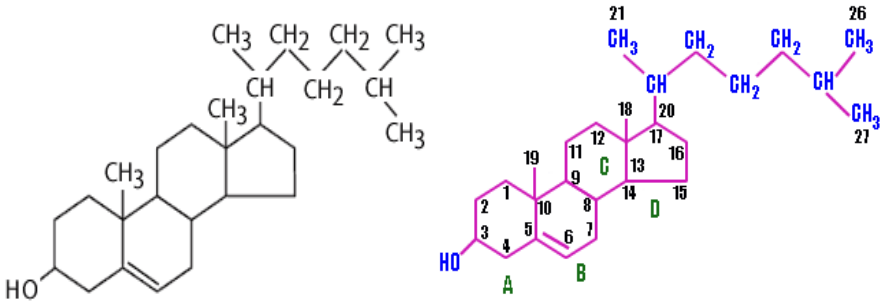
(Cholesterol)

Cholesterol and significance

Cholesterol

- Cholesterol, exclusively found in animals, is the most abundant animal sterol.
- It is widely distributed in all cells and is a major component of cell membranes and lipoproteins.
- Cholesterol (Creek: chole-bile) was first isolated from bile.

- Cholesterol literally means 'solid alcohol from bile.'



Functions of Cholesterol

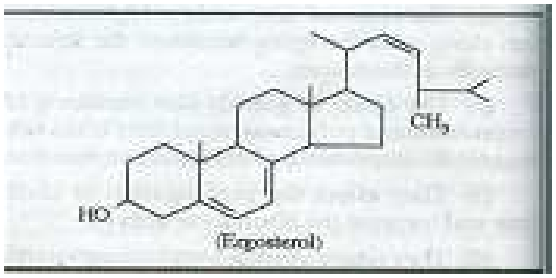
- Cholesterol is a poor conductor of heat and electricity, since it has a high dielectric constant.
- It is present in abundance in nervous tissues.
- It appears that cholesterol functions as an insulating cover for the transmission of electrical impulses in the nervous tissue.
- Cholesterol performs several other biochemical functions which include its role in membrane structure and function, in the synthesis of bile acids, hormones (sex and cortical) and vitamin D.

Erogesrol

(i) It occurs in ergot and yeast.

(ii) It is the precursor of vitamin 0.

(iii) It acquires antirachitic properties with the opening of ring B when irradiated with ultraviolet light.



Coprosterol

It occurs in feces as a result of the reduction by bacteria in the intestine of the double

bond between C_5, and C_6 of cholesterol.

Important Tests

(1) Greese spot test: A drop of oil placed over a piece of ordinary paper. A translucent spot is visible. This indicates the presence of fat.

(2) Emulsification test: 2 ml water is taken in one test tube and 2 ml of diluted bile salt solution in another test tube. Add 3 drops of the given oil to each test tube and shake vigorously. Note the stability of the emulsification formed.

(3) Saponification test: Take 10 drops of coconut oil in a test tube. Add 20 drops of 40% NaOH and 2 ml of glycerol to it. Gently boil for about 3 minutes until complete saponification occurs. If oil globules are visible, boiling must be continued. Divide the solution into 3 parts to carry the following experiments in test tube 1, 2, 3.

To test tube No. 1 add saturated solution of NaCl. Note that the soap separates out and floats to the surface (salting out process).

To test tube No.2 add a few drops of cone. HCl. An oily layer of the fatty acids rises to the surface.

To test tube No.3 add a few drops of $CaCl_2$ solution. The insoluble calcium soap is precipitated. . .

Unsaturation test

Add 10 drops of Huble's iodine reagent to 10 rnl of chloroform. The chloroform assumes a pink colour due to the free iodine. The solution is divided equally into three test tubes as (a), (b) and (c) and three types of oil are added.

Add the oil No. 1 to the test tube (a) drop by drop shaking the tube vigorously after each addition till the pink colour of the solution just disappears. The number of oil drops required are noted. The experiment is repeated by oil 2 and 3 adding to test tubes (b) and (c), respectively. The more the number of drops required to discharge the pink colour, the less is the unsaturation.

Colour Reactions to Detect Sterols

Liebermann-Burchard Reaction: A chloroform solution of a sterol when treated with acetic anhydride and sulphuric acid gives a green colour .This reaction is the basis of a colorimetric estimation of blood cholesterol.

Salkowski test: A red to purple colour appears when a chloroform solution 0; the sterol is treated with an equal volume of concentrated sulphuric acid. .

Clinical Orientation

- The high concentration of polyunsaturated fatty acids in the lipids of gonads are important in reproductive function. .
- The essential fatty acid deficiency causes swelling of mitochondrial membrane resulting. in the reduction in efficiency of oxidative phosphorylation producing increased heat.
- Docosahexenoic acid formed from dietary linolenic acids enhances the electrical response of the photoreceptors to illumination. Therefore; linolenic acid of the diet is essential for optimal vision.
- The deficiency of essential fatty acids causes skin lesions, abnormal pregnancy and lactation in adult females, fatty liver, kidney damage.
- The genetic deficiency of lecithin cholesterol acyl transferase (LCAT) .causes Norum's Disease.
- Sitosterol decreases the intestinal absorption of exogenous and endogenous cholesterol and thereby lowers the blood cholesterol level.
- The deficiency of the enzyme sphingomyelinase. Causes the large accumulations of sphingomyelins in brain, liver and spleen of children resulting in the Niemann-Pick disease with the symptoms of enlarged abdomen, liver, spleen and mental deterioration.
- Absence of dipalmityl lecithin {DPL} in premature foetus produces respiratory distress syndrome (Hyaline-membrane disease).
- The inherited Gaucher's Disease in infancy and childhood is caused by the deficiency of the enzyme glucocerebrosldase involving the large accumulations of glucocereqrosides (usually Kerasin) in the liver, spleen, bone marrow, and brain with the manifestations of weight loss, failure in growth, and progressive mental retardation.
- The autosomal recessive Tay-Sach's Disease (GM_2 Gangliosidosis) results in the accumulation of large amounts of gangliosides in the brain and nervous tissues due

to the absence of the enzyme hexosaminidase A with the association of progressive development of idiocy and blindness in infants soon after birth.

- The inherited disorder Metachromatic Leukodystrophy (MLD) happens on the salfatide,

  formed from galactocerebroside, accumulation in various tissues owing to the deficiency of the enzyme sulfatase (Aryl salfatase) with the symptoms of weakness, ataxia, defects in locomotion, paralysis, difficulties in speech in children before three years of age and psychiatric manifestation including progressive dementia in adults.

- Obesity and atherosclerosis are distinctly related to the concentrations of cholesterol and polyunsaturated fatty acids in the body.

All possible questions:

1. Write the short notes on Acid number and Iodine number?
2. Define lipids?
3. Differences between saturated and unsaturated fatty acids? Give an example?
4. What is saponification?
5. Write the structure of cholesterol.

Detailed questions:

1. Describe the structure, functions and properties of phosphoglycerides?
2. Describe the structure of phosphatidylethanolamine and phosphatidylcholine.

3. Give a detailed account on saponification number and iodine number of oil and lipid?

4. Describe the structure and function of sphingolipids

5. Explain in detail the structure, functions and properties of tricayl glycerols.

6. Explain in detail the various function of lipids.?

7. How the lipids is classified?

8. Write the short notes on Free fatty acid? Saponification? Polenske number?

9. Explain i) Phospholipid ii) Sphingolipids iii) Glycolipid.

10. Describe about Beta oxidation of fatty acids?

Questions	Option A	Option B	Option C	Option D	Answers
An example of a hydroxy fatty acid is	Ricinoleic acid	Crotonic acid	Butyric acid	Oleic acid	**Ricinoleic acid**
An example of a saturated fatty acid is	Palmitic acid	Oleic acid	Linoleic acid	Erucic acid	**Palmitic acid**
If the fatty acid is esterified with an alcohol of high molecular weight instead of glycerol, the resulting compound is	Lipositol	Plasmalogen	Wax	Cephalin	**Wax**
A fatty acid which is not synthesized in the body and has to be supplied in the diet is	Palmitic acid	Lauric acid	Linolenic acid	Palmitoleic acid	**Linolenic acid**
Essential fatty acid:	Linoleic acid	Linolenic acid	Arachidonic acid	All these	**All these**
The fatty acid present in cerebrosides is	Lignoceric acid	Valeric acid	Caprylic acid	Behenic acid	**Lignoceric acid**
The number of double bonds in arachidonic acid is	1	2	4	6	**4**
In humans, a dietary essential fatty acid is	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	**Linoleic acid**
A lipid containing alcoholic amine residue is	Phosphatidic acid	Ganglioside	Glucocerebroside	Sphingomyelin	**Sphingomyelin**
Cephalin consists of	Glycerol, fatty acids, phosphoric acid and choline	Glycerol, fatty acids, phosphoric acid and ethanolamine	Glycerol, fatty acids, phosphoric acid and inositol	Glycerol, fatty acids, phosphoric acid and serine	**Glycerol, fatty acids, phosphoric acid and ethanolamine**
In mammals, the major fat in adipose tissues is	Phospholipid	Cholesterol	Sphingolipids	Triacylglycerol	**Triacylglycerol**
Glycosphingolipids are a combination of	Ceramide with one or more sugar residues	Glycerol with galactose	Sphingosine with galactose	Sphingosine with phosphoric acid	**Ceramide with one or more sugar residues**

The importance of phospholipids as constituent of cell membrane is because they possess	Fatty acids	Both polar and nonpolar groups	Glycerol	Phosphoric acid	**Both polar and nonpolar groups**
In neutral fats, the unsaponificable matter includes	Hydrocarbons	Triacylglycerol	Phospholipids	Cholsesterol	**Hydrocarbons**
Higher alcohol present in waxes is	Benzyl	Methyl	Ethyl	Cetyl	**Cetyl**
Kerasin consists of	Nervonic acid	Lignoceric acid	Cervonic acid	Clupanodonic acid	**Lignoceric acid**
Gangliosides are complex glycosphingolipids found in	Liver	Brain	Kidney	Muscle	**Brain**
Unsaturated fatty acid found in the cod liver oil and containing 5 double bonds is	Clupanodonic acid	Cervonic acid	Elaidic acid	Timnodonic acid	**Timnodonic acid**
Phospholipid acting as surfactant is	Cephalin	Phosphatidyl inositol	Lecithin	Phosphatidyl serine	**Lecithin**
An oil which contains cyclic fatty acids and once used in the treatment of leprosy is	Elaidic oil	Rapeseed oil	Lanoline	Chaulmoogric oil	**Chaulmoogric oil**
Unpleasant odours and taste in a fat (rancidity	Lead	Copper	Tocopherol	Ergosterol	**Tocopherol**
Gangliosides derived from glucosylceramide contain in addition one or more molecules of	Sialic acid	Glycerol	Diacylglycerol	Hyaluronic acid	**Sialic acid**
'Drying oil', oxidized spontaneously by atmospheric oxygen at ordinary temperature and forms a hard water proof material is	Coconut oil	Peanut oil	Rape seed oil	Linseed oil	**Linseed oil**
Deterioration of food (rancidity	Cholesterol	Vitamin E	Peroxidation of lipids	Phenolic compounds	**Peroxidation of lipids**

The number of ml of N/10 KOH required to neutralize the fatty acids in the distillate from 5 gm of fat is called	Reichert-Meissel number	Polenske number	Acetyl number	Non volatile fatty acid number	**Reichert-Meissel number**
Molecular formula of cholesterol is	C27H45OH	C29H47OH	C29H47OH	C23H41OH	**C27H45OH**
The cholesterol molecule is	Benzene derivative	Quinoline derivative	Steroid	Straight chain acid	**Steroid**
Salkowski test is performed to detect	Glycerol	Cholesterol	Fatty acids	Vitamin D	**Cholesterol**
Palmitic, oleic or stearic acid ester of cholesterol used in manufacture of cosmetic creams is	Elaidic oil	Lanoline	Spermaceti	Chaulmoogric oil	**Lanoline**
Dietary fats after absorption appear in the circulation as	HDL	VLDL	LDL	Chylomicron	**Chylomicron**
Free fatty acids are transported in the blood	Combined with albumin	Combined with fatty acid binding protein	Combined with β - lipoprotein	In unbound free salts	**Combined with albumin**
Long chain fatty acids are first activated to acetyl-CoA in	Cytosol	Microsomes	Nucleus	Mitochondria	**Cytosol**
The enzyme acyl-CoA synthase catalyses the conversion of a fatty acid of an active fatty acid in the presence of	AMP	ADP	ATP	GTP	**ATP**
Carnitine is synthesized from	Lysine and methionine	Glycine and arginine	Aspartate and glutamate	Proline and hydroxyproline	**Lysine and methionine**
The enzymes of β-oxidation are found in	Mitochondria	Cytosol	Golgi apparatus	Nucleus	**Mitochondria**

Long chain fatty acids penetrate the inner mitochondrial membrane	Freely	As acyl-CoA derivative	As carnitine derivative	Requiring Na dependent carrier	**As carnitine derivative**
Dietary fibres are rich in	Cellulose	Glycogen	Starch	Proteoglycans	**Cellulose**
The end products of saponification:	glycerol	acid	soap	Both (A and (C	**Both (A and (C**
Triglycerides are	Heavier than water	Major constituents of membranes	Non-polar	Hydrophilic	**Non-polar**
Cerebronic acid is present in	Glycerophospholipids	Sphingophospholipids	Galactosyl ceramide	Gangliosides	**Galactosyl ceramide**
Acylsphingosine is also known as	Sphingomyelin	Ceramide	Cerebroside	Sulphatide	**Ceramide**
The highest phospholipids content is found in	Chylomicrons	VLDL	LDL	HDL	**HDL**
The major lipid in chylomicrons is	Triglycerides	Phospholipids	Cholesterol	Free fatty acids	**Triglycerides**
Number of carbon atoms in cholesterol is	17	19	27	30	**27**
The lipoprotein richest in cholesterol is	Chylomicrons	VLDL	LDL	HDL	**LDL**
The nitrogenous base in lecithin is	Ethanolamine	Choline	Serine	Betaine	**Choline**
All the following are omega-6-fatty acids except	Linoleic acid	α-Linolenic acid	γ-Linolenic acid	Arachidonic acid	**α-Linolenic acid**
All the following have 18 carbon atoms except	Linoleic acid	Linolenic acid	Arachidonic acid	Stearic acid	**Arachidonic acid**
A 20-carbon fatty acid among the following is	Linoleic acid	α - Linolenic acid	β - Linolenic acid	Arachidonic acid	**Arachidonic acid**

Predominant fatty acids in meat are	Saturated	Monounsaturated	Polyunsaturated	Mono and poly-unsaturated	**Saturated**
Cholesterol is present in all of the following except	Egg	Fish	Milk	Pulses	**Pulses**
Which of the following has the highest cholesterol content?	Meat	Fish	Butter	Milk	**Butter**
Cholesterol is a	Animal sterol	M.F. C27 H46O	5 methyl groups	All of these	**All of these**
Lieberman-Burchard reaction is performed to detect	Cholesterol	Glycerol	Fatty acid	Vitamin D	**Cholesterol**
Fatty acids are oxidized by	α - oxidation	β - oxidation	ω - oxidation	All of these	**All of these**
Which of the following is not an unsaturated fatty acid?	Oleic acid	Stearic acid	Linaoleic acid	Palmitic acid	**Stearic acid**
Calorific value of lipids per gm is	4 Kcal	8 Kcal	9 Kcal	None of these	**9 Kcal**
Saponification:	Hydrolysis of fats by alkali	Hydrolysis of glycerol by liposes	Esterification	Reduction	**Hydrolysis of fats by alkali**
In cephalin, choline is replaced by	Serine	Ethanolamine	Betaine	Sphingosine	**Ethanolamine**
A fatty acid not synthesized in man is	Oleic	Palmitic	Linoleic	Stearic	**Linoleic**

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Pollachi Main Road, Eachanari Post, Coimbatore – 641 021. INDIA
Phone: 0422-6471113-5, 6453777; Fax No: 0422-2980022-3
Email: info@karpagam.com; Web: www.kahedu.edu.in

DEPARTMENT OF MICROBIOLOGY

Subject	**:**	**Biochemistry**	**Semester**	**:**	**I**
Subject code	**:**	**19MBU103**	**Class**	**:**	**I B.Sc Microbiology**

UNIT-III: COURSE MATERIAL

Unit-III

Classification and functions of proteins and amino acids, structure of amino acids and concept of zwitterions. Ninhydrin reaction. Natural modifications of amino acids in proteins. Non-protein amino acids, Oligopeptides: Structure and functions of glutathione, insulin and aspartame. Primary and secondary structure of proteins-alpha helix, beta pleated sheet. Tertiary and quaternary structures of proteins. Human haemoglobin structure.

Suggest Readings

1. Nelson, D.L and Cox, M.M. (2008). Lehninger Principles of Biochemistry, 5th edition. W.H. Freeman and Company.

Proteins

Proteins are important macromolecules of the cells, formed by the polymerization of amino acids. Proteins are the mode of expression of the genetic information. They performs various biology functions in the cells, such as they act as the structural components of cells, enzymes, hormones, pigments, storage proteins and some toxins in the cells.

Classification of proteins

Proteins are classified based on the following criterions:

(1) Structure

(2) Composition

(3) Function

Structure

Based on the structure, proteins are classified into 3 groups

(a) Fibrous proteins

(b) Globular Proteins

(c) Intermediate proteins

Fibrous Proteins

- They are linear in shape
- Secondary structure is the most important functional structure of fibrous proteins
- Usually, these proteins do not have tertiary structures
- Physically fibrous proteins are very tough and strong
- They are insoluble in the water
- Long parallel polypeptide chains cross linked at regular intervals
- Fibrous proteins form long fibres or sheaths.

Functions of fibrous proteins

- Perform the structural functions in the cells

Example: Collagen, Myosin, Silk and keratin

Globular Proteins

- Globular proteins are spherical or globular in shape
- The polypeptide chain is tightly folded into spherical shapes
- Tertiary structure is the most important functional structure in globular proteins

- Physically they are soft than fibrous proteins.
- They are readily soluble in water.
- Most of the proteins in the cells belong to the category of globular proteins.

Functions

- Form enzymes, antibodies, and some hormones.

Example: Insulin, Haemoglobin, DNA polymerase and RNA polymerase.

Intermediate proteins

- Their structure is intermediate to linear and globular structures.
- They are short and more or less linear shaped proteins
- Unlike Fibrous proteins, they are soluble in water.

Functions:

- Blood clotting proteins

Example: Fibrinogen

Composition

They are broadly divided into two types

(a) Simple proteins

(b) Conjugated proteins

Simple proteins

Simple proteins composed of only amino acids

Proteins may be fibrous or globular

They possess relatively simple structural organization

Example: Collagen, Myosin, Insulin, Keratin

Conjugated proteins

- Conjugated proteins are complex proteins
- They contain one or more non-amino acid components.
- Here the protein is tightly or loosely bound to one or more non-protein parts.
- The non-protein parts of these proteins are called prosthetic groups.
- The prosthetic group may be metal ions, carbohydrates, lipids, phosphoric acids, nucleic acids and FAD.
- The prosthetic group is essential for the biological functions of these proteins.

- Conjugated proteins are usually globular in shape and are soluble in water.
- Most of the enzymes are conjugated proteins.

Based on the nature of prosthetic groups, the conjugated proteins are further classified as follows.

Phosphoprotein: Prosthetic group is phosphoric acid, Example-casein of milk, vitellin of egg yolk.

Glycoproteins: Prosthetic group is carbohydrates, Example- most of the membrane proteins, mucin (Component of saliva).

Nucleoprotein: Prosthetic group is nucleic acid, Example- proteins in chromosomes, structural proteins of ribosome.

Chromoproteins: Prosthetic group is pigment of chrome, Example: Haemoglobin, Phytochrome and Cytochrome.

Lipoproteins: Prosthetic group is lipids, Example: Membrane proteins.

Flavoproteins: Prosthetic group is FAD (Flavin Adenine Dinucleotide), Example: Proteins of Electron Transport System (ETS).

Metalloproteins: Prosthetic group is metal ions, Example: Nitrate reductase.

Functions

Structural proteins

- Form the component of the connective tissue, bone, tendons, cartilage, skin, feathers, nails, hairs and horn
- Most of them are fibrous proteins and are insoluble in water.

Example: Collagen, Keratin and Elastin

Enzymes

- They are the biological catalysts
- Enzymes reduce the activation energy of reactants and speed up the metabolic reactions in the cells.
- Most of them are globular conjugated proteins.

Hormones

- They include the proteinaceous hormones in the cells.

Example: Insulin, Glucagon

Respiratory pigments

- They are coloured proteins
- All of them are conjugated proteins and they contain pigments (chrome) as their prosthetic group.

Example: Haemoglobin, Myoglobin

Transport proteins

- They transport the materials in the cells
- They form channels in the plasma membrane
- They also form one of the components of blood and lymph in animals

Example: Serum albumin

Contractile proteins

- They are the force generators of muscles
- They can contract with the expense of energy from ATP molecules.

Example: Actin, Myosin

Storage proteins

- They act as the store of metal ions and amino acids in the cells
- Found in seeds, egg and milk
- Abundantly seen in pulses (Legume seeds).

Example: Ferritin which stores iron, casein

Toxins

- They are toxic proteins

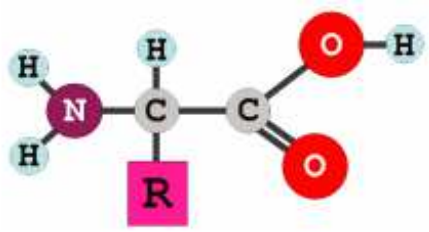
Example: Snake venom

Protein Class	Functions	Examples
Structural Proteins	They are used as bricks and mortars to construct the biological buildings and machineries	α-Keratin of fur, feathers, hairs and claws; collagens of skin, bone and cartilage
Carrier Proteins	They carry metabolites from one site to the other to make biological processes a reality	Haemoglobin carries oxygen; transferrin transports iron
Storage Proteins/ Nutrient Proteins	They serve as biological store houses to preserve nutritional proteins which act as source of essential amino acids	Casein of milk, ovalbumin and ovomucoid of egg, and glutelin of wheat, ferritin
Enzyme Proteins	They act as biological catalysts and make an otherwise slow or improbable reaction fast and feasible	Digestive enzymes trypsin and pepsin, papain from papaya and ribonucleases
Hormone Proteins	They act as biological signals; mediate and regulate physiological processes	Insulin, glucagons and adrenocorticotrophic hormone
Defense Proteins	Protect against foreign invaders like bacteria and viruses, make survival possible under hostile conditions	Antibodies, thrombin, antifreeze proteins and lysozyme in tears
Proteins as Toxins/ Poisons	They are toxic/poisonous to others but provide a defense tool to organisms they belong to	Snake venoms, diphtheria toxin, ricin in caster bean, gossypin of cotton seed

Functions of proteins

Amino acids

Amino acids as the building blocks of proteins. Amino acids is defined as a molecule containing an amine group (-NH2), carboxyl group (-COOH) and the variable group denoted as R, different among different amino acids. R groups is also called the side chain, The overall amino acid formula can be represented as : R-CH(NH2)-COOH. An average molecular weight is about 135 daltons.



Classification of Amino acids

Amino acids are broadly classified into four types

(a) Non-Polar

(b) Polar

(c) Acidic

(d) Basic

Other type of classifications

(a) Essential

(b) Non-essential

(c) Semi-essential

Non-polar Amino Acids

- The non-polar amino acids contain mostly hydrocarbon R groups that do not bear positive or negative charges.
- Non-polar (i.e., hydrophobic) amino acids play an important role in maintaining the three-dimensional structures of proteins, because they interact poorly with water.

Two types of hydrocarbon side chains are found in this group:

(a) Aromatic

(b) Aliphatic

Aromatic amino acids

- Aromatic amino acid contains aromatic ring in their structure
- Benzene is one of the simplest aromatic amino acid.

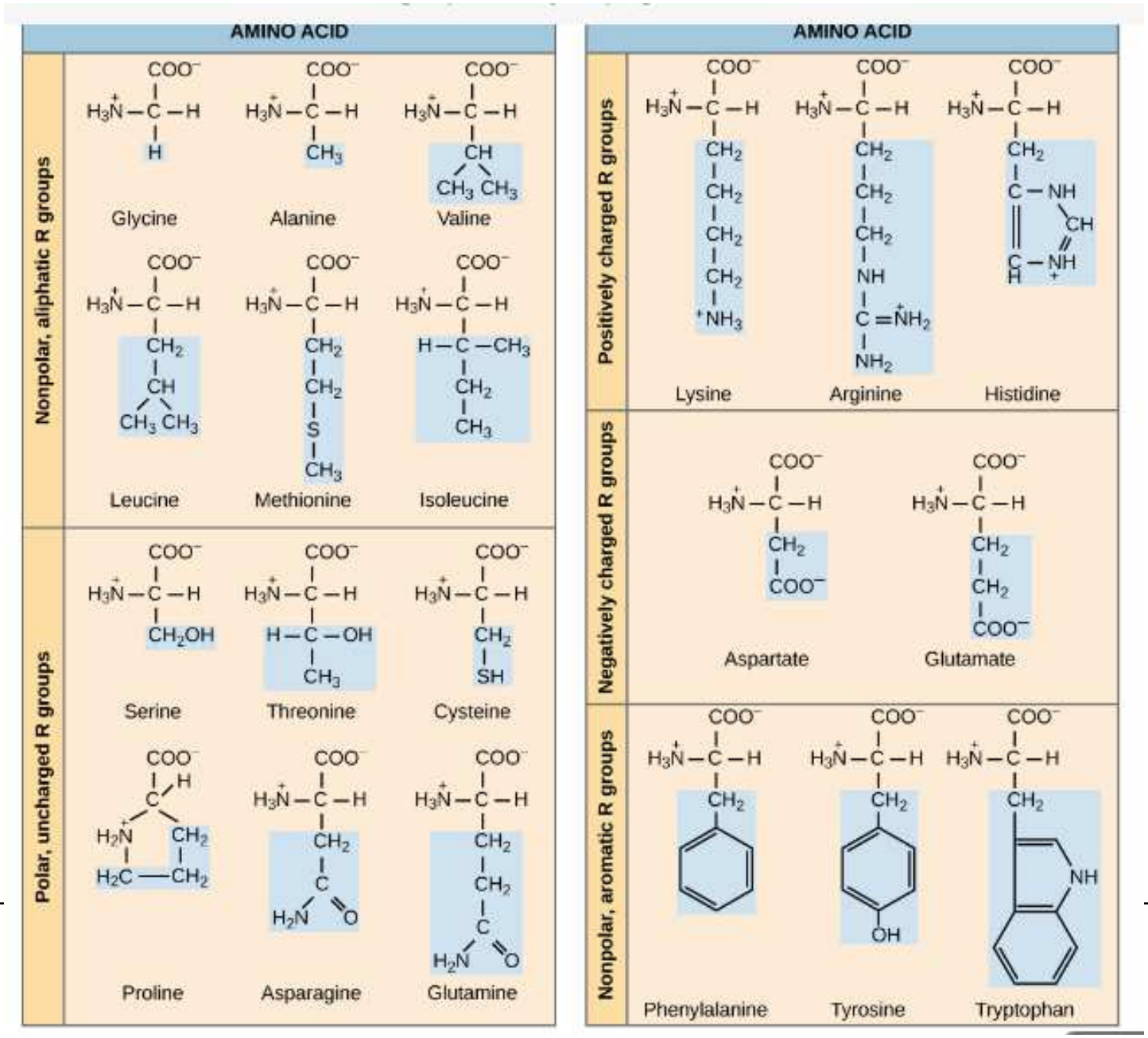
Aliphatic amino acids

- The term aliphatic refers to non-aromatic amino acids such as methane and cyclohexane.
- Phenylalanine and tryptophan contain aromatic ring structures.
- Glycine, alanine, valine, leucine, isoleucine, and proline have aliphatic R groups.

- A sulfur atom appears in the aliphatic side chains of methionine and cysteine. Methionine contains a thioether group (—S—CH3) in its side chain.

Polar Amino Acids

- Polar amino acids have functional groups capable of hydrogen bonding, they easily interact with water.
- Polar amino acids are described as hydrophilic, or "water-loving."
- Serine, threonine, tyrosine, asparagine, and glutamine belong to this category.
- Serine, threonine, and tyrosine contain a polar hydroxyl group, which enables them to participate in hydrogen bonding, an important factor in protein structure.
- The hydroxyl groups serve other functions in proteins.



Acidic Amino Acids

- Two standard amino acids have side chains with carboxylate groups.
- Because the side chains of aspartic acid and glutamic acid are negatively charged at physiological pH, they are often referred to as aspartate and glutamate.

Basic Amino acids

- Basic amino acids bear a positive charge at physiological pH.
- They can therefore form ionic bonds with acidic amino acids.
- Lysine, which has a side chain amino group, accepts a proton from water to form the conjugate acid (—NH3).

Classification based on nutritional requirements

Essential amino acids:

These amino acids cannot be synthesized in the body and have to be present essentially in the diet.

Examples: Valine, Isoleucine, Leucine, Lysine, Methionine, Threonin, Tryptophan and Phenylalanine.

Semi-essential amino acids

These amino acids can be synthesized in the body but the rate of synthesis is lesser than the requirement (e.g. during growth, repair or pregnancy)

Example: Arginine and Histidine

Non-essential amino acids

These amino acids are synthesized in the body, thus their absence in the diet does not adversely affect the growth.

Example: Glycine, Alanine and the other remaining amino acids.

Physical Properties of amino acids

- Colourless
- Crystalline
- May be sweet (Glycine, Alanine, Valine)
- Tasteless (Leucine)
- Bitter (Arginine, Isoleucine).
- Soluble in water, acids, but insoluble in organic solvents.

- High melting point (More than 200° C).

Chemical Properties of amino acids

Reaction due to NH2 group

- Reaction with acids to form salt
- Reaction with nitrous acids to liberate Nitrogen
- Reaction with CO2 to form carbamino compounds

Reaction due to COOH group

- Reaction with strong alkalies to form salt
- Reaction with alcohols to form esters

Reaction due to both NH2 and COOH group

- Amino acids condense with each other by COOH group at one amino acids with NH2 of other amino acid to form peptide bond.

Functions of amino acids

Histidine

- Found in high concentrations in hemoglobin.
- Useful in treating anemia due to relationship to hemoglobin.
- Has been used to treat rheumatoid arthritis.
- Precursor to histamine.
- Associated with allergic response and has been used to treat allergy.
- Assists in maintaining proper blood pH.

Isoleucine

- Muscle tissue uses Isoleucine as an energy source.
- Required in the formation of hemoglobin.

Leucine

- Potent stimulator of insulin.
- Helps with bone healing.
- Helps promote skin healing.
- Modulates release of Enkephalins, which are natural pain-reducers.

Lysine

- Helps form collagen, the connective tissue present in bones, ligaments, tendons, and joints.
- Assists in the absorption of calcium.
- Essential for children, as it is critical for bone formation.
- Involved in hormone production.
- Lowers serum triglyceride levels.

Methionine

- Assists in breakdown of fats.
- Precursor of the amino acids Cysteine and Taurine.
- Helps reduce blood cholesterol levels.
- Antioxidant.
- Assists in the removal of toxic wastes from the liver.
- Helps prevent disorder of hair, skin, and nails due to sulfur and anti-oxidant activity.
- Required for synthesis of RNA and DNA.
- Natural chelating agent for heavy metals, such as lead and mercury.

Phenylalanine

- Precursor to the hormone, Thyroxin.
- Enhances mood, clarity of thought, concentration, and memory.
- Suppresses appetite.
- Major part of collagen formation.
- Powerful anti-depressant.
- Used in the treatment of Parkinson's Disease.

Threonine

- Required for formation of collagen.
- Helps prevent fatty deposits in the liver.
- Aids in production of antibodies.
- Can be converted to Glycine (a neurotransmitter) in the central nervous system.
- Acts as detoxifier.
- Needed by the GI (gastrointestinal) tract for normal functioning.

Tryptophan

- Precursor to the key neurotransmitter, serotonin, which exerts a calming effect.
- Effective sleep aid, due to conversion to serotonin.
- Effective in some forms of depression.
- Treatment for migraine headaches.
- Stimulates growth hormone.
- Tryptophan must compete with 5 other amino acids to pass through the blood-brain barrier and enter the brain. Those 5 are: tyrosine, phenylalanine, leucine, isoleucine, and valine and are called Large Neutral Amino Acids (LNAA).

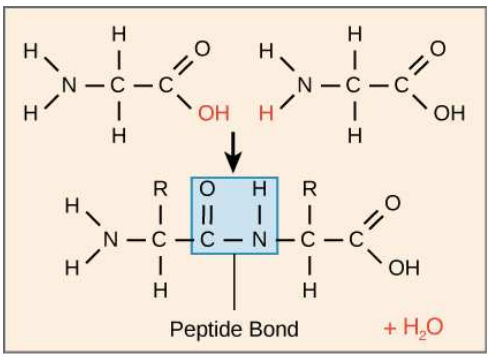
Valine

- Competes with Tyrosine and Tryptophan in crossing the blood-brain barrier.
- The higher the Valine level, the lower the brain levels of Tyrosine and Tryptophan.
- Actively absorbed and used directly by muscle as an energy source.

Peptide bonds

Each protein in your cells consists of one or more polypeptide chains. Each of these polypeptide chains is made up of amino acids, linked together in a specific order. The chemical properties and order of the amino acids are key in determining the structure and function of the polypeptide

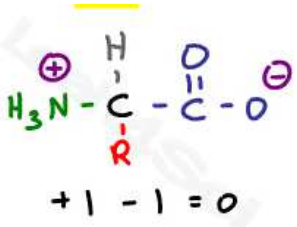
The amino acids of a polypeptide are attached to their neighbors by covalent bonds known as peptide bonds. Each bond forms in a dehydration synthesis (condensation) reaction. During protein synthesis, the carboxyl group of the amino acid at the end of the growing polypeptide chain reacts with the amino group of an incoming amino acid, releasing a molecule of water. The resulting bond between amino acids is a peptide bond.



At one end, the polypeptide has a free amino group, and this end is called the amino terminus (or N-terminus). The other end, which has a free carboxyl group, is known as the carboxyl terminus (or C-terminus).

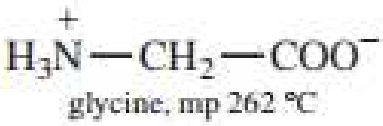
Concept of Zwitterions

Zwitterion is the dipolar form of an amino acid which occurs when H^+ ion is transferred from an acid group to an amine group.



The dipolar nature of amino acids gives them some unusual properties:

1. Amino acids have high melting points, generally over 200° C.



2. Amino acids are more soluble in water than they are in ether, dichloromethane, and other common organic solvents.
3. Amino acids have much larger dipole moments than simple amines or simple acids.
4. Amino acids are less acidic than most carboxylic acids and less basic than most amines.

Ninhydrin reaction

The reaction between alpha-amino acid and ninhydrin involved in the development of color.

alpha-amino acid + ninhydrin ---> reduced ninhydrin + alpha-amino acid + H_2O

alpha-amino acid + H_2O ---> alpha-keto acid +NH_3

alpha-keto acid + NH_3 ---> aldehyde + CO_2

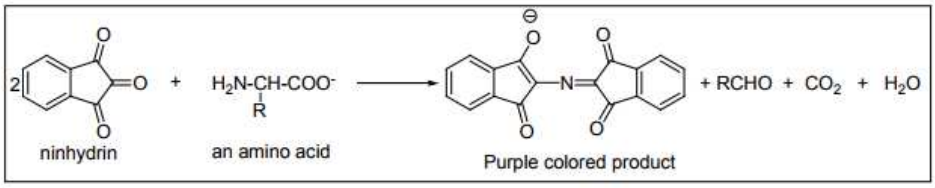
Step 1: it is an oxidative deamination reaction that removes two hydrogen from the alpha amino acid to yield an alpha-imino acid. Simultaneously, the original ninhydrin is reduced and loses an oxygen atom with the formation of a water molecule.

In Step 2: The NH group in the alpha imino acid is rapidly hydrolyzed to form an alpha keto acid with the production of an ammonia molecules.

Step 3: This alpha-keto acid further undergoes decarboxylation reaction under a heated condition to form an aldehyde that has one less carbon atom than the original amino acid. A carbon dioxide molecule is produced.

Further the overall, reaction becomes

alpha-amino acid + 2 ninhydrin ---> CO_2 + aldehyde + final complex(BlUE) + $3H_2O$



Non-Non-Protein amino acids

The amino acid which are not involved in the protein synthesis are called as non protein amino acids.

These non-protein amino acids are classified into two types

(a) Alpha

(b) Non-alpha

Alpha amino acids

- Ornithine
- Citrulline
- Thyroxine
- S-adenosylmethionine
- Homecysteine
- Ovathiol
- Azaserine

Non-Alpha amino acids

- Beta-alanine
- Beta-aminoisobutyric acid

- Gama-aminobutyric acid
- Aminolevulinic acid
- taurine

Alpha Amino acid

Ornithine

- Ornithine is precursors of polyamine
- Ornithine enters liver, mitochondria and participates in urea synthesis.

Citrulline

- Citrulline is intermediates in the biosynthesis of urea.
- L-ornithine transcarbamoylase catalyzes transfer of the carbamoyl group carbamoyl phosphate to ornithine, forming cirtulline and orthophosphate. While the reaction occurs in the mitochondrial matrix, both the formation of ornithine and the subsequent metabolism of citrulline take place in the cytosol.

Thyrosine

- Tyrosine forms norepinephrine and epinephrine and following iodination the thyroid hormones triiodothyronine and thyroxine.
- Use of measurement of blood thyroxine or thyroid stimulating hormone (TSH) in the neonatal diagnosis of congenital hypothyroisidm.
- The amino acid thyrosine is the starting point in the synthesis of the catecholamines and of the thyroid hormones tetraiodothyronine (thyroxine; T4) and triiodothyronine (T3).

Ovathiol

- Sulfur containing amino acid found in fertilized eggs, and acts as an antioxidant.

Azaserine

- Purine deficiency states, while rare in humans, generally reflect a deficiency of folic acid.
- Compounds that inhibit formation of tetrahydrofolates and therefore block purine synthesis have been used in cancer chemotherapy.
- Inhibitory compounds and the reactions they inhibit include, azaserine, diazanorleucine, 6-mercaptopurine and mycophenolic acid.

Non-alpha amino acids

Beta-Alanine and Aminoisobutyrate

- Alanine and aminoisobutyrate are formed during catabolism of the pyrimidines uracil and thymine.
- Traces of alanine also result from the hydrolysis of alanyl dipeptides by the enzyme carnosinase.
- Aminoisobutyrate also arises by transamination of methylmalonate semialdehyde, a catabolite of L-valine.
- The initial reaction of alanine catabolism is transamination to malonate semialdehyde.
- Subsequent transfer of coenzyme A from succinyl-CoA forms malonyl-CoA semialdehyde which is then oxidized to malonyl-CoA and decarboxylated to the amphibolic intermediate acetyl-CoA.

Beta-Alanyl Dipeptides

- The alanyl dipeptides carnosine and anserine (N-methylcarnosine) activate myosin ATPase chelate copper, and enhance copper uptake.
- Analyl-imidazole buffers the pH of anaerobically contracting skeletal muscle.
- Biosynthesis of carnosine is catalyzed by carnosine synthetase in a two-stage reaction that involves initial formation of an enzyme-bound acyl-adenylate of alanine and subsequenct transfer of the alanyl moiety to L-histidine.
- Hydrolysis of carnosine to alanine and L-histidine is catalyzed by carnosinase. The heritable disorder carnosinase deficiency is characterized by carnosinuria.
- Homocarnosine present in human brain at higher levels than carnosine is synthesized in brain tissue by carnosine synthetase. Serum carnosinase does not hydrolyze homocarnosine.
- Homocarnosinosis, a rare genetic disorder, is associated with progressive spastic paraplegia and mental retardation.

Gama-Aminobutyrate

- Gama-Aminobutyrate (GABA) functions in brain tissue as an inhibitory neurotransmitter by altering transmembrane potential differences.

- GABA is formed by decarboxylation of glutamate by L-glutamate decarboxylase.
- Trasamination of aminobutyrate forms succinate semialdehyde which can be reduced to hydroxybutyrate by L-lactate dehydrogenase,or be oxidized to succinate and thence via the citric and cycle to CO_2 and H_2O.
- A rare genetic disorder of GABA metabolism involves a defective GABA amino transferase, an enzyme that participate in the catabolism of GABA subsequent to its postsynpatic release in brain tissue.
- Defects in succinic semialdehyde dehydrogenase are responsible for another rare metabolic disorder of aminobutyrate catabolism characterized by 4-hydroxybutyric aciduria.

Structure and functions of Glutathione

Glutathione (GSH) is often referred to as the body's master antioxidant.

Composted of three amino acids

- Cysteine
- Glycine
- Glutamate

Glutathione can be found in virtually every cell of the human body.

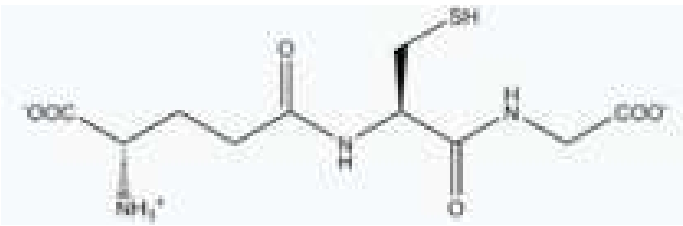
The highest concentration of glutathione is in the liver, making it critical in the body's detoxification process.

Glutathione is also an essential component to the body's natural defense system.

Viruses, bacteria, heavy metal toxicity, radiation, certain medications, and even the normal process of aging can all cause free-radical damage to healthy cells and deplete glutathione.

Glutathione depletion has been correlated with lower immune function and increased vulnerability to infection due to the liver's reduced ability to detoxify.

As the generation of free radicals exceeds the body's ability to neutralize and eliminate them, oxidative stress occurs.

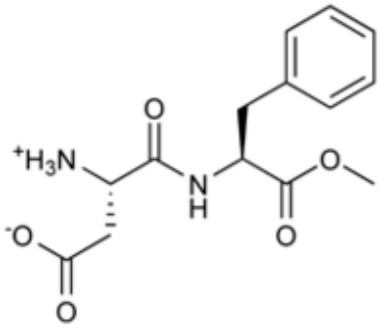


Functions of Glutathione

- It maintains levels of reduced glutaredoxin and glutathione peroxidase.
- It is one of the major endogenous antioxidants produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds
- Regulation of the nitric oxide cycle is critical for life, but can be problematic if unregulated
- It is used in metabolic and biochemical reactions such as DNA synthesis and repair, protein synthesis, prostaglandin synthesis, amino acid transport, and enzyme activation.
- Thus, every system in the body can be affected by the state of the glutathione system, especially the immune system, the nervous system, the gastrointestinal system, and the lungs.
- It has a vital function in iron metabolism.
- It has roles in progression of the cell cycle, including cell death.
- GSH levels regulate redox changes to nuclear proteins necessary for the initiation of cell differentiation.
- Differences in GSH levels also determine the expressed mode of cell death, being either apoptosis or cell necrosis.
- Manageably low levels result in the systematic breakage of the cell whereas excessively low levels result in rapid cell death.

Aspartame

- Aspartame is an artificial sweeter.
- Aspartame is 180 to 200 times sweeter than normal sugar.
- Aspartame is not suitable for baking because if often breaks down when heated and loses much of its wetness and at temperatures above 90̊ Fa component of it can covert to formaldehyde.



These aspartame is synthesized from two amino acids

- Aspartic acid
- Phenylalanine

Aspartame has the chemical formula of $C_{14}H_{18}N_2O_5$.

Upon ingestion, aspartame breaks down into several residual chemicals, including

- Aspartic acid
- Phenylalanine
- Methanol
- Formaldehyde

Methanol and Formaldehyde

- Approximately 10% of aspartame is broken down into methanol in the small intestine. Most of the methanol is absorbed and quickly converted into formaldehyde.
- High concentration, formaldehyde can kill cells and tissues.

Phenylalanine

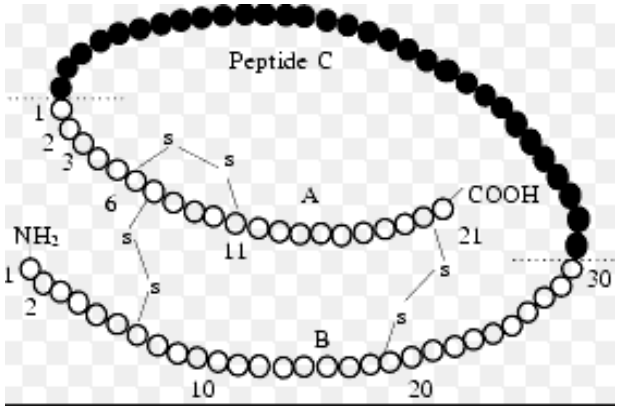
- One of the functional groups in aspartame is phenylalanine, which is unsafe for those born with phenylketonuria, a rare genetic conditions.
- Approximately 50% of aspartame is broken down into phenylalanine, which is completely safe for everyone except sufferers of phenylketonuria.

Aspartic acid

- Approximately 40 percent of aspartame is broken down into aspartic acid.
- Aspartic acid belongs to a class of chemicals that in high concentrations act as an excitotoxin, damage on brain and nerve cell.

Structure of Insulin

Insulin is a hormone secreted by the pancreas that regulates glucose levels in the blood. Without insulin, cells cannot use the energy from glucose to carry out functions within the body. Insulin was first discovered in 1921 by Frederick Grant Banting and Charles. The FDA approved insulin in 1939.



Insulin is composed of two peptide chains referred to as the A chain and B chain. A and B chains are linked together by two disulfide bonds, and an additional disulfide is formed within the A chain. In most species, the A chain consists of 21 amino acids and the B chain of 30 amino acids.

Although the amino acid sequence of insulin varies among species, certain segments of the molecule are highly conserved, including the positions of the three disulfide bonds, both ends of the A chain and the C-terminal residues of the B chain. These similarities in the amino acid sequence of insulin lead to a three dimensional conformation of insulin that is very similar among species, and insulin from one animal is very likely biologically active in other species. Indeed, pig insulin has been widely used to treat human patients.

Insulin molecules have a tendency to form dimers in solution due to hydrogen-bonding between the C-termini of B chains. Additionally, in the presence of zinc ions, insulin dimers associate into hexamers.

Functions of insulin

- Insulin is made in the pancreas by beta cells.
- After the body takes in food, these beta cells release insulin, which enables cells in the liver, muscles and fat tissues to take up glucose and either store it as glycogen or allow blood to transfer it to organs in the body for use as an energy source.

- This process stops the use of fat as a source of energy.
- When glucose levels are elevated in the blood, insulin is produced at higher rates by the pancreas in order to maintain normal sugar concentrations in the blood.
- Without insulin, the body cannot process glucose effectively and glucose begins to build up in the blood stream instead of being transported to different cells.
- In contrast with elevated levels of glucose in the blood, when there is a deficit of glucose available to the body, alpha cells in the pancreas release glucagon, a hormone that causes the liver to convert stored glycogen into usable glucose which is then released into the bloodstream.

Some of the effects of the insulin on the metabolism include:

- Controlling cell intake of substances like glucose in many organs like muscles and adipose tissues.
- Controlling amino acid uptake, thus increasing DNA replication and protein synthesis
- Altering the activity of enzymatic cells

Other Cellular effects of insulin include:

- Increasing synthesis of glycogen. Glycogen is a type of storage for glucose and is stored in the liver. Levels of blood glucose determine whether glucose is stored as glycogen or is excreted. Low levels of glucose cause the liver to excrete glucose, while higher levels of glucose allow glucose to be stored as glycogen.
- Increasing the synthesis and esterification of fatty acids. This is caused by the insulin causing fat cells to convert blood lipids to triglycerides. Esterification is caused when the insulin causes the adipose tissue to convert fats from fatty acid esters.
- Increasing the esterification of fatty 4. Decreasing protein breakdown (proteolysis) 5. Reducing lipolysis 6. Increasing uptake of substances like amino acid and potassium 7. Relaxing wall of arteries of muscles, which vasodilation 8. Increasing secretion of HCl into the stomach.

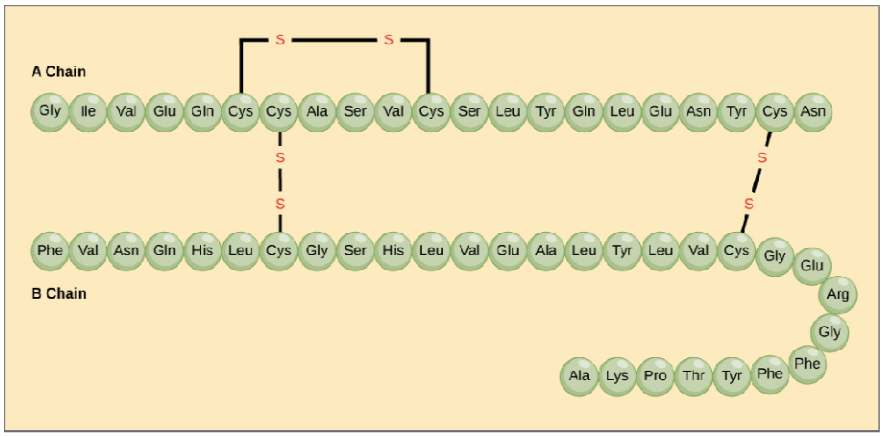
Protein structure

- Egg whites contain large amounts of proteins called albumins, and the albumins normally have a specific 3D shape, thanks to bonds formed between different amino acids in the protein. Heating causes these bonds to break and exposes hydrophobic (water-hating) amino acids usually kept on the inside of the protein. The hydrophobic amino acids, trying to get away from the water surrounding them in the egg white, will stick to one another, forming a protein network that gives the egg white structure while turning it white and opaque.
- The shape of a protein is very important to its function.
- To understand how a protein gets its final shape or conformation, we need to understand the four levels of protein structure: primary, secondary, tertiary, and quaternary.

Primary structure

- The simplest level of protein structure, **primary structure**, is simply the sequence of amino acids in a polypeptide chain.
- For example, the hormone insulin has two polypeptide chains, A and B, shown in diagram below. (The insulin molecule shown here is cow insulin, although its structure is similar to that of human insulin.)
- Each chain has its own set of amino acids, assembled in a particular order.

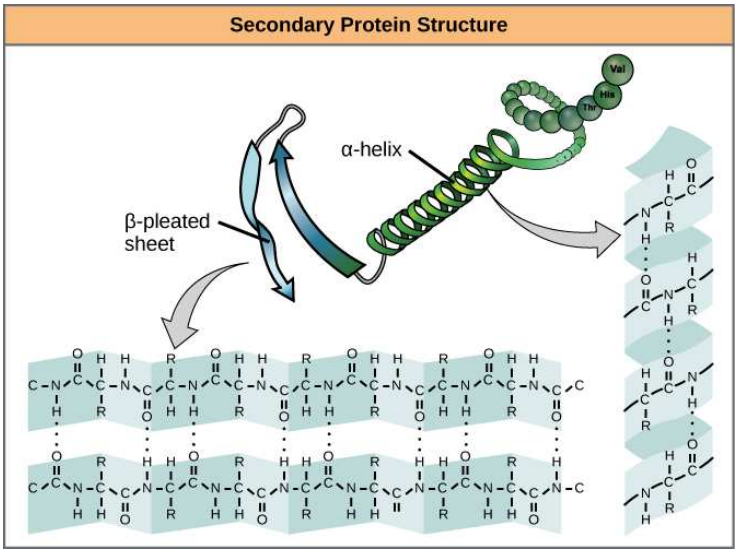
- For instance, the sequence of the A chain starts with glycine at the N-terminus and ends with asparagine at the C-terminus, and is different from the sequence of the B chain.



- Insulin consists of an A chain and a B chain. They are connected to one another by disulfide bonds (sulfur-sulfur bonds between cysteines).
- The A chain also contains an internal disulfide bond. The amino acids that make up each chain of insulin are represented as connected circles, each with the three-letter abbreviation of the amino acid's name.

Secondary structure

- The next level of protein structure, **secondary structure**, refers to local folded structures that form within a polypeptide due to interactions between atoms of the backbone. (The backbone just refers to the polypeptide chain apart from the R groups – so all we mean here is that secondary structure does not involve R group atoms).
- The most common types of secondary structures are the α helix and the β pleated sheet. Both structures are held in shape by hydrogen bonds, which form between the carbonyl O of one amino acid and the amino H of another.

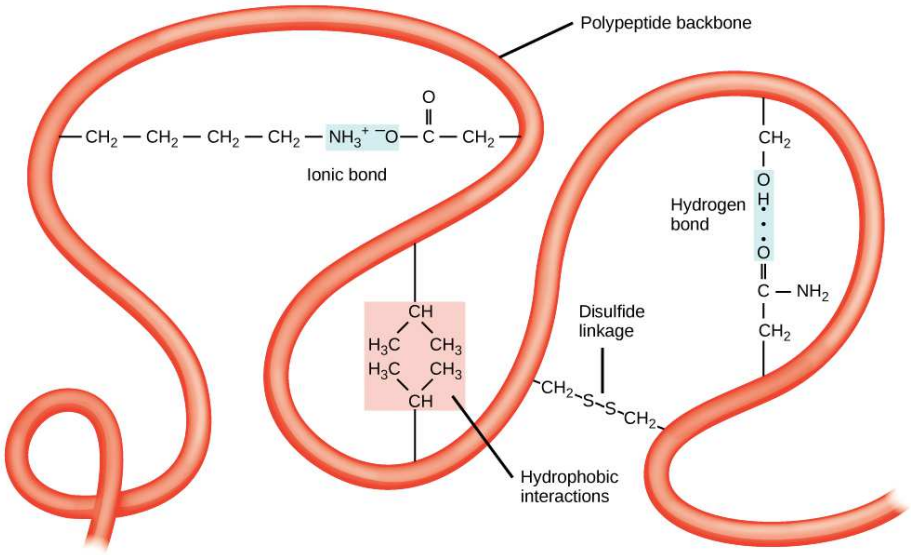


- In an **α helix**, the carbonyl (C=O) of one amino acid is hydrogen bonded to the amino H (N-H) of an amino acid that is four down the chain. (E.g., the carbonyl of amino acid 1 would form a hydrogen bond to the N-H of amino acid 5).
- This pattern of bonding pulls the polypeptide chain into a helical structure that resembles a curled ribbon, with each turn of the helix containing 3.6 amino acids.
- The R groups of the amino acids stick outward from the α helix, where they are free to interact.
- In a **β pleated sheet**, two or more segments of a polypeptide chain line up next to each other, forming a sheet-like structure held together by hydrogen bonds.
- The hydrogen bonds form between carbonyl and amino groups of backbone, while the R groups extend above and below the plane of the sheet.
- The strands of a β pleated sheet may be **parallel**, pointing in the same direction (meaning that their N- and C-termini match up), or **antiparallel**, pointing in opposite directions (meaning that the N-terminus of one strand is positioned next to the C-terminus of the other).

Tertiary structure

- The overall three-dimensional structure of a polypeptide is called its **tertiary structure**. The tertiary structure is primarily due to interactions between the R groups of the amino acids that make up the protein.

- R group interactions that contribute to tertiary structure include hydrogen bonding, ionic bonding, dipole-dipole interactions.
- For example, R groups with like charges repel one another, while those with opposite charges can form an ionic bond. Similarly, polar R groups can form hydrogen bonds and other dipole-dipole interactions. Also important to tertiary structure are **hydrophobic interactions**, in which amino acids with nonpolar, hydrophobic R groups cluster together on the inside of the protein, leaving hydrophilic amino acids on the outside to interact with surrounding water molecules.
- Finally, there's one special type of covalent bond that can contribute to tertiary structure: the disulfide bond. **Disulfide bonds**, covalent linkages between the sulfur-containing side chains of cysteines, are much stronger than the other types of bonds that contribute to tertiary structure.



Quaternary structure

- Many proteins are made up of a single polypeptide chain and have only three levels of structure. However, some proteins are made up of multiple polypeptide chains, also known as subunits. When these subunits come together, they give the protein its **quaternary structure**.

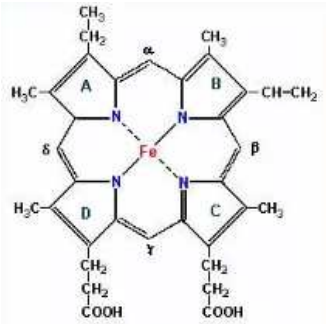
- one example of a protein with quaternary structure: hemoglobin. hemoglobin carries oxygen in the blood and is made up of four subunits, two each of the α and β types. In general, the same types of interactions that contribute to tertiary structure (mostly weak interactions, such as hydrogen bonding and London dispersion forces) also hold the subunits together to give quaternary structure.

Hemoglobin

- Hemoglobin, a chromo protein, found exclusively in red blood cells is actually a conjugated protein containing heme as prosthetic group and globin as the protein part apoprotein.
- The normal concentration of Hb in an adult varies from 14.0 to 16.0 gm%.Approximately 90 mg/kg of Hb is produced and destroyed in the body every day.
- Hb has a molecular weight of about 67,000.
- Each gram of Hb contains 3.4 mg of iron.
- Heme is present as a prosthetic group in hemoglobin as well as in myoglobin, cytochromes, peroxidases, catalases and tryptophan pyrrolases etc.
- Heme is produced by the combination of iron with a porphyrin ring.
- The heme protion is alike in all forms of hemoglobin

Structure of Heme

- Heme is a derivative of porphyrin, porphyrins are cyclic compounds formed by the fusion of 4 pyrrole rings linked by methenyl bridges.
- Since an atom of iron is present heme is called ferroprotoporphyrin.
- These rings are names as I,II,III, IV and the bridges are names as alpha, beta, gamma and delta.
- Porphyrins contain side chains attached to each of the other four pyrrole rings.
- Different porphyrins vary in nature of the side chains that are attached to each of the pyrrole rings.



Heme Molecule

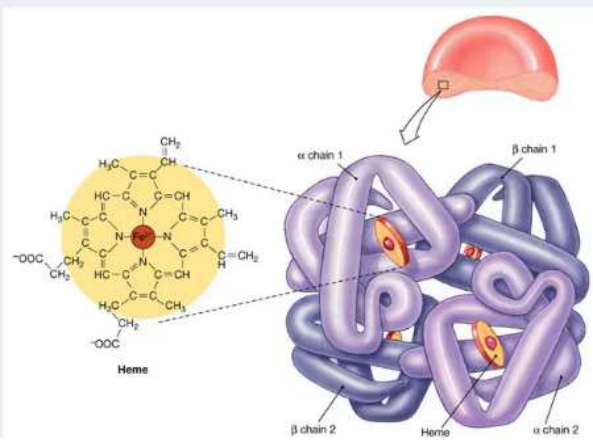
- Heme consists of one ferrous atom (Fe++) that is co-ordinated in the centre of the tetra pyrrole ring of protoporphyrin IX.
- The double bonds are resonating and therefore keep shifting in their position.
- When the ferrous atom in heme gets oxidized to ferric form. Hematin is formed, which loses the property of carrying oxygen and is brown in color, as compared to that of heme which is red in color.

Structure of Globin

1. Different hemoglobins are produced during embryonic, fetal and adult life.
2. Each consists of a tetramer of globin polypeptide chains.
3. The major adult hemoglobin HbA has the structure $\alpha_2\beta_2$.

Polypeptide chains

- Each polypeptide chain contains heme in the heme pocket. Thus one Hb molecule contains 4 Heme units.
- The subunits of hemoglobin are arranged array with a tight spherical overall appearance and each individual polypeptide is folded in such a manner to maximize polar residues being on the exposed surface and non-polar interactions being internal, making this large protein water soluble. The interior surface of the molecule lined with non-polar groups froms a hydrophobic pocket into which heme is inserted.



- The arrangement of polypeptides is held together by hydrogen bonding, hydrophobic interactions and multiple ionic interactions that take place at the contact points between subunits.
- These subunits interactions play a critical role in the binding of oxygen to hemoglobin.
- In the amino acid sequence of each polypeptide chain, certain residues appear to be critical to stability and function.
- Such residues are usually the same in α or β chains.
- The NH2 terimal valines of the beta chains are important in 2,3-BPG interactions. The C-terminal residues are important in the salt bridges.
- Each heme moiety can bind a single oxygen molecule, a molecule of hemoglobin can transport up to four oxygen molecules.
- Each heme unit holds an iron ion is such a way that the iron can interact with an oxygen molecule, forming oxyhemoglobin.
- Blood containg RBCs filled with oxyhemoglobin is bright red.
- The iron oxygen interaction is very weak; the two can easily be separated without damaging the heme unit or the oxygen molecules.
- The binding of an oxygen molecule to the iron in a heme unit is therefore completely reversible.
- A hemoglobin molecule in which the iron has separated from the oxygen molecule is called deoxyhemoglobin.

Primary structure of hemoglobin

- Normal alpha chain contains 141 AA residues in linear sequence.
- The non-alpha chains are all 146 amino acids in length; the beta chain begins with valine and histidine.
- The C-terminal residues are Tyrb145 and Hisb146.The delta chain differs from the beta chain in only 10 residues.
- The first eight residues are the C-terminal residues (127-146) are the same in the delta and beta chains. Tetramers of beta chains maybe found in a thalassemia.
- The gamma chain of fetal hemoglobin differs from the beta chain by 39 residues.
- The N-terminal residues of the gamma chain and beta chain are glycine and valine respectively, while the C-terminal residues.
- Try145 and His146 are the same as in gamma and beta chains. Appreciable quantities of free gamma are found in the red cells of some infants with a thalassemia, free gamma chains like beta chains can form homotetramers known as hemoglobin barts.

Secondary structure of hemoglobin

- About 75 percent of the amino acids in α or β chains are in a helical arrangement.
- All studied hemoglobins have a similar helical content.
- Eight helical arease lettered A to H, occur in the β chains.
- Hemoglobin nomenclature specifies that amino acids within helices are designated by the amino acid number and the helix letter, while amino acids between helices bear the number of the amino acid and the letters of the two helices. Thus residues EF3 is the third residue of the segment connecting the E and F helices, while residues F8 is the eighth residue of the F helix. Alignment according to helical designation makes homology evident; residue F8 is the proximal heme-linked histidine and the histidine on the distal side of the heme is E7.

Tertiary structure

- The tertiary folding of each globin chain forms an approximate sphere. Tertiary folding fives rise to at least 3 functionally important characteristics of the hemoglobin molecules.

- Polar or charged side chains tend to be directed to the outside surface of the subunit and conversely, non-polar structure tend to the directed inwards. The effect of this is to make the surface of the molecule hydrophilic and the interior hydrophobic
- An open toped cleft in the surface of the subunit known as haem pocket iscreated.
- This hydrophobic cleft protects the ferrous ion from oxidation.
- The amino acids which form the inter-subunit bonds responsible for maintaining the quaternary structure and thus the function of the haemoglobin molecule are brought into the correct orientation to permit these bonds to form.

Quarternary structure

T-form

- The deoxy form of hemoglobin is called the "T" form or taut or tense form. In this form the two αβ dimmers interact through a network of ionic bonds and hydrogen bonds that constrain the movement of the polypeotide chains. The T form is the low oxygen affinity form of hemoglobin.

R form

- The binding of hemoglobin causes rupture of some the ionic bonds and hydrogen bonds between the αβ dimmers. This leads to a structure called "R" or relaxed form, in which the polypeptide chains have more freedom of movement. The R form is the high affinity form of hemoglobin.

Functions of hemoglobin

Hemoglobin as oxygen carrier

- The main function of hemoglobin is to carry oxygen from the lungs to all the tissues of the body. This is due to the affinity of hemoglobin for oxygen. When hemoglobin comes in contact with oxygen, it combines with it and form oxy-hemoglobin. This is a weak bond. When blood reaches to tissues, where oxygen is deficient, the bond is broken and oxygen diffuses out to tissues.

Hemoglobin as carbon dioxide carrier

- Some of carbon dioxide is transported from tissues to lungs through hemoglobin. Although the majority of it is transported via plasma but still it carries some of CO2 to lungs.

Color of blood

- The red color of blood is due to hemoglobin. When red blood cells are separated from the blood, the red color disappears. This means that the red color of blood is due to red blood cells. Hence the name red blood cells is given to it. And as we know that hemoglobin is present inside red blood cells, therefore it gives red coloration to RBCs

Buffering action

- Hemoglobin also acts as a buffer. Buffer means to resist change in pH.Blood has 7.4 pH and it remains in the narrow range. Because, if it changes the life of the person may be endangered. Therefore, hemoglobin plays very important role in keeping the pH of blood constant.

Erythrocyte metabolism

- Hemoglobin plays an important role in the modulation of erythrocyte metabolism.

Interaction with drugs

- Not only for oxygen, but hemoglobin act a very important role the transport of various drugs to their site of action.

Physiological active catabolites

- Hemoglobin is a source of various physiological active catabolites.

ALL POSSIBLE QUESTIONS:

1. Define protein?
2. What is the functions of proteins?
3. What is zwitterions?
4. What is D and L amino acid?
5. What is the importance of ninhydrin reaction.?
6. What is globular proteins?
7. What is holoprotein?
8. What is transition state?

Detailed questions:

1. Explain in detail about the classification of protein?
2. Explain in detail about the secondary structure of proteins with an example?
3. Describe the tertiary structure of proteins?
4. Write the significance of protein ?
5. Explain about the fibrous and globular protein?
6. Explain in detail the classification of amino acid?
7. Write the significance of Haemoglobin?
8. Describe the structure and functions of insulin?
9. Explain in detail the structure and functions of haemoglobin.

Questions	Option A	Option B	Option C	Option D	Answers
Proteins contain	Only L- α - amino acids	Only D-amino acids	DL-Amino acids	Both A) and B)	**Only L- α -amino acids**
The optically inactive amino acid is	Glycine	Serine	Threonine	Valine	**Glycine**
At neutral pH, a mixture of amino acids in solution would be predominantly:	Dipolar ions	Nonpolar molecules	Positive and monovalent	Hydrophobic	**Dipolar ions**
The true statement about solutions of amino acids at physiological pH is	All amino acids contain both positive and negative charges	All amino acids contain positively charged side chains	Some amino acids contain only positive charge	All amino acids contain negatively charged side chains	**All amino acids contain both positive and negative charges**
pH (isoelectric pH) of alanine is	6.02	6.6	6.8	7.2	**6.02**
Since the pK values for aspartic acid are 2.0, 3.9 and 10.0, it follows that the isoelectric (pH) is	3	3.9	5.9	6	**3**
Sulphur containing amino acid is	Methionine	Leucine	Valine	Asparagine	**Methionine**
All the following are sulphur containing amino acids found in proteins except	Cysteine	Cystine	Methionine	Threonine	**Threonine**
An aromatic amino acid is	Lysine	Tyrosine	Taurine	Arginine	**Tyrosine**
The functions of plasma albumin are	Osmosis	Transport	Immunity	both A)and B)	**Osmosis**
Amino acid with side chain containing basic groups is	2-Amino 5-guanidovaleric acid	2-Pyrrolidine carboxylic acid	2-Amino 3-mercaptopropanoic acid	2-Aminopropanoic acid	**2-Amino 5-guanidovaleric acid**
An essential amino acid in man is	Aspartate	Tyrosine	Methionine	Serine	**Methionine**

Non essential amino acids	Are not components of tissue proteins	May be synthesized in the body from essential amino acids	Have no role in the metabolism	May be synthesized in the body in diseased states	**May be synthesized in the body from essential amino acids**
An example of polar amino acid is	Alanine	Leucine	Arginine	Valine	**Arginine**
The amino acid with a nonpolar side chain is	Serine	Valine	Asparagine	Threonine	**Asparagine**
A ketogenic amino acid is	Valine	Cysteine	Leucine	Threonine	**Cysteine**
An amino acid that does not form an α-helix is	Valine	Proline	Tyrosine	Tryptophan	**Proline**
An amino acid not found in proteins is	β-Alanine	Proline	Lysine	Histidine	**β-Alanine**
In mammalian tissues serine can be a biosynthetic precursor of	Methionine	Glycine	Tryptophan	Phenylalanine	**Glycine**
A vasodilating compound is produced by the decarboxylation of the amino acid:	Arginine	Aspartic acid	Glutamine	Histidine	**Histidine**
Biuret reaction is specific for	–CONH- linkages	–CSNH2 group	–(NH)NH2 group	All of these	**–CONH-linkages**
Sakaguchi's reaction is specific for	Tyrosine	Proline	Arginine	Cysteine	**Arginine**
Million-Nasse's reaction is specific for the amino acid:	Tryptophan	Tyrosine	Phenylalanine	Arginine	**Tyrosine**
Ninhydrin with evolution of CO2 forms a blue complex with	Peptide bond	α -Amino acids	Serotonin	Histamine	**α -Amino acids**
Which of the following is a dipeptide?	Anserine	Glutathione	Glucagon	β -Lipoprotein	**Anserine**
Which of the following is a tripeptide?	Anserine	Oxytocin	Glutathione	Kallidin	**Glutathione**
Casein, the milk protein is	Nucleoprotein	Chromoprotein	Phosphoprotein	Glycoprotein	**Phosphoprotein**
An example of phosphoprotein present in egg yolk is	Ovoalbumin	Ovoglobulin	Ovovitellin	Avidin	**Ovovitellin**
A simple protein found in the nucleoproteins of the sperm is	Prolamine	Protamine	Glutelin	Globulin	**Protamine**

Histones are	Identical to protamine	Proteins rich in lysine and arginine	Proteins with high molecular weight	Insoluble in water and very dilute acids	**Proteins rich in lysine and arginine**
The protein present in hair is	Keratin	Elastin	Myosin	Tropocollagen	**Keratin**
Both α-helix and β-pleated sheet conformation of proteins were proposed by	Watson and Crick	Pauling and Corey	Waugh and King	Y.S.Rao	**Pauling and Corey**
Each turn of α-helix contains the amino acid residues (number):	3.6	3	4.2	4.5	**3.6**
Distance traveled per turn of α−helix in nm is	0.53	0.54	0.44	0.48	**0.54**
Along the α-helix each amino acid residue advances in nm by	0.15	0.1	0.12	0.2	**0.15**
The number of helices present in a collagen molecule is	1	2	3	4	**3**
In proteins the α-helix and β-pleated sheet are examples of	Primary structure	Secondary structure	Tertiary structure	Quaternary structure	**Secondary structure**
The α-helix of proteins is	A pleated structure	Made periodic by disulphide bridges	A non-periodic structure	Stabilised by hydrogen bonds between NH and CO groups of the main chain	**A non-periodic structure**
Tertiary structure of a protein describes	The order of amino acids	Location of disulphide bonds	Loop regions of proteins	The ways of protein folding	**The ways of protein folding**
In a protein molecule the disulphide bond is not broken by	Reduction	Oxidation	Denaturation	X-ray diffraction	**Denaturation**

Denaturation of proteins results in	Disruption of primary structure	Breakdown of peptide bonds	Destruction of hydrogen bonds	Irreversible changes in the molecule	**Destruction of hydrogen bonds**
The enzyme trypsin is specific for peptide bonds of	Basic amino acids	Acidic amino acids	Aromatic amino acids	Next to small amino acid residues	**Basic amino acids**
Chymotrypsin is specific for peptide bonds containing	Uncharged amino acid residues	Acidic amino acids	Basic amino acid	Small amino acid residues	**Uncharged amino acid residues**
The end product of protein digestion in G.I.T. IS	Dipeptide	Tripeptide	Polypeptide	Amino acid	**Amino acid**
At isoelectric pH, an amino acid exists as	Anion	Cation	Zwitterion	None of these	**Zwitterion**
At a pH below the isoelectric point, an amino acid exists as	Cation	Anion	Zwitterion	Undissociated molecule	**Cation**
An amino acid having a hydrophilic side chain is	Alanine	Proline	Methionine	Serine	**Serine**
An amino acid that does not take part in α helix formation is	Histidine	Tyrosine	Proline	Tryptophan	**Proline**
Primary structure of a protein is formed by	Hydrogen bonds	Peptide bonds	Disulphide bonds	All of these	**Peptide bonds**
α-Helix is formed by	Hydrogen bonds	Hydrophobic bonds	Electrostatic bonds	Disulphide bonds	**Hydrogen bonds**
Aromatic amino acids can be detected by	Sakaguchi reaction	Millon-Nasse reaction	Hopkins-Cole reaction	Xanthoproteic reaction	**Xanthoproteic reaction**
Two amino groups are present in	Leucine	Glutamate	Lysine	Threonine	**Lysine**
During denaturation of proteins, all of the following are disrupted except	Primary structure	Secondary structure	Tertiary structure	Quaternary structure	**Secondary structure**
All the following are branched chain amino acids except	Isoleucine	Alanine	Leucine	Valine	**Alanine**

Millon’s test is for identification of	Tyrosine	Tryptophan	Proline	Arginine	**Tyrosine**
Hopkins-Cole test is for identification of	Tyrosine	Tryptophan	Arginine	Cysteine	**Tryptophan**
Collagen is very rich in	Glycine	Serine	Aspartic acid	Glutamic acid	**Glycine**
In glutathione (a tripeptide) is present apart from Glutamic acid and cysteine:	Serine	Glycine	Leucine	Phenyl alanine	**Glycine**
2-Amino 3-OH propanoic acid is	Glycine	Alanine	Valine	Serine	**Serine**
All amino acids have one asymmetric carbon atom, except	Arginine	Asparagine	Histidine	Glycine	**Glycine**

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(*Deemed to be University Established Under Section 3 of UGC Act 1956*) Pollachi
Main Road, Eachanari Post, Coimbatore – 641 021. INDIA
Phone: 0422-6471113-5, 6453777; Fax No: 0422-2980022-3
Email: info@karpagam.com; Web: www.kahedu.edu.in

DEPARTMENT OF MICROBIOLOGY

Subject	**:**	**Biochemistry**	**Semester**	**:**	**I**
Subject code	**:**	**19MBU103**	**Class**	**:**	**I B.Sc Microbiology**

UNIT-IV: COURSE MATERIAL

Unit-IV

Structure and classification of enzymes,specificity of enzymes.Michaelis menten equation. Km, V_{max}. isoenzymes Allosteric enzyme and its mechanism. Multienzyme complex, enzyme inhibition.

Suggest Readings

1. Nelson, D.L and Cox, M.M. (2008). Lehninger Principles of Biochemistry, 5th edition. W.H. Freeman and Company.

Enzymes

Enzymes are soluble, colloidal, organic catalyst formed by living cells that catalyze a specific biochemical reaction by lowering the activation energy and in the process they remain unchanged.

Types of Enzymes

1. Exo-enzymes

Enzymes that function outside the cell are called so, e.g. zymase, lysozyme, digestive enzymes.

2. Endo-enzyme

Enzymes that function inside the cell are called so, e.g. enzymes of glycolysis, Krebs cycle, protein biosynthesis etc.

3. Zymogens

These are inactive precursors or pro-enzymes forms of exo-enzymes. They become activated prior to enzymatic action, e.g., proteases.

4. Constitute or housekeeping enzymes

Those enzymes are always present and synthesized in cell, e.g., glycolytic enzymes.

5. Inducible enzymes

Most enzymes are synthesized only when they are needed e.g. Nitric oxide synthase, cycloxygenase, aldehyde dehydrogenase etc.

6. Isoenzymes (isozymes)

These are the different forms of the same enzymes which catalyze the same chemical reaction but, differ each other chemically, immunologically, and electrophoretically and in kinetic properties. For example, in maize 18 isozymes found for peroxidase. In plants aspertate kinase exist in two isozyme forms. Aspertate kinase catalyzes the amino acid biosynthesis from aspertate, LDH (Lactic acid dehydrogenase)

7. Ribozyme or RNA Enzymes

e.g. ribonuclease-P (RNAase-P), Peptidyl transferase (23S rRNA of larger subunit of ribosome) etc.

8. Abzymes

These are the antibodies that act as enzymes.

Structure (Chemical Nature) of Enzyme

All enzymes are generally globular proteins except some RNA enzymes like

Ribonuclease-P, ribozyme and peptidyl transferase.

On the basis of number of polypeptide chains, enzymes are of 2 types

(a) Monomeric enzymes:

Consist of one polypeptide chain (subunit), e.g., ribonuclease, lysozyme, hexokinase etc. These are functional in their 3 dimensional or tertiary structures.

(b) Oligomeric enzymes:

Consist of more than one olgolypeptide chain. They are functional in their quaternary structure. For example, aldolase consists of 4 chains (tetrameric), Rubisco of Calvin cycle consists of 24 chains, and Enolase is a dimmer.

On the basis of chemical nature, enzymes are also 2 types

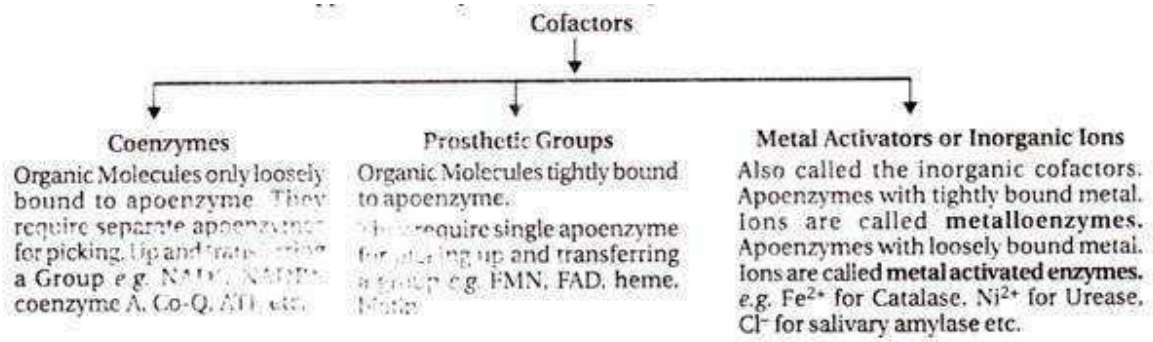
(i) Simple enzymes

They consist of only proteins, e.g. urease, lysozyme, pepsin, trypsin etc.

(ii) Holoenzyme or Conjugate enzyme

These enzymes consist of proteinous part called apoenzyme and non-proteinous part called co-factor.

Holoenzyme = Apoenzyme + Co-factor (active) (Proteinous part) (Non-proteinous part). The cofactors are of 3 types: Co-enzymes, Prosthetic groups and inorganic ions.



The surface of a functional enzyme contains 2 sites *i.e. catalytic site* and *allosteric sites.*

Catalytic Site or Active Site or Active Spot

It is a small three-dimensional (3D) area on or near enzyme surface that binds the specific substrate(s) and convert into products. The unique 3D shape of a catalytic site may alter by denaturation (unfolding) through high temperature or exposure to extremes of pH. This results in the loss of catalytic activity.

In monomeric enzymes, the catalytic site is often a cleft or crevice, but in multimeric enzymes, it resides at the interface between polypeptides. In some enzymes, the catalytic site is rigid or non- flexible to accommodate a substrate. But in most cases, the binding of substrate induces a conformational change in the catalytic site, e.g., glucose (substrate) induces a conformational change of hexokinase. An enzyme may have one or more active sites. Each active site consists of 3-12 amino acids that come together by folding of polypeptides. In a holoenzyme, the catalytic site also contains cofactor for its function.

The amino acids residues of catalytic site have 4 roles

Provide charged R-groups to attract substrate,

Some act as template for holding substrate,

Some provide functional groups that perform chemical changes by lowering

activation energy

A few residues determine substrate specificity

Enzymes : Amino Acid residues in active site

Pepsin : Tyrosine only

Aldolase : Glycine – Histidine – Alanine

Allosteric Sites

These are special sites on enzyme surface other than catalytic site, which when bind with effectors or modulators alter the conformation of the catalytic site. The enzymes having allosteric sites are called allosteric enzymes. Allosteric sites are of two types: activator site and inhibitor site. An allosteric activator when binds to activator site increase the enzyme activity while an allosteric inhibitor decreases the enzyme activity by binding the inhibitor site.

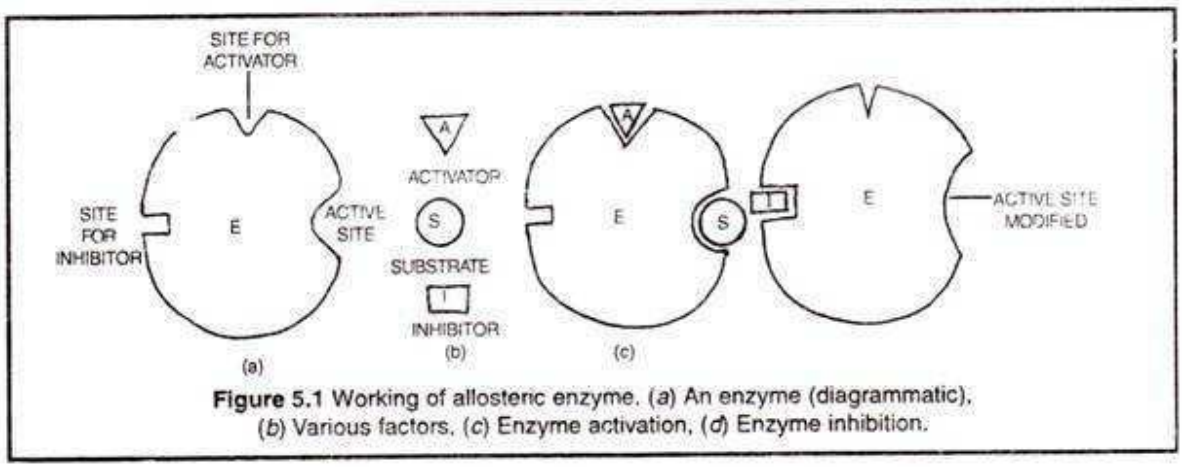


Figure 5.1 Working of allosteric enzyme. (*a*) An enzyme (diagrammatic), (*b*) Various factors. (*c*) Enzyme activation, (*d*) Enzyme inhibition.

Cofactors

Cofactors, mostly metal ions or coenzymes, are inorganic and organic chemicals that assist enzymes during the catalysis of reactions.

Coenzymes are non-protein organic molecules that are mostly derivatives of vitamins soluble in water by phosphorylation; they bind apoenzyme to proteins to produce an active holoenzyme.

Apoenzymes are enzymes that lack their necessary cofactor(s) for proper functioning; the binding of the enzyme to a coenzyme forms a holoenzyme. Holoenzymes are the active form of an apoenzyme.

Cofactors can be metals or coenzymes, and their primary function is to assist in enzyme activity.

They are able to assist in performing certain, necessary, reactions the enzyme cannot perform alone.

They are divided into coenzymes and prosthetic groups.

A holoenzyme refers to a catalytically active enzyme that consists of both apoenzyme (enzyme without its cofactor(s)) and cofactor.

There are two groups of cofactors: metals and small organic molecules called coenzymes.

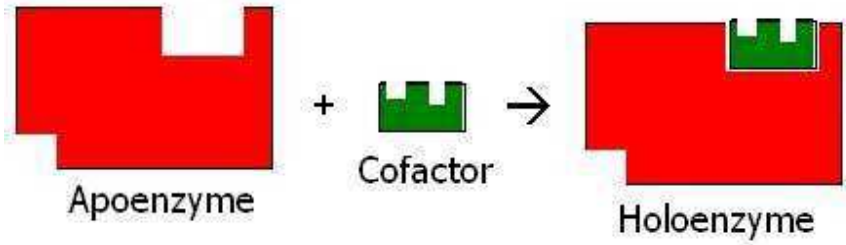
Coenzymes are small organic molecules usually obtained from vitamins.

Prosthetic groups refer to tightly bound coenzymes, while co-substrates refer to loosely bound coenzymes that are released in the same way as substrates and products.

Loosely bound coenzymes differ from substrates in that the same coenzymes may be used by different enzymes in order to bring about proper enzyme activity.

Enzymes without their necessary cofactors are called apoenzymes, which are the inactive form of an enzyme. Cofactors with an apoenzyme are called a holoenzyme, which is the active form.

General formula



Metal cofactors

Metal ions are known as the common cofactors.

In some enzymes, the function as a catalyst cannot be carried out if a metal ion is not available to be bound the active site.

In daily nutrition, this kind of cofactor plays a role as the essential trace elements such as: iron (Fe^{3+}), manganese (Mn^{2+}), cobalt (Co^{2+}), copper (Cu^{2+}), zinc (Zn^{2+}), selenium (Se^{2+}), and molybdenum (Mo^{5+}).

For example, Mg2 is used in glycolysis. In the first step of converting glucose to glucose 6-phosphate, before ATP is used to give ADP and one phosphate group, ATP is bonded to Mg2 which stabilizing the other two phosphate groups so it is easier to release only one phosphate group without resonate with other two.

In some bacteria such as genus Azotobacter and Pyrococcus furiosus, metal cofactors are also discovered to play an important role. An example of cofactors in action is the zinc-mediated function of carbonic anhydrase or the magnesium-mediated function of restriction endonuclease.

Enzyme Classification

The International Union of Biochemistry (IUB, 1961) adopted a scheme for systematic functional classification and nomenclature of enzymes.

The recommendations of IUB are as follows

All known enzymes have been grouped into six major classes on the basis of reaction type they catalyze,

Each class further sub-divided into subclasses and sub-subclasses,

Each enzyme is assigned two names i.e., recommended (trivial) name and systematic name,

Each enzyme is identified by a unique four digit classification number.

For example, hexokinase is recommended name, its systematic name is glucose phosphotransferase and its classification number in EC 2.7.1.1. Here, "EC" stands for Enzyme commission, the first number (2) stands for the major class, the second number (7) stands for the sub class, the third number (1) indicates sub-class and the fourth number (1) denotes the serial number assigned in its sub- classes.

Table 5.2. IUB classification of enzymes

Major Class (Type of reaction catalyzed)	Common exmaples	Kind of reaction	Specific Example
1. ***Oxidoreductases*** *(Transfer of electrons)*	Oxidases Reductases Dehydrogenase	$A^{+3} + B^{+2} \rightarrow A^{+2} + B^{+3}$	Alcohol + NAD ↓ *Alcohol dehydrogenase* Aldehyde + $NADH_2$
2. ***Transferases*** *(Transfer of functional groups)*	Transaminase Transketolase Transaldolase	$A - X + B \rightarrow A + B - X$	Glucose + ATP ↓ *Glukokinase or hexokinase* Glucose-6-Phosphate + ADP
3. ***Hydrolases*** *(Hydrolysis Reactions)*	Amylases Lipases Proteases Nucleases	$A–B+H_2O \rightarrow A–OH + B–H$	Sucrose ↓ *Sucrase* Glucose + Fructose
4. **Lyases or *Desmolases*** *(Group elimination to form double bonds without hydrolysis)*	Aldolase Decarboxylase Fumarase Citrate synthase	$A - B \rightarrow A = B + X - Y$ X Y	Histidine ↓ *Histidine decarboxylase* Histidine + CO_2
5. ***Isomerases*** *(Transfer of Groups within a molecule*	Isomerase Mutase Epimerase	$A - B \rightarrow A - B$ Y X X Y	Glucose – 6-Phosphate ↓ *Isomerase* Fructose-6-Phosphate
6. ***Ligases or Synthetases*** *(Bond formation couples with ATP hydrolysis)*	Synthetases Carboxylases	$A + B + ATP \rightarrow A - B + ADP + Pi$	Pyruvate + CO_2 + ATP ↓ *Pyruvate carboxylase* Oxaloacetate + ADP + Pi

1. Oxidoreductases catalyze a variety of oxidation-reduction reactions. Common names include dehydrogenase, oxidase, reductase and catalase.

2. Transferases catalyze transfers of groups (acetyl, methyl, phosphate, etc.). Common names include acetyltransferase, methylase, protein kinase and polymerase. The first three subclasses play major roles in the regulation of cellular processes. The polymerase is essential for the synthesis of DNA and RNA.

3. Hydrolases catalyze hydrolysis reactions where a molecule is split into two or more smaller molecules by the addition of water. Common examples are given below.

Proteases split protein molecules. Examples: HIV protease and caspase. HIV protease is essential for HIV replication. Caspase plays a major role in apoptosis.

Nucleases split nucleic acids (DNA and RNA). Based on the substrate type, they are divided into RNase and DNase. RNase catalyzes the hydrolysis of RNA and DNase acts on DNA. They may also be divided into exonuclease and endonuclease. The exonuclease progressively splits off single nucleotides from one end of DNA or RNA. The endonuclease splits DNA or RNA at internal sites.

Phosphatase catalyzes dephosphorylation (removal of phosphate groups). Example: calcineurin. The immunosuppressive drugs FK506 and Cyclosporin A are the inhibitors of calcineurin.

4. Lyases catalyze the cleavage of C-C, C-O, C-S and C-N bonds by means other than hydrolysis or oxidation. Common names include decarboxylase and aldolase.

5. Isomerases catalyze atomic rearrangements within a molecule. Examples include rotamase, protein disulfide isomerase (PDI), epimerase and racemase.

6. Ligases catalyze the reaction which joins two molecules. Examples include peptide synthase, aminoacyl-tRNA synthetase, DNA ligase and RNA ligase.

The IUBMB committee also defines subclasses and sub-subclasses. Each enzyme is assigned an EC (Enzyme Commission) number. For example, the EC number of catalase is EC1.11.1.6. The first digit indicates that the enzyme belongs to oxidoreductase (class 1). Subsequent digits represent subclasses and sub-subclasses.

Mechanism of Enzyme Action:

Arrhenius first pointed out that, all the molecules in a given population do not have the same kinetic energy some molecules are energy poor and other are energy rich. Higher is the energy barrier the grater is the inactiveness of reaction. This energy

barrier can be overcome by the enzymes and making the molecule active with available energy level.

To explain the velocity of enzymatic reaction Leonor Michaels and Moud Menten (1913) proposed following assumptions.

Only a single substrate and a single product are formed in enzymatic reaction.

The process continued essentially to its completion.

Concentration of substrate is much greater than the enzyme in the system.

An intermediate enzyme substrate complex is formed.

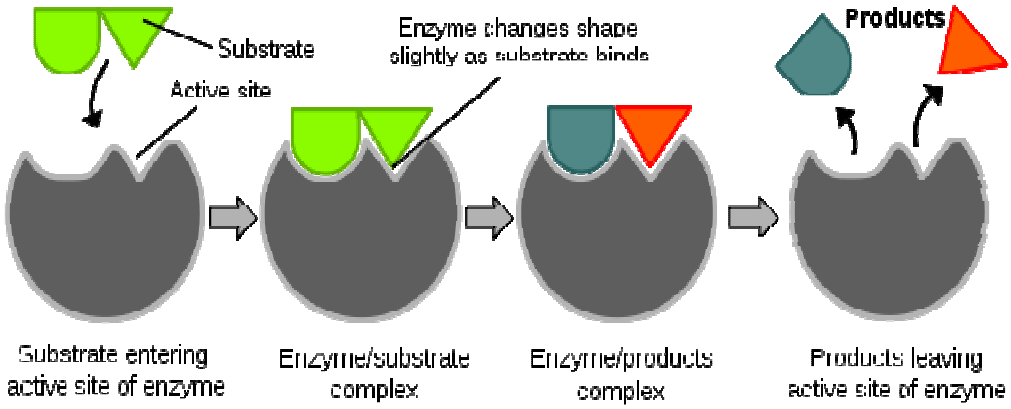
The rate of decomposition of the substrate is proportional to the concentration of the enzyme substrate complex.

They proposed an equation popularly accepted as Michaelis. Menten's equation, which concerned the velocity of enzymatic reaction.

Where Km is the Michaelis constant 'S' is the substrate concentration, Vmax - maximum velocity of the reaction and V0 is the initial velocity.

Km value is constant for all enzymes up to the half of the maximum velocity of reaction. Greater is the ES complex period the lower is the Km value.

There are several theories has been put forwarded by different biochemists to explain the mechanism of the enzyme action.



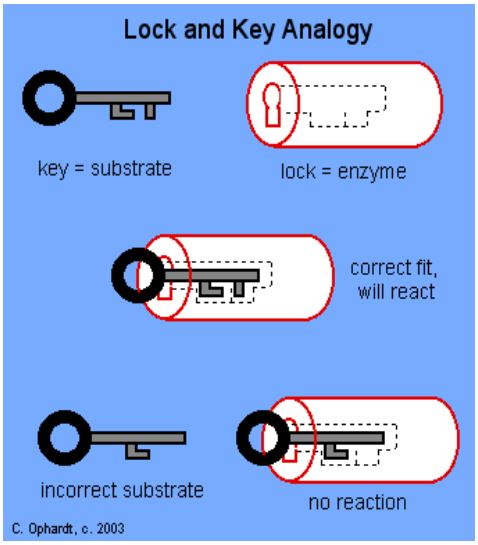
(i) Lock and key thoery

In the **lock-and-key model** of enzyme action:

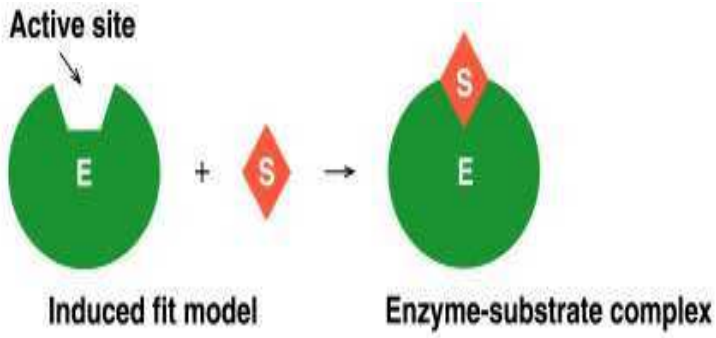
- the active site has a rigid shape
- only substrates with the matching shape can fit

- the substrate is a key that fits the lock of the active site

This is an older model, however, and does not work for all enzymes



(II) Inducted fit Theory: In the induced-fit model of enzyme action



the active site is flexible, not rigid

the shapes of the enzyme, active site, and substrate adjust to maximize the fit, which improves catalysis

there is a greater range of substrate specificity

This model is more consistent with a wider range of enzymes

Enzyme Catalyzed Reactions

- When a substrate (S) fits properly in an active site, an enzyme-substrate (ES) complex is formed:

  E + S ⇌ ES

- Within the active site of the ES complex, the reaction occurs to convert substrate to product (P):

  ES E + P

- The products are then released, allowing another substrate molecule to bind the enzyme

  - this cycle can be repeated millions (or even more) times per minute

- The overall reaction for the conversion of substrate to product can be written as follows:

  E + S ES E + P

Factors affecting Enzyme Activity

The activity of an Enzyme is affected by its environmental conditions. Changing these alter the rate of reaction caused by the enzyme. In nature, organisms adjust the conditions of their enzymes to produce an Optimum rate of reaction, where necessary, or they may have enzymes which are adapted to function well in extreme conditions where they live.

Temperature

Increasing temperature increases the Kinetic Energy that molecules possess. In a fluid, this means that there are more random collisions between molecules per unit time.

Since enzymes catalyse reactions by randomly colliding with Substrate molecules, increasing temperature increases the rate of reaction, forming more product.

However, increasing temperature also increases the Vibrational Energy that molecules have, specifically in this case enzyme molecules, which puts strain on the bonds that hold them together.

As temperature increases, more bonds, especially the weaker Hydrogen and Ionic bonds, will break as a result of this strain. Breaking bonds within the enzyme will cause the Active Site to change shape.

This change in shape means that the Active Site is less Complementary to the shape of the Substrate, so that it is less likely to catalyse the reaction. Eventually, the enzyme will become Denatured and will no longer function.

As temperature increases, more enzymes' molecules' Active Sites' shapes will be less Complementary to the shape of their Substrate, and more enzymes will be Denatured. This will decrease the rate of reaction.

In summary, as temperature increases, initially the rate of reaction will increase, because of increased Kinetic Energy. However, the effect of bond breaking will become greater and greater, and the rate of reaction will begin to decrease.

The temperature at which the maximum rate of reaction occurs is called the enzyme's Optimum Temperature. This is different for different enzymes. Most enzymes in the human body have an Optimum Temperature of around 37.0 °C.

pH - Acidity and Basicity

pH measures the Acidity and Basicity of a solution. It is a measure of the Hydrogen Ion (H+) concentration, and therefore a good indicator of the Hydroxide Ion (OH-) concentration. It ranges from pH1 to pH14. Lower pH values mean higher H+ concentrations and lower OH- concentrations.

Acid solutions have pH values below 7, and Basic solutions (alkalis are bases)

have pH values above 7. Deionised water is pH7, which is termed 'neutral'.

H+ and OH- Ions are charged and therefore interfere with hydrogen and ionic bonds that hold together an enzyme, since they will be attracted or repelled by the charges created by the bonds. This interference causes a change in shape of the enzyme, and importantly, its Active Site.

Different enzymes have different Optimum pH values. This is the pH value at which the bonds within them are influenced by H+ and OH- Ions in such a way that the shape of their Active Site is the most Complementary to the shape of their Substrate. At the Optimum pH, the rate of reaction is at an optimum.

Any change in pH above or below the Optimum will quickly cause a decrease in the rate of reaction, since more of the enzyme molecules will have Active

Sites whose shapes are not (or at least are less) Complementary to the shape of their Substrate.

Small changes in pH above or below the Optimum do not cause a permanent change to the enzyme, since the bonds can be reformed. However, extreme changes in pH can cause enzymes to Denature and permanently lose their function.

Enzymes in different locations have different Optimum pH values since their environmental conditions may be different. For example, the enzyme Pepsin functions best at around pH2 and is found in the stomach, which contains Hydrochloric Acid (pH2).

Concentration

Changing the Enzyme and Substrate concentrations affect the rate of reaction of an enzyme-catalysed reaction. Controlling these factors in a cell is one way that an organism regulates its enzyme activity and so its Metabolism.

Changing the concentration of a substance only affects the rate of reaction if it is the limiting factor: that is, it the factor that is stopping a reaction from preceding at a higher rate.

If it is the limiting factor, increasing concentration will increase the rate of reaction up to a point, after which any increase will not affect the rate of reaction. This is because it will no longer be the limiting factorand another factor will be limiting the maximum rate of reaction.

As a reaction proceeds, the rate of reaction will decrease, since the Substrate will get used up. The highest rate of reaction, known as the Initial Reaction Rate is the maximum reaction rate for an enzyme in an experimental situation.

Substrate Concentration

Increasing Substrate Concentration increases the rate of reaction. This is because more substrate molecules will be colliding with enzyme molecules, so more product will be formed.

However, after a certain concentration, any increase will have no effecton the rate of reaction, since Substrate Concentration will no longer be the limiting

factor. The enzymes will effectively become saturated, and will be working at their maximum possible rate.

Enzyme Concentration

Increasing Enzyme Concentration will increase the rate of reaction, as more enzymes will be colliding with substrate molecules.

However, this too will only have an effect up to a certain concentration, where the Enzyme Concentration is no longer the limiting factor.

Allosteric enzymes

Allosteric enzymes are enzymes that change their conformation upon binding of an effector. An allosteric enzyme is an oligomer whose biological activity is affected by *altering* the conformation(s) of its quaternary structure. Allosteric enzymes tend to have several subunits. These subunits are referred to as protomers. In a given conformational state, these enzymes can bind substrate (S), inhibitor (l), and activator (A).

Whereas enzymes with single active sites display normal Michaelis-Menten kinetics, allosteric enzymes have multiple active sites and show cooperative binding. As a result, allosteric enzymes display a sigoidal dependence on the concentration of their substrates, allowing them to greatly vary catalytic output in response to small changes in effector concentration. Effector molecules, which may be the substrate itself (homotropic effectors) or some other small molecule (heterotropic effector), may cause the enzyme to become more active or less active. The binding sites for heterotropic effectors, called allosteric sites, are separate from the active site.

Properties of Allosteric Enzymes:

Allosteric or Regulatory enzymes have multiple subunits (Quaternary Structure) and multiple active sites. Allosteric enzymes have active and inactive shapes differing in 3D structure. Allosteric enzymes often have multiple inhibitor or activator binding sites involved in switching between active and inactive shapes. Allosteric enzymes have characteristic “S”-shaped curve for reaction rate vs. substrate concentration. Because the substrate binding is “Cooperative.” And the binding of first substrate at first active site stimulates active shapes, and promotes binding of second substrate.

A modulator is a metabolite, when bound to the allosteric site of an enzyme, alters its kinetic characteristics. The modulators for allosteric enzyme may be either stimulatory or inhibitory. A stimulator is often the substrate itself. The regulatory enzymes for which substrate and modulator are identical are called homo-tropic. When the modulator has a structure different then the substrate, the enzyme is called heterotropic. Some enzymes have more then one modulators. The allosteric enzymes also have one or more regulatory or allosteric sites for binding the modulator. Enzymes with several modulators generally have different specific binding sites for each.

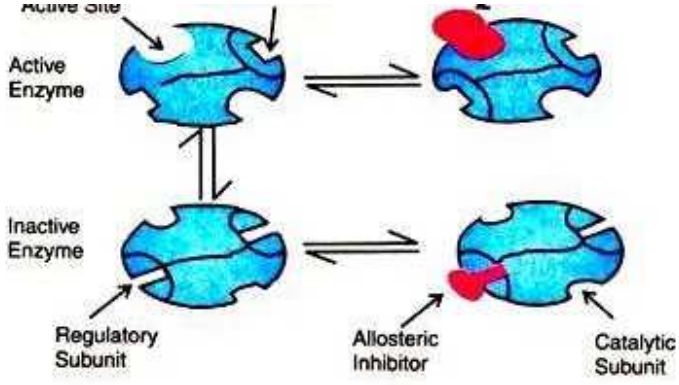


Fig. 12.16: Mechanisms of allosteric effect.

Mechanism of Action of Allosteric Enzymes:

Two general models for the inter-conversion of inactive and active forms of allosteric enzymes have been proposed:

Simple sequential model:

This model was proposed by Koshland Jr. in the year 1966. According to this theory, the aliosteric enzyme can exist in only two conformational changes individually. Consider an aliosteric enzyme consisting of two identical subunits, each containing an active site.

The T (tense) form has low affinity and the R (relaxed) form has high affinity for substrate. In this model, the binding of substrate to one of the subunits induces a

T

→ R transition in that subunit but not in the other subunits.

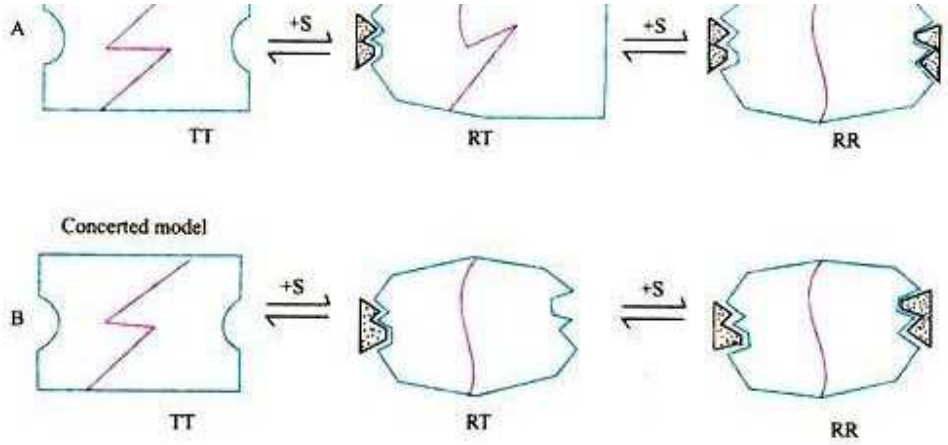


Fig. 12.17: Kinetic models of allosteric enzymes (A) : Simple sequential model (B) Concerted or symmetry model.

Concerted or Symmetry Model

This model was proposed by Jacques Monod and his colleagues in 1965. According to them, an allosteric enzyme can exist in still two conformations, active and relaxed or inactive form.

All subunits are either in the active form or all are in inactive form. Every substrate molecule that binds with enzyme increases the probability of transition from the inactive to the active site. The effect of allosteric activators and inhibitors can be explained quite easily by this model.

An allosteric inhibitor binds preferably to the T form whereas an allosteric activator binds to the R form (Fig. 12.17B). An allosteric inhibitor shifts The R → T conformational equilibrium towards T. Whereas an allosteric activator shifts it toward R.

The result is that an allosteric activator increases the binding to substrate of the enzyme, whereas an allosteric inhibitor decreases substrate binding. Symmetry is conserved in this model but not in the sequential model.

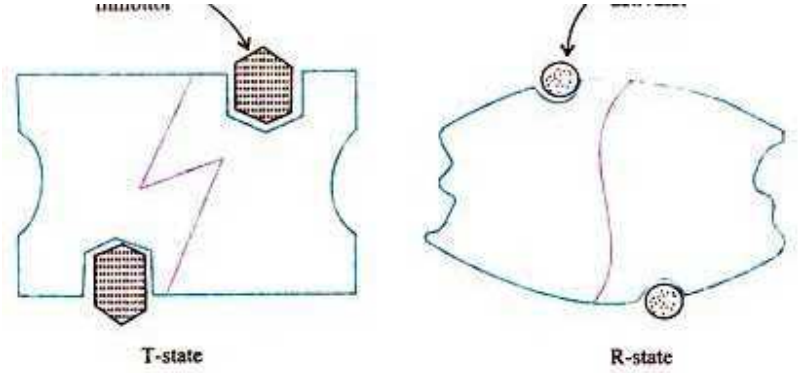


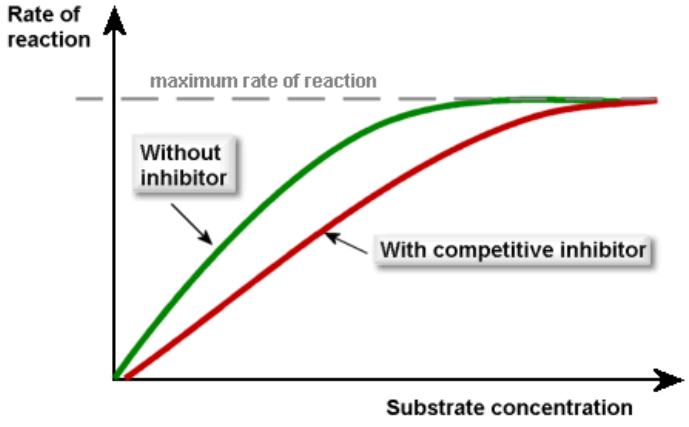
Fig. 12.18: Effect of activator and inhibitor on substrate binding.

Enzyme Inhibitors

Enzyme Inhibitors reduce the rate of an enzyme catalyzed reaction by interfering with the enzyme in some way. This effect may be permanent or temporary.

Competitive Enzyme Inhibitors work by preventing the formation of Enzyme- Substrate Complexes because they have a similar shape to the substrate molecule. This means that they fit into the Active Site, but remain unreacted since they have a different structure to the substrate. Therefore less substrate molecules can bind to the enzymes so the reaction rate is decreased.

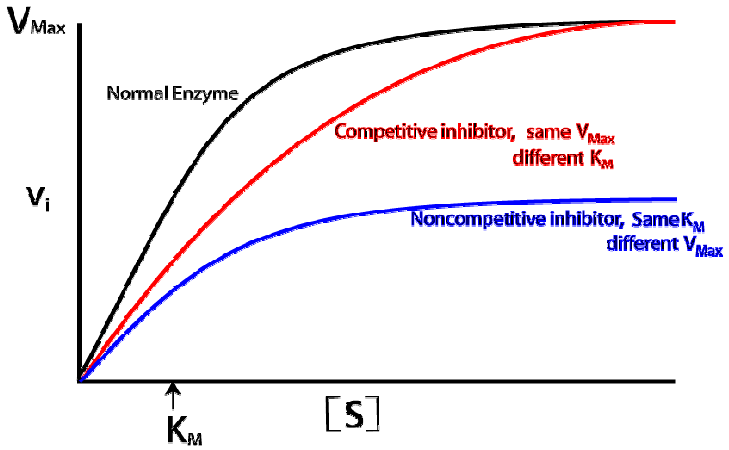
Competitive Inhibition is usually temporary, and the Inhibitor eventually leaves the enzyme. This means that the level of inhibition depends on the relative concentrations of substrate and Inhibitor, since they are competing for places in enzyme Active Sites.



Non-competitive Enzyme Inhibitors work not by preventing the formation of Enzyme-Substrate Complexes, but by preventing the formation of Enzyme-Product Complexes. So they prevent the substrate from reacting to form product.

Usually, Non-competitive Inhibitors bind to a site other than the Active Site, called an Allosteric Site. Doing so distorts the 3D Tertiary structure of the enzyme, such that it can no longer catalyse a reaction.

Since they do not compete with substrate molecules, Non-competitive Inhibitors are not affected by substrate concentration.



Many Non-competitive Inhibitors are irreversible and permanent, and effectively denature the enzymes which they inhibit. However, there are a lot of non-permanent and reversible Non-competitive Inhibitors which are vital in controlling Metabolic functions in organisms.

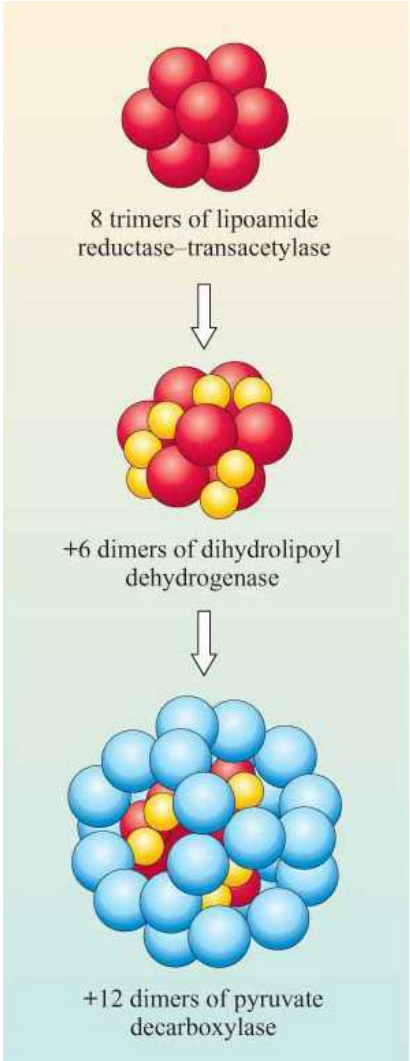
Enzyme Inhibitors by organisms are used in controlling metabolic reactions. This allows product to be produced in very specific amounts.

Multienzyme complexes

In free solution, the rate of an enzyme-catalysed reaction depends on the concentration of the enzyme and the concentration of its substrate. For an enzyme operating at suboptimal concentrations, the reaction is said to be *diffusion-limited*, since it depends on the random collision of the enzyme and substrate. If we consider a metabolic pathway, the product of one reaction is the substrate for the next enzyme in the pathway. Direct transfer of a metabolite from one enzyme to another would avoid

dilution of the metabolite in the bulk aqueous environment and would increase the rate of reaction.

In the cell, enzymes of a particular pathway are frequently organised spatially so that such *metabolic channelling* can occur. Some enzymes are associated with other enzymes involved in a particular pathway to form **multienzyme complexes**. For the enzymes in such complexes, the diffusion of the substrate is not rate-limiting. Pyruvate dehydrogenase is a complex of three different enzymes that collectively catalyse the oxidation of pyruvate. In fact, in eukaryotic cells, most enzymes do not diffuse freely in the cytosol but are effectively concentrated in particular parts of the cell along with other enzymes or proteins involved in related processes. Concentration of enzymes in this way can be achieved by specific protein–protein interactions.



Pyruvate dehydrogenase is a multienzyme complex comprising multiples of three different enzymes: eight of lipoamide reductase–transacetylase (a trimer), six of dihydrolipoyl

dehydrogenase (a dimer) and 12 of pyruvate decarboxylase (a dimer), giving a total of 60 polypeptide chains per complex.

Michaelis-Menten Equation.

First derivation, we start with kinetic mechanism.

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

E is enzyme, S is substrate, ES is the enzyme-substrate complex, and P is product. This equation includes the assumption that during the early stages of the reaction, so little product is formed that the reverse reaction (product combining with enzyme and re-forming substrate) can be ignored). Another assumption is that the concentration of substrate is much greater than that of total enzyme ([S]>>[Et]), so it can essentially be treated as a constant.

From general chemistry we can equate that rate of this process (k3[ES]) to the change in product concentration as a function of time (d[P]/dt),or equivalently, we can designate the rate with an italicized v (v) as follows.

$$\frac{d[P]}{dt} = v = k_3[ES]$$

Because the concentration of the enzyme-substrate complex ([ES]) cannot be measured experimentally, we need an alternative expression for this term. Because the enzyme that we add to the reaction will either be unbound (E) or bound (ES) we can express the fraction of bound enzyme as follows.

$$\frac{[ES]}{[E_t]} = \frac{[ES]}{[ES]+[E]}$$

If we multiply the numerator and denominator of the right-hand side of the above equation. We are in effect, multiplying by one and we do not change the value of this expression. When we do this we obtain.

$$[ES] = \frac{[E_t]}{1 + \frac{[E]}{[ES]}}$$

We have almost achieved our goal of isolating [ES], Next we need to come up with an alternative expression for the ration [E]/[ES]. We do this by recalling that a major assumption in enzyme kinetics is the steady state assumption. Basically, it says the rate of change of [ES] as a function of time is zero: d[ES]/dt=0. Another way to express the steady state assumption is that the rate of formation of ES equals the rate of breakdown of ES.

$$k_1[E][S] = k_2[ES] + k_3[ES] = (k_2 + k_3)[ES]$$

The left hand side of the equation expresses the rate of formation of ES and the right hand side expresses the two ways that ES can breakdown.

We can rearrange the equation to isolate the ration [E]/[ES].

$$\frac{[E]}{[ES]} = \frac{(k_2 + k_3)}{k_1[S]}$$

We now define a new constant, the Michaelis constant (Km)

$$K_m = \frac{(k_2 + k_3)}{k_1}$$

If we substitute Km back into equation we obtain

$$\frac{[E]}{[ES]} = \frac{K_m}{[S]}$$

We now substitute the ration Km/[S] from equation in place of the ratio [E]/[ES] and we obtain

$$[ES] = \frac{[E_t]}{1 + \frac{K_m}{[S]}}$$

If we multiply the numerator and denominator of the right hand side of equation by [S], we are in effect, multiplying by one and we do not change the value of this expression.

When we do this we obtain

$$[ES] = \frac{[E_t][S]}{[S] + K_m} = \frac{[E_t][S]}{K_m + [S]}$$

Now we have achieved our goal of isolating [ES] and we can substitute this alternative expression of [ES] into equation. We obtain

$$v = \frac{k_3[E_t][S]}{K_m + [S]}$$

Next, we imagine what happens to equation [S]>>Km as follow

$$v \approx \frac{k_3[E_t][S]}{[S]} = k_3[E_t] = k_{cat}[E_t]$$

The constant K_{cat} in the right hand most term of equation is used to signify that k_3 is considered the catalytic constant. Under such conditions, when [S] is said to saturating, the enzyme in functioning as fast as it can and we define $k_3[Et]$ (or kcat[Et]) to be equal to V_{max} the maximum velocity that can be obtained. Therefore the equation can be rewritten into the familiar form of Michaelis-Menten equation. **Unit 4:**

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

ALL POSSIBLE QUEESTIONS:

1. Define an enzyme.
2. Define in the enzyme unit?
3. What is monomeric and Oligomeric enzymes?

Detailed questions:

1. Describe about the classification of enzymes?
2. Explain the types of Enzymes?
3. Write in detail about the Michaelis-Menten equation.
4. Describe in detail about the allosteric enzyme and its mechanism?

Questions	Option 1	Option 2	Option 3	Option 4	Answers
A Holoenzyme is	Functional unit	Apo enzyme	Coenzyme	All of these	**All of these**
Example of an extracellular enzyme is	Lactate dehydrogenase	Cytochrome oxidase	Pancreatic lipase	Hexokinase	**Pancreatic lipase**
Enzymes, which are produced in inactive form in the living cells, are called	Papain	Lysozymes	Apoenzymes	Proenzymes	**Proenzymes**
An example of ligases is	Succinate thiokinase	Alanine racemase	Fumarase	Aldolase	**Succinate thiokinase**
An example of lyases is	Glutamine synthetase	Fumarase	Cholinesterase	Amylase	**Fumarase**
The enzyme which can add water to a carbon-carbon double bond or remove water to create a double bond without breaking the bond is	Hydratase	Hydroxylase	Hydrolase	Esterase	**Hydratase**
Fischer's 'lock and key' model of the enzyme action implies that	The active site is complementary in shape to that of substance only after interaction.	The active site is complementary in shape to that of substance	Substrates change conformation prior to active site interaction	The active site is flexible and adjusts to substrate	**The active site is complementary in shape to that of substance**

From the Lineweaver-Burk plot of Michaelis-Menten equation, Km and Vmax can be determined when V is the reaction velocity at substrate concentration S, the X- expressed as axis experimental data are	1/V	V	1/S	S	**1/S**
A sigmoidal plot of substrate concentration ([S] verses reaction velocity (V) may indicate	Michaelis-Menten kinetics	Co-operative binding	Competitive inhibition	Non-competitive inhibition	**Co-operative binding**
The kinetic effect of purely competitive inhibitor of an enzyme	Increases Km without affecting Vmax	Decreases Km without affecting Vmax	Increases Vmax without affecting Km	Decreases Vmax without affecting Km	**Increases Km without affecting Vmax**
An inducer is absent in the type of enzyme:	Allosteric enzyme	Constitutive enzyme	Co-operative enzyme	Isoenzymic enzyme	**Constitutive enzyme**
In reversible non-competitive enzyme activity inhibition	Vmax is increased	Km is increased	Km is decreased	Concentration of active enzyme is reduced	**Concentration of active enzyme is reduced**
In competitive enzyme activity inhibition	The structure of inhibitor generally resembles that of the substrate	Inhibitor decreases apparent Km	Km remains unaffective	Inhibitor decreases Vmax without affecting Km	**The structure of inhibitor generally resembles that of the substrate**
In enzyme kinetics Vmax reflects	The amount of an active enzyme	Substrate concentration	Half the substrate concentration	Enzyme substrate complex	**The amount of an active enzyme**

In enzyme kinetics Km implies	The substrate concentration that gives one half Vmax	The dissocation constant for the enzyme substrate comples	Concentration of enzyme	Half of the substrate concentration required to achieve Vmax	**The substrate concentration that gives one half Vmax**
In non competitive enzyme activity inhibition, inhibitor	Increases Km	Decreases Km	Does not effect Km	Increases Km	**Does not effect Km**
Factors affecting enzyme activity:	Concentration	pH	Temperature	All of these	**All of these**
The isoenzymes LDH5 is elevated in	Myocardial infarction	Peptic ulcer	Liver disease	Infectious diseases	**Liver disease**
LDH1 and LDH2 are elevated in	Myocardial infarction	Liver disease	Kidney disease	Brain disease	**Myocardial infarction**
The pH optima for salivary analyse is	between 6.6–6.8	between 2.0–7.5	Above 7.9	between 8.6-12.3	**between 6.6–6.8**
The pH optima for pancreatic analyse is	4	7.1	7.9	8.6	**8.6**
The substrate for amylase is	Cane sugar	Starch	Lactose	Ribose	**Starch**
Coenzymes are	Heat stable, dialyzable, non protein organic molecules	Soluble, colloidal, protein molecules	Structural analogue of enzymes	Different forms of enzymes	**Heat stable, dialyzable, non protein organic molecules**
Enzyme involved in joining together two substrates is	Glutamine synthetase	Aldolase	Gunaine deaminase	Arginase	**Glutamine synthetase**
An example of group transferring coenzyme is	NAD+	NADP+	FAD	CoA	**CoA**
An example of hydrogen transferring coenzyme is	CoA	NAD+	Biotin	TPP	**NAD+**

Some enzymes act on closely related substrates which is known as	Relative substrate specificity	Broad specificity	Reaction specificity	Stereo specificity			**Broad specificity**
Coenzymes are often regarded as	First substrate	Second substrate	Third substrate	Fourth substrate			**Second substrate**
Competitive inhibitor	Increases km	Decreases km	Decreases Vmax	Increases Vmax			**Increases km**
The pH optima of most of the enzymes is	Between 2 and 4	Between 5 and 9	Between 8 and 12	Above 12			**Between 5 and 9**
---------- is the multifunctional enzyme	Lactate dehydrogenase	Fatty acid synthetase	Aminoacid oxidase	Lipase			**Fatty acid synthetase**
The thermostability of an enzyme can increased by	adding disulfide bonds	changing non-essential aspargine residues	changing non-essential glutamine residues	changing non-essential serine residues			**adding disulfide bonds**
The steady state theory was proposed by	Michaelis and mentan	Hill	Eadie	Brigges and Handane			**Michaelis and mentan**
Multiple forms of the same enzymes are known as	Zymogens	Isoenzymes	Proenzymes	Pre-enzymes			**Isoenzymes**
Who got Nobel Prize in 1978 for working on enzymes?	Koshland	Arber and Nathans	Nass and Nass	H.G. Khorana			**Koshland**
Site of enzyme synthesis in a cell is	Ribosomes	Rough endoplasmic reticulum	Golgi bodies	mitochondria			**Rough endoplasmic reticulum**
Inactive precursors of enzymes are known as	Apoenzymes	Coenzymes	Proenzymes	Holoenzymes			**Proenzymes**
Kinetics of an allosteric enzyme are explained by	Michaelis-Menten equation	Lineweaver-Burk plot	Hill plot	linear plot			**Hill plot**
Ribozymes_____ _	RNA enzyme	Non-protein enzymes	Catalyst function	all options			**all options**

The following reaction is characteristic of what type of enzymes? $2H_2O_2 \rightarrow 2H_2O + O_2$	Peroxides	Catalase	Dehydrogenase	Copper containing oxidases			**Catalase**
Enzyme that function inside the cells are called_____	Exoenzymes	Endoenzymes	Ribozymes	Isoenzyme			**Endoenzymes**
Abzymes are	Antibodies that acts as enzyme	Antigen that acts as enzyme	Coenzyme that acts as enzyne	haloenzyme that acts as enzyme			**Antibodies that acts as enzyme**
On the basis of polypeptide chains, enzymes are of _____types	one	two	three	Four			**two**
Apoenzyme and cofactor_____	Haloenzyme	Proenzyme	Coenzyme	exoenzyme			**Haloenzyme**
Deionized water the pH is_____	four	seven	ten	twelve			**seven**
Who first Pointed out that, all the molecules in a given population do not have the samekinetic energy some molecules are energy poor and other are energy rich_____	Arrhenius	Michaelis-Menten	Wilhelm Kuhne	Fisher			**Arrhenius**
The first used the term enzyme is _____	Arrhenius	Michaelis-Menten	Wilhelm Kuhne	Fisher			**Wilhelm Kuhne**
EC stands for _____	Enzyme commission	Enzyme connection	Enzyme correction	Enzyme collection			**Enzyme commission**
Co enzymes are small organic molecules usually obtained from	Vitamins	Carbohydrates	lipid	proteins			**Vitamins**
Allosteric sites are of _____ types	one	two	three	four			**two**
Enzyme that function outside the cells are called_____	Exoenzymes	Endoenzymes	Ribozymes	Isoenzyme			**Exoenzymes**

Nuclease split nucleid acid into _____	DNA and RNA	Cell and tissue	larger molecule and smaller molecules	hormone and proteins			**DNA and RNA**
The word enzyme is derived from the Greek meaning in _____	Bacteria	Microbes	Yeast	Fungi			**Yeast**
Enzymes can be precipitated by _____	Ammonium Sulphate	Ammonium Oxalate	Ammonium Chloride	Ammonium oxide			**Ammonium Sulphate**
_____ is the inorganic chemical component that is required for enzyme activity	Coenzyme	Protein	Aminoacids	Cofactor			**Cofactor**
In _____ the international union of Biochemistrty gave the classification and naming system of enzymes on the basis of overall reaction catalysed.	1923	1961	1941	1963			**1961**
IUPAC is _____	International Unit of Pure and Applied Chemistry	International Union of Pure and Applied Chemistry	Indian Unit of Pure and Applied Chemistry	Indian Union of Pure and Applied Chemistry			**International Union of Pure and Applied Chemistry**
An example for a animal enzyme is _____	Rennet	α-amylase	Pullulanase	Raffinase			**Rennet**
An example for a plant enzyme is _____	Rennet	Lipoxygenase	Lipase	Pullulanase			**Lipoxygenase**
An example for a yeast enzyme is _____	Rennet	Lipase	Lactase	Raffinase			**Lipase**

UNIT-V STUDY MATERIALS

Nucleic acids:

Nucleic Acids-Purines &Pyrimidines nucleotides, RNA, & DNA base pairing schemes, types of RNA: mRNA, rRNA, tRNA, Secondary structure of DNA, Watson and Crick model.

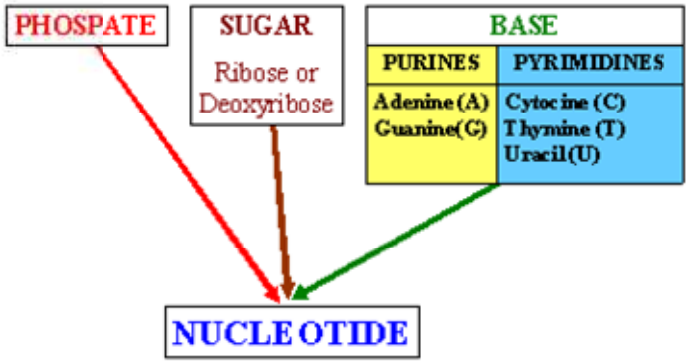
NUCLEIC ACIDS:

Nucleic acids consist of nucleotides that have a sugar, nitrogen base, and phosphate. Two types of nucleic acid are found.

- Deoxyribonucleic acid (DNA)
- Ribonucleic acid (RNA)

Nucleic Acid Structure

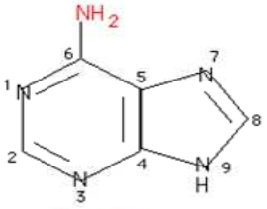
- Polymers of four nucleotides
- Linked by alternating sugar-phosphate bonds
- RNA: ribose and A, G, C, U
- DNA: deoxyribose and A,G,C,T
- Nucleic acids are polynucleotides
- Their building blocks are nucleotides

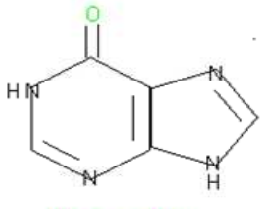


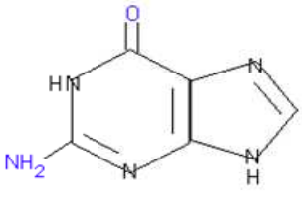
STRUCTURE OF PURINES AND PYRIMIDINES:

PURINES:

- A purine is a heterocyclic aromatic organic compound, consisting of a pyrimidine ring fused to an imidazole ring.
- Adenine = 6-amino purine
- Guanine = 2-amino-6-oxy purine
- Hypoxanthine = 6-oxy purine
- Xanthine = 2,6-dioxy purine



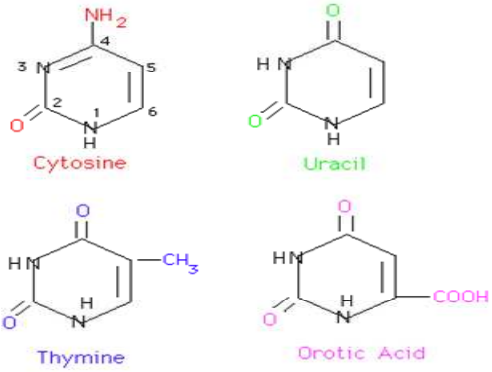




- Adenine and guanine are found in both DNA and RNA.
- Hypoxanthine and xanthine are not incorporated into the nucleic acids as they are being synthesized but are important intermediates in the synthesis and degradation of the purine nucleotides.

PYRIMIDINES:

- Uracil = 2,4-dioxy pyrimidine
- Thymine = 2,4-dioxy-5-methyl pyrimidine
- Cytosine = 2-oxy-4-amino pyrimidine
- Orotic acid = 2,4-dioxy-6-carboxy pyrimidine

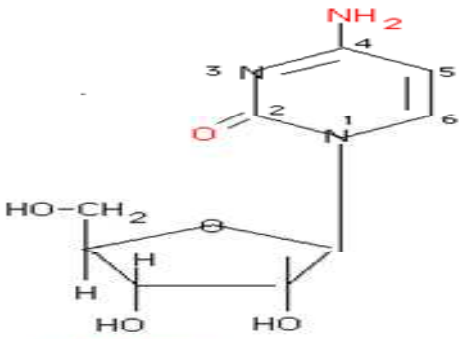


- Cytosine is found in both DNA and RNA.
- Uracil is found only in RNA.
- Thymine is normally found in DNA.
- Sometimes tRNA will contain some thymine as well as uracil.

NUCLEOSIDES:

- If a sugar, either ribose or 2-deoxyribose, is added to a nitrogen base, the resulting compound is called a nucleoside.
- Carbon 1 of the sugar is attached to nitrogen 9 of a purine base or to nitrogen 1 of a pyrimidine base.

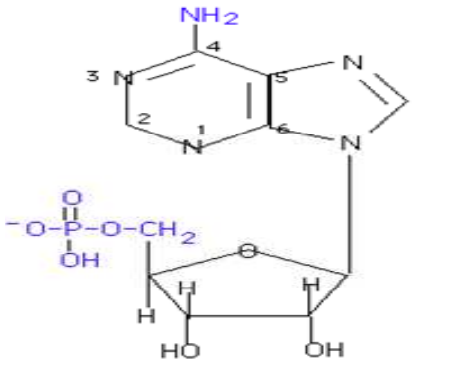
- The names of purine nucleosides end in -osine and the names of pyrimidine nucleosides end in -idine.
- The convention is to number the ring atoms of the base normally and to use l', etc. to distinguish the ring atoms of the sugar.
- Unless otherwise specificed, the sugar is assumed to be ribose.
- To indicate that the sugar is 2'-deoxyribose, a d- is placed before the name.
  - Adenosine
  - Guanosine
  - Inosine - the base in inosine is hypoxanthine
  - Uridine
  - Thymidine
  - Cytidine



Cytidine

NUCLEOTIDES:

- Adding one or more phosphates to the sugar portion of a nucleoside results in a nucleotide.
- Generally, the phosphate is in ester linkage to carbon 5' of the sugar.
- If more than one phosphate is present, they are generally in acid anhydride linkages to each other.
- If such is the case, no position designation in the name is required.
- If the phosphate is in any other position, however, the position must be designated.
- For example, 3'-5' cAMP indicates that a phosphate is in ester linkage to both the 3' and 5' hydroxyl groups of an adenosine molecule and forms a cyclic structure.
- 2'-GMP would indicate that a phosphate is in ester linkage to the 2' hydroxyl group of a guanosine. Some representative names are:
  - AMP = adenosine monophosphate = adenylic acid
  - CDP = cytidine diphosphate
  - dGTP = deoxy guanosine triphosphate
  - dTTP = deoxy thymidine triphosphate (more commonly designated TTP)
  - cAMP = 3'-5' cyclic adenosine monophosphate



Adenosine Monophosphate
AMP

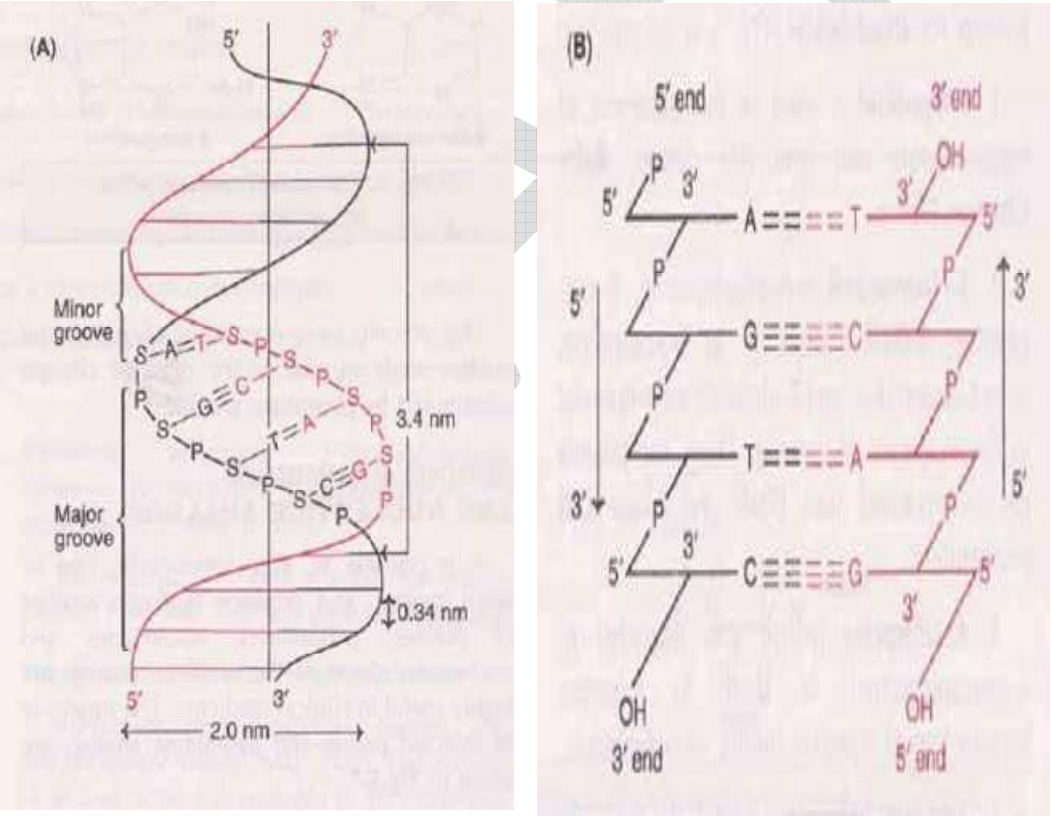
DNA:

- DNA is a polymer of deoxyribonucleotides (or simply deoxynucleotides).

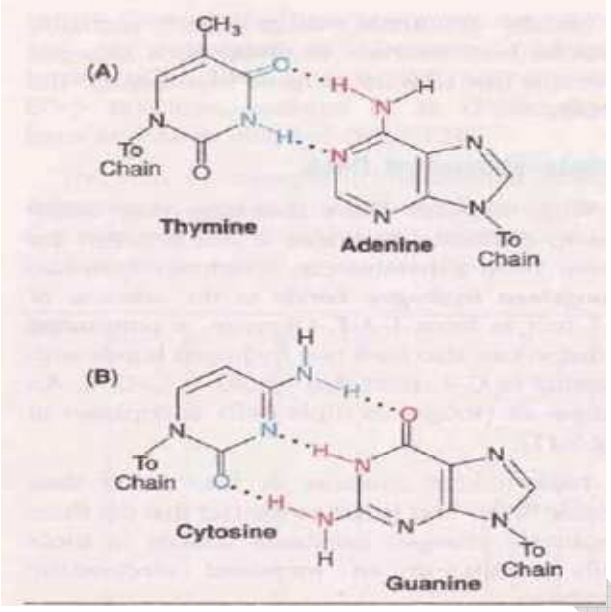
- It is composed of monomeric units namely deoxyadenylate (dAMP), eoxyguanylate (dGMP), deoxycytidylate(dCMP) and deoxythymidylate(d TMP) (It may be noted here that some authors prefer to use TMP for deoxythymidylate, since it is found only in DNA).
- The details of the nucleotide structure are given above.

DNA DOUBLE HELIX:

- The double helical structure of DNA was proposed by lames Watson and Francis Crick in 1953 (Nobel Prize, 1962).
- The elucidation of DNA structure is considered as a milestone in the era of modern biology.
- The structure of DNA double helix is comparable to a twisted ladder.
- The salient features of Watson - Crick Model of DNA (now known as B-DNA) are described next.



(A) Watson-Crick model of DNA helix (B) Complementary base pairing in DNA helix.



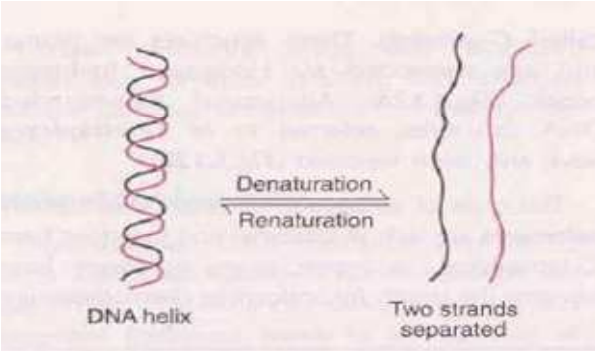
Complementary base paring in DNA
(A) Thymine pairs with adenine by 2 hydrogen bonds
(B) Cytosine pairs with guanine by 3 hydrogen bonds.

- The DNA is a right handed double helix.
- It consists of two polydeoxyribonucleotide chains (strands) twisted around each other on a common axis.
- The two strands are antiparallel, i.e., one strand runs in the 5' to 3' direction while the other in 3'to 5'direction.T his is comparable to two parallel adjacent roads carrying traffic in opposite direction.
- The width (or diameter) of a double helix is 20 A^o (2 nm).
- Each turn (pitch) of the helix is 34 A" (3.4 nm) with 10 pairs of nucleotides each pair placed at a distance of about 3.4 A^o.
- Each strand of DNA has a hydrophilic deoxyribose phosphate backbone (3'-5' phosphor diester bonds)o n the outside (periphery) of the molecule while the hydrophobic bases are stacked inside (core).
- The two polynucleotide chains are not identical but complementary to each other due to base pairing.

- The two strands are held together by hydrogen bonds formed by complementary base pairs.
- The A-T pair has 2 hydrogen bonds while G-C pair has 3 hydrogen bonds. The G = C is stronger by about 50% than A=T.
- The hydrogen bonds are formed between a purine and a pyrimidine only.
- If two purines face each other, they would not fit into the allowable space. And two pyrimidines would be too far to form hydrogen bonds.
- The only base arrangement possible in DNA structure, from spatial considerations is A-T, T-A, G-C and C-C.
- The complementary base pairing in DNA helix proves Chargaffs rule.
- The content of adenine equals to that of thymine (A = T) and guanine equals to that of cytosine (G = C). 10.
- The genetic information resides on one of the two strands known as template strand or sense strand.
- The opposite strand is antisense strand.
- The double helix has (wide) major grooves and (narrow) minor grooves along the phosphodiester backbone.
- Proteins interact with DNA at these grooves, without disrupting the base pairs and double helix.

DENATURATION OF DNA STRANDS:

- The two strands of DNA helix are held together by hydrogen bonds.
- Disruption of hydrogen bonds (by change in pH or increase in temperature) results in the separation of polynucleotide strands.
- This phenomenon of loss of helical structure of DNA is known as denaturation.
- The phosphodiester bonds are not broken by denaturation.
- Loss of helical structure can be measured by increase in absorbance at 260 nm (in a spectrophotometer).



denaturation and renaturation of DNA.

- Melting temperature (Tm) is defined as the temperature at which half of the helical structure of DNA is lost.
- Since C-C base pairs are more stable (due to 3 hydrogen bonds) than A-T base pairs(2 hydrogen bonds), the Tm is greater for DNAs with higher C-C content.
- Thus, the Tm is 65°C for 35% G-C content while it is 70°C for 50% G-C content.
- Formamide destabilizes hydrogen bonds of base pairs and, therefore, lowers Tm.
- This chemical compound is effectively used in recombinant DNA experiments.

RENATURATION:

- Renaturation or reannealing is the process in which the separated complementary DNA strands can form a double helix.

RNA:

- **Ribonucleic acid** or **RNA**, is one of the three major macromolecules (along with DNA and proteins) essential for all known forms of life.

TYPES OF RNA:

- The three major types of RNAs with their respective cellular composition are given below
  1. Messenger RNA (mRNA): 5-1O"/"
  2. Transfer RNA (tRNA): 10-200/"
  3. Ribosomal RNA (rRNA): 50-80%

Messenger RNA (mRNA)

- The mRNA is synthesized in the nucleus (in eukaryotes) as heterogeneous nuclear RNA (hnRNA).
- hnRNA, on processing, liberates the functional mRNA which enters the cytoplasm to participate in protein synthesis.
- mRNA has high molecular weight with a short half-life.
- The eukaryotic mRNA is capped at the S'-terminal end by 7- methylguanosine triphosphate.
- It is believed that this cap helps to prevent the hydrolysis of mRNA by 5'-exonucleases.
- Further, the cap may be also involved in the recognition of mRNA for protein synthesis.
- The 3'-terminal end of mRNA contains a polymer of adenylate residues (20-250 nucleotides) which is known as poly (A) tail.
- This tail may provide stability to mRNA, besides preventing it from the attack of 3'-exonucleases.mRNA molecules often contain certain modified bases such as 6-methyladenylatesin the internal structure.

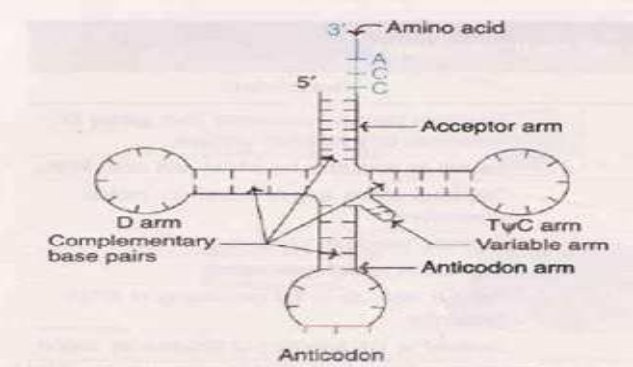
Transfer RNA (tRNA)

- Transfer RNA (soluble RNA) molecule contains 71-80 nucleotides (mostly 75) with a molecular weight of about 25,000.
- There are at least2 0 species of tRNAs, corresponding to 20 amino acids present in protein structure.
- The structure of tRNA (for alanine) was first elucidated by Holley.
- The structure of IRNA, depicted in resembles that of a clover leaf tRNA contains mainly four arms, each arm with a base paired stem.

  1. The acceptor arm: This arm is capped with a sequence C CA (5'to 3'). The amino acid is attached to the acceptor arm.

  2. The anticodon arm: This arm, with the three specific nucleotide bases (anticodon), is

responsible for the recognition of triplet codon of mRNA. The codon and anticodon are complementary to each other.



Structure of transfer RNA.

3. The D arm : It is so named due to the presence of dihydrouridine.

4. The TΨC arm : This arm contains a sequence of T, pseudouridine (represented by Psi, Ψ) and C.

5. The variable arm : This arm is the most variable in tRNA. Based on this variability, tRNAs are classified into 2 categories :

(a) Class I tRNAs : The most predominant (about 75"/") form with 3-5 base pairs length"

(b) Class ll tRNAs : They contain 13-20 base pair long arm.

- Base pairs in tRNA : The structure of tRNA is maintained due to the complementary base pairing in the arms.
- The four arms with their respective base pairs are given below
  - The acceptor arm - 7 bp
  - The TΨC arm - 5 bp
  - The anticodon arm - 5 bp
  - The Darm -4bp

Ribosomal RNA (rRNA)

- The ribosomes are the factories of protein synthesis.

- The eukaryotic ribosomes are composed of two major nucleoprotein complexes-60S subunit and 40S subunit.
- The 605 subunit contains 28S rRNA, 55 rRNA and 5.8S rRNA while the 40S subunit contains 18S rRNA.
- The function of rRNAs in ribosomes is not clearly known.
- It is believed that they play a significant role in the binding of mRNA to ribosomes and protein synthesis.

STRUCTURE:

- RNA is a polymer of ribonucleotides held together by 3',5'-phosphodiester bridges.
- Although RNA has certain similarities with DNA structure, they have specific differences.
- **Pentose:** The sugar in RNA is ribose in contrast to deoxyribose in DNA.
- **Pyrimidine :** RNA contains the pyrimidines uracil in place of thymine (in DNA).
- **Single strand:** RNA is usually a singlestranded polynucleotide. However, this strand may fold at certain places to give a doublestranded structure, if complementary base pairs are in close proximity.
- **Chargaff's rule-not obeyed:** Due to the single-stranded nature, there is no specific relation between purine and pyrimidines contents. Thus the guanine content is not equal to cytosine (as is the case in DNA).
- **Susceptibility to alkali hydrolysis:** Alkali can hydrolyse RNA to 2',3'-cyclic diesters. This is possible due to the presence of a hydroxyl group at 2' position. DNA cannot be subjected to alkali hydrolysis due to lack of this group.
- **Orcinol colour reaction :** RNAs can be histologically identified by orcinol colour reaction due to the presence of ribose.

FUNCTIONS OF RNA

Table 5.3 Cellular RNAs and their function(s)

Type of RNA	*Abbreviation*	*Function(s)*
Messenger RNA	mRNA	Transfers genetic information from genes to ribosomes to synthesize proteins.
Heterogeneous nuclear RNA	hnRNA	Serves as precursor for mRNA and other RNAs.
Transfer RNA	tRNA	Transfers amino acid to mRNA for protein biosynthesis.
Ribosomal RNA	rRNA	Provides structural framework for ribosomes.
Small nuclear RNA	snRNA	Involved in mRNA processing.
Small nucleolar RNA	snoRNA	Plays a key role in the processing of rRNA molecules.
Small cytoplasmic RNA	scRNA	Involved in the selection of proteins for export.
Transfer–messenger RNA	tmRNA	Mostly present in bacteria. Adds short peptide tags to proteins to facilitate the degradation of incorrectly synthesized proteins.

sn RNA

A small nuclear RNA (snRNA) is one of many small RNA species confined to the nucleus; several of the snRNAs are involved in splicing or other RNA processing reactions. Small cytoplasmic RNAs(scRNA) are present in the cytoplasm and (sometimes are also found in the nucleus).

mall nuclear ribonucleic acid (snRNA), also commonly referred to as U-RNA, is a class of small RNA molecules that are found within the splicing speckles and Cajal bodies of the cell nucleus in eukaryotic cells. The length of an average snRNA is approximately 150 nucleotides. They are transcribed by either RNA polymerase II or RNA polymerase III. Their primary function is in the processing of pre-messenger RNA (hnRNA) in the nucleus. They have also been shown to aid in the regulation of transcription factors (7SK RNA) or RNA polymerase II (B2 RNA), and maintaining the telomeres.

snRNA are always associated with a set of specific proteins, and the complexes are referred to as small nuclear ribonucleoproteins (snRNP, often pronounced "snurps"). Each snRNP particle is composed of a snRNA component and several snRNP-specific proteins (including Sm proteins, a family of nuclear proteins). The most common snRNA components of these complexes are known, respectively, as: U1 spliceosomal RNA, U2 spliceosomal RNA, U4 spliceosomal RNA, U5 spliceosomal RNA, and U6 spliceosomal RNA. Their nomenclature derives from their high uridine content.

snRNAs were discovered by accident during a gel electrophoresis experiment in 1966. An unexpected type of RNA was found in the gel and investigated. Later analysis has shown that these RNA were high in uridylate and were established in the nucleus.

A large group of snRNAs are known as small nucleolar RNAs (snoRNAs). These are small RNA molecules that play an essential role in RNA biogenesis and guide chemical modifications of ribosomal RNAs (rRNAs) and other RNA genes (tRNA and snRNAs). They are located in the nucleolus and the Cajal bodies of eukaryotic cells (the major sites of RNA synthesis), where they are called scaRNAs (small Cajal body-specific RNAs).

siRNA

Small interfering RNA (siRNA), sometimes known as short interfering RNA or silencing RNA, is a class of double-stranded RNAmolecules, 20-25 base pairs in length, similar to miRNA, and operating within the RNA interference (RNAi) pathway. It interferes with the expression of specific genes with complementary nucleotide sequences by degrading mRNA after transcription, preventing translation.

siRNA can also act in RNAi-related pathways as an antiviral mechanism or play a role in the shaping of the chromatin structure of a genome. siRNAs and their role in post-transcriptional gene silencing (PTGS) were first discovered in plants by David Baulcombe's group at the Sainsbury Laboratory in Norwich, England and reported in *Science* in 1999.[2] Thomas Tuschl and colleagues soon reported in *Nature* that synthetic siRNAs could induce RNAi in mammalian cells. This discovery led to a surge in interest in harnessing RNAi for biomedical research and drug development. Significant developments in siRNA therapies have been made with both organic (carbon based) and inorganic (non-carbon based) nanoparticles, such as these which have been successful in drug delivery to the brain, offering promising methods of delivery into human subjects. However, significant barriers to successful siRNA therapies remain, the most significant of which is off-targeting.

hn RNA

Unlike prokaryotic mRNA, eukaryotic mRNAs are monocistronic. The primary transcript in eukaryotes is much larger than the mature mRNA and is called Heterogeneous nuclear RNA (hnRNA). It contains unique sequences and has about 10 times as many sequences as the mature mRNA. hnRNA undergoes processing and finally the mRNA is produced and therefore, it is called "mRNA precursor" or "pre-mRNA".

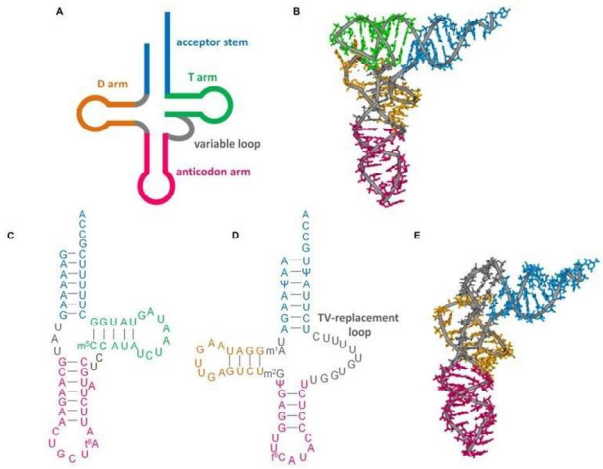
Biological functions

One of these active processes is protein synthesis, a universal function where RNA molecules direct the assembly of proteins on ribosomes. This process uses transfer RNA (tRNA) molecules to deliver amino acids to the ribosome, where ribosomal RNA (rRNA) then links amino acids together to form proteins.

Secondary and tertiary structure of tRNA

A transfer RNA (abbreviated tRNA and formerly referred to as sRNA, for soluble RNA[1]) is an adaptor molecule composed of RNA, typically 76 to 90 nucleotides in length,[2] that serves as the physical link between the mRNA and the amino acid sequence of proteins. tRNA does this by carrying an amino acid to the protein synthetic machinery of a cell (ribosome) as directed by a three-nucleotide sequence (codon) in a messenger RNA (mRNA). As such, tRNAs are a necessary

component of translation, the biological synthesis of new proteins in accordance with the genetic code.



Secondary and tertiary structure of rRNA

Ribosomal ribonucleic acid (rRNA) is the RNA component of the ribosome, and is essential for protein synthesis in all living organisms. It constitutes the predominant material within the ribosome, which is approximately 60% rRNA and 40% protein by weight, or 3/5 of ribosome mass. Ribosomes contain two major rRNAs and 50 or more proteins. The ribosomal RNAs form two subunits, the large subunit (LSU) and small subunit (SSU). The LSU rRNA acts as a ribozyme, catalyzing peptide bond formation. rRNA sequences are widely used for working out evolutionary relationships among organisms, since they are of ancient origin and are found in all known forms of life.

Structure

The ribosomal RNAs complex with proteins to form two subunits, the large subunit (LSU) and small subunit (SSU). During translation, mRNA is sandwiched between the small and large subunits, and the ribosome catalyzes the formation of a peptide bond between the two amino acids that are contained in the rRNA.

A ribosome also has three binding sites called A, P, and E.

- The A site in the ribosome binds to an aminoacyl-tRNA (a tRNA bound to an amino acid).
- The amino (NH_2) group of the aminoacyl-tRNA, which contains the new amino acid, attacks the ester linkage of peptidyl-tRNA (contained within the P site), which contains the last amino acid of the growing chain, forming a new peptide bond. This reaction is catalyzed by peptidyl transferase.
- The tRNA that was holding onto the last amino acid is moved to the E site, and what used to be the aminoacyl-tRNA is the peptidyl-tRNA.

A single mRNA can be translated simultaneously by multiple ribosomes.

POSSIBLE QUESTIONS

PART-B (2 MARKS)

1. Draw the structure of purine?
2. Draw the structure of purine and pyrimidine?
3. Differentiate between the purines and pyrimidines?
4. List out the different types of RNA?
5. List out the different types of DNA?
6. Differentiate between the Nucleotides and nucleosides?
7. Write the short notes on:
   1) A forms of DNA
   2) Z forms of DNA
8. write the notes on :
   i. mRNA ii. snRNA

Detailed questions:

9. Describe the DNA base pairing scheme?
10. Describe the RNA base pairing scheme?
11. Explain in detail about the Watson and Crick model?
12. Write in detail about the different types of RNA?
13. Elaborate on the physical and chemical properties of nucleic acid?
14. Give a detail account on secondary structure of tRNA?
15. Explain in detail about siRNA and hnRNA
16. Explain chemical synthesis of DNA?
17. Describe the structure and biological functions of hn RNA
18. Explain DNA supercoiling ?
19. Give on detail account of secondary and tertiary structure of rRNA?

Questions	Option 1	Option 2	Option 3	Option 4			Answer
A nucleoside consists of	Nitrogenous base	Purine or pyrimidine base + sugar	Purine or pyrimidine base + phosphorous	Purine + pyrimidine base + sugar + Phosphorous			**Purine or pyrimidine base + sugar**
RNA does not contain_____	Uracil	Adenine	Thymine	Ribose			**Thymine**
The pyrimidine base of the DNA is	cytosine	guanine	uracil	adenine			**cytosine**
Adenine is	6-Amino purine	2-Amino-6-oxypurine	2-Oxy-4-aminopyrimidine	2, 4-Dioxypyrimidine			**6-Amino purine**
The width (helical diameter) of the double helix in B-form DNA in nm is_____	one	two	three	four			**two**
Nucleic acid show strong absorption at the wavelength of _____	80nm	260nm	340nm	420nm			**420nm**
The sugar found in DNA is	Ribose	Ribulose	Erythrose	Deoxyribose			**Deoxyribose**
The most abundant free nucleotide in mammalian cells is	ATP	UTP	UDPG	CMP			**ATP**
A DNA molecule contains 20% Adenine. What is the amount of thyamine in it?	20%	30%	40%	50%			**20%**
A purine nucleotide is	AMP	UMP	CMP	TMP			**AMP**
A pyrimidine nucleotide is	GMP	AMP	CMP	IMP			CMP
The chemical name of guanine is	2,4-Dioxy 5-methylpyrimidine	2-Amino-6-oxypurine	2-Oxy-4-aminopyrimidine	2, 4-Dioxypyrimidine			**2-Amino-6-oxypurine**
The first true pyrimidine ribonucleotide synthesized is	UMP	UDP	TMP	CTP			**UMP**
De novo synthesis of purine nucleotide occurs in	Mitochondria	Cytosol	Microsmes	Ribosomes			**Cytosol**
The enzyme common to catabolism of all the purines is	Adenosine deaminase	Purine nucleosidase phosphorylase	Guanase	Aspartase			**Purine nucleoside phosphorylase**
Dietary purines are catabolised in	Liver	Kidneys	Intesitnal mucosa	brain			**Intesitnal mucosa**
In humans purine are catabolised to uric acid due to lack of the	Urease	Uricase	Xanthine oxidase	Guanase			**Uricase**

The first true pyrimidine ribonucleotide synthesized is	UMP	UDP	TMP	CTP			**UMP**
Purine biosynthesis is inhibited by	Aminopterin	Tetracyclin	Methotrexate	Chloramphenicol			**Aminopterin**
DNA does not contain	Thymine	Adenine	Uracil	Deoxyribose			**Uracil**
Uracil and ribose form	Uridine	Cytidine	Guanosine	Adenosine			**Uridine**
The pyrimidine nucleotide acting as the high energy intermediate is	ATP	UTP	UDPG	CMP			**UDPG**
End product of purine metabolism is	urea	uric acid	sulphuric acid	glucose			**uric acid**
Ribonucleases cleave	Ribosomes	RNA	DNA	Protein			**RNA**
Deoxyribonuclease cleave	Ribosomes	RNA	DNA	Protein			**DNA**
The unit of genetic information is the_____ or cistron.	Genom	Codon	Gene	Anticodon			**Gene**
Each transfer RNA molecule contains the number of nucleotides _____	J.B.Sumner	Koshland	Menten	Fisher			**J.B.Sumner**
DNA is refered as _____	Transforming factor	Range constants	Transplantation factor	Heterogenous factor			**Transforming factor**
Messenger RNA has a molecuar weight of_____	15000 to 30000	20000 to 35000	25000 to 40000	30000 to 50000			**30000 to 50000**
DNA is denatured by _____	Acid	Alkali	Heat	All the above			**All the above**
Double helical structure model of the DNA was proposed by	Pauling and Corey	Peter Mitchell	Watson and Crick	King and Wooten			**Watson and Crick**
Uracil and ribose form	Uridine	Cytidine	Guanosine	Adenosine			**Uridine**
A pyrimidine nucleotide is	GMP	AMP	CMP	IMP			**CMP**
DNA rich in G-C pairs have	1 Hydrogen bond	2 Hydrogen bonds	3 Hydrogen bonds	4 Hydrogen bonds			**3 Hydrogen bonds**
Left handed double helix is present in_____	Z-DNA	A-DNA	B-DNA	E-DNA			**Z-DNA**
The double standard DNA molecule loses its viscosity upon	Denaturation	Filteration	Sedimentation	Concentration			**Denaturation**
Each turn of DNA structure has a pitch in nm of	1.4	2.4	3.4	4.4			**3.4**
----------are formed							

The structure of tRNA (for alanine) was first elucidated by____	Holley	Peter Mitchell	Watson and Crick	King and Wooten	**Holley**
RNAs can be histologically identified by orcinol colour reaction due to the presence of_____	ribose	mannose	fructose	galactose	**ribose**
Loss of helical structure of DNA is known as _____.	coagulation	precipitation	denaturation	sedimentation	**denaturation**
The salient features of Watson - Crick Model of DNA now known as _____	B-DNA	A-DNA	Z-DNA	E-DNA	**B-DNA**