KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)
(Established Under Section 3 of UGC Act 1956)
Coimbatore - 641021.
(For the candidates admitted from 2018 onwards)

DEPARTMENT OF BIOCHEMISTRY

SUBJECT : C**HEMISTRY OF BIOPOLYMERS**
SEMESTER : **I**
SUBJECT CODE: **18BCP101** **CLASS**: **I M.Sc., BIOCHEMISTRY**

Course objectives

- To know the structure and role of water in biological system
- To understand the structure and organization of carbohydrate , lipids , proteins and nucleic acids
- To realize the interactions nucleic acid with proteins

Course outcome

- Able to understand the importance of water in biological system
- Understand the structure and organization of storage and structural polysaccharides, basics behind the four level organization of proteins
- Explain the role of lipids in membrane and their associated function as signal molecule
- Structure and organization of DNA, RNA and their properties
- Exploit the interaction of nucleic acid with proteins and their consequences

UNIT I

Polysaccharides: Brief review of carbohydrates, classification. Occurrence, structure and biological functions of cellulose, chitin, starch and glycogen. Fructans, arabinans and galactans (brief account). Dietary fibre. Occurrence, structure, and biological functions of bacterial cell wall polysaccharides and blood group antigens. Structure and significance of glycoconjucates -Glycosaminoglycans – structure and biological role of hyaluronic acid, chondroitin sulfate and heparin, sialic acid; glycoproteins and glycolipids.

UNIT II

Proteins: Review of structure and classification of aminoacids. Orders of protein structure. Primary structure – determination of amino acid sequence of proteins. The peptide bond – The Ramachandran plot. Secondary structures – α-helix, β-sheet and β-turns. Fibrous proteins- Collagen triple helix-Structure and assembly. Globular proteins-forces involved, folding process and folding patterns. Tertiary structure – Myoglobin organisation. Quarternary structure of proteins-Structure of haemoglobin. Models for haemoglobin allostery. Quintinary structure-basics only. Protein function as enzymes, defensive and transport.

UNIT III

Lipids: Introduction, classification, structure and functions of simple lipid, compound lipids-phospholipids, glycolipids, storage lipids and choesterol. Eicosanoids- porstaglandins, thromboxanes and leucotriens. Properties of lipids-Mice lles, bilayers and liposomes. Significance of lipid anchored protein-prenylated, fatty acylated and GPI anchored proteins. Lipoproteins – classification, composition and biological functions. Lipids as signals, cofactors and pigments (Brief account). Lipid peroxidation and antioxidants.

UNIT IV

Nucleic acids: DNA double helical structure – Watson and Crick model. A, B and Z forms of DNA. Tertiary and quadraplex structures of DNA. DNA supercoiling and linking number. Properties of DNA – DNA bending, buoyant density, viscosity, denaturation and renaturation – The cot curve – Chemical synthesis of DNA.
Major classes of RNA – mRNA, rRNA, tRNA, sn RNA, siRNA, hn RNA – structure and biological functions. Secondary and tertiary structure of tRNA and rRNA.

UNIT V

Nucleic acid interaction with proteins: DNA binding motifs in proteins – the basic
helix loop helix (bHLH) motif, zinc finger, the leucine zipper, helix-loop helix and homeo domain. RNA binding motifs in proteins. Molecular aspects of protein-nucleic acid binding – direct interactions. Techniques characterizing nucleic acid-protein complex – chromatin immunoprecipitation assay, DNase I footprinting.

REFERENCES

Nelson, D., and Cox, M. W.H. (2012) Lehninger Principles of Biochemistry (4th Ed.) New York, Freeman and Company.

Murray, R.K., Bender, D.A., Botham, K.M., and Kennelly, P.J., (2012). Harper's illustrated Biochemistry, 29th Edition. McGraw-Hill Medical. London.

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Nucleic acid structure and recognition. Neidle, Oxford University Press, 2002

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Rawn, .J.D.,(2004). Biochemistry, First Indian reprint, Panima Publishing Corporation, New Delhi.

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DEPARTMENT OF BIOCHEMISTRY

LESSON PLAN

STAFF NAME: Dr. Rajesh Pandiyan

SUBJECT NAME: Chemistry of Biopolymers

SUB.CODE: 18BCP101

SEMESTER: I **CLASS: I M.Sc., BIOCHEMISTRY**

S. No.	Duration	Topics to be covered	Books and webs referred with Page No.
1	1	Brief review of carbohydrates, classification	R1: 40-52
2	1	Occurrence, structure and biological functions o cellulose, chitin, starch and glycogen	R1: 44-52
3	1	Fructans, arabinans, and Glactans (Brief account)	R1: 247-254
4	1	Dietary fibre, occurrence, structure, and biological functions of bacterial cell wall polysaccharides	R1: 252 R1: 775-778
5	1	Blood group antigens	R1: 353
6	1	Structure and significance of glycoconjucates-Glycosaminoglycans	R1: 353-354
7	1	Structure and biochemical role of Hyaluronic acid, chondroitin sulfate and heparin, sialic acid	R1: 255-256 R1: 253-254
8	1	Glycoproteins and glycolipids	R1:88 R1: 258-260
9	1	Revision and possible QP discussion	
Total No. of Hours planned for Unit I: 9 hours			

1	2	Review of structure and classification of amino acids	R1: 88
2	2	Orders of protein structure	R1: 88
3	2	Primary structure – determination of amino acid sequence of proteins	R1: 85-86 R1: 120
4	2	The peptide bond – The Ramachandran plot. Secondary structures – α-helix, β-sheet and β- turns	R1: 125-129
5	2	Fibrous proteins- Collagen triple helix-Structure and assembly	R1: 125-141
6	2	Globular proteins-forces involved, folding process and folding patterns	R1: 132-134
7	2	Tertiary structure –Myoglobin organisation	R1: 88, 125 R1: 140-144
8	2	Quarternary structure of proteins- Structure of haemoglobin. Models for haemoglobin allostery	R1:170, 171 R1: 173
9	2	Quintinary structure-basics only. Protein function as enzymes, defensive and transport	R1: 76 R1: 157-186
Total No. of Hours planned for Unit II: 9 hours			
1	3	Introduction, classification, structure and functions of simple lipid, compound lipids-phospholipids	R1: 343-348
2	3	Structure and functions of storage lipids and cholesterol	R1: 56, 290
3	3	Eicosanoids-prostaglandins, thrombaoxanes and leucotriens	R2: 56
4	3	Properties of lipids-Micelles, bilayers and liposomes	R1: 348-355
5	3	Significance of lipid anchored protein prenylated fatty acylated and GPI anchored proteins	R3: 358-359
6	3	Lipoproteins - Classifications, composition and biological functions	R1: 821-823 R1: 378-379

7	3	Lipids as signals cofactor and pigments (Brief account)	R1: 580
8	3	Lipid peroxidation and anti-oxidants	W1
9	3	Revision and possible QP discussion	
Total No. of Hours planned for Unit III: 9 hours			
1	4	DNA double helical structure – Watson and Crick model	R1: 279-280
2	4	A, B and Z forms of DNA	R1: 273-278
3	4	Tertiary and quadraplex structures of DNA	R2: 89-94
4	4	DNA supercoiling and linking number	R1: 930-931
5	4	Properties of DNA – DNA bending, buoyant density, viscosity, denaturation, and renaturation- The cot curve – chemical synthesis of DNA	R1: 932-933 R1: 956, 599
6	4	Major classes of snRNA,	R1: 291-293 R1: 1008-1020
7	4	siRNA, hnRNA-structure and biological functions	R1: 97-100
8	4	Secondary and tertiary structure of tRNA and rRNA	R1: 30-35
9	4	Revision and possible QP discussion	
Total No. of Hours planned for Unit IV: 9 hours			
1	5	DNA binding motifs in proteins	R1: 1089-1090
2	5	The basic helix loop helix (bHLH) motif zinc finger	R1: 1090-1091
3	5	The leucine zipper, helix-loop helix and homeo domain	R2: 1091-1092
4	5	RNA binding motifs in proteins	R1: 998-1001

5	5	Molecular aspects of protein – nucleic acid binding – direct interactions	R1: 157-184
6	5	Techniques characterizing nucleic acid – protein complex	R1: 185-186
7	5	Chromatin immuno-precipitation assay	R1: 938
8	5	DNase I foot printing	R1: 952
9	5	Revision and possible QP discussion	
Total No. of Hours planned for Unit V: 9 hours			
1	1	Previous year ESE question paper discussion	
2	1	Objective questions discussion	
3	1	Revision	
Total hours planned: 45 + 3 : 48			

REFERENCES

R1: Nelson, D., and Cox, M. W.H. (2012) Lehninger Principles of Biochemistry (4th Ed.) New York, Freeman and Company.

R2: Deb. C., Fundamentals of biochemistry, 9th edition, new central book agency Calcutta.

R3: Murray, R.K., Bender, D.A., Botham, K.M., and Kennelly, P.J., (2012). Harper's illustrated Biochemistry, 29th Edition. McGraw-Hill Medical. London.

W1: www.wikipida.org

UNIT-I

SYLLABUS

Polysaccharides: Brief review of carbohydrates, classification. Occurrence, structure and biological functions of cellulose, chitin, starch and glycogen. Fructans, arabinans and galactans (brief account). Dietary fibre. Occurrence, structure, and biological functions of bacterial cell wall polysaccharides and blood group antigens. Structure and significance of glycoconjucates -Glycosaminoglycans – structure and biological role of hyaluronic acid, chondroitin sulfate and heparin, sialic acid; glycoproteins and glycolipids.

CARBOHYDRATES

Introduction:

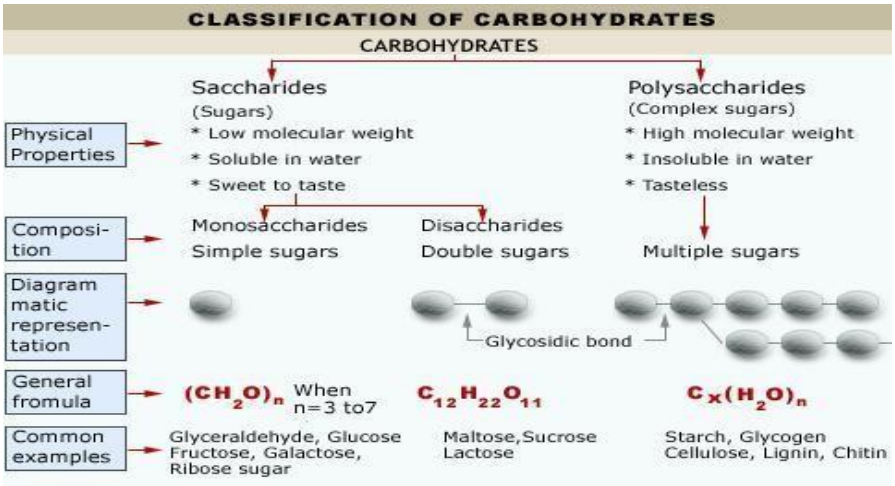
A carbohydrate is an organic compound with the empirical formula C_m (H_2O) $_n$; that is, consists only of carbon, hydrogen, and oxygen, with hydrogen: oxygen atom ratio of 2:1 (as in water).

Functions of Carbohydrates:

Carbohydrates participate in a wide range of functions

- ☐ They are the most abundant dietary source of energy (a Cal/S) for all organisms.
- ☐ Carbohydrates are precursors for many organic compounds (fats, amino acids).
- ☐ Carbohydrates (as glycoproteins and glycol-lipids) participate in the structure of cell membrane and cellular functions such as cell growth, adhesion and fertilization.
- ☐ They are structural components of many organisms. These include the fiber (cellulose) of plants, exoskeleton of some insects and the cell wall of microorganisms.
- ☐ Carbohydrates also serve as the storage form of energy (glycogen) to meet the immediate energy demands of the body.

CLASSIFICATION:



MONOSACCHARIDES:

- ☐ Monosaccharides (G reek: mono-one) are the simplest group of carbohydrates and are often referred to as simple sugars.
- ☐ They have the general formula $Cn(H_2O)_n$, and they cannot be further hydrolysed.
- ☐ The monosaccharides are divided into different categories, based on the functional group and the number of carbon atoms.

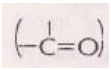
Monosaccharides (empirical formula)	*Aldose*	*Ketose*
Trioses ($C_3H_6O_3$)	Glyceraldehyde	Dihydroxyacetone
Tetroses ($C_4H_8O_4$)	Erythrose	Erythrulose
Pentoses ($C_5H_{10}O_5$)	Ribose	Ribulose
Hexoses ($C_6H_{12}O_6$)	Glucose	Fructose
Heptoses ($C_7H_{14}O_7$)	Glucoheptose	Sedoheptulose

Classification of monosaccharide with selected examples

Aldoses :

When the functional group in monosaccharides in aldehyde  they are known as aldoses e.g. glyceraldehyde, 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

STEREOCHEMISTRY:

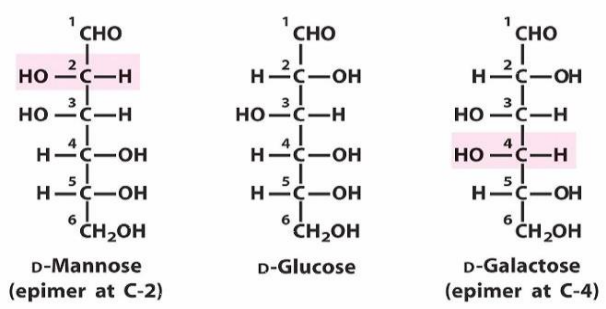
- ☐ Carbon 2 of glyceradehyde is a chiral center.
- ☐ There are thus 3 steroisomers of glyceraldehydes: D-glyceraldehyde and L-glyceraldehyde.
- ☐ By convention, sugars are written with the most oxidized carbon (i.e. aldehyde or ketone)
  - o at the top.

- 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 tp 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 substituents above and below are out of the plane directed away from the viewer.

Epimers

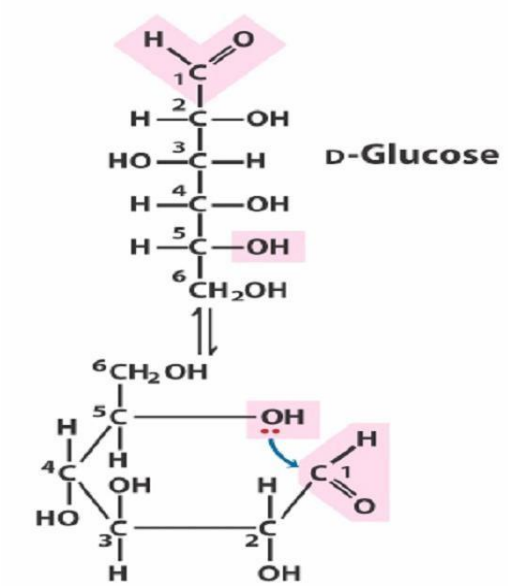
Carbohydrates that differ only in their stereochemistry at one position are called Epimers.

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



CYCLIC STRUCTURE AND ANOMERIC FORMS:

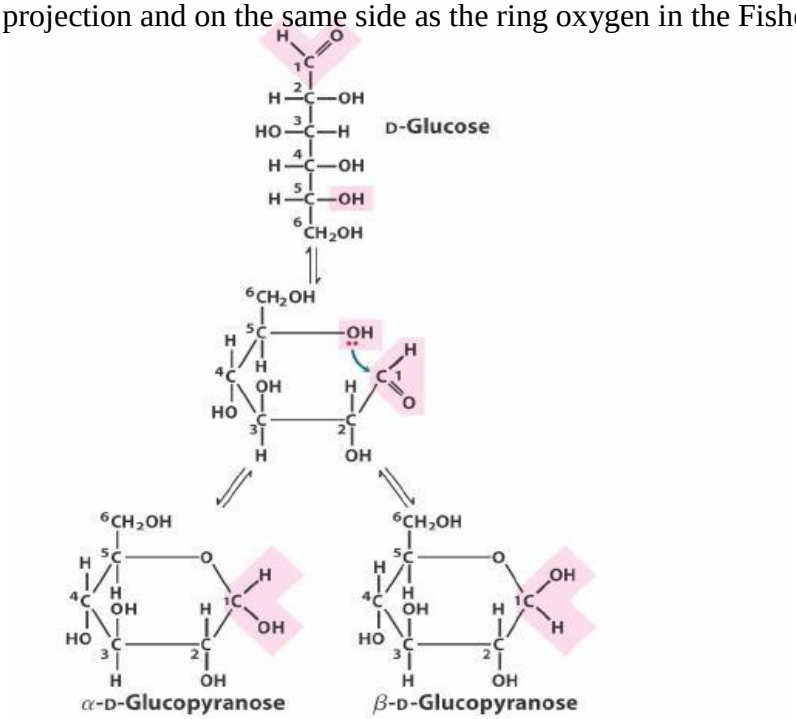
- ☐ In aqueous solution, D-glucose exists in one of 2 forms: α-D-glucose and ß-D- glucose.
- ☐ This is because Aldehydes can react with alcohols to form a hemiacetal.
- ☐ In this case, the hydroxyl oxygen attacking the molecule it is an interamolecular reaction, which results in formation of a ring.
- ☐ 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

- ☐ The different stereo isomers (α and ☐) are called Anomers.
- ☐ 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 α 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:

1. If the ring closes on a hydroxyl which points to the right (which it always does), then the hydroxymethyl (hydroxyalkyl) group point up. If the ring closes on a hydroxyl which points to the lift, then the hydroxymethyl (hydroxyalkyl) group points town.
2. The hydroxyls that point to the right in the Fischer projection, point down in the Haworth projection. The hydroxyls that point to the left in the Fischer projection point up in the Haworth projection.
3. For the D series: If the hydroxyl on the anomeric carbon points down, then it is α. It points up, and then the sugar is ß.

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, Trehalose.

MALTOSE:

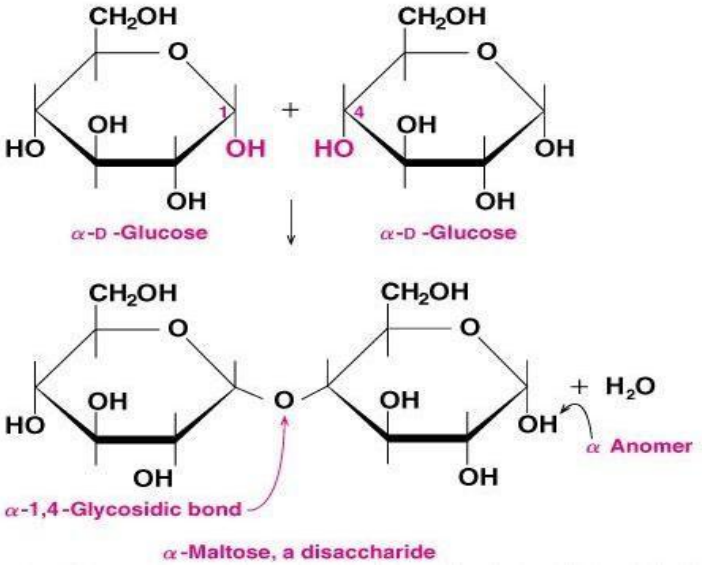
☐ Maltose (malt sugar or corn sugar) consists of two glucose molecules linked by an ☐-1, 4- glycosidic bond.

☐ It comes from partial hydrolysis of starch by the enzyme amylase, which is in saliva and also in grains (like barley).

☐ Maltose can be fermented by yeast to produce ethanol.

☐ Maltose is also used in cereals, candies and malted milk.

☐ Because one of the glucose molecules is a hemiacetal, it can undergo mutorotation, and so maltose is a reducing sugar.



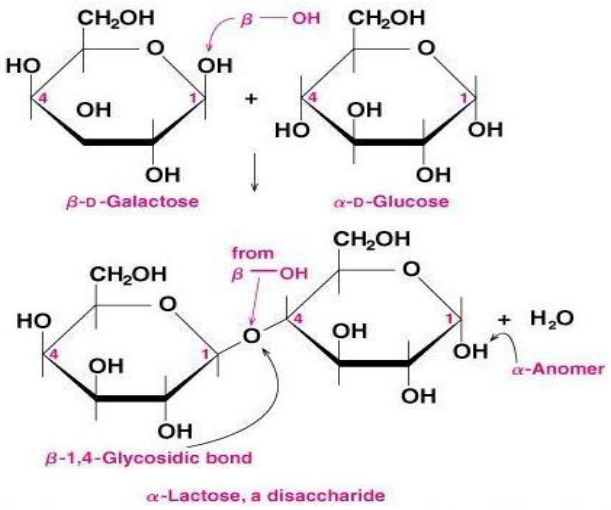
SUCROSE:

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

- Sucrose is the most abundant disaccharide and is commercially produced from sugar cane and sugar beets.
- Because the glycosidic bond in sucrose involves both anomeric carbons, neither monosaccharide can undergo mutorotation, and so sucrose is not a reducing sugar.

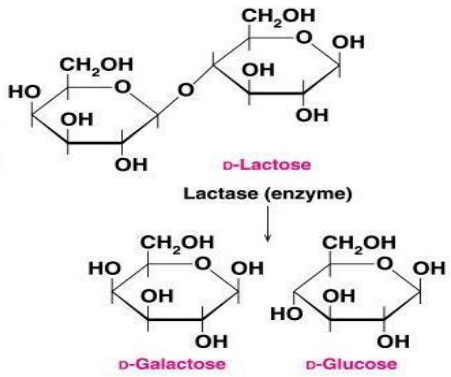
LACTOSE:

- **Lactose** (milk sugar) consists of one glucose molecule and one galactose molecule linked by alpha-1,4 glycosidic bond.
- It comes from milk products (about 4-5% of cow's milk).
- Because the glucose is a hemiacetal, it can undergo mutorotation, and so lactose is a reducing sugar.



Hydrolysis of Lactose:

- 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.



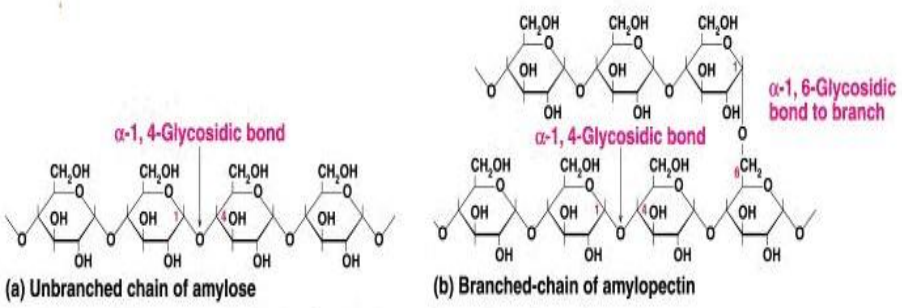
OLIGOSACCHARIDES:

- ☐ Oligosaccharides (Greek: oligo-few) contain 2-10 monosaccharide molecules which are liberated on hydrolysis.
- ☐ Based on the number of monosaccharide units present, the oligosaccharides are further subdivided to disaccharides, trisaccharides etc.

POLYSACCHARIDES:

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

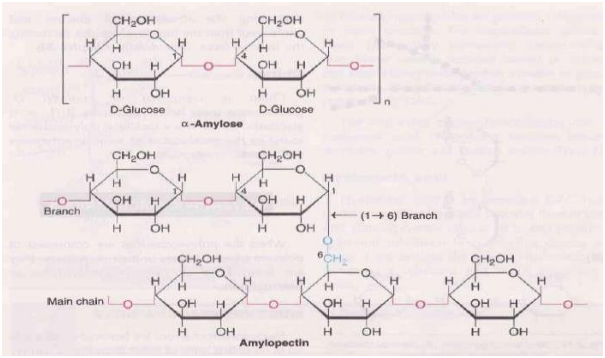
- Cellulose is a structural material used in formation of cell walls in plants Plant Starch
    - (Amylose and Amylopectin)



STORAGE POLYSACCHARIDES:

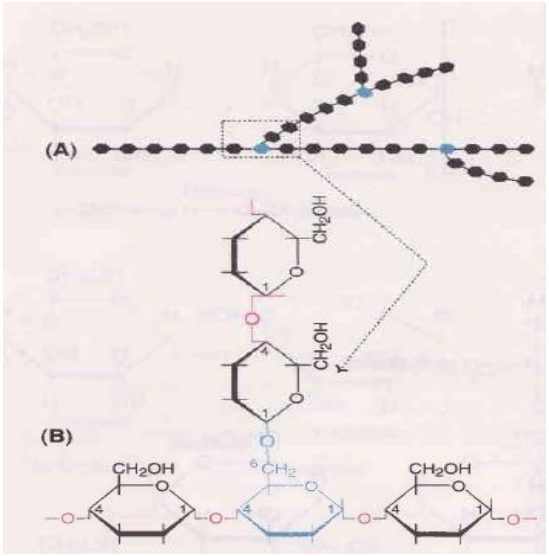
STARCH:

- Starch is the carbohydrate reserve of plants which is the most important dietary source for higher animals, including man.
- High content of starch is found in cereals, roots, tubers, vegetables etc.
- 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%).
- Chemically, amylose is a long unbranched chain with 200-1,00O D-glucose units held by (1-4) glycosidic linkages.
- Amylopectin on the other hand, is a branched chain with (1-6) glycosidic bonds at the branching points and (1-4) linkages everywhere else Amylopectin molecule containing a few thousand glucose units looks like a branched tree (20-30 glucose units per branch).
- Starches are hydrolysed by amylase (pancreatic or salivary) to liberate dextrins, and finally maltose and glucose units.
- Amylase acts specifically on a (1-4) glycosidic bonds.



GLYCOGEN:

- Glycogen is the carbohydrate reserve in animals, hence often referred to as animal starch.
- It is present in high concentration in liver, followed by muscle, brain etc. Glycogen is also found in plants that do not possess chlorophyll (e.g. yeast, fungi).
- The structure of glycogen is similar to that of amylopectin with more number of branches.
- Glucose is the repeating unit in glycogen joined together by ☐ (1 ☐ 4) glycosidic bonds, and ☐(1 ☐ 6) glycosidic bonds at branching points.
- The molecular weight (up to 1 x 10^8) and the number of glucose units (up to 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

STRUCTURAL POLYSACCHARIDES:

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

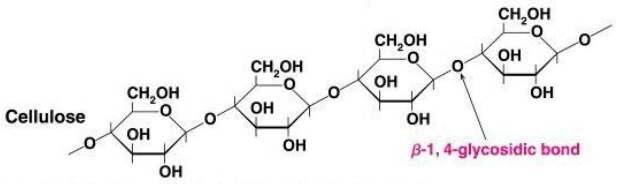
Eg. Cellulose - in plants

Chitin - found in outer skeleton of insects and crabs

Lignin - wood

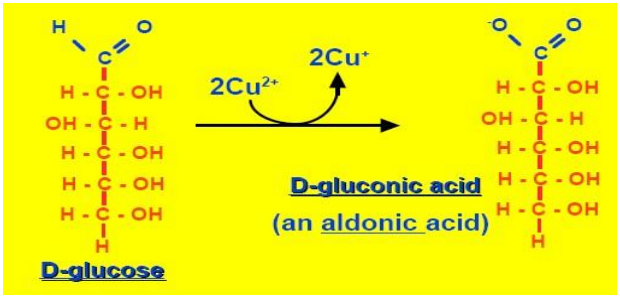
CELLULOSE:

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

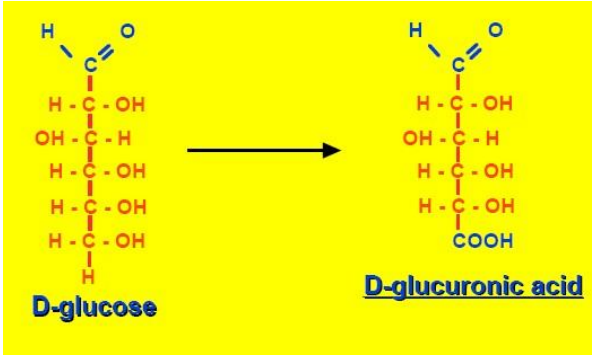


REACTIONS OF MONOSACCHARIDE:

☐ Can be oxidized by mild oxidizing agents (i.e., ferric or cupric ions). Carbonyl goup is oxidized to a carboxylic acid.



☐ Oxidation of the primary alcohol group yields a uronic acid:



- ☐ Aldoses can be oxidized at both c1 and c6 to yield aldaric acids.
- ☐ Aldoses and ketoses can be reduced to yield alditols.
- ☐ Sorbitol, mannitol (gum sweetener); glycerol (component of lipids)

Important Reactions of Sugars:

1) Oxidation of Aldehydes

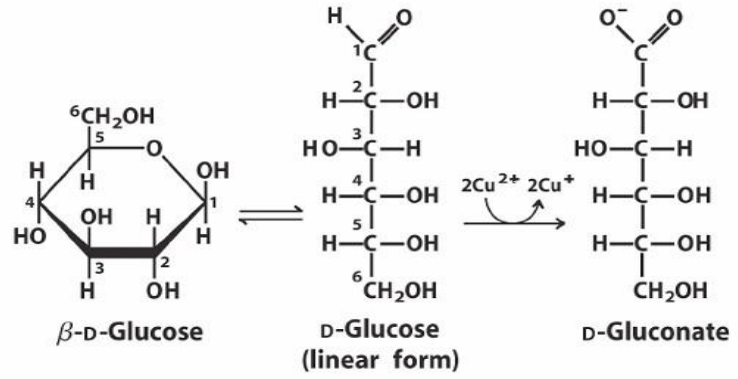
Aldehydes can be oxidized to carboxylic acids. Thus, aldoses are reducing agents. Any sugar that has (or potentially has) a free aldehyde is referred to as a reducing sugar. The name is made by changing the –ose ending to – onic acid (or onate).

Tests for reducing sugars:

a) Fehling's reaction [Cu (II)☐ Cu (I)]:

$R\text{-}CHO + 2Cu^{2+} + 5\ OH^{-} \square\ R\text{-}CO_2^{-} + Cu_2O + 3\ H_2O$

Visualizad as deposition of Cu_2O (red).



b) **Tollen's reaction** [Ag(I) ☐ Ag(o)]:

$R\text{-}CHO + 2\ Ag\ (NH_3)_2^{+} + 2\ OH^{-} \square\ R\text{-}Co_2^{-} + 2\ Ag + 3\ NH_3 + NH_4^{+}$

(Visualized as deposition of metabolic silver).

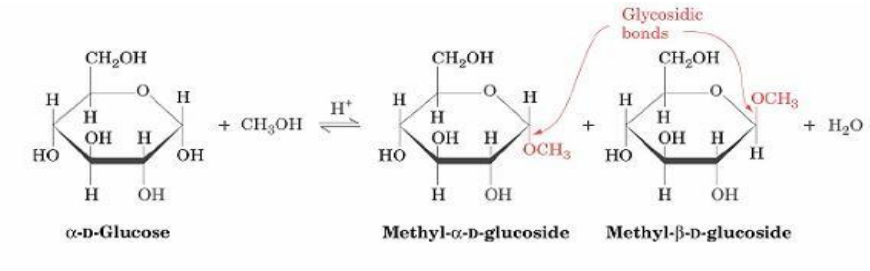
c) Aldehyde can also be oxidized to carboxylic acid by Br_2.

2) Oxidation of primary alcohol:

The CH_2OH group can be oxidized with dilute HNO_3 to a carboxylic acid. These are named with the root of the sugar plus – uronic acid or – aric acid. (e.g. glucuronic acid is formed by oxidation of glucose).

3) Reaction with alcohols: formation of acetals:

This is one of the most important reactions of sugars, because this is the way they are linked together to form polymers. In its simplest form, one can consider the reaction of methanol with a pyranose:



L - Sugars:

- It is important to realize that the identity stereoisomer is determined by the relative orientations of its substituents (i.e. hydroxyls, etc). The L carbohydrate series represent mirror images to the D sugars.
- To get the configuration of L sugar, start with the D configuration and reverse it:
- Fischer projection: Change the orientation of every chiral center – if the
  - Hydroxyl points to the left, point it to the right and vice-versa.
- Haworth projection: Move tham from up to down and vice versa. (The rules for α and β Anomers are reversed: the hydroxyl points up in α and down in β).
- Thus the relative orientation of the substituents is maintained, but you have a mirror image: the enantiomer.

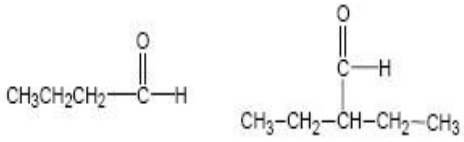
CHARACTERISTICS OF ALDEHYDE AND KETO GROUPS:

Aldehyde:

☐ Select the longest carbon chain containing the carbonyl carbon.

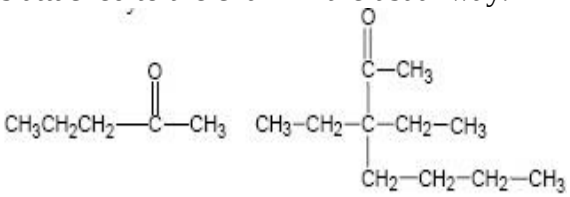
☐ The -e ending of the parent alkane name is replaced by the suffix -al.

- ☐ The carbonyl carbon is always numbered "1." (It is not necessary to include the number in the name.)
- ☐ Name the substituents attached to the chain in the usual way.



Ketones

- ☐ Select the longest carbon chain containing the carbonyl carbon.
- ☐ The -e ending of the parent alkane name is replaced by the suffix -one.
- ☐ Number the chain starting with the end closest to the ketone group (i.e., the carbonyl carbon should have the lowest possible number).
- ☐ Name the substituents attached to the chain in the usual way.



ACTION OF ACIDS AND ALKALIES ON SUGARS:

- ☐ A sugar loses water on being heated with strong mineral acid, and forms furtural derivatives.
- ☐ These furfurals may from colored complexes with α-naphthol, thymol, resorcinol, orinol and phloroglucinol.
- ☐ On treatment with dilute aqucous alkali solutions, both aldose and ketose are changed to enediols which are good reducing agents.
- ☐ Only sugars with a free aldehyde or ketose group can form enediols because the reaction involves the free aldehyde or ketonyl C= O group.
- ☐ Glucose and fructose form a common 1,2 enediol in dilute alkaline solutions.

Dietary fiber

Dietary fiber or roughage is the portion of plant-derived food that cannot be completely broken down by digestive enzymes. It has two main components:

- Fermentable, soluble fiber – which dissolves in water – is readily fermented in the colon into gases and physiologically active by-products, such as short-chain fatty acids produced in the colon by gut bacteria; it is viscous, may be called prebiotic fiber, and delays gastric emptying which, in humans, can result in an extended feeling of fullness.
- Insoluble fiber – which does not dissolve in water – is inert to digestive enzymes in the upper gastrointestinal tract and provides bulking. Some forms of insoluble fiber, such as resistant starches, can be fermented in the colon. Bulking fibers absorb water as they move through the digestive system, easing defecation.

Dietary fiber consists of non-starch polysaccharides and other plant components such as cellulose, resistant starch, resistant dextrins, inulin, lignins, chitins, pectins, beta-glucans, and oligosaccharides

Dietary fibers can act by changing the nature of the contents of the gastrointestinal tract and by changing how other nutrients and chemicals are absorbed. Some types of soluble fiber absorb water to become a gelatinous, viscous substance which may or may not be fermented by bacteria in the digestive tract. Some types of insoluble fiber have bulking action and are not fermented. Lignin, a major dietary insoluble fiber source, may alter the rate and metabolism of soluble fibers. Other types of insoluble fiber, notably resistant starch, are fermented to produce short-chain fatty acids, which are physiologically active and confer health benefits.

Food sources of dietary fiber have traditionally been divided according to whether they provide soluble or insoluble fiber. Plant foods contain both types of fiber in varying amounts, according to the plant's characteristics of viscosity and fermentability. Advantages of consuming fiber depend upon which type of fiber is consumed and which benefits may result in the gastrointestinal system Bulking fibers – such as cellulose, hemicellulose and psyllium – absorb and hold water, promoting regularity. Viscous fibers – such as beta-glucanand psyllium – thicken the fecal mass. Fermentable fibers – such as resistant starch and inulin – feed the bacteria and microbiota of

the large intestine, and are metabolized to yield short-chain fatty acids, which have diverse roles in gastrointestinal health.

Biological functions of bacterial cell wall polysaccharides

A major function is to act as pressure vessels, preventing over-expansion of the cell when water enters. The composition of cell walls varies between species and may depend on cell type and

developmental stage. The primary cell wall of land plants is composed of the polysaccharides cellulose, hemicelluloses and pectin.

A cell wall is a structural layer surrounding some types of cells, just outside the cell membrane. It can be tough, flexible, and sometimes rigid. It provides the cell with both structural support and protection, and also acts as a filtering mechanism. Cell walls are present in most prokaryotes (except mycoplasma bacteria), in algae, plants and fungi but rarely in other eukaryotes including animals. A major function is to act as pressure vessels, preventing over- expansion of the cell when water enters.

The composition of cell walls varies between species and may depend on cell type and developmental stage. The primary cell wall of land plants is composed of the polysaccharides cellulose, hemicelluloses and pectin. Often, other polymers such as lignin, suberin or cutin are anchored to or embedded in plant cell walls. Algae possess cell walls made of glycoproteins and polysaccharides such as carrageenan and agar that are absent from land plants. In bacteria, the cell wall is composed of peptidoglycan. The cell walls of archaea have various compositions, and may be formed of glycoprotein S-layers, pseudopeptidoglycan, or polysaccharides. Fungi possess cell walls made of the N-acetylglucosamine polymer chitin. Unusually, diatoms have a cell wall composed of biogenic silica.

Blood group antigens

Depending on which of these genetically determined proteins or antigens, known as red blood cell antigens, you inherited; you will have one of four blood types: type A, type B, type AB or type O. If you have type A blood, your red blood cells have the A antigen, and your body will produce anti B-antibodies.

The ABO blood group system is used to denote the presence of one, both, or neither of the A and B antigens on erythrocytes. In human blood transfusions it is the most important of the 36 different blood type (or group) classification systems currently recognized. A very rare (in modern medicine) mismatch in this, or any other serotype, can cause a serious, potentially fatal, adverse reaction after a transfusion, or a contra-indicated immune response to an organ transplant. The associated anti-A and anti-B antibodies are usually IgM antibodies, which are produced in the first years of life by sensitization to environmental substances, such as food, bacteria, and viruses.

Structure and significance of glycoconjugates

Glycoconjugates is the general classification for carbohydrates covalently linked with other chemical species such as proteins, peptides, lipids and saccharides. Glycoconjugates are formed in processes termed glycosylation. Glycoconjugates are very important compounds in biology and consist of many different categories such as glycoproteins, glycopeptides, peptidoglycans, glycolipids, glycosides and lipopolysaccharides. They are involved in cell–cell interactions, including cell–cell recognition; in cell– matrix interactions; in detoxification processes.

Generally the carbohydrate part(s) play an integral role in the function of a glycoconjugate; prominent examples of this are NCAM and blood proteins where fine details in the carbohydrate structure determine cell binding or not or lifetime in circulation.

Although the important molecular species DNA, RNA, ATP, cAMP, cGMP, NADH, NADPH, and coenzyme A all contain a carbohydrate part, generally they are not considered as glycoconjugates. Glycocojugates is covalent linking of carbohydrates antigens to protein scaffolds with goal of achieving a long term immunological response in body. Immunization with glycoconjugates successfully induced long term immune memory against carbohydrates antigens. Glycoconjugate vaccines introduced since the 1990s have yielded effective results against influenza and meningococcus.

Structure and function of glycosaminoglycans

Glycosaminoglycans are essential molecules in the body. They can covalently connect to proteins in order to form proteoglycans. ... These proteoglycans are integral parts of connective tissue such as tendons and cartilage, they can act as anticoagulants, and they are also a component of the fluid that lubricates joints.

Functions

CSGAGs

Endogenous heparin is localized and stored in secretory granules of mast cells. Histamine that is present within the granules is protonated (H_2A^{2+}) at pH within granules (5.2–6.0), thus it is believed that heparin, which is highly negatively charged, functions to electrostatically retain and store histamine. In the clinic, heparin is administered as an anticoagulant and is also the first line choice for thromboembolic diseases. Heparan sulfate (HS) has numerous biological activities and functions, including cell adhesion, regulation of cell growth and proliferation, developmental processes, cell surface binding of lipoprotein lipase and other proteins, angiogenesis, viral invasion, and tumor metastasis.

CSGAGs interact with heparin binding proteins, specifically dermatan sulfate interactions with fibroblast growth factor FGF-2 and FGF-7 have been implicated in cellular proliferation and wound repair while interactions with hepatic growth factor/scatter factor (HGF/SF) activate the HGF/SF signaling pathway (c-Met) through its receptor. Other biological functions for which CSGAGs are known to play critical functions in include inhibition of axonal growth and regeneration in CNS development, roles in brain development, neuritogenic activity, and pathogen infection.

Keratan sulfates

One of the main functions of the third class of GAGs, keratan sulfates, is the maintenance of tissue hydration. Within the normal cornea, dermatan sulfate is fully hydrated whereas keratan sulfate is only partially hydrated suggesting that keratan sulfate may behave as a dynamically controlled buffer for hydration. In disease states such as macular corneal dystrophy, in which GAGs levels such as KS are altered, loss of hydration within the corneal stroma is believed to be the cause of corneal haze, thus supporting the long-held hypothesis that corneal transparency is a dependent on proper levels of keratan sulfate. Keratan sulfate GAGs are found in many other tissues besides the cornea, where they are known to regulate macrophage adhesion, form barriers to neurite growth, regulate embryo implantation in the endometrial uterine lining during menstrual cycles, and affect the motility of corneal endothelial cells. In summary, KS plays an anti-adhesive role, which suggests very important functions of KS in cell motility and attachment as well as other potential biological processes.

Hyaluronic acid

Hyaluronic acid is a major component of synovial tissues and fluid, as well as other soft tissues, and endows their environments with remarkable rheological properties. For example, solutions of hyaluronic acid are known to be viscoelastic, and viscosity changes with shear stress. At low shear stress, a solution of 10 g/L of hyaluronic acid may have a viscosity 10^6 times the viscosity of the solvent, while under high shear stress, viscosity may drop by as much as 10^3 times. The aforementioned rheological properties of solutions of hyaluronic acid make it ideal for lubricating joints and surfaces that move along each other, such as cartilage. *In vivo*, hyaluronic acid forms hydrated coils that form randomly kinked coils that entangle to form a network. Hyaluronan networks retard diffusion and form a diffusion barrier that regulates transport of substances through intercellular spaces. For example, hyaluronan takes part in the partitioning of plasma proteins between vascular and extravascular spaces, and it is this excluded volume phenomenon that affects solubility of macromolecules in the interstitium, changes chemical equilibria, and stabilizes the structure of collagen fibers. Other functions include matrix interactions with hyaluronan binding proteins such as hyaluronectin, glial hyaluronan binding protein, brain enriched hyaluronan binding protein, collagen VI, TSG-6, and inter-alpha-trypsin inhibitor. Cell surface interactions involving hyaluronan are its well-known coupling with CD44, which may be related to tumor progression, and also with RHAMM (Hyaluronan-mediated motility receptor), which has been implicated in developmental processes, tumor metastasis, and pathological reparative processes. Fibroblasts, mesothelial cells, and certain types of stem cells surround themselves in a pericellular "coat", part of which is constructed from hyaluronan, in order to shield themselves from bacteria, red blood cells, or other matrix molecules. For example, with regards to stem cells, hyaluronan, along with chondroitin sulfate, helps to form the stem cell niche. Stem cells are protected from the effects of

growth factors by a shield of hyaluronan and minimally sulfated chondroitin sulfate. During progenitor division, the daughter cell moves outside of this pericellular shield where it can then be influenced by growth factors to differentiate even further.

Structure and biological role of hyaluronic acid

Hyaluronic acid (HA; conjugate base hyaluronate), also called hyaluronan, is an anionic, nonsulfated glycosaminoglycandistributed widely throughout connective, epithelial, and neural tissues. It is unique among glycosaminoglycans in that it is nonsulfated, forms in the plasma membrane instead of the Golgi apparatus, and can be very large, with its molecular weightoften reaching the millions. One of the chief components of the extracellular matrix, hyaluronan contributes significantly to cell proliferation and migration, and may also be involved in the progression of some malignant tumors.

The average 70 kg (154 lb) person has roughly 15 grams of hyaluronan in the body, one-third of which is turned over (degraded and synthesized) every day. Hyaluronic acid is also a component of the group A streptococcal extracellular capsule, and is believed to play a role in virulence.

Until the late 1970s, hyaluronic acid was described as a "goo" molecule, a ubiquitous carbohydrate polymer that is part of the extracellular matrix. For example, hyaluronic acid is a major component of the synovial fluid, and was found to increase the viscosity of the fluid. Along with lubricin, it is one of the fluid's main lubricating components.

Hyaluronic acid is an important component of articular cartilage, where it is present as a coat around each cell (chondrocyte). When aggrecan monomers bind to hyaluronan in the presence of HAPLN1 (hyaluronanic acid and proteoglycan link protein 1), large, highly negatively charged aggregates form. These aggregates imbibe water and are responsible for the resilienceof cartilage (its resistance to compression). The molecular weight (size) of hyaluronan in cartilage decreases with age, but the amount increases. A lubricating role of hyaluronan in muscular connective tissues to enhance the sliding between adjacent tissue layers has been suggested. A particular type of fibroblasts, embedded in dense fascial tissues, has been proposed as being cells specialized for the biosynthesis of the hyaluronan-rich matrix. Their related activity could be involved in regulating the sliding ability between adjacent muscular connective tissues.

Hyaluronic acid is also a major component of skin, where it is involved in tissue repair. When skin is exposed to excessive UVB rays, it becomes inflamed (sunburn) and the cells in the dermis stop producing as much hyaluronan, and increase the rate of its degradation. Hyaluronan degradation products then accumulate in the skin after UV exposure. While it is abundant in extracellular matrices, hyaluronan also contributes to tissue hydrodynamics, movement and proliferation of cells, and participates in a number of cell surface receptor interactions, notably those including its primary receptors, CD44 and RHAMM. Upregulation of CD44 itself is widely accepted as a marker of cell activation in lymphocytes. Hyaluronan's contribution to tumor growth may be due to its interaction with CD44. Receptor CD44 participates in cell adhesion interactions required by tumor cells.

Although hyaluronan binds to receptor CD44, there is evidence hyaluronan degradation products transduce their inflammatory signal through toll-like receptor 2 (TLR2), TLR4, or both TLR2 and TLR4 in macrophages and dendritic cells. TLR and hyaluronan play a role in innate immunity.

There are limitations including the in vivo loss of this compound limiting the duration of effect.

Cancer

In some cancers, hyaluronic acid levels correlate well with malignancy and poor prognosis. Hyaluronic acid is, thus, often used as a tumor marker for prostate and breast cancer. It may also be used to monitor the progression of the disease.

Figure 1. The process of cancer metastasis in which HA-associated molecules play a role in the steps. Abbreviations: hyaluronic acid (HA), hyaluronic acid synthase (HAS), hyaluronic acid receptor (HAR), hyaluronidase (HAase)

As shown in Figure 1, the various types of molecules that interact with hyaluronan can contribute to many of the stages of cancer metastasis, i.e. further the spread of cancerHyaluronic acid synthases (HAS) play roles in all of the stages of cancer metastasis. By producing anti- adhesive HA, HAS can allow tumor cells to release from the primary tumor mass, and if HA associates with receptors such as CD44, the activation of Rho GTPases can promote epithelial- mesenchymal transition (EMT) of the cancer cells. During the processes of intravasation or extravasation, the interaction of HAS produced HA with receptors such as CD44 or RHAMM promote the cell changes that allow for the cancer cells to infiltrate the vascular or lymphatic systems. While traveling in these systems, HA produced by HAS protects the cancer cell from physical damage. Finally, in the formation of a metastatic lesion, HAS produces HA to allow the cancer cell to interact with native cells at the secondary site and to produce a tumor for itself. HA fragments promote angiogenesis and hyaluronidases produce these fragments. Hypoxia also increases production of HA and activity of hyaluronidases. The hyaluronic acid receptors, CD44 and RHAMM, are most thoroughly studied in terms of their roles in cancer metastasis. Increased clinical CD44 expression has been positively correlated to metastasis in a number of tumor types. In terms of mechanics, CD44 affects adhesion of cancer cells to each other and to endothelial cells, rearranges the cytoskeleton through the Rho GTPases, and increases the activity of ECM degrading enzymes. Increased RHAMM expression has also been clinically correlated with cancer metastasis. In terms of mechanics, RHAMM promotes cancer cell motility through a number of pathways including focal adhesion kinase (FAK), Map kinase (MAPK), pp60(c-src), and the downstream targets of Rho kinase (ROK). RHAMM can also cooperate with CD44 to promote angiogenesis toward the metastatic lesion.

Wound repair

Skin provides a mechanical barrier to the external environment and acts to prevent the ingress of infectious agents. Once injured, the tissues beneath are exposed to infection; therefore, rapid and effective healing is of crucial significance to reconstruct a barrier function. Skin wound healing is a complex process, and includes many interacting processes initiated by haemostasis and the release of platelet-derived factors. The following stages are inflammation, granulation tissue formation, reepithelization and remodeling. HA is likely to play a multifaceted role in mediation of these cellular and matrix events. The proposed roles of HA in this sequence of skin wound healing events are detailed below.

Hyaluronic acid has also been used in the synthesis of biological scaffolds for wound-healing applications. These scaffolds typically have proteins such as fibronectin attached to the hyaluronan to facilitate cell migration into the wound. This is particularly important for individuals with diabetes suffering from chronic wounds.

Inflammation

In the early inflammatory phase of wound repair, wounded tissue is abundant in HA, probably a reflection of increased synthesis HA acts as a promoter of early inflammation, which is crucial in the whole skin wound-healing process. In a murine air pouch model of carrageenan/IL-1-induced inflammation, HA was observed to enhance cellular infiltration. showed a dose-dependent increase of the proinflammatory cytokines TNF-α and IL-8 production by human uterine fibroblasts at HA concentrations of 10 μg/mL to 1 mg/mL via a CD44-mediated mechanism. Endothelial cells, in response to inflammatory cytokines such as TNF-α, and bacterial lipopolysaccharide, also synthesize HA, which has been shown to facilitate primary adhesion of cytokine-activated lymphocytes expressing the HA-binding variants of CD44 under laminar and static flow conditions. It is interesting to note that HA has contradictory dual functions in the inflammatory process. It not only can promote the inflammation, as stated above, but also can moderate the inflammatory response, which may contribute to the stabilization of granulation tissue matrix, as described in the following part.

Although inflammation is an integral part of granulation tissue formation, for normal tissue repair to proceed, inflammation needs to be moderated. The initial granulation tissue formed is highly inflammatory with a high rate of tissue turnover mediated by matrix degrading enzymes and reactive oxygen metabolites that are products of inflammatory cells Stabilization of granulation tissue matrix can be achieved by moderating inflammation. HA functions as an important moderator in this moderation process, which contradicts its role in inflammatory stimulation, as described above. HA can protect against free-radical damage to cells. This may attribute to its free- radical scavenging property, a physicochemical characteristic shared by large polyionic polymers. In a rat model of free-radical scavenging property, HA has been shown to reduce damage to the granulation tissue.

In addition to the free-radical scavenging role, HA may also function in the negative feedback loop of inflammatory activation through its specific biological interactions with the

biological constituents of inflammation TNF-α, an important cytokine generated in inflammation, stimulates

the expression of TSG-6 (TNF-stimulated gene 6) in fibroblasts and inflammatory cells. TSG-6, a HA-binding protein, also forms a stable complex with the serum proteinase inhibitor IαI (Inter-α-inhibitor) with a synergistic effect on the latter's plasmin-inhibitory activity. Plasmin is involved in activation of the proteolytic cascade of matrix metalloproteinases and other proteinases leading to inflammatory tissue damage. Therefore, the action of TSG-6/ IαI complex, which may be additionally organized by binding to HA in the extracellular matrix, may serve as a potent negative feedback loop to moderate inflammation and stabilize the granulation tissue as healing progresses. In the murine air pouch model of carragenan/IL-1 (Interleukin-1β)-induced inflammation, where HA has been shown to have a proinflammatory property, reduction of inflammation can be achieved by administrating TSG-6, and the result is comparable with systemic dexamethasone treatment.

Granulation

Granulation tissue is the perfused, fibrous connective tissue that replaces a fibrin clot in healing wounds. It typically grows from the base of a wound and is able to fill wounds of almost any size it heals. HA is abundant in granulation tissue matrix. A variety of cell functions that are essential for tissue repair may attribute to this HA-rich network. These functions include facilitation of cell migration into the provisional wound matrix, cell proliferation and organization of the granulation tissue matrix. Initiation of inflammation is crucial for the formation of granulation tissue, therefore the pro-inflammatory role of HA as discussed above also contributes to this stage of wound healing.

Cell migration

Cell migration is essential for the formation of granulation tissue. The early stage of granulation tissue is dominated by a HA-rich extracellular matrix, which is regarded as a conducive environment for migration of cells into this temporary wound matrix. Contributions of HA to cell migration may attribute to its physicochemical properties as stated above, as well as its direct interactions with cells. For the former scenario, HA provides an open hydrated matrix that facilitates cell migration, whereas, in the latter scenario, directed migration and control of the cell locomotory mechanisms are mediated via the specific cell interaction between HA and cell surface HA receptors. As discussed before, the three principal cell surface receptors for HA are CD44, RHAMM, and ICAM-1. RHAMM is more related to cell migration. It forms links with several protein kinases associated with cell locomotion, for example, extracellular signal-regulated protein kinase (ERK), p125fak, and pp60c-src. During fetal development, the migration path through which neural crest cells migrate is rich in HA. HA is closely associated with the cell migration process in granulation tissue matrix, and studies show that cell movement can be inhibited, at least partially, by HA degradation or blocking HA receptor occupancy.

By providing the dynamic force to the cell, HA synthesis has also been shown to associate with cell migration Basically, HA is synthesized at the plasma membrane and released directly into the

extracellular environment. This may contribute to the hydrated microenvironment at sites of synthesis, and is essential for cell migration by facilitating cell detachment.

Skin healing

HA plays an important role in the normal epidermis. HA also has crucial functions in the reepithelization process due to several of its properties. It serves as an integral part of the extracellular matrix of basal keratinocytes, which are major constituents of the epidermis; its free- radical scavenging function and its role in keratinocyte proliferation and migration.

In normal skin, HA is found in relative high concentrations in the basal layer of the epidermis where proliferating keratinocytes are found. CD44 is collocated with HA in the basal layer of epidermis where additionally it has been shown to be preferentially expressed on plasma membrane facing the HA-rich matrix pouches. Maintaining the extracellular space and providing an open, as well as hydrated, structure for the passage of nutrients are the main functions of HA in epidermis. A report found HA content increases at the presence of retinoic acid (vitamin A). The proposed effects of retinoic acid against skin photo-damage and aging may be correlated, at least in part, with an increase of skin HA content, giving rise to increase of tissue hydration. It has been suggested the free-radical scavenging property of HA contributes to protection against solar radiation, supporting the role of CD44 acting as a HA receptor in the epidermis.

Epidermal HA also functions as a manipulator in the process of keratinocyte proliferation, which is essential in normal epidermal function, as well as during reepithelization in tissue repair. In the wound healing process, HA is expressed in the wound margin, in the connective tissue matrix, and collocating with CD44 expression in migrating keratinocytes. Kaya et al. found suppression of CD44 expression by an epidermis-specific antisense transgene resulted in animals with defective HA accumulation in the superficial dermis, accompanied by distinct morphologic alterations of basal keratinocytes and defective keratinocyte proliferation in response to mitogen and growth factors. Decrease in skin elasticity, impaired local inflammatory response, and impaired tissue repair were also observed. Their observations are strongly supportive of the important roles HA and CD44 have in skin physiology and tissue repair.

Fetal wound healing

Lack of fibrous scarring is the primary feature of fetal wound healing. Even for longer periods, HA content in fetal wounds is still higher than that in adult wounds, which suggests that HA may, at least in part, reduce collagen deposition and therefore lead to reduced scarring. This suggestion is in agreement with the research of West et al., who showed in adult and late gestation fetal wound healing, removal of HA results in fibrotic scarring.

chondroitin sulfate

Chondroitin sulfate is a sulfated glycosaminoglycan (GAG) composed of a chain of alternating sugars (N-acetylgalactosamine and glucuronic acid). It is usually found attached to proteins as

part of a proteoglycan. A chondroitin chain can have over 100 individual sugars, each of which can be sulfated in variable positions and quantities. Chondroitin sulfate is an important structural component of cartilage and provides much of its resistance to compression.[1] Along with glucosamine, chondroitin sulfate has become a widely used dietary supplement for treatment of osteoarthritis.

Mechanisms of action

The effect of chondroitin sulfate in people with osteoarthritis is likely the result of a number of reactions including its anti-inflammatory activity, the stimulation of the synthesis of proteoglycans and hyaluronic acid, and the decrease in catabolic activity of chondrocytes inhibiting the synthesis of proteolytic enzymes, nitric oxide, and other substances that contribute to damage cartilage matrix and cause death of articular chondrocytes. A recent review summarizes data from relevant reports describing the biochemical basis of the effect of chondroitin sulfate on osteoarthritis articular tissues.

Bioavailability and pharmacokinetics

Pharmacokinetic studies performed on humans and experimental animals after oral administration of chondroitin sulfate revealed that it can be absorbed orally. Chondroitin sulfate shows first-order kinetics up to single doses of 3,000 mg. multiple doses of 800 mg in people with osteoarthritis do not alter the kinetics of chondroitin sulfate. The bioavailability of chondroitin sulfate ranges from
15% to 24% of the orally administered dose. More particularly, on the articular tissue, chondroitin sulfate is not rapidly absorbed in the gastro-intestinal tract and a high content of labeled chondroitin sulfate is found in the synovial fluid and cartilage.

Heparin

Heparin, also known as unfractionated heparin (UFH), is medication which is used as an anticoagulant (blood thinner). Specifically it is used to treat and prevent deep vein thrombosis, pulmonary embolism, and arterial thromboembolism. It is also used in the treatment of heart attacks and unstable angina. It is given by injection into a vein. Other uses include inside test tubes and kidney dialysis machines. Common side effects include bleeding, pain at the injection site, and low blood platelets. Serious side effects include heparin induced thrombocytopenia. Greater care is needed in those with poor kidney function Heparin appears to be relatively safe for use during pregnancy and breastfeeding. Heparin is a naturally occurring glycosaminoglycan.

Heparin is a naturally occurring anticoagulant produced by basophils and mast cells. In therapeutic doses, it acts as an anticoagulant, preventing the formation of clots and extension of existing clots within the blood. While heparin does not break down clots that have already formed (unlike tissue plasminogen activator), it allows the body's natural clot lysis mechanisms to work normally to break down clots that have formed. Heparin is generally used for anticoagulation for the following conditions:

- ☐ Acute coronary syndrome, e.g., NSTEMI
- ☐ Atrial fibrillation
- ☐ Deep-vein thrombosis and pulmonary embolism
- ☐ Cardiopulmonary bypass for heart surgery
- ☐ ECMO circuit for extracorporeal life support
- ☐ Hemofiltration
- ☐ Indwelling central or peripheral venous catheters

Heparin and its low-molecular-weight derivatives (e.g., enoxaparin, dalteparin, tinzaparin) are effective in preventing deep vein thromboses and pulmonary emboli in people at risk, but no evidence indicates any one is more effective than the other in preventing mortality.

Sialic acid

Sialic acid is a generic term for the *N*- or *O*-substituted derivatives of neuraminic acid, a monosaccharide with a nine-carbon backbone. It is also the name for the most common member of this group, N-acetylneuraminic acid (Neu5Ac or NANA). Sialic acids are found widely distributed in animal tissues and to a lesser extent in other organisms, ranging from fungi to yeasts and bacteria, mostly in glycoproteins and gangliosides (they occur at the end of sugar chains connected to the surfaces of cells and soluble proteins). That is because it seems to have appeared late in evolution. However, it has been observed in *Drosophila* embryos and other insects and in the capsular polysaccharides of certain strains of bacteria. Generally, plants do not contain or display sialic acids. In humans the brain has the highest sialic acid concentration, where these acids play an important role in neural transmission and ganglioside structure in synaptogenesis. In general, the amino group bears either an acetyl or a glycolyl group, but other modifications have been described. These modifications along with linkages have shown to be tissue specific and developmentally regulated expressions, so some of them are only found on certain types of glycoconjugates in specific cells. The hydroxyl substituents may vary considerably; acetyl, lactyl, methyl, sulfate, and phosphate groups have been found.

Role

Sialic acid-rich glycoproteins (sialoglycoproteins) bind selectin in humans and other organisms. Metastatic cancer cells often express a high density of sialic acid-rich glycoproteins. This overexpression of sialic acid on surfaces creates a negative charge on cell membranes. This creates repulsion between cells (cell opposition) and helps these late-stage cancer cells enter the blood stream.

Many bacteria also use sialic acid in their biology, although this is usually limited to bacteria that live in association with higher animals (deuterostomes). Many of these incorporate sialic acid

into cell surface features like their lipopolysaccharide and capsule, which helps them evade the innate immune response of the host. Other bacteria simply use sialic acid as a good nutrient source, as it contains both carbon and nitrogen and can be converted to fructose-6-phosphate, which can then enter central metabolism.

Sialic acid-rich oligosaccharides on the glycoconjugates (glycolipids, glycoproteins, proteoglycans) found on surface membranes help keep water at the surface of cells. The sialic acid- rich regions contribute to creating a negative charge on the cells' surfaces. Since water is a polar molecule with partial positive charges on both hydrogen atoms, it is attracted to cell surfaces and membranes. This also contributes to cellular fluid uptake.

Sialic acid can "hide" mannose antigens on the surface of host cells or bacteria from mannose-binding lectinThis prevents activation of complement. Sialic acid in the form of polysialic acid is an unusual posttranslational modification that occurs on the neural cell adhesion molecules (NCAMs). In the synapse, the strong negative charge of the polysialic acid prevents NCAM cross-linking of cells.

Administration of estrogen to castrated mice leads to a dose-dependent reduction of the sialic acid content of the vagina. Conversely, the sialic acid content of mouse vagina is a measure of the potency of the estrogen. Reference substances are estradiol for subcutaneous application and ethinylestradiol for oral administration.

Glycoproteins

Glycoproteins are proteins which contain oligosaccharide chains (glycans) covalently attached to amino acid side-chains. The carbohydrate is attached to the protein in a cotranslational or posttranslational modification. This process is known as glycosylation. Secreted extracellular proteins are often glycosylated.

In proteins that have segments extending extracellularly, the extracellular segments are also often glycosylated. Glycoproteins are also often important integral membrane proteins, where they play a role in cell–cell interactions. It is importantto distinguish endoplasmic reticulum-based glycosylation of the secretory system from reversible cytosolic-nuclear glycosylation. Glycoproteins of the cytosol and nucleus can be modified through the reversible addition of a single GlcNAc residue that is considered reciprocal to phosphorylation and the functions of these are likely to be additional regulatory mechanism that controls phosphorylation-based signalling. contrast, classical secretory glycosylation can be structurally essential. For example, inhibition of asparagine-linked, i.e. N-linked, glycosylation can prevent proper glycoprotein folding and full inhibition can be toxic to an individual cell. In contrast, perturbation of glycan processing (enzymatic removal/addition of carbohydrate residues to the glycan), which occurs in both the endoplastic reticulum and Golgi apparatus, is dispensable for isolated cells (as evidence by survival with glycosides inhibitors) but can lead to human disease (congenital disorders of glycosylation) and can be lethal in animal models. It is therefore likely that the fine processing of glycans is important for endogenous functionality, such as cell trafficking, but that this is likely to have been secondary to its role in host-pathogen interactions. A famous example of this latter effect is the ABO blood group system.

Function	Glycoproteins
Structural molecule	Collagens
Lubricant and protective agent	Mucins
Transport molecule	Transferrin, ceruloplasmin
Immunologic molecule	Immunoglobulins, histocompatibility antigens
Hormone	Human chorionic gonadotropin (HCG), thyroid-stimulating hormone (TSH)
Enzyme	Various, e.g., alkaline phosphatase, patatin
Cell attachment-recognition site	Various proteins involved in cell–cell (e.g., sperm–oocyte), virus–cell, bacterium–cell, and hormone–cell interactions
Antifreeze protein	Certain plasma proteins of coldwater fish
Interact with specific carbohydrates	Lectins, selectins (cell adhesion lectins), antibodies
Receptor	Various proteins involved in hormone and drug action
Affect folding of certain proteins	Calnexin, calreticulin

Glycolipids

Glycolipids are lipids with a carbohydrate attached by a glycosidic bond or covalently bonded. Their role is to maintain the stability of the cell membrane and to facilitate cellular recognition, which is crucial to the immune response and in the connections that allow cells to connect to one another to form tissues. Glycolipids are found on the surface of all eukaryotic cell membranes, where they extend from the phospholipid bilayer into the extracellular environment.

Functions

Cell–cell Interactions

The main function of glycolipids in the body is to serve as recognition sites for cell–cell interactions. The saccharide of the glycolipid will bind to a specific complementary carbohydrate or to a lectin (carbohydrate-binding protein), of a neighboring cell. The interaction of these cell surface markers is the basis of cell recognitions, and initiates cellular responses that contribute to activities such as regulation, growth, and apoptosis.

Immune Responses

An example of how glycolipids function within the body is the interaction between leukocytes and endothelial cells during inflammation. Selectins, a class of lectins found on the surface of leukocytes and endothelial cells bind to the carbohydrates attached to glycolipids to initiate the immune response. This binding causes leukocytes to leave circulation and congregate near the site of inflammation. This is the initial binding mechanism, which is followed by the expression of integrins which form stronger bonds and allow leukocytes to migrate toward the site of inflammation. Glycolipids are also responsible for other responses, notably the recognition of host cells by viruses.

Blood types

Blood types are an example of how glycolipids on cell membranes mediate cell interactions with the surrounding environment. The four main human blood types (A, B, AB, O) are determined by the oligosaccharide attached to a specific glycolipid on the surface of red blood cells, which acts as an antigen. The unmodified antigen, called the H antigen, is the characteristic of type O, and is present on red blood cells of all blood types. Blood type A has an N-acetylgalactosamine added as the main determining structure, type B has a galactose, and type AB has all three of these antigens. Antigens which are not present in an individual's blood will cause antibodies to be produced, which will bind to the foreign glycolipids. For this reason, people with blood type AB can receive transfusions from all blood types (the universal acceptor), and people with blood type O can act as donors to all blood types (the universal donor).

POSSIBLE QUESTIONS

UNIT-I

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Give a detail account on structure and biological functions of cellulose
2. Explain in detail about Chitin
3. Discuss in detail about bacterial cell wall polysaccharides
4. Give a detail account on structure and significance of gylcoconjucates

PART-C (8 MARKS)

` 1. Give a detail account on structure and significance of gylcoconjucates
2. Discuss in detail about glycosaminoglycans
3. Explain in detail about glycoproteins
4. Give a detail account on starch and sucrose
5. Discuss in detail about fructans and galactans
6. Give a detail account glycolipids
7. Explain in detail account on blood group antigens
8. Discuss in detail about structure and biological role of hyaluronic acid

Karpagam Academy of Higher Education
Department of Biochemistry
I M.Sc., Biochemistry
18BCP101- Chemistry of Biopolymers

S. No.	Unit I	Question	Option I	Option II	Option III	Option IV	Answer
1	1	Digitonin is a	Protein	Glycoside	lipid	Alkaloid	Glycoside
2	1	The following sugar exhibits inversion of optical rotation	Sucrose	glucose	fructose	Lactose	Lactose
3	1	Hydrolysis of sucrose yields	Glucose	Glucose	Maltose	Fructose + glucose	Fructose +
4	1	Osazone formation is due to	Presence of	presence of	presence of	presence of	presence of
5	1	Raffinose is composed of	Glucose,	glucose,	glucose,	glucose	glucose,
6	1	A reducing disaccharide containing glucose is	maltose	lactose	trehalose	Furanose	trehalose
7	1	The reagent used for distinguishing a reducing	Benedict's	Barfoed's	Fehling's reagent		Benedict's
8	1	The arrangements of sugars into D&L configuration is	Glyceraldehy	lactic acid	glucose		Glyceraldehydes
9	1	Starch is composed of repeating unit of	Maltose	Glucose	Cellobiose		Maltose
10	1	β (1-4) linkage is present in	Starch	Glycogen	cellulose		cellulose
11	1	Amylose contains glucose units	100-200	200-300	300-400		300-400
12	1	Each branch of amylopectin is an interval of glucose units	14-20	24-30	34-40		24-30
13	1	The end product of hydrolysis of strach by amylase is	Soluble	Glucose	dextrin		Soluble starch
14	1	The component present in starch that gives blue colour	Amylase	amylopecti	amylose		Amylase
15	1	Amylopectins are present in	Hyaluronic	strach	sucrose		Hyaluronic acid
16	1	The general formula for polysaccharides	$(C_6H_{10}O_5)n$	$(C_6H_{12}O_6)n$	$(C_6H_{12}O_5)n$	$(C_6H_{10}O_6)n$	$(C_6H_{10}O_5)n$
17	1	The simplest of aldoses is	Glucose	Arabinose	Glyceraldehyde	Ribose	Glyceraldehyde
18	1	The number of isomers of glucose	4	8	12	16	16
19	1	The epimers of glucose	Fructose	galactose	ribose	deoxy ribose	galactose
20	1	The reducing property of glucose is due to	OH group	Hemiacetal	Aldehyde group	Acid group	Aldehyde group
21	1	Oxidation of glucose with hydrogen peroxide gives	Glucuronic	Glucaric	Gluconic acid	Tartaric acid	Glucuronic acid
22	1	Human heart muscle contains	D- Arabinose	D- Ribose	D-Xylose	L-Xylose	D-Xylose
23	1	Lobry de Bruyn Alberda Van Ekenstein transformation is	Glucose with	Lactose	sucrose with dil.	starch with	Glucose with
24	1	Fucose is a	Glycoside	Hexose	Triose	methyl pentose	Hexose
25	1	Example for a fructosan is	Starch	inulin	cellulose	Chitin	Starch
26	1	Glucose reacts with phenyl hydrazine to give	osazone	glucocyano hydrin	gluconic acid	None	osazone
27	1	The dissacharide which does not show mutarotation	Sucrose	Lactose	Maltose	Cellobiose	Sucrose
28	1	Glycoside are found in many	Vitamins	drugs	Minerals	Nucleoproteins	drugs
29	1	Trehalose is a	Disaccharide	trisacharide	poly saccharide		Disaccharide
30	1	Galactose on oxidation with conc. HNO3 produces	Gluconic	saccharic	mucic acid	both a and b	mucic acid
31	1	Chitin is composed of	fructofuranos e	D-glucuronic	N-acetyl glucoseamine	Fructose + glucose	N-acetyl glucoseamine
32	1	the mucopolysaccharide which serve as lubricant and shock absorbent in joints is	heparin	chondroitin sulphate	hyaluronic acid	cellulose	hyaluronic acid
33	1	All carbohydrates contain carbon. This can be shown by heating with	sodium hydroxide solution	burning in air	heating with conc. Sulphuric acid	Hydrochloric acid	heating with conc. Sulphuric acid
34	1	The buffer acting in the osazone reaction is	phenyl hydrazine	acetic acid +phenyl	sodium acetate +acetic acid	sodium acetate	phenyl hydrazine
35	1	The monosaccharide units of hyaluronic acid are	glucuronic acid & N- acetyl D-glucosamine	glucuronic acid & N-acetyl galactosem ine	iduronic acid & N-Acetyl galactoseamine		glucuronic acid & N-acetyl D-glucosamine
36	1	NASA has launched a satellite named _____to study the effect of water in various forms on climate.	*Aqua*	*Hydro*	*Hi*	*Water*	*Aqua*
37	1	Water is	Tasteless	Odorless	both a and b		both a and b
38	1	The epimers of glucose	Fructose	galactose	ribose	deoxy ribose	galactose
39	1	The dissacharide which does not show mutarotation	sucrose	lactose	maltose	cellobiose	sucrose
40	1		Eight	Ten	Six	five	Ten
41	1	Stereoisomer classified into	Two	Three	Four	Seven	Two
42	1	D isomer rotate the plane polarized light to	Left	Right	DL mixture	Interchange	Right
43	1	Isomers formed by the interchange of H and OH groups on Carbon atom2, 3 and 4 are known as	Monomer	Dimer	Epimers	Tetramer	Epimers
44	1	Sugars forming six member ring are known as	Furanose		mannose	Pyranose	Pyranose
45	1	Maltose is composed of					
46	1	Erythrodextrin gives colour with iodine	blue	violet	red		red
47	1	Cellulose is made up of	α-glucose	β-glucose	fructose		β-glucose
48	1	Hyaluronidase is the enzyme, which acts on	homopolysac charide	heteropolys accharide	disaccharide		heteropolysacch aride
49	1	In place of glucuronic acid chondroitin sulphate B contains	gluconic acid	glucamic acid	iduronic acid		iduronic acid
50	1	Heparin has a molecular weight of about	14,000	15,000	16,000		17,000
51	1	Blood group subtances consists of	lactose	maltose	fucose		fucose

52	1	The component of cartilage & cornea is		chondroitin sulphate	cadmium sulphate		Keratosulphate
53	1	The compound which is an acid mucopolysacchride	dicoumarol	EDTA	Hyaluronic acid		Hyaluronic acid
54	1	A polymer of N-acetylated glucosamine is	dextran	heparin	chitin		chitin
55	1	Change in optical rotation is	specific rotation	mutarotation	epimerism		mutarotation
56	1	The polymer of fructose is	Strach	glycogen	cellulose		insulin
57	1	The glycosaminoglycan which acts as an anticoagulant is	Heparin	Hyaluronic acid	dextrin		Heparin
58	1	The glycosaminoglycan which is present in synovial fluid	Heparin	Hyaluronic	dextrin		Hyaluronic acid
59	1	2 carbon epimer of glucose	Fructose	galactose	Mannose		Mannose
60	1	The mirror images are	Enantiomers	Anomers	Epimers		Epimers

UNIT-II

SYLLABUS

Proteins: Review of structure and classification of aminoacids. Orders of protein structure. Primary structure – determination of amino acid sequence of proteins. The peptide bond – The Ramachandran plot. Secondary structures – α-helix, β-sheet and β-turns. Fibrous proteins- Collagen triple helix-Structure and assembly. Globular proteins-forces involved, folding process and folding patterns. Tertiary structure –Myoglobin organisation. Quarternary structure of proteins-Structure of haemoglobin. Models for haemoglobin allostery. Quintinary structure-basics only. Protein function as enzymes, defensive and transport.

AMINO ACIDS:

Definition:

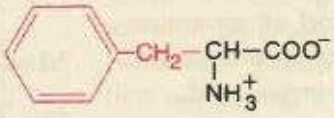
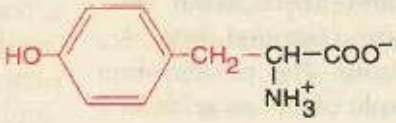
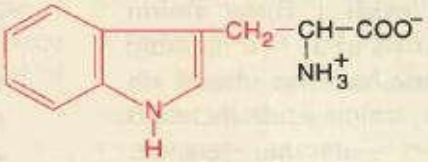
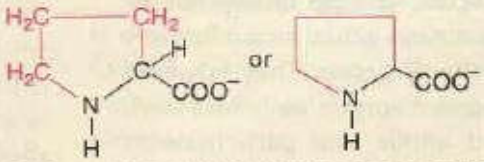
Amino acids are a group of organic compounds containing two functional groups amino and carboxyl. The amino group (-NH2) is basic while the carboxyl group (-COOH) is acidic in nature.

CLASSIFICATION:

There are different ways of classifying the amino acids based on the structure and chemical nature nutritional requirement, metabolic fate etc.

A. Amino acid classification based on the structure:

- A comprehensive classification of amino acids is based on their structure and chemical nature.
- Each amino acid is assigned a 3 letter or 1 letter symbol.
- These symbols are commonly used to represent the amino acids in protein structure.
- The 20 amino acids found in proteins are divided into seven distinct groups.
- The different groups of amino acids, their symbols and structures are given.
- The salient features of different groups are described next.

Name	Symbol		Structure	Special group present
	3 letters	1 letter		
VI. Aromatic amino acids				
17. Phenylalanine	Phe	F		Benzene or phenyl
18. Tyrosine	Tyr	Y		Phenol
19. Tryptophan	Trp	W		Indole
VII. Imino acid				
20. Proline	Pro	P		Pyrrolidine

(Note : R group is shown in red)

- **Amino acids with aliphatic side chains:** These are monoamino monocarboxylic acids.

  This group consists of the most simple amino acids-glycine, alanine, valine, leucine and isoleucine. The last three amino acids (Leu, lle, Val) contain branched aliphatic side chains, hence they are referred to as branched chain amino acids.

- **Hydroxyl group containing amino acids:** Serine, threonine and tyrosine are hydroxyl group containing amino acids. Tyrosine-being aromatic in nature-is usually considered under aromatic amino acids.

- **Sulfur containing amino acids**: Cysteine with sulfhydryl group and methionine with

  thioether group are the two amino acids incorporated during the course of protein synthesis.

Cystine, another important sulfur containing amino acid, is formed by condensation of two molecules of cysteine.

- **Acidic amino acids and their amides:** Aspartic acid and glutamic acids are dicarboxylic monoamino acids while asparagine and glutamine are their resoective amide derivatives. All these four amino acids possess distinct codons for their incorporation into proteins.
- **Basic amino acids**: The three amino acids lysine, arginine (with guanidino group) and histidine (with imidazole ring) are dibasic monocarboxylic acids. They are highly basic in character.
- **Aromatic amino acids:** Phenylalanine, tyrosine and tryptophan (with indole ring) are aromatic amino acids. Besides these, histidine may also be considered under this category.
- **lmino acids:** Proline containing pyrrolidine ring is a unique amino acid. It has an amino group (=NH), instead of an amino group (-NH2) found in other amino acids. Therefore proline is an aimino acid.

B. Classification of amino acids based on polarity:

- Amino acids are classified into 4 groups based on their polarity. The polarity in turn reflects the functional role of amino acids in protein structure.
- **Non-polar amino acids :** These amino acids are also referred to as hydrophobic (water hating). They have no charge on the 'R' group. The amino acids included in this group are - alanine, leucine, isoleucine, valine, methionine, phenylalanine, tryptophan and proline.
- **Polar amino acids with no charge on 'R' group:** These amino acids, as such, carry no charge on the 'R'group. They however possess groups such as hydroxyl, sulfhydryl and amide and participate in hydrogen bonding of protein structure. The simple amino acid glycine (where R = H) is also considered in this category. The amino acids in this group are glycine, serine, threonine, cysteine, glutamine, asparagine and tyrosine.
- **Polar amino acids with positive 'R' group:** The three amino acids lysine, arginine and histidine are included in this group.
- **Polar amino acids with negative 'R'group:** The dicarboxylic monoamino acids aspartic acid and glutamic acid are considered in this group.

C. Nutritional classification of amino acids:

- The twenty amino acids are required for the synthesis of variety of proteins, besides other biological functions.
- However, all these 20 amino acids need not be taken in the diet. Based on the nutritional requirements amino acids are grouped into two classes essential and nonessential.
- **Essential or indispensable amino acids:** The amino acids which cannot be synthesized by the body and, therefore, need to be supplied through the diet are called essential amino acids. They are required for proper growth and maintenance of the individual. The ten amino acids listed below are essential for humans.
- **Non - essential or dispensable amino acids :** The body can synthesize about '10 amino acids to meet the biological needs, hence they need not be consumed in the diet. These are- glycine, alanine, serine, cystein e, aspartate,a sparagnie, glutamate, glutamine, tyrosine and proline.

D. Amino acid classification based on their metabolic fate:

- The carbon skeleton of amino acids can serve as a precursor for the synthesis of glucose.
- From metabolic view point, amino acids are divided into three
- **Glycogenic amino acids:** These amino acids can serve as precursors for the formation of glucose or glycogen. e.g. alanine, aspartate, glycine, methionine etc.
- **Ketogenic amino acids**: Fat can be synthesized from these amino acids. Two amino acids leucine and lysine are exclusively ketogenic.
- **Glycogenic and ketogenic amino acids:** The four amino acids isoleucinep, henylalanine, tryptophan, tyrosine are precursors for synthesis of glucose as well as fat.

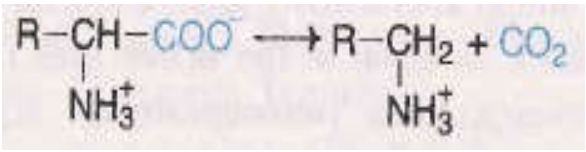
CHEMICAL REACTIONS OF AMINO ACIDS:

- The general reactions of amino acids are mostly due to the presence of two functional groups namely carboxyl (-COOH) group and amino (-NH2) group.

REACTIONS DUE TO -COOH GROUP:

1. Amino acids form salts (-COONa) with bases and esters (-COOR') with alcohols.

2. **Decarboxylation:** Amino acids undergo decarboxylation to produce corresponding amines.



- This reaction assumes significance in the living cells due to the formation of many biologically important amines.
- These include histamine, tyramine and y-amino butyric acid (CABA) from the amino acids histidine, tyrosine and glutamate, respectively.

3. Reaction with ammonia: The carboxyl group of dicarboxylic amino acids reacts with NH3 to form amide

- Aspartic acid + NH, ------ Asparagine
- Glutamic acid + NH. ------Glutamine

REACTIONS DUE TO -NH_2 GROUP:

4. The amino groups behave as bases and combine with acids (e.g. HCI) to form salts (-NHiCl-).

5. **Reaction with ninhydrin :** The a-amino acids react with ninhydrin to form a purple, blue or pink colour complex (Ruhemann's purple).

- Amino acid + Ninhydrin ----- Keto acid + NH_3+CO_2+Hydrindantin
- Hydrindantin + NH_3 + Ninhydrin- ---- Ruhemann's purple
- Ninhydrin reaction is effectively used for the quantitative determination of amino acids and proteins.

6. **Colour reactions of amino acids:** Amino acids can be identified by specific colour reactions

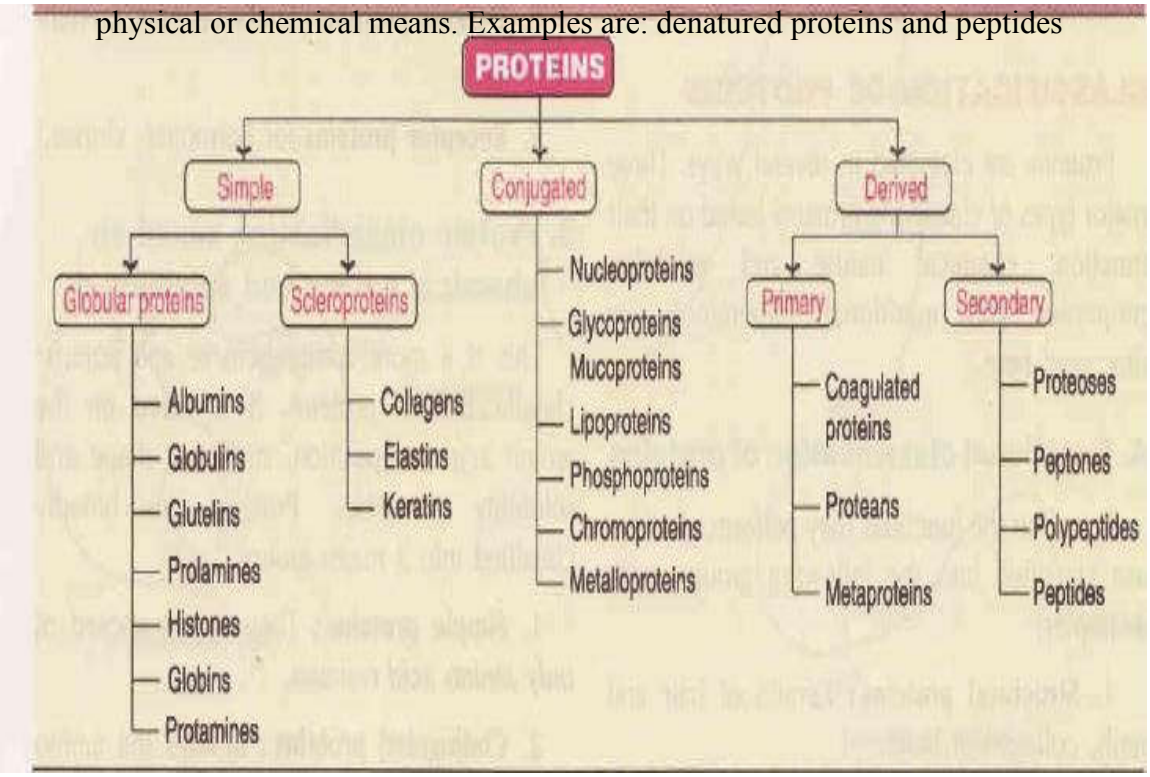
7. Transamination: Transfer of an amino group from an amino acid to a keto acid to form a new amino acid is a very important reaction in amino acid metabolism.

8. Oxidative deamination: The amino acids undergo oxidative deamination to liberate free ammonia.

PROTEINS:

CLASSIFICATION OF PROTEINS:

- Proteins are classified in several ways.
- Three major types of classifying proteins based on their function, chemical nature and solubility properties and nutritional importance are discussed here.
- **Simple proteins:** On hydrolysis they yield only the amino acids and occasional small carbohydrate compounds. Examples are: albumins, globulins, glutelins, albuminoids, histones and protamines.
- **Conjugated proteins:** These are simple proteins combined with some non-protein material in the body. Examples are: nucleoproteins, glycoproteins, phosphoproteins, haemoglobins and lecithoproteins.
- **Derived proteins:** These are proteins derived from simple or conjugated proteins by physical or chemical means. Examples are: denatured proteins and peptides



PROPERTIES:

- Proteins can also be characterized by their chemical reactions. Most proteins are soluble in water, in alcohol, in dilute base or in various concentrations of salt solutions.
- Proteins have the characteristic coiled structure which is determined by the sequence of amino acids in the primary polypeptide chain and the stereo configuration of the radical groups attached to the alpha carbon of each amino acid.
- Proteins are heat labile exhibiting various degrees of lability depending upon type of protein, solution and temperature profile.
- Proteins can be reversible or irreversible, denatured by heating, by salt concentration, by freezing, by ultrasonic stress or by aging. Proteins undergo characteristic bonding with other proteins in the so-called plastein reaction and will combine with free aldyhyde and hydroxy groups of carbohydrates to form Maillard type compounds.

DENATURATION:

- The phenomenon of disorganization of native protein structure is known as denaturation.
- Denaturation results in the loss of secondary, tertiary and quaternary structure of proteins.
- This involves a change in physical, chemical and biological properties of protein molecules.

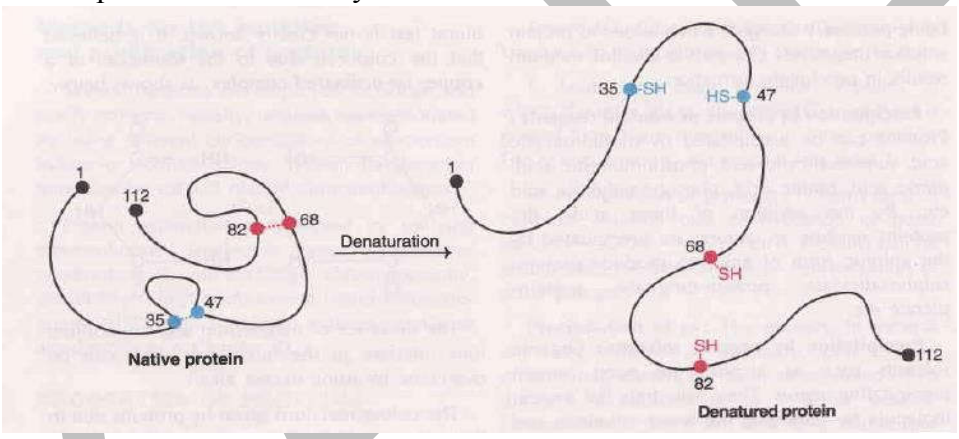
Agents of denaturation:

- **Physical agents**: Heat, violent shaking, X-rays, UV radiation.
- **Chemical agents:** Acids, alkalies, organic solvents (ether, alcohol), salts of heavy metals (Pb, Hg), urea, salicylate.

Characteristics of denaturation:

- The native helical structure of protein is lost.

- The primary structure of a protein with peptide linkages remains intact i.e., peptide bonds are not hydrolyzed.
- The protein loses its biological activity.
- Denatured protein becomes insoluble in the solvent in which it was originally soluble.
- The viscosity of denatured protein (solution) increases while its surface tension decreases.
- Denaturation is associated with increase in ionizable and sulfhydryl groups of protein.
- Denatured protein is more easily digested.
- Denaturation is usually irreversible.
- Careful denaturation is sometimes reversible (known as renaturation).
- Denatured protein cannot be crystallized.



Denaturation of protein

RENATURATION:

- The original structure of a protein is a three-dimensional structure.
  - The process of returning a denatured protein structure to its original structure and normal level of biological activity, or simply the remodification or folding of an unfolded polypeptide chain of proteins to its normal three-dimensional structure is known as reconstitution of protein.
  - This reconstitution of a protein structure is also known as renaturation of protein.
  - In other words, renaturation of proteins is technically the opposite of denaturation of proteins.

- In a renatured protein, the primary structure of the biopolymer remains the same, but the protein which had been denatured (with the help of such agents as chaotropic agents, detergents, heat or reagents) gets restored back to its former native structure (that is the native structure of the protein before it was denatured) and is able to function as effectively as before, because a renatured protein merely undergoes the process of reversal of a denatured protein.
- In fact, a renatured protein is able to carry out its functions better, faster and more efficiently, because it is able to pinpoint the level of biological activity that it was going through prior to the process of denaturation.

Orders of protein structure

Protein structure is the three-dimensional arrangement of atoms in an amino acid-chain molecule. Proteins are polymers – specifically polypeptides – formed from sequences of amino acids, the monomers of the polymer. A single amino acid monomer may also be called a residue indicating a repeating unit of a polymer. Proteins form by amino acids undergoing condensation reactions, in which the amino acids lose one water molecule per reaction in order to attach to one another with a peptide bond. By convention, a chain under 30 amino acids is often identified as a peptide, rather than a protein. To be able to perform their biological function, proteins fold into one or more specific spatial conformations driven by a number of non-covalent interactions such as hydrogen bonding, ionic interactions, Van der Waals forces, and hydrophobic packing. To understand the functions of proteins at a molecular level, it is often necessary to determine their three-dimensional structure. This is the topic of the scientific field of structural biology, which employs techniques such as X-ray crystallography, NMR spectroscopy, and dual polarisation interferometry to determine the structure of proteins.

Protein structures range in size from tens to several thousand amino acids. By physical size, proteins are classified as nanoparticles, between 1–100 nm. Very large aggregates can be formed from protein subunits. For example, many thousands of actin molecules assemble into a microfilament.

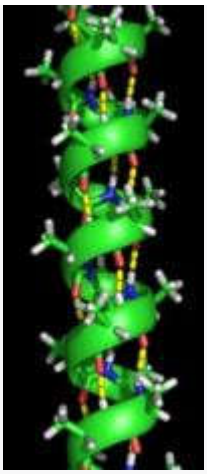
A protein generally undergo reversible structural changes in performing its biological function. The alternative structures of the same protein are referred to as different conformational isomers, or simply, conformations, and transitions between them are called conformational changes.

Primary structure

The primary structure of a protein refers to the sequence of amino acids in the polypeptide chain. The primary structure is held together by peptide bonds that are made during the process of protein biosynthesis. The two ends of the polypeptide chain are referred to as the carboxyl terminus (C- terminus) and the amino terminus (N-terminus) based on the nature of the free group on each

extremity. Counting of residues always starts at the N-terminal end (NH_2-group), which is the end where the amino group is not involved in a peptide bond. The primary structure of a protein is determined by the gene corresponding to the protein. A specific sequence of nucleotides in DNA is transcribed into mRNA, which is read by the ribosome in a process called translation. The sequence of amino acids in insulin was discovered by Frederick Sanger, establishing that proteins have defining amino acid sequences.[3][4] The sequence of a protein is unique to that protein, and defines the structure and function of the protein. The sequence of a protein can be determined by methods such as Edman degradation or tandem mass spectrometry. Often, however, it is read directly from the sequence of the gene using the genetic code. It is strictly recommended to use the words "amino acid residues" when discussing proteins because when a peptide bond is formed, a water molecule is lost, and therefore proteins are made up of amino acid residues. Post-translational modification such as disulfide bond formation, phosphorylations and glycosylations are usually also considered a part of the primary structure, and cannot be read from the gene. For example, insulin is composed of 51 amino acids in 2 chains. One chain has 31 amino acids, and the other has 20 amino acids.

Secondary structure



A α-helix with hydrogen bonds (yellow dots)

Secondary structure refers to highly regular local sub-structures on the actual polypeptide backbone chain. Two main types of secondary structure, the α-helixand the β-strand or β-sheets, were suggested in 1951 by Linus Pauling and coworkers. These secondary structures are defined by patterns of hydrogen bondsbetween the main-chain peptide groups. They have a regular geometry, being constrained to specific values of the dihedral angles ψ and φ on the Ramachandran plot. Both the α-helix and the β-sheet represent a way of saturating all the hydrogen bond donors and acceptors in the peptide backbone. Some parts of the protein are ordered but do not form any regular structures. They should not be confused with random coil, an unfolded polypeptide chain lacking any fixed three-dimensional structure. Several sequential secondary structures may form a "supersecondary unit".

Tertiary structure

Tertiary structure refers to the three-dimensional structure of monomeric and multimeric protein molecules. The α-helixes and β-pleated-sheets are folded into a compact globular structure. The folding is driven by the *non-specific* hydrophobic interactions, the burial of hydrophobic residues from water, but the structure is stable only when the parts of a protein domain are locked into place by *specific* tertiary interactions, such as salt bridges, hydrogen bonds, and the tight packing of side chains and disulfide bonds. The disulfide bonds are extremely rare in cytosolic proteins, since the cytosol (intracellular fluid) is generally a reducing environment.

Quaternary structure

Quaternary structure is the three-dimensional structure consisting of the aggregation of two or more individual polypeptide chains (subunits) that operate as a single functional unit. In this context, the quaternary structure is stabilized by the same non-covalent interactions and disulfide bonds as the tertiary structure. Complexes of two or more polypeptides (i.e. multiple subunits) are called multimers. Specifically it would be called a dimer if it contains two subunits, a trimer if it contains three subunits, a tetramer if it contains four subunits, and a pentamer if it contains five subunits. The subunits are frequently related to one another by symmetry operations, such as a 2- fold axis in a dimer. Multimers made up of identical subunits are referred to with a prefix of "homo- " (e.g. a homotetramer) and those made up of different subunits are referred to with a prefix of "hetero-", for example, a heterotetramer, such as the two alpha and two beta chains of hemoglobin.

Fibrous proteins

Fibrous proteins, also called scleroproteins, are long filamentous protein molecules

Fibrous proteins are only found in animals.

Fibrous proteins form 'rod' or 'wire' -like shapes and are usually inert structural or storage proteins. They are generally water-insoluble. Fibrous proteins are usually used to construct connective tissues, tendons, bone and muscle fiber.

Examples of fibrous proteins include keratins, collagens and elastins.

Fingernails and claws are made up of the common fibrous proteins, Keratin.

Collagen triple helix-Structure and assembly

The alpha helix (α-helix) is a common motif in the secondary structure of proteins and is a righthand-spiral conformation (i.e. helix) in which every backbone N−H group donates
a hydrogen bond to the backbone C=O group of the amino acid located three or four residues earlier along the protein sequence.

The alpha helix is also called a classic Pauling–Corey–Branson α-helix. The name 3.6_{13}-helix is also used for this type of helix, denoting the average number of residues per helical turn, with 13 atoms being involved in the ring formed by the hydrogen bond.

Among types of local structure in proteins, the α-helix is the most regular and the most predictable from sequence, as well as the most prevalent.

Globular proteins

Globular proteins or spheroproteins are spherical ("globe-like") proteins and are one of the common protein types (the others being fibrous, disordered and membrane proteins). Globular proteins are somewhat water-soluble (forming colloids in water), unlike the fibrous or membrane proteins. There are multiple fold classes of globular proteins, since there are many different architectures that can fold into a roughly spherical shape.

The term globin can refer more specifically to proteins including the globin fold.

Role

Unlike fibrous proteins which only play a structural function, globular proteins can act as:

- Enzymes, by catalyzing organic reactions taking place in the organism in mild conditions and with a great specificity. Different esterases fulfill this role.
- Messengers, by transmitting messages to regulate biological processes. This function is done by hormones, i.e. insulin etc.
- Transporters of other molecules through membranes
- Stocks of amino acids.
- Regulatory roles are also performed by globular proteins rather than fibrous proteins.
- Structural proteins, e.g., actin and tubulin, which are globular and soluble as monomers, but polymerize to form long, stiff fibers.

Models for haemoglobin allostery

Haemoglobin is an allosteric protein. This means that the binding of oxygen to one of the subunits is affected by its interactions with the other subunits. In fact the binding of oxygen to one haemoglobin subunit induces conformational changes (discussed before) that are relayed to the other subunits, making them more able to bind oxygen by raising their affinity for this molecule. Thus the binding of oxygen to haemoglobin is said to be cooperative. In contrast the binding of oxygen to the single polypeptide chain of myoglobin is noncooperative. This is clearly shown by

The curve for haemoglobin is said to be sigmoidal, which reflects its cooperative binding, whereas that for myoglobin is hyperbolic which reflects noncooperative binding. From the oxygen dissociation curve it can also be seen that for any particular oxygen partial pressure the degree of saturation of myoglobin is always higher than haemoglobin. Myoglobin therefore has a higher affinity for oxygen than does haemoglobin. This reflects the different functions of the two oxygen binding proteins. For example in blood capillaries (partial pressure of oxygen is approx 20 mmHg) haemoglobin will release its oxygen to myoglobin for storage there.

Fetal haemoglobin also has a high affinity for oxygen to supply the developing fetus with sufficient oxygen from the mothers blood.

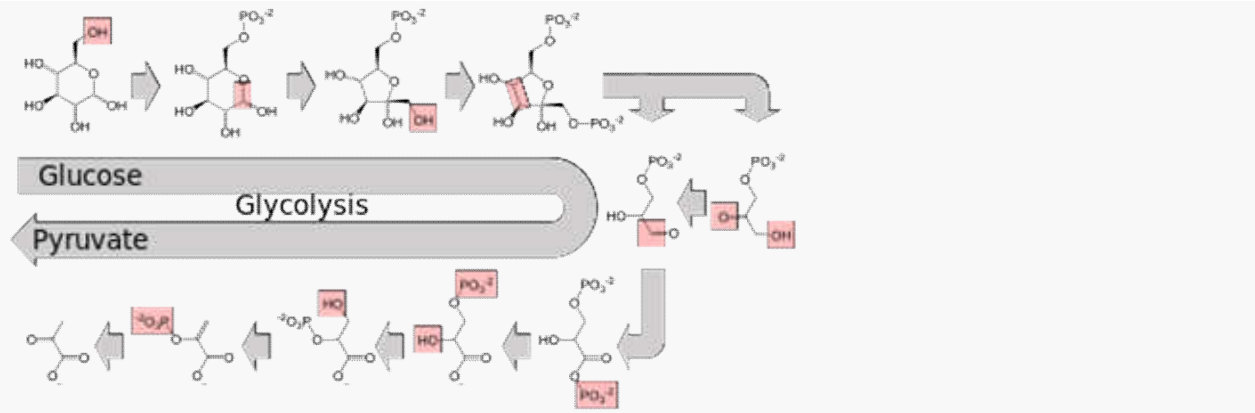
Protein function as enzymes

Enzymes serve a wide variety of functions inside living organisms. They are indispensable for signal transduction and cell regulation, often via kinases and phosphatases. They also generate movement, with myosin hydrolyzing ATP to generate muscle contraction, and also transport cargo around the cell as part of the cytoskeleton. Other ATPases in the cell membrane are ion pumps involved in active transport. Enzymes are also involved in more exotic functions, such as luciferase generating light in fireflies. Viruses can also contain enzymes for infecting cells, such as the HIV integrase and reverse transcriptase, or for viral release from cells, like the influenza virus neuraminidase.

An important function of enzymes is in the digestive systems of animals. Enzymes such as amylases and proteases break down large molecules (starch or proteins, respectively) into smaller ones, so they can be absorbed by the intestines. Starch molecules, for example, are too

large to be absorbed from the intestine, but enzymes hydrolyze the starch chains into smaller molecules such as maltose and eventually glucose, which can then be absorbed. Different enzymes digest different food substances. In ruminants, which have herbivorousdiets, microorganisms in the gut produce another enzyme, cellulase, to break down the cellulose cell walls of plant fiber.

Metabolism



The metabolic pathway of glycolysis releases energy by converting glucose to pyruvate via a series of intermediate metabolites. Each chemical modification (red box) is performed by a different enzyme.

Several enzymes can work together in a specific order, creating metabolic pathways. In a metabolic pathway, one enzyme takes the product of another enzyme as a substrate. After the catalytic reaction, the product is then passed on to another enzyme. Sometimes more than one enzyme can catalyze the same reaction in parallel; this can allow more complex regulation: with, for example, a low constant activity provided by one enzyme but an inducible high activity from a second enzyme.

Enzymes determine what steps occur in these pathways. Without enzymes, metabolism would neither progress through the same steps and could not be regulated to serve the needs of the cell. Most central metabolic pathways are regulated at a few key steps, typically through enzymes whose activity involves the hydrolysis of ATP. Because this reaction releases so much energy, other reactions that are thermodynamically unfavorable can be coupled to ATP hydrolysis, driving the overall series of linked metabolic reactions.

Defensive

Defensive proteins are know as antibodies and are found in the immune system. What would happen without this protein? Without this protein you are more likely to get sick and get diseases. The defensive proteins, also known as antibodies are produced by the body to fight diseases and prevent injury.

Transport Protein

A transport protein (variously referred to as a transmembrane pump, transporter, escort protein, acid transport protein, cation transport protein, or anion transport protein) is

a protein that serves the function of moving other materials within an organism. Transport proteins are vital to the growth and life of all living things. There are several different kinds of transport proteins.

Carrier proteins are proteins involved in the movement of ions, small molecules, or macromolecules, such as another protein, across a biological membrane. Carrier proteins are integral membrane proteins; that is, they exist within and span the membrane across which they transport substances. The proteins may assist in the movement of substances by facilitated diffusion (i.e., passive transport) or active transport. These mechanisms of movement are known as carrier-mediated transport. Each carrier protein is designed to recognize only one substance or one group of very similar substances. Research has correlated defects in specific carrier proteins with specific diseases. A membrane transport protein (or simply *transporter*) is a membrane protein[4] that acts as such a carrier.

A vesicular transport protein is a transmembrane or membrane associated protein. It regulates or facilitates the movement by vesicles of the contents of the cell.

POSSIBLE QUESTIONS

UNIT-II

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Give a detail about simple lipid
2. Explain in detail about glycolipids
3. Discuss about phospholipids

PART-C (8 MARKS)

` 1. Discuss in detail about GPI anchored proteins
2. Discuss in detail about lipoproteins classification
3. Explain in detail about cofactors and pigments
4. Discuss in detail about Apo lipoproteins
5. Explain in detail about antioxidants

Karpagam Academy of Higher Education
Department of Biochemistry
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18BCP101- Chemistry of Biopolymers

S. No.	II	Question	Option I	Option II	Option III	Option IV	Answer
1	2	The following are the structural proteins except	Collagen	Elastin	α- keratin	trypsin	trypsin
2	2	"The working horses" of the cell which perform dynamic	proteins	carbohydrates	lipids	nucleic acids	proteins
3	2	The content of nitrogen in protein is	15%	16%	25%	36%	16%
4	2	The three dimensional structure of a functional protein is called as	primary structure	secondary	tertiary	quarternary	tertiary structure
5	2	The spatial arrangement of two or more polypeptide chains is	primary structure	secondary	tertiary	quarternary	quarternary
6	2	The amino acids are held together in a protein by a ----------	covalent – peptide bond	covalent glycosidic bond	hydrogen bond	ionic bond	covalent – peptide bond
7	2	Pauling and Corey proposed	primary structure	secondary	tertiary	quarternary	secondary structure
8	2	The number of aminoacids in each turn of α-helix is	3.8	3.6	4.2	5	3.6
9	2	The spacing of each aminoacid in α-helix is	0.25nm	0.28nm	0.15nm	0.32nm	0.15nm
10	2	The most stable form of α-helix is	left handed α-helix	right handed α-	circular helix	spiral helix	right handed α-
11	2	The α-helix structure is disrupted by	Arginine	Lysine	Leucine	proline	proline
12	2	The N-terminal aminoacid of A-chain in Insulin molecule is	Glysine	Asparagines	phenyl alanine	alanine	Glysine
13	2	The C-terminal aminoacid of A-chain in Insulin molecule is	Glysine	Asparagine	phenyl alanine	alanine	Asparagine
14	2	The N-terminal aminoacid of B-chain in Insulin molecule is	Glysine	Asparagines	phenyl alanine	alanine	phenyl alanine
15	2	The C-terminal aminoacid of B-chain in Insulin molecule is	Glysine	Asparagines	phenyl alanine	alanine	alanine
16	2	The following is one of the transport protein	Keratin	Collagen	serum albumin	insulin	serum albumin
17	2	The connective tissue protein collagen lacks	Tyrosine	Tryptophan	Histidine	Glysine	Tryptophan
18	2	Human hair keratin contains about	14% cysteine	14% cystine	14% histidine	14% tryptophan	14% cysteine
19	2	The mucoprotein in saliva is	Ovomucoid	Mucin	Casein	Vitelline	Mucin
20	2	The following are complete proteins	gelatin and wheat protein	gelatin and zein	wheat and rice protein	egg albumin and milk casein	egg albumin and milk casein
21	2	In quaternary structure subunits are linked by	peptide bonds	disulphide	covalent bonds	non-covalent	non-covalent bonds
22	2	The largest protein amongst the following is	Fibrinogen	Globulin	Albumin	Hemoglobin	Fibrinogen
23	2	Among the following, an essential aminoacid is	Phenylalanine	Tyrosine	Proline	Hydroxyproline	Phenylalanine
24	2	A disulphide bond can be formed between	two glycine residues	two cysteine residues	an alanine and a cysteine	a proline and a cysteine residues	two cysteine residues
25	2	An aminoacid that does not takes part in a-helix formation is	Alanine	Leucine	Proline	Isoleucine	Proline
26	2	A protein rich in cysteine is	Collagen	Keratin	Haemoglobin	Gelatin	Keratin
27	2	A protein rich in proline is	Protamine	Prothrombin	Procollagen	Proinsulin	Procollagen
28	2	Primary structure of a protein is broken by	Heat	ammonium	pepsin	amylase	pepsin
29	2	Electrostatic bonds can be formed between the side chains of	alanine and leucine	leucine and	aspartate and	lysine and	lysine and aspartate
30	2	The following is a metalloprotein	Hemoglobin	Myoglobin	Ferritin	insulin	Ferritin
31	2	Quarternary structure is present in	coagulated proteins	denatured	haemoglobin	myoglobin	haemoglobin
32	2	Primary structure of a protein is formed by	hydrogen bonds	peptide bonds	electrostatic	vanderwaals forces	peptide bonds
33	2	α-helix is formed by	hydrogen bonds	hydrophobic	electrostatic	sulphide bonds	hydrogen bonds
34	2	Glutelins are present in	Milk	Eggs	Meat	cereals	cereals
35	2	The α-helix and β-pleated sheet are examples of	primary structure	secondary	tertiary	quarternary	secondary structure
36	2	The following protein has a tertiary structure	Insulin	Myoglobin	Keratin	Collagen	Myoglobin
37	2	The following proteins has a tertiary structure except	Proinsulin	myoglobin	ribonuclease	collagen	collagen
38	2	The metal ion present in ceruloplasmin is	Cu	Zn	Fe	Co	Cu
39	2	A metal ion present in carbonic anhydrase is	Cu	Zn	Fe	Co	Zn
40	2	The rice protein lacks	Lysine	tryptophan	phenylalanine	tyrosine	Lysine
41	2	The most abundant protein in mammals is	Albumin	Hemoglobin	Collagen	Elastin	Collagen
42	2	The repeating units of proteins are	Glucose	amino acids	fattyacids	peptides	amino acids
43	2	Amino acids are joined by	peptide bonds	hydrophobic	hydrogen bond	Elastin	peptide bonds
44	2	The primary structure of protein represents	Linear sequence of am	sub unit structu	helical structure	3-dimensional struct	Linear sequence of am
45	2	Peptide bond is	rigid with partial doubl	planar, covalent	covalent	all of the above	all of the above
46	2	Enzymes are	proteins	carbohydrates	nucleic acids	DNA molecule	proteins
47	2	The first protein sequenced by Frederick Sanger is	Haemoglobin	myoglobin	insulin	myosin	insulin
48	2	A dipeptide has	2 amino acids and 1 pe	2 amino acids an	2 amino acids and	2 amino acids and 4	2 amino acids and 1 p
49	2	The most common secondary structure is	α-helix	β-pleated sheet	β-pleated sheet p	β-pleated sheet non	α-helix
50	2	Myoglobin is a	protein with primary s	protein with sec	protein with terti	protein with quatern	protein with tertiary s
51	2	Fibrous protein such as silk fibroin consists of polypeptide chains arrang	α-helix	β-pleated sheet	β-helix	none of these	β-pleated sheet
52	2	α-helix has	3.4 amino acid residue	3.6 amino acid r	3.8 amino acid re	3.0 amino acid residu	3.6 amino acid residue
53	2	Tertiary structure is maintained by	peptide bond	hydrogen bond	di-sulphide bond	all of the above	all of the above
54	2	The 3-D structure of protein can be determined by	Nuclear magnetic reso	X-ray	both a and b	Spectroscopy	both a and b
55	2	. Disulphide bonds are formed between	cysteine residues that	cystine residues	proline residues t	histidine residues tha	cysteine residues that
56	2	Haemoglobin has	primary structure	secondary struc	tertiary structure	quaternery structure	quaternery structure
57	2	Which of the following statements about amino acids is correct?	Amino acids are clas	Amino acids ar	Amino acids in p	twenty four amino	Amino acids are clas
58	2	Which term below best defines the 'quaternary structure' of a prot	The arrangement of	he folding of the		The interaction of a	he arrangement of t
59	2	Which of the following statements about collagen is correct?	Collagen contains a	Collagen is a g	Post-translation	he structure of coll	Collagen contains a
60	2	Which of the following statements about haemoglobin is correct?	2,3-Bisphosphoglyce	Deoxygenated	Haemoglobin h	One molecule of ha	Deoxygenated haem

UNIT-III

SYLLABUS

Lipids: Introduction, classification, structure and functions of simple lipid, compound lipids-phospholipids, glycolipids, storage lipids and choesterol. Eicosanoids-porstaglandins, thromboxanes and leucotriens. Properties of lipids-Micelles, bilayers and liposomes. Significance of lipid anchored protein-prenylated, fatty acylated and GPI anchored proteins. Lipoproteins – classification, composition and biological functions. Lipids as signals, cofactors and pigments (Brief account). Lipid peroxidation and antioxidants.

Lipids

Definition

- Lipids 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.
- The main biological functions of lipids include energy storage, as structural components of cell membranes, and as important signaling molecules.

CLASSIFICATION OF LIPIDS:

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

1. Simple lipids:

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

(a) 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.

(b) 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.

2. 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

(a) 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.

(b) 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.

(c) Lipoproteins:

- Macromolecular complexes of lipids with proteins.

(d) Other complex lipids:

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

3. 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.

4. 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 LIPIDS:

FATS:

- Fat, any substance of plant or animal origin that is nonvolatile, insoluble in water, and oily or greasy to the touch. 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.

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.*

COMPOUND LIPIDS:

PHOSPHOLIPIDS

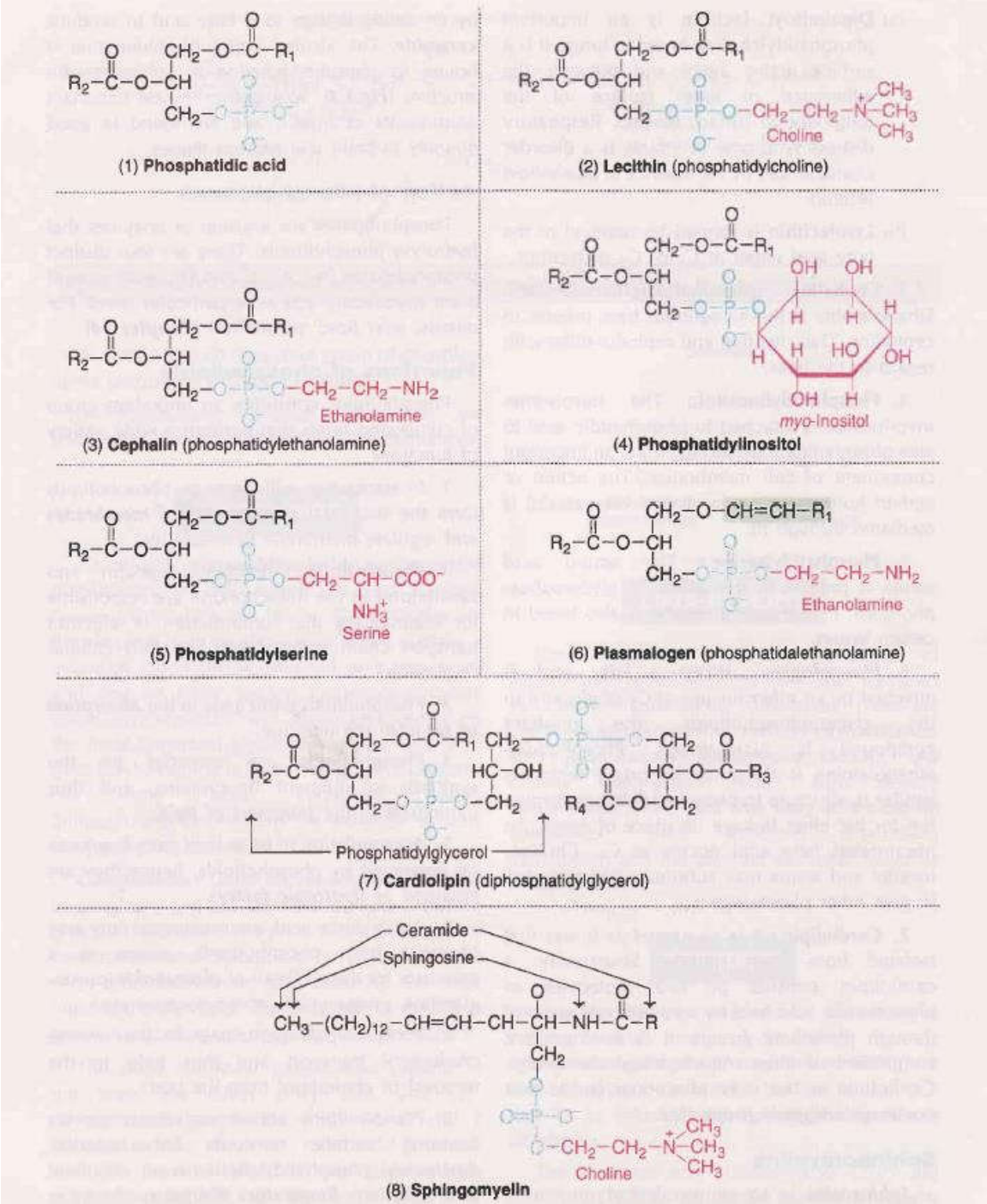
- These are complex or compound lipids containing phosphoric acid, in addition to fatty acids, nitrogenous base and alcohol.
- There are two classes of phospholipids
- Glycerophospholipids (or phosphoglycerides) that contain glycerol as the alcohol.
- Sphingophospholipid (or sphingomyelins) that contains phingosine as the alcohol

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.

- **Cephafins (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 plasmaloge.
- **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

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 eicosanoid s (prostaglandins, prostacyclinst, hromboxanese tc.).
- 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 is 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.

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, from 4 to 28.
- Fatty acids are usually derived from triglycerides or phospholipids.
- When they are not attached to other molecules, they are known as "free" fatty acids.
- 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

SATURATED AND UNSATURATED FATTY ACIDS:

- Saturated fatty acids do not contain double bonds, while unsaturated fatty acids contain one or more double bonds.
- Both saturated and unsaturated fatty acids almost equally occur in the natural lipids.
- Fatty acids with one double bond are monounsaturated and those with 2 or more double bonds are collectively known as polyunsaturated fatty acids (PIJFA).

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.

SIGNIFICANCE:

- Essential Fatty Acids (EFA's) are considered the building blocks of the membranes for every cell in our body.
- The term essential means that our bodies do not produce these acids; therefore we must consume them in the foods we eat.
- Twenty different fatty acids that our body's need. Surprisingly they are all made from two basic acids, Linoleic Acid and Linolenic Acid. Linoleic Acid is part of the Omega-6 acids and Linolenic Acid is part of the Omega-3 acids.
- These two fatty acids are needed by our bodies to create and maintain the integrity of our cell membranes, regulate chemical processes that occur in our cells, and to maintain proper kidney functions.
- One part of the definition of oleic means derived from oil.
- Most people get plenty of Omega-6 fatty acids in their diet by consuming approximately a tablespoon of polyunsaturated plant oils per day.
- It would be easy if simply consuming Omega-3 and Omega-6 fatty acids would get us the needed nutrition for cell functions.

- Unfortunately studies on these important acids indicate that a proper balance or ratio of Omega-3 to Omega-6 is needed by our bodies to use them efficiently.

- An easy way to check if you are getting enough EFA's in your diet is to monitor the dryness of your skin.

- If your skin is too dry (watch during the changing of the seasons), your body may be indicating to you that it needs more Essential Fatty Acids.
- If you're getting enough EFA's your skin should be soft to the touch.

Eicosanoids are signaling molecules made by the enzymatic or non-enzymatic oxidation of arachidonic acid or other polyunsaturated fatty acids (PUFAs) that are, similar to arachidonic acid, 20 carbon units in length. Eicosanoids are a sub-category of oxylipins, i.e. oxidized fatty acids of diverse carbon units in length, and are distinguished from other oxylipins by their overwhelming importance as cell signaling molecules. Eicosanoids function in diverse physiological systems and pathological processes such as: mounting or inhibiting inflammation, allergy, fever and other immune responses; regulating the abortion of pregnancy and normal childbirth; contributing to the perception of pain; regulating cell growth; controlling blood pressure; and modulating the regional flow of blood to tissues. In performing these roles, eicosanoids most often act as autocrine signaling agents to impact their cells of origin or as paracrine signaling agents to impact cells in the proximity of their cells of origin. Eicosanoids may also act as endocrine agents to control the function of distant cells. There are multiple subfamilies of eicosanoids, including most prominently the prostaglandins, thromboxanes, leukotrienes, lipoxins, resolvins, and eoxins. For each subfamily, there is the potential to have at least 4 separate series of metabolites, two series derived from ω-6 PUFAs (arachidonic and dihomo-gamma-linolenic acids), one series derived from the ω-3 PUFA (eicosapentaenoic acid), and one series derived from the ω-9 PUFA (mead acid). This subfamily distinction is important. Mammals, including humans, are unable to convert ω-6 into ω- 3 PUFA. In consequence, tissue levels of the ω-6 and ω-3 PUFAs and their corresponding eicosanoid metabolites link directly to the amount of dietary ω-6 versus ω-3 PUFAs consumed. Since certain of the ω-6 and ω-3 PUFA series of metabolites have almost diametrically opposing physiological and pathological activities, it has often been suggested that the deleterious consequences associated with the consumption of ω-6 PUFA-rich diets reflects excessive production and activities of ω-6 PUFA-derived eicosanoids while the beneficial effects associated with the consumption of ω-3 PUFA-rich diets reflect the excessive production and activities of ω-3 PUFA-derived eicosanoids. In this view, the opposing effects of ω-6 PUFA-derived and ω-3 PUFA-derived eicosanoids on key target cells underlie the detrimental and beneficial effects of ω-6 and ω-3 PUFA-rich diets on inflammation and allergy reactions, atherosclerosis, hypertension, cancer growth, and a host of other processes.

Properties of lipids

Chemical Properties:

1. Saponification number:

The number of milligrams of KOH required to saponify 1 gm. of fat or oil

2. Iodine Value:

The iodine value is the number which expresses in grams the quantity of Iodine, which is absorbed by 100 g of the substance.

3. Acid Value

The acid value is the number of mg of potassium hydroxide required to neutralize the free acid in 1 g of the substance.

4. Acetyl Value:

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

5. Polenske number:

The number of milliliters of 0.1 normal KOH required to neutralize the insoluble fatty acids from 5 gms of fat.

6. Reichert-Miessel 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.

7. Rancidity:

Nearly all natural fats are oxidized when exposed to air, light, moisture, particularly, if warm. It develops an unpleasant odour and taste.

Lipoproteins

A lipoprotein is a biochemical assembly whose purpose is to transport hydrophobic lipid (a.k.a. fat) molecules in water, as in blood or extracellular fluid. They have a single- layer phospholipid and cholesterol outer shell, with the hydrophilic portions oriented outward toward the surrounding water and lipophilic portions of each molecule oriented inwards toward the lipids molecules within the particles. Apolipoproteins are embedded in the membrane, both stabilising the complex and giving it functional identity determining its fate. Thus the complex serves to emulsify the fats. Many enzymes, transporters, structural proteins, antigens, adhesions, and toxins are lipoproteins. Examples include the plasma lipoprotein particles classified as HDL, LDL, IDL, VLDL and ULDL (a.k.a. chylomicrons) lipoproteins, according to density / size (an inverse relationship), compared with the surrounding plasma water. These complex protein capsules enable fats to be carried in all extracellular water, including the blood stream (an example of emulsification), subgroups of which are primary drivers / modulators of atherosclerosis, the transmembrane proteins of mitochondrion, chloroplast, and bacterial lipoproteins. Proteolipids are a different kind of protein-lipid combination that are insoluble in water. Proteolipids are abundant in brain tissue, and are also present in many other animal and plant tissues.

Lipid peroxidation

Lipid peroxidation is the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene bridges ($-CH_2-$) that possess especially reactive hydrogen atoms. As with any radical reaction, the reaction consists of three major steps: initiation, propagation, and termination. The chemical products of this oxidation are known as lipid peroxides or lipid oxidation products (LOPs).

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., catalase and superoxide dismutase), produced internally, or the dietary antioxidants vitamin A, vitamin C, and vitamin E.

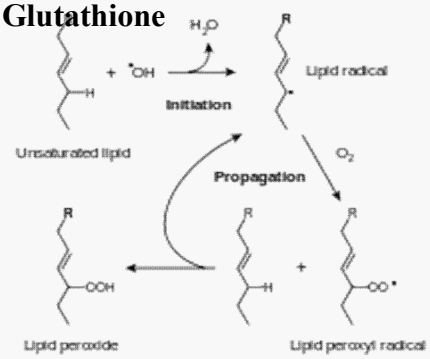
The term "antioxidant" is mostly used for two entirely different groups of substances: industrial chemicals that are added to products to prevent oxidation, and naturally occurring compounds that are present in foods and tissue. The former, industrial antioxidants, have diverse uses: acting as preservatives in food and cosmetics, and being oxidation-inhibitors in fuels.

Vitamin C

Ascorbic acid or "vitamin C" is a monosaccharide oxidation-reduction (redox) catalyst found in both animals and plants. As one of the enzymes needed to make ascorbic acid has been lost by mutation during primate evolution, humans must obtain it from the diet; it is therefore a vitamin.

Most other animals are able to produce this compound in their bodies and do not require it in their diets. Ascorbic acid is required for the conversion of the procollagen to collagen by oxidizing proline residues to hydroxyproline. In other cells, it is maintained in its reduced form by reaction with glutathione, which can be catalysed by protein disulfide isomerase and glutaredoxins. Ascorbic acid is a redox catalyst which can reduce, and thereby neutralize, reactive oxygen species such as hydrogen peroxide. In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the redox enzyme ascorbate peroxidase, a function that is particularly important in stress resistance in plants Ascorbic acid is present at high levels in all parts of plants and can reach concentrations of 20 millimolar in chloroplasts.

Glutathione



The free radical mechanism of lipid peroxidation.

Glutathione is a cysteine-containing peptide found in most forms of aerobic life. It is not required in the diet and is instead synthesized in cells from its constituent amino acids. Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems, such as ascorbate in the glutathione-ascorbate cycle, glutathione peroxidases and glutaredoxins, as well as reacting directly with oxidants. Due to its high concentration and its central role in maintaining the cell's redox state, glutathione is one of the most important cellular antioxidants. In some organisms glutathione is replaced by other thiols, such as by mycothiol in the Actinomycetes, bacillithiol in some Gram-positive bacteria, or by trypanothione in the Kinetoplastids.

Vitamin E

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins with antioxidant properties. Of these, α-tocopherol has been most studied as it has the highest bioavailability, with the body preferentially absorbing and metabolising this form. It has been claimed that the α-tocopherol form is the most important lipid-soluble antioxidant, and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. This removes the free radical intermediates and prevents the propagation reaction from continuing. This reaction produces oxidised α-tocopheroxyl radicals that can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol. This is in line with findings showing that α-tocopherol, but not

water-soluble antioxidants, efficiently protects glutathione peroxidase 4 (GPX4)-deficient cells from cell death. GPx4 is the only known enzyme that efficiently reduces lipid-hydroperoxides within biological membranes.

However, the roles and importance of the various forms of vitamin E are presently unclear, and it has even been suggested that the most important function of α-tocopherol is as a signaling molecule, with this molecule having no significant role in antioxidant metabolism. The functions of the other forms of vitamin E are even less well understood, although γ-tocopherol is a nucleophile that may react with electrophilic mutagens, and tocotrienols may be important in protecting neurons from damage.

As with the chemical antioxidants, cells are protected against oxidative stress by an interacting network of antioxidant enzymes. Here, the superoxide released by processes such as oxidative phosphorylation is first converted to hydrogen peroxide and then further reduced to give water. This detoxification pathway is the result of multiple enzymes, with superoxide dismutases catalysing the first step and then catalases and various peroxidases removing hydrogen peroxide. As with antioxidant metabolites, the contributions of these enzymes to antioxidant defenses can be hard to separate from one another, but the generation of transgenic mice lacking just one antioxidant enzyme can be informative.

Superoxide dismutase, catalase, and peroxiredoxins

Superoxide dismutases (SODs) are a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide. SOD enzymes are present in almost all aerobic cells and in extracellular fluids. Superoxide dismutase enzymes contain metal ion cofactors that, depending on the isozyme, can be copper, zinc, manganese or iron. In humans, the copper/zinc SOD is present in the cytosol, while manganese SOD is present in the mitochondrion. There also exists a third form of SOD in extracellular fluids, which contains copper and zinc in its active sites. The mitochondrial isozyme seems to be the most biologically important of these three, since mice lacking this enzyme die soon after birth. In contrast, the mice lacking copper/zinc SOD (Sod1) are viable but have numerous pathologies and a reduced lifespan (see article on superoxide), while mice without the extracellular SOD have minimal defects (sensitive to hyperoxia). In plants, SOD isozymes are present in the cytosol and mitochondria, with an iron SOD found in chloroplasts that is absent from vertebrates and yeast.

Catalases are enzymes that catalyse the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor. This protein is localized to peroxisomes in most eukaryotic cells. Catalase is an unusual enzyme since, although hydrogen peroxide is its only substrate, it follows a ping-pong mechanism. Here, its cofactor is oxidised by one molecule of hydrogen peroxide and then regenerated by transferring the bound oxygen to a second molecule of substrate. Despite its apparent importance in hydrogen peroxide removal, humans with genetic deficiency of catalase — "acatalasemia" — or mice genetically engineered to lack catalase completely, suffer few ill effects.

Decameric structure of AhpC, a bacterial 2-cysteine peroxiredoxin from *Salmonella typhimurium*.[141]

Peroxiredoxins are peroxidases that catalyze the reduction of hydrogen peroxide, organic hydroperoxides, as well as peroxynitrite.[142] They are divided into three classes: typical 2-cysteine peroxiredoxins; atypical 2-cysteine peroxiredoxins; and 1-cysteine peroxiredoxins.[143]These enzymes share the same basic catalytic mechanism, in which a redox-active cysteine (the peroxidatic cysteine) in the active site is oxidized to a sulfenic acid by the peroxide substrate.[144] Over-oxidation of this cysteine residue in peroxiredoxins inactivates these enzymes, but this can be reversed by the action of sulfiredoxin. Peroxiredoxins seem to be important in antioxidant metabolism, as mice lacking peroxiredoxin 1 or 2 have shortened lifespan and suffer from hemolytic anaemia, while plants use peroxiredoxins to remove hydrogen peroxide generated in chloroplasts.

Thioredoxin and glutathione systems

The thioredoxin system contains the 12-kDa protein thioredoxin and its companion thioredoxin reductase. Proteins related to thioredoxin are present in all sequenced organisms. Plants, such as *Arabidopsis thaliana,* have a particularly great diversity of isoforms.The active site of thioredoxin consists of two neighboring cysteines, as part of a highly conserved CXXC motif, that can cycle between an active dithiol form (reduced) and an oxidized disulfide form. In its active state, thioredoxin acts as an efficient reducing agent, scavenging reactive oxygen species and maintaining other proteins in their reduced state. After being oxidized, the active thioredoxin is regenerated by the action of thioredoxin reductase, using NADPH as an electron donor. The glutathione system includes glutathione, glutathione reductase, glutathione peroxidases, and glutathione *S*-transferases. This system is found in animals, plants and microorganisms. Glutathione peroxidase is an enzyme containing four selenium-cofactors that catalyzes the breakdown of hydrogen peroxide and organic hydroperoxides. There are at least four different glutathione peroxidase isozymes in animals. Glutathione peroxidase 1 is the most abundant and is a very efficient scavenger of hydrogen peroxide, while glutathione peroxidase 4 is most active with lipid hydroperoxides. Surprisingly, glutathione peroxidase 1 is dispensable, as mice lacking this enzyme have normal lifespans, but they are hypersensitive to induced oxidative stress. In addition, the glutathione *S*-transferases show high activity with lipid peroxides. These enzymes are at particularly high levels in the liver and also serve in detoxification metabolism.

POSSIBLE QUESTIONS

UNIT-III

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Give a detail about simple lipid
2. Explain in detail about glycolipids
3. Discuss about phospholipids

PART-C (8 MARKS)

` 1. Discuss in detail about GPI anchored proteins
2. Discuss in detail about lipoproteins classification
3. Explain in detail about cofactors and pigments
4. Discuss in detail about Apo lipoproteins
5. Explain in detail about antioxidants

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S. No.	III	Question	Option I	Option II	Option III	Option IV	Answer
1	3	Fats are solids at	10^0C	20^0C	30^0C	40^0C	20^0C
2	3	Hydrolysis of vegetable oil gives	fatty acid & glycine	fatty acid & glycol	fatty acid & glycerol	fatty acid & glyoxal	fatty acid & glycerol
3	3	Fatty acid 18:1:9 is	Staric acid	Linoleic acid	Palmitic acid	Oleic acid	Oleic acid
4	3	Essential fatty acid is	lenoleic acid	Palmitic acid	Stearic acid	Myristic acid	lenoleic acid
5	3	The lipid which is also having hormonal function is	Vitamin A	Vitamin D	Stearic acid	Etanolamine	Vitamin D
6	3	Oils which do not solidify at low temperature contain	More of saturated fatty acids	more of unsaturated fatty acids	less ofunsaturated fatty acid	saturated	more of unsaturated fatty acids
7	3	The melting point of a fat is higher when it has	higher unsaturated fatty acids	higher short chain fatty acids	higher saturated fatty acids	lower saturated fatty acids	higher saturated fatty acids
8	3	The structure of lenoleic acid is represented by the symbol	18:00	18:19	$_{18:2}{}^{9,12}$	$_{8:3}{}^{9,12,15}$	$_{18:2}{}^{9,12}$
9	3	Butter contains	more of saturated fatty acid	more of unsaturated fatty acids	arachidonic acid	fatty acid & glyoxal	more of saturated fatty acid
10	3	The combination of an amino alcohol, fatty acid & sialic acid form	Phospholipids	sulpholipids	Glycolipids	amino lipids	Glycolipids
11	3	The rate of fatty acid oxidation is increased by	Phospholipids	Glyco lipids	amino lipids	all of the above	Phospholipid s
12	3	Cardiolipin found in mitochondria is formed from	Lipositol	Phosphotidyl ethanolamine	Phosphatidyl Glycerol	glycerol	Phosphatidyl Glycerol
13	3	Iodine value of an shows the extent of	Polymerisation	molecular size	unsaturation	esterification	esterification
14	3	Hydrolysis of fat by alkali is called	Saponification	acid number	iodine number	both b and c	Saponificatio n
15	3	The number of milliliters of 0.1N KOH required to neutralize the insoluble fatty acids from 5gms of fat is called	Acid number	Acetyl number	Halogen number	Polenske number	Polenske number
16	3	Lecithin contains a nitrogenous base	Ethanolamine	choline	inositol	aminogroup	choline
17	3	Lecithin contains an unsaturated fatty acid at position	α	α+β	αβ	β	β
18	3	Lecithins are soluble in ordinary fat solvents except	Benzene	ethyl alcohol	methanol	metyl alcohol	ethyl alcohol
19	3	When lecithins are exposed to air	Black	Brown	yellow	Red	Brown
20	3	The most abundant group of phospholipids in the	phosphatidic acid	lecithins	cephalins	phosphatidyl serine	lecithins
21	3	Sphingosine contains	18 carbon	12 carbon	13 carbon	15 carbon	18 carbon
22	3	Lecithins combine to protein to form	phospoho protein	muco protein	lipo protein	glycoprotein	lipo protein
23	3	Phosphotidyl inositol is found in	Cabbage	soybean	cauliflower	Apples	soybean
24	3	Spingosine is present in	cardiolipin	diacylglycerol	ceramide	Lecithin	cardiolipin
25	3	The nitrogen base in the sphingomyelin is	choline	serine	ethanolamine	a complex amino alcohol	choline
26	3	The concentrations of sphingomyelins are increased in	Gaucher's disease	fabry' disease	febrile disease	Niemann-pick disease.	Niemann-pick disease
27	3	Spingomyelins contain a complex amino alcohol named as	Seddddrine	lyso lecithin	spingosine	Glycol	spingosine
28	3	Glcolipids contain an amino alcohol	spingosinse	iso spingosine	both	all of the above	all of the above
29	3	kerasin contains	nervonic acid	hydroxy nervonic acid	cerebronic acid	lignoceric acid	nervonic acid
30	3	Oxynervon contains	nervonic acid	hydroxy nervonic acid	cerebronic acid	lignoceric acid	hydroxy nervonic acid
31	3	Gaucher's disease is characterized by the eye in	Lignoceric acid	nervonic acid	cerebronic acid	hydroxy nervonic acid	cerebronic acid
32	3	Gangliosides are the glycolipids occurring in	Liver	Brain	Kidney	Muscle	Brain
33	3	The most abundant lipid is	Triglycerides	Waxes	phospholipid	Cholesterol	Triglycerides
34	3	Gangliosides are	Glycospingolipids	lipoproteins	glycophospholipid	waxes	Glycospingol ipids
35	3	The lipoprotein which has the largest size and least density is	LDL	HDL	VLDL	Chylomicrons	Chylomicrons
36	3	Lipoproteins precent in cell membrane is by nature	hydrophobic	Hydrophilic	both	all of the above	all of the above
37	3	The density of lipoproteins increases as the protein content	rises	decreases	highly decreases	slightly & promptly decreas	rises
38	3	Lipo proteins may be identified more accurately by means of	Electrophoresis	centrifugation	Immuno electrophoresis	Ultra centrifugation	Immuno electrophores is
39	3	Very low density lipo proteins are also known as	β-lipoprot - β -lipeins	preoproteins	α- lipo proteins	beta lipo protein	preoproteins
40	3	Adults need ___milligrams of phosphorus daily.	700	500	600	400	700
41	3	The protein moiety of lipoprotein is known as	Apoprotein	preprotein	post protein	Pseudoprotein	Apoprotein
42	3	The β-lipoprotein fraction increases in severe	Diabetes mellitus	Uremia	nephritis	muscular dystrophy	Diabetes mellitus
43	3	Adultration of butter can be tested by	Acid number	iodine number	RM number	All	RM number
44	3	Bee wax contains	Stearic acid	Myricyl palmitate	Oleic acid	Linolenic acid	Myricyl palmitate
45	3	Rancidity occurs when fats are exposed to	air	moisture	light	All	All
46	3	A lipid containing steroid ring is	Plasmalogens	sphingomyelin	cholesterol	none	cholesterol
47	3	NANA is a component of	spingomyelin	ganglioside	cardiolipin	Plasmalogen	cardiolipin
48	3	The phospholipid containing an ether linkage at C1 is known as	Plasmalogens	Cardiolipins	Cephalin	Lecithin	Plasmalogen
49	3	Which one of the following lipid has detergent activity	Triacylglycerides	Glycolipid	phospholipid	Cholesterol	Triacylglyceri des
50	3	The degree of adulteration of given oil is determined by measuring	saponification num	Acid number	RM number	iodine number	RM number
51	3	Fat serve as a efficient source of	metabolic activity	energy storage	enezyme activity	starch	energy storage
52	3	Lipid serve as a structural compound of	cell membrane	signaling	both a & b	cell membfane	both a & b
53	3	Hydrocarbons may be formed at	low temperature	high temperature	low boiling point	high boiling point	high temperature
54	3	Sphingomyleins are found in large quantities in	brain	nerves tissues	both a & b	kidney	both a & b
55	3	Gangliosides are glycolipids occur in	kidney	liver	brain	spleen	brain
56	3	Predominant lipids in cholesterol	LDL	HDL	VLDL	sterol	LDL
57	3	Predominant lipids in phospholipids	LDL	HDL	VLDL	sterol	HDL

UNIT-IV

SYLLABUS

Nucleic acids: DNA double helical structure – Watson and Crick model. A, B and Z forms of DNA. Tertiary and quadraplex structures of DNA. DNA supercoiling and linking number. Properties of DNA – DNA bending, buoyant density, viscosity, denaturation and renaturation
– The cot curve – Chemical synthesis of DNA.
Major classes of RNA – mRNA, rRNA, tRNA, sn RNA, siRNA, hn RNA – structure and biological functions. Secondary and tertiary structure of tRNA and rRNA.

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)

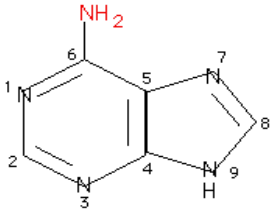
Nucleic Acid Structure

- 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

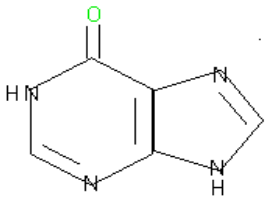
STRUCTURE OF PURINES AND PYRIMIDINES:

PURINES:

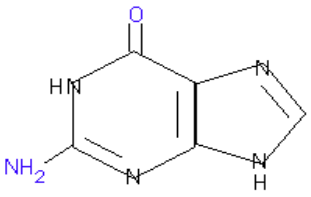
- A purine is a heterocyclic aromatic organic compound, consisting of a pyrimidine ring fused to an imidazole ring.
- Adenine = 6-amino purine
- Guanine = 2-amino-6-oxy purine
- Hypoxanthine = 6-oxy purine
- Xanthine = 2,6-dioxy purine



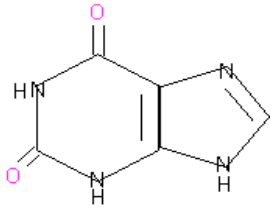
Adenine



Hypoxanthine



Guanine

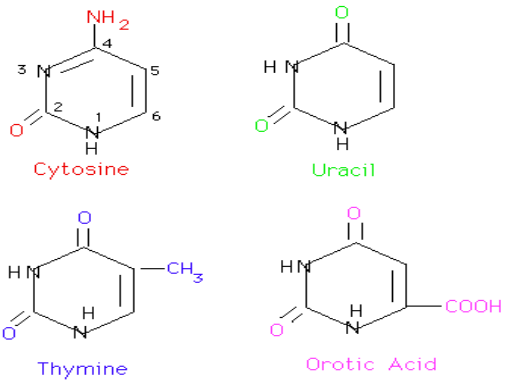


Xanthine

- 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

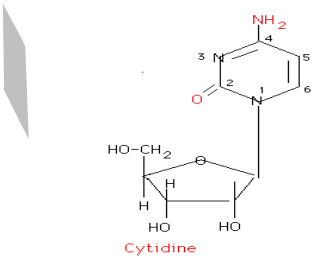


- Cytosine is found in both DNA and RNA.
- Uracil is found only in RNA.
- Thymine is normally found in DNA.
- Sometimes tRNA will contain some thymine as well as uracil.

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.

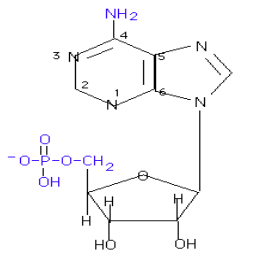
- The names of purine nucleosides end in -osine and the names of pyrimidine nucleosides end in -idine.
- The convention is to number the ring atoms of the base normally and to use l', etc. to distinguish the ring atoms of the sugar.
- Unless otherwise specificed, the sugar is assumed to be ribose.
- 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

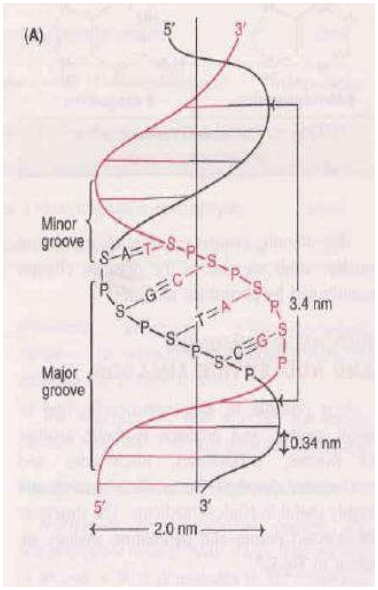
DNA:

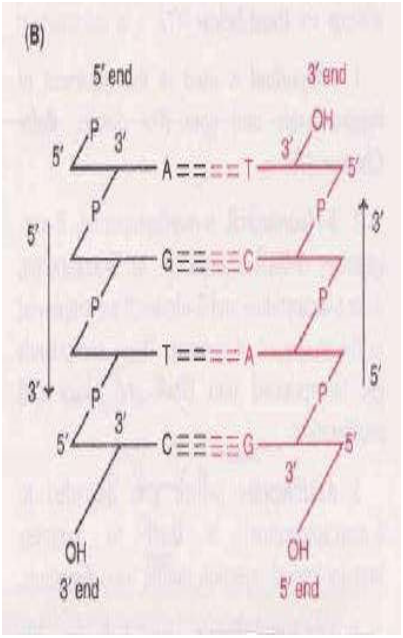
- DNA is a polymer of deoxyribonucleotides (or simply deoxynucleotides).

- 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.

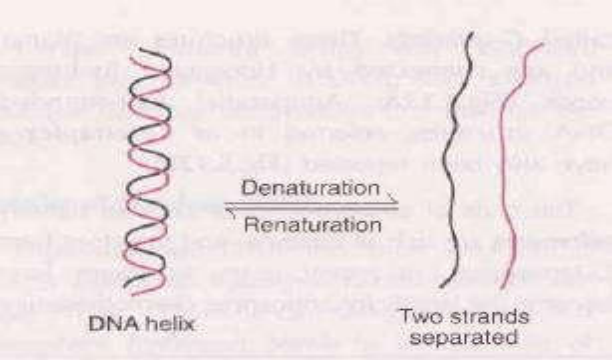
Complementary base paring in DNA (A) Thymine pairs with adenine by 2 hydrogen bonds (B) Cytosine pairs with guanine by 3 hydrogen bonds.

- 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%

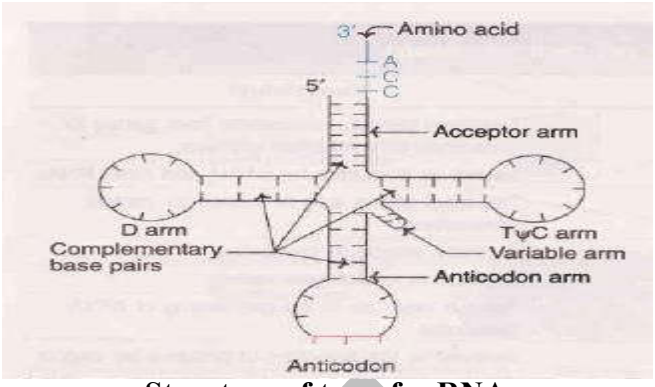
Messenger RNA (mRNA)

- 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,t he 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 T· C arm : This arm contains a sequence of T, pseudouridine (represented by Psi, ·) 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 T· C 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”.

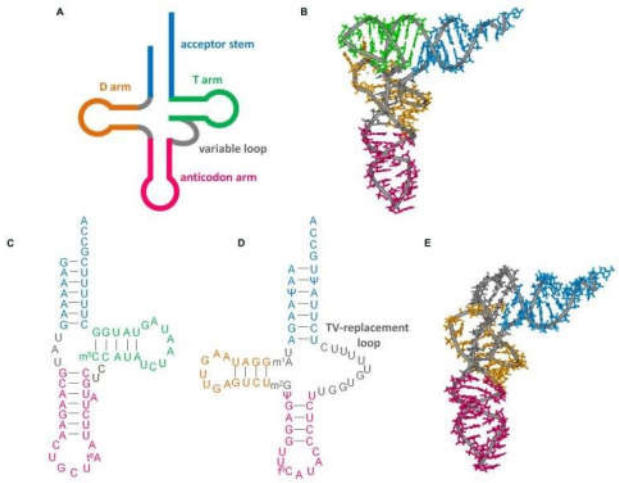
Biological functions

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

UNIT-IV

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Discuss in detail about Watson and Crick model
2. Give a detail account on secondary structure of tRNA
3. Explain in detail about different forms of DNA 4. Write notes on:
   i. A forms of DNA
   ii. Z forms of DNA

PART-C (8 MARKS)

` 1. Explain in detail about siRNA and hnRNA
2. Explain chemical synthesis of DNA.
3. Describe the structure and biological functions of hn RNA
4. Write notes on: i. mRNA ii. snRNA
5. Explain DNA supercoiling 10. Discuss in detail about Watson and Crick model
6. Give a detail account on secondary and tertiary structure of rRNA

Karpagam Academy of Higher Education
Department of Biochemistry
I M.Sc., Biochemistry
18BCP101- Chemistry of Biopolymers

Question number	Unit IV	Question	Option I	Option II	Option III	Option IV	Answer	
1	4	Proofreading activity to maintain the fidelity of DNA synthesis	occurs after the synthesis has been completed	is a function of the 3'-5' exonuclease activity of the DNA polymerases	requires the presence of an enzyme separate from the DNA polymerases	occurs in prokaryotes but not eukaryotes	is a function of the 3'-5' exonuclease activity of the DNA polymerases	
2	4	Both strands of DNA serve as templates concurrently in	replication	excision repair	mismatch repair	Transcription	replication	
3	4	In E.coli, which enzyme synthesizes the RNA primer for Okazaki fragments?	DnaA	DnaC	DnaG	DnaB	DnaG	
4	4	During which of the following process a new copy of a DNA molecule is precisely synthesized?	Transformatio n	Transcription	Translation	Replication	Replication	
5	4	DNA gyrase is inhibited by	tetracycline	nalidixic acid	tetracycline and nalidixic acid	Cephalosporin	nalidixic acid	
6	4	DNA replication rates in prokaryotes are approximately of the order of	10 bases per second	1,00 bases per second		1,000 bases per second	10,000 bases per second	1,000 bases per second
7	4	What DNA sequence is required for termination of replication in E. coli?	oriC	ter		Tus	Fork	ter
8	4	What is the major difference between T1 and T2 ter sequences?	Tus binds to T1 but not T2.	Tus binds to T2 but not T1.		T1 stops counterclockwis e-moving forks, T2 stops clockwise- moving forks.	T2 stops counterclockwise- moving forks, T1 stops only clockwise-moving forks.	T1 stops counterclockwise- moving forks, T2 stops clockwise-moving forks.
9	4	Which enzyme performs decatenation?	Polymerase	Topoisomeras e		Telomerase	Decatenase	Topoisomerase
10	4	For initiation ___subunit of RNA pol required	Alpha	beta		gamma	Sigma	sigma
11	4	The RNA polymerase of E. coli complex holoenzyme composed of 5 polypeptide subunits	$2\alpha,2\beta$ and one sigma factor	2α 2β and one rho factor		2α $1\beta,1\beta$ and one sigma factor	$2\alpha,1\beta,1\beta$ and one rho factor	2α $1\beta,1\beta$ and one sigma factor
12	4	Pribnow box consists of the following 6 nucleotide bases	TATAAT	TATATA		TTAAAT	TAATAT	TATAAT
13	4	The process of making copy of RNA from DNA is	Replication		Transcription	Translation	Reverse transcription	Transcription
14	4	RNA polymerase utilizes the following RNA triphosphates for the formation of RNA	ATP, GTP, CTP and TTP		ATP, GTP, CTP and UTP	ATP, CTP, UTP and UTP	ATP, CTP, GTP and TTP	ATP, GTP, CTP and UTP
15	4	Transfer RNA perform	Amino acid sequence by gene		Read information in mRNA	Synthesize proteins	Read information in tRNA	Read information in mRNA
16	4							
17	4	RNA self splicing was discovered by	Watson & Crick		McClintock	Sanger	Thomas Cech	Thomas Cech
18	4	DNA replication rates in prokaryotes are approximately of the order of	10 bases per second	1,00 bases per second		1,000 bases per second	10,000 bases per second	1,000 bases per second
19	4	What DNA sequence is required for termination of replication in E. coli?	oriC	ter		Tus	Fork	ter
20	4	What is the major difference between T1 and T2 ter sequences?	Tus binds to T1 but not T2.	Tus binds to T2 but not T1.		T1 stops counterclockwis e-moving forks, T2 stops clockwise- moving	T2 stops counterclockwise- moving forks, T1 stops only clockwise-moving forks.	T1 stops counterclockwise- moving forks, T2 stops clockwise-moving forks.
21	4							
22	4	What enzyme performs decatenation?	Polymerase	Topoisomeras e		Telomerase	Decatenase	Topoisomerase
23	4	For initiation ___subunit of RNA pol required	Alpha	beta		gamma	Sigma	sigma
24	4	The RNA polymerase of E. coli complex holoenzyme composed of 5 polypeptide subunits	$2\alpha,2\beta$ and one sigma factor	2α 2β and one rho factor		2α $1\beta,1\beta$ and one sigma factor	$2\alpha,1\beta,1\beta$ and one rho factor	2α $1\beta,1\beta$ and one sigma factor
25	4	Pribnow box consists of the following 6 nucleotide bases	TATAAT	TATATA		TTAAAT	TAATAT	TATAAT
26	4	The process of making copy of RNA from DNA is	Replication		Transcription	Translation	Reverse transcription	Transcription
27	4	RNA polymerase utilizes the following RNA triphosphates for the formation of RNA	ATP, GTP, CTP and TTP		ATP, GTP, CTP and UTP	ATP, CTP, UTP and UTP	ATP, CTP, GTP and TTP	ATP, GTP, CTP and UTP
28	4	Transfer RNA perform	Amino acid sequence by gene		Read information in mRNA	Synthesize proteins	Read information in tRNA	Read information in mRNA
29	4	RNA self splicing was discovered by	Watson & Crick		McClintock	Sanger	Thomas Cech	Thomas Cech
30	4	DNA polymerase can add new nucleotides to the free _____of a nucleotide strand.	1' end		3' end	5' end	1 ' end and 3 ' end	3' end
31	4	Which of the following possesses both 5'-3' and 3'-5' exonuclease activity?	Kornberg enzyme	DNA polymerase III		Taq DNA polymerase	DNA gyrase	Kornberg enzyme
32	4	The enzyme that catalyzes the synthesis of DNA is called	DNA polymerase	DNA gyrase		DNA ligase	Helicase	DNA polymerase
33	4	Which of the following repairs nicked DNA by forming a phosphodiester bond between adjacent nucleotides?	Helicase	DNA gyrase		Topoisomerases	DNA ligase	DNA ligase
34	4	DNA synthesis can be specifically measured by estimating the incorporation of radiolabeled	Uracil	Thymine	Adenine	Deoxyribose sugar	Thymine	
35	4	The elongation of the leading strand during DNA synthesis	Progresses away from the replication fork	Occur in 3 ' -5 ' direction	Produce Okazaki fragment	Depend on the action of DNA polymerase	Depend on the action of DNA polymerase	

36	4	Eukaryotes differ from prokaryote in mechanism of DNA replication due to:	Different enzyme for synthesis of lagging and leading strand	Use of DNA primer rather than RNA primer	Unidirectional rather than bidirectional replication	Discontinuous rather than semidiscontinuous replication	Discontinuous rather than semidiscontinuous replication			
37	4	The biological information flows from DNA to RNA and from RNA to	lipids	carbohydrates		proteins	thiamin	nucleotides	A	proteins
38	4	The total genetic information contained in a DNA is referred to as	gene	genome		Okazaki piece	Rickets	ribozyme	B	genome
39	4	The DNA base pairing is based on _____rules	Watson & Crick	Arther Kornberg		Stahl & Meselson	a2 globulin	McClintock	B	Watson & Crick
40	4	Pseudogenes are	Related to non functional	Transcribed into mRNA		Translated in to functional proteins	iron	Transcribed into tRNA	A	Related to non functional genes
41	4									
42	4	Mobile genetic elements were visualized by	T.H Morgan		Barbara McClintock	G Khorana	vitamin E	C.B Bridge	C	Barbara McClintock
43	4	The last DNA to be replicated in the eukaryotic chromosome is	Telomeres at the end of the chromosomes		Heterochroma tin	Euchromatin in the arms of the chromosome		Facultative heterochromatin		Heteroch romatin
44	4	In which phase of the cell cycle does DNA replication occur?	G0		G1	S		G2		S
45	4	The enzyme responsible for initiating DNA replication in prokaryotes is	DNA polymerase I		DNA polymerase III	Polymerase beta		Primase		Primase
46	4	The enzyme responsible for continuing DNA replication in prokaryotes, once it is initiated is:	DNA polymerase I	DNA polymerase III	Polymerase beta	Polymerase delta	DNA polymerase III			
47	4	The enzyme _____ unzips and unwinds the DNA molecule.	DNA polymerase	helicase	primase	DNA ligase	helicase			
48	4	Looped rolling circle mode of DNA replication is seen in	E. coli	Chloroplast	θx174	Mitochondria	θx174			
49	4	DNA replication results in:	2 completely new DNA molecules	2 DNA molecules that each contain a strand of the original	1 new DNA molecule, 1 old molecule is conserved	1 new molecule of RNA	2 DNA molecules that each contain a strand of the original			
50	4	During replication, what enzyme adds complimentary bases?	helicase	synthesase	replicase	polymerase	polymerase			
51	4	The backbone of nucliec acid structure is contributed by	Hydrophobic forces	hydrogen bonds	phosphodiester linkages	ionic bonds	covlent bond	phosphodiester linkages		
52	4	The pyrimidine base of the DNA is	cytosine	Guanine	Uracil	Adenine	thymine	cytosine		
53	4	There are three hydrogen bonds between Cytosine and	Adenine	guanine	cytosine	thymine	thymine	thymine		
54	4	An increased melting temperature for duplex DNA results from a high content of	Adenine & Guanine	Cytosine &thymine	Adenine &thymine	Cytosine &guanine	Cytosine &guanine	Cytosine &guanine		
55	4	The base which is absent in RNA is	Cytosine	Uracil	thymine	Adenine	Adenine	thymine		
56	4	Z-DNA was discovered by	Watson&Cric k	Hoogsten	Chargaff	warg &Rich		Hoogsten		
57	4	Ribose is linked with purine by	C_1 to N_1	C_1 to N_9	C_5 to N_9	C_1 to N	C_1 to N_9			
58	4	Melting temperature or Tm is high in DNA containing	rich in A, T pair	rich in G,C pair	Both in equal ratio	poor in G,C pair	rich in G,C pair			
59	4	The double stranded DNA molecule loses its viscosity upon	Denaturation	Filteration	sedimentation	purification	Denaturation			
60	4	A nucleotide consists of	a sugar, a base and a phosphate	a sugar and a phosphate	paired bases	a sugar, a base and three phosphates	a sugar, a base and a phosphate			
61	4	Which of the following is found on RNA but not DNA?	Uracil	Deoxyribose	Phosphate	Adenine	Uracil			
62	4	The most stabilizing force for nucleic acids is	hydrogen bonds	electrostatic bond	Van der Waals	conformational entropy	hydrogen bonds			
63	4	Oligonucleotides are formed from 2 to 10	strands	hydrogen bonds	nucleosides	nucleotides	nucleotides			
64	4	The variable portion of DNA is the sequence of _____.	phosphoric acids	sugars	bases	phosphates	bases			
65	4	The following facts are true of all transfer (t)RNA except that	the 5' end is phosphorylated	they are single chains	methylated bases are found	methylate d bases are found	the anticodon loop is identical			the anticodon loop is identical

UNIT-V

SYLLABUS

Nucleic acid interaction with proteins: DNA binding motifs in proteins – the basic helix loop helix (bHLH) motif, zinc finger, the leucine zipper, helix-loop helix and homeo domain. RNA binding motifs in proteins. Molecular aspects of protein-nucleic acid binding – direct interactions. Techniques characterizing nucleic acid-protein complex – chromatin immunoprecipitation assay, DNase I footprinting.

DNA binding motifs in proteins

A DNA-binding domain (DBD) is an independently folded protein domain that contains at least one structural motif that recognizes double- or single-stranded DNA. A DBD can recognize a specific DNA sequence (a recognition sequence) or have a general affinity to DNA. Some DNA-binding domains may also include nucleic acids in their folded structure.

Function

One or more DNA-binding domains are often part of a larger protein consisting of further protein domains with differing function. The extra domains often regulate the activity of the DNA-binding domain. The function of DNA binding is either structural or involves transcription regulation, with the two roles sometimes overlapping.

DNA-binding domains with functions involving DNA structure have biological roles in DNA
replication, repair, storage, and modification, such as methylation.

Many proteins involved in the regulation of gene expression contain DNA-binding domains. For example, proteins that regulate transcription by binding DNA are called transcription factors. The final output of most cellular signaling cascades is gene regulation.

The DBD interacts with the nucleotides of DNA in a DNA sequence-specific or non-sequence-specific manner, but even non-sequence-specific recognition involves some sort of molecular complementarity between protein and DNA. DNA recognition by the DBD can occur at the major or minor groove of DNA, or at the sugar-phosphate DNA backbone (see the structure of DNA). Each specific type of DNA recognition is tailored to the protein's function. For example, the DNA- cutting enzyme DNAse I cuts DNA almost randomly and so must bind to DNA in a non-sequence- specific manner. But, even so, DNAse I recognizes a certain 3-D DNA structure, yielding a somewhat specific DNA cleavage pattern that can be useful for studying DNA recognition by a technique called DNA footprinting.

Many DNA-binding domains must recognize specific DNA sequences, such as DBDs of transcription factors that activate specific genes, or those of enzymes that modify DNA at specific sites, like restriction enzymes and telomerase. The hydrogen bonding pattern in the DNA major groove is less degenerate than that of the DNA minor groove, providing a more attractive site for sequence-specific DNA recognition.

The specificity of DNA-binding proteins can be studied using many biochemical and biophysical techniques, such as gel electrophoresis, analytical ultracentrifugation, calorimetry, DNA mutation, protein structure mutation or modification, nuclear magnetic resonance, x-ray crystallography, surface plasmon resonance, electron paramagnetic resonance, cross- linking and microscale thermophoresis (MST).

The basic helix loop helix (bHLH) motif

A basic helix-loop-helix (bHLH) is a protein structural motif that characterizes one of the largest families of dimerizing transcription factors. It should not be confused with the helix-turn-helix domain.

The motif is characterized by two α-helices connected by a loop. In general, transcription factors including this domain are dimeric, each with one helix containing basic amino acid residues that facilitate DNA binding. In general, one helix is smaller, and, due to the flexibility of the loop, allows dimerization by folding and packing against another helix. The larger helix typically contains the DNA-binding regions. bHLH proteins typically bind to a consensus sequence called an E-box, CANNTG. The canonical E-box is CACGTG (palindromic), however some bHLH transcription factors, notably those of the bHLH-PAS family, bind to related non-palindromic sequences, which are similar to the E-box. bHLH TFs may homodimerize or heterodimerize with other bHLH TFs and form a large variety of dimers, each one with specific functions.

Zinc finger

A zinc finger is a small protein structural motif that is characterized by the coordination of one or more zinc ions (Zn^{2+}) in order to stabilize the fold. Originally coined to describe the finger-like appearance of a hypothesized structure from *Xenopus laevis*transcription factor IIIA, the zinc finger name has now come to encompass a wide variety of differing protein structures *Xenopus laevis* TFIIIA was originally demonstrated to contain zinc and require the metal for function in 1983, the first such reported zinc requirement for a gene regulatory protein.

Proteins that contain zinc fingers (*zinc finger proteins*) are classified into several different structural families. Unlike many other clearly defined supersecondary structures such as Greek keys or β hairpins, there are a number of types of zinc fingers, each with a unique three- dimensional architecture. A particular zinc finger protein's class is determined by this three- dimensional structure, but it can also be recognized based on the primary structure of the protein or the identity of the ligands coordinating the zinc ion. In spite of the large variety of these proteins, however, the vast majority typically function as interaction modules that bind DNA, RNA, proteins, or other small, useful molecules, and variations in structure serve primarily to alter the binding specificity of a particular protein.

Since their original discovery and the elucidation of their structure, these interaction modules have proven ubiquitous in the biological world and may be found in 3% of the genes of the human genome.[4] In addition, zinc fingers have become extremely useful in various therapeutic and research capacities. Engineering zinc fingers to have an affinity for a specific sequence is an area of active research, and zinc finger nucleases and zinc finger transcription factors are two of the most important applications of this to be realized to date.

Leucine zipper

A leucine zipper (or leucine scissors) is a common three-dimensional structural motif in proteins. They were first described by Landschulz and collaborators in 1988 when they found that an enhancer binding protein had a very characteristic 30-amino acid segment and the display of these amino acid sequences on an idealized alpha helix revealed a periodic repetition of leucine residues at every seventh position over a distance covering eight helical turns. The polypeptide segments containing these periodic arrays of leucine residues were proposed to exist in an alpha-helical conformation and the leucine side chains from one alpha helix interdigitate with those from the alpha helix of a second polypeptide, facilitating dimerization.

Leucine zippers are a dimerization domain of the bZIP (Basic-region leucine zipper) class of eukaryotic transcription factors. The bZIP domain is 60 to 80 amino acids in length with a highly conserved DNA binding basic region and a more diversified leucine zipper dimerization region. The leucine zipper is a common three-dimensional structural motif in proteins and it has that name because leucines occur every seven amino acids in the dimerization domain. The localization of the leucines are critical for the DNA binding to the proteins. Leucine zippers are present in both eukaryotic and prokaryotic regulatory proteins, but are mainly a feature of eukaryotes. They can also be annotated simply as ZIPs, and ZIP-like motifs have been found in proteins other than transcription factors and are thought to be one of the general protein modules for protein–protein interactions.

The mechanism of transcriptional regulation by bZIP proteins has been studied in detail. Most bZIP proteins show high binding affinity for the ACGT motifs, which include CACGTG (G box), GACGTC (C box), TACGTA (A box), AACGTT (T box), and a GCN4 motif, namely TGA(G/C)TCA A small number of bZIP factors such as OsOBF1 can also recognize palindromic sequences. However, the others, including LIP19, OsZIP-2a, and OsZIP-2b, do not bind to DNA sequences. Instead, these bZIP proteins form heterodimers with other bZIPs to regulate transcriptional activities.

Homeo domain

A homeobox is a DNA sequence, around 180 base pairs long, found within genes that are involved in the regulation of patterns of anatomical development (morphogenesis) in animals, fungi and plants. These genes encode homeodomain protein products that are transcription factors sharing a characteristic protein fold structure that binds DNA. The "homeo-" prefix in the words "homeobox" and "homeodomain" stems from the mutational phenotype known as "homeosis", which is frequently observed when these genes are mutated in animals. Homeosis is a term coined by William Bateson to describe the outright replacement of a discrete body part with another body part. Homeobox genes are not only found in animals, but have also been found in fungi, for example the unicellularyeasts, in plants, and numerous single cell eukaryotes.

A homeobox is about 180 DNA base pairs long and encodes a protein domain that binds DNA. The following shows the consensus homeodomain (~60 amino acid residue chain):

Structure

The characteristic homeodomain protein fold consists of a 60-amino acid long domain composed of three alpha helixes. Helix 2 and helix 3 form a so-called helix-turn-helix (HTH) structure, where the two alpha helices are connected by a short loop region. The N-terminal two helices of the homeodomain are antiparallel and the longer C-terminal helix is roughly perpendicular to the axes established by the first two. It is this third helix that interacts directly with DNA via a number of hydrogen bonds and hydrophobic interactions, as well as indirect interactions via water molecules, which occur between specific side chains and the exposed bases within the major groove of the DNA.

Homeodomain proteins are found in eukaryotes.[4] Through the HTH motif, they share limited sequence similarity and structural similarity to prokaryotic transcription factors, such as lambda phage proteins that alter the expression of genes in prokaryotes. The HTH motif shows some sequence similarity but a similar structure in a wide range of DNA-binding proteins (e.g., cro and repressor proteins, homeodomain proteins, etc.). One of the principal differences between HTH motifs in these different proteins arises from the stereo-chemical requirement for glycine in the turn which is needed to avoid steric interference of the beta-carbon with the main chain: for cro and repressor proteins the glycine appears to be mandatory, whereas for many of the homeotic and other DNA-binding proteins the requirement is relaxed.

Sequence specificity

Homeodomains can bind both specifically and nonspecifically to B-DNA with the C-terminal recognition helix aligning in the DNA's major groove and the unstructured peptide "tail" at the N- terminus aligning in the minor groove. The recognition helix and the inter-helix loops are rich in arginine and lysine residues, which form hydrogen bonds to the DNA backbone; conserved hydrophobic residues in the center of the recognition helix aid in stabilizing the helix packing. Homeodomain proteins show a preference for the DNA sequence 5'-TAAT-3'; sequence-independent binding occurs with significantly lower affinity.

Biological function

Through the DNA-recognition properties of the homeodomain, homeoproteins are believed to regulate the expression of targeted genes and direct the formation of many body structures during early embryonic development. Many homeodomain proteins induce cellular differentiation by initiating the cascades of coregulated genes required to produce individual tissues and organs.

Other proteins in the family, such as NANOG are involved in maintaining pluripotency. Homeobox genes are critical in the establishment of body axes during embryogenesis.

Homeoprotein transcription factors typically switch on cascades of other genes. The homeodomain binds DNA in a sequence-specific manner. However, the specificity of a single homeodomain protein is usually not enough to recognize only its desired target genes. Most of the time, homeodomain proteins act in the promoter region of their target genes as complexes with other transcription factors. Such complexes have a much higher target specificity than a single homeodomain protein. Homeodomains are encoded both by genes of the Hox gene clusters and by other genes throughout the genome.

The homeobox domain was first identified in a number of *Drosophila* homeotic and segmentation proteins, but is now known to be well-conserved in many other animals, including vertebrates. Specific members of the Hox family have been implicated in vascular remodeling, angiogenesis, and disease by orchestrating changes in matrix degradation, integrins, and components of the ECM. HoxA5 is implicated in atherosclerosis. HoxD3 and HoxB3 are proinvasive, angiogenic genes that upregulate b3 and a5 integrins and Efna1 in ECs, respectively. HoxA3 induces endothelial cell (EC) migration by upregulating MMP14 and uPAR. Conversely, HoxD10 and HoxA5 have the opposite effect of suppressing EC migration and angiogenesis, and stabilizing adherens junctions by upregulating TIMP1/downregulating uPAR and MMP14, and by upregulating Tsp2/downregulating VEGFR2, Efna1, Hif1alpha and COX-2, respectively. HoxA5 also upregulates the tumor suppressor p53 and Akt1 by downregulation of PTEN. Suppression of HoxA5 has been shown to attenuate hemangioma growth. HoxA5 has far-reaching effects on gene expression, causing ~300 genes to become upregulated upon its induction in breast cancer cell lines. HoxA5 protein transduction domain overexpression prevents inflammation shown by inhibition of TNFalpha-inducible monocyte binding to HUVECs.

RNA binding motifs in proteins

RNA-binding proteins (often abbreviated as RBPs) are proteins that bind to the double or single stranded RNA in cells and participate in forming ribonucleoprotein complexes. RBPs contain various structural motifs, such as RNA recognition motif (RRM), dsRNA binding domain, zinc finger and others They are cytoplasmic and nuclear proteins. However, since most mature RNA is exported from the nucleus relatively quickly, most RBPs in the nucleus exist as complexes of protein and pre-mRNA called heterogeneous ribonucleoprotein particles (hnRNPs). RBPs have crucial roles in various cellular processes such as: cellular function, transport and localization. They especially play a major role in post-transcriptional control of RNAs, such as: splicing, polyadenylation, mRNA stabilization, mRNA localization and translation. Eukaryotic cells encode diverse RBPs, approximately 500 genes, with unique RNA-binding activity and protein–protein interaction. During evolution, the diversity of RBPs greatly increased with the increase in the number of introns. Diversity enabled eukaryotic cells to utilize RNA exons in various arrangements, giving rise to a unique RNP (ribonucleoprotein) for each RNA. Although RBPs have a crucial role in post-transcriptional regulation in gene expression, relatively few RBPs have been studied systematically.

RNA processing and modification

Alternative splicing

Alternative splicing is a mechanism by which different forms of mature mRNAs (messengers RNAs) are generated from the same gene. It is a regulatory mechanism by which variations in the incorporation of the exons into mRNA leads to the production of more than one related protein, thus expanding possible genomic outputs. RBPs function extensively in the regulation of this process. Some binding proteins such as neuronal specific RNA-binding proteins, namely NOVA1, control the alternative splicing of a subset of hnRNA by recognizing and binding to a specific sequence in the RNA (YCAY where Y indicates pyrimidine, U or C). These proteins then recruit splicesomal proteins to this target site. SR proteins are also well known for their role in alternative splicing through the recruitment of snRNPs that form the splicesome, namely U1 snRNP and U2AF snRNP. However, RBPs are also part of the splicesome itself. The splicesome is a complex of snRNA and protein subunits and acts as the mechanical agent that removes introns and ligates the flanking exons. Other than core splicesome complex, RBPs also bind to the sites of *Cis*-acting RNA elements that influence exons inclusion or exclusion during splicing. These sites are referred to as exonic splicing enhancers (ESEs), exonic splicing silencers (ESSs), intronic splicing enhancers (ISEs) and intronic splicing silencers (ISSs) and depending on their location of binding, RBPs work as splicing silencers or enhancers

RNA editing

The most extensively studied form of RNA editing involves the ADAR protein. This protein functions through post-transcriptional modificationof mRNA transcripts by changing the nucleotide content of the RNA. This is done through the conversion of adenosine to inosine in an enzymatic reaction catalyzed by ADAR. This process effectively changes the RNA sequence from that encoded by the genome and extends the diversity of the gene products. The majority of RNA editing occurs on non-coding regions of RNA; however, some protein-encoding RNA transcripts have been shown to be subject to editing resulting in a difference in their protein's amino acid sequence. An example of this is the glutamate receptor mRNA where glutamine is converted to arginine leading to a change in the functionality of the protein.

Polyadenylation

Polyadenylation is the addition of a "tail" of adenylate residues to an RNA transcript about 20 bases downstream of the AAUAAA sequence within the three prime untranslated region. Polyadenylation of mRNA has a strong effect on its nuclear transport, translation efficiency, and stability. All of these as well as the process of polyadenylation depend on binding of specific RBPs. All eukaryotic mRNAs with few exceptions are processed to receive 3' poly (A) tails of about 200 nucleotides. One of the necessary protein complexes in this process is CPSF. CPSF binds to the 3' tail (AAUAAA) sequence and together with another protein called poly(A)-binding protein, recruits and stimulates the activity of poly(A) polymerase. Poly(A) polymerase is inactive on its own and requires the binding of these other proteins to function properly.

Export

After processing is complete, mRNA needs to be transported from the cell nucleus to cytoplasm. This is a three-step process involving the generation of a cargo-carrier complex in the nucleus followed by translocation of the complex through the nuclear pore complex and finally release of the cargo into cytoplasm. The carrier is then subsequently recycled. TAP/NXF1:p15 heterodimer is thought to be the key player in mRNA export. Over-expression of TAP in *Xenopus laevis* frogs increases the export of transcripts that are otherwise inefficiently exported. However TAP needs adaptor proteins because it is unable interact directly with mRNA. Aly/REF protein interacts and binds to the mRNA recruiting TAP.

mRNA localization

mRNA localization is critical for regulation of gene expression by allowing spatially regulated protein production. Through mRNA localization proteins are transcribed in their intended target site of the cell. This is especially important during early development when rapid cell cleavages give different cells various combinations of mRNA which can then lead to drastically different cell fates. RBPs are critical in the localization of this mRNA that insures proteins are only transcribed in their intended regions. One of these proteins is ZBP1. ZBP1 binds to beta- actin mRNA at the site of transcription and moves with mRNA into the cytoplasm. It then localizes this mRNA to the lamella region of several asymmetric cell types where it can then be translated. FMRP is another RBP involved in RNA localization. It was shown that in addition to other functions for FMRP in RNA metabolism, FMRP is involved in the stimulus-induced localization of several dendritic mRNAs in neuronal dendrites.

Translation

Translational regulation provides a rapid mechanism to control gene expression. Rather than controlling gene expression at the transcriptional level, mRNA is already transcribed but the recruitment of ribosomes is controlled. This allows rapid generation of proteins when a signal activates translation. ZBP1 in addition to its role in the localization of B-actin mRNA is also involved in the translational repression of beta-actin mRNA by blocking translation initiation. ZBP1 must be removed from the mRNA to allow the ribosome to properly bind and translation to begin.

Molecular aspects of protein-nucleic acid binding – direct interactions

DNA-binding proteins are proteins that have DNA-binding domains and thus have a specific or general affinity for single- or double-stranded DNA Sequence-specific DNA-binding proteins generally interact with the major groove of B-DNA, because it exposes more functional groups that identify a base pair. However, there are some known minor groove DNA-binding ligands such as netropsin, distamycin, Hoechst 33258, pentamidine, DAPI and others.

Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (**ChIP**) is a type of immunoprecipitation experimental technique used to investigate the interaction between proteins and DNA in the cell. It aims to determine whether specific proteins are associated with specific genomic regions, such as transcription factors on promoters or other DNA binding sites, and possibly defining cistromes. ChIP also aims to determine the specific location in the genome that various histone modifications are associated with, indicating the target of the histone modifiers.

Briefly, the conventional method is as follows:

1. DNA and associated proteins on chromatin in living cells or tissues are crosslinked (this step is omitted in Native ChIP).
2. The DNA-protein complexes (chromatin-protein) are then sheared into ~500 bp DNA fragments by sonication or nuclease digestion.
3. Cross-linked DNA fragments associated with the protein(s) of interest are selectively immunoprecipitated from the cell debris using an appropriate protein-specific antibody.
4. The associated DNA fragments are purified and their sequence is determined. Enrichment of specific DNA sequences represents regions on the genome that the protein of interest is associated with *in vivo*.

There are mainly two types of ChIP, primarily differing in the starting chromatin preparation. The first uses reversibly cross-linkedchromatin sheared by sonication called cross-linked ChIP (XChIP). Native ChIP (NChIP) uses native chromatin sheared by micrococcal nuclease digestion.

Cross-linked ChIP (XChIP)

Cross-linked ChIP is mainly suited for mapping the DNA target of transcription factors or other chromatin-associated proteins, and uses reversibly cross-linked chromatin as starting material. The agent for reversible cross-linking could be formaldehyde or UV light. Then the cross-linked chromatin is usually sheared by sonication, providing fragments of 300 - 1000 base pairs (bp) in length. Mild formaldehyde crosslinking followed by nuclease digestion has been used to shear the chromatin. Chromatin fragments of 400 - 500bp have proven to be suitable for ChIP assays as they cover two to three nucleosomes.

Cell debris in the sheared lysate is then cleared by sedimentation and protein–DNA complexes are selectively immunoprecipitated using specific antibodies to the protein(s) of interest. The antibodies are commonly coupled to agarose, sepharose or magnetic beads. Alternatively, chromatin-antibody complexes can be selectively retained and eluted by inert polymer discs. The immunoprecipitated complexes (i.e., the bead–antibody–protein–target DNA sequence complex) are then collected and washed to remove non-specifically bound chromatin, the protein– DNA cross-link is reversed and proteins are removed by digestion with proteinase K. An epitope- tagged version of the protein of interest, or *in vivo*biotinylation can be used instead of antibodies to the native protein of interest.

The DNA associated with the complex is then purified and identified by polymerase chain reaction (PCR), microarrays (ChIP-on-chip), molecular cloning and sequencing, or direct high- throughput sequencing (ChIP-Seq).

Native ChIP (NChIP)

Native ChIP is mainly suited for mapping the DNA target of histone modifiers. Generally, native chromatin is used as starting chromatin. As histones wrap around DNA to form nucleosomes, they are naturally linked. Then the chromatin is sheared by micrococcal nuclease digestion, which cuts DNA at the length of the linker, leaving nucleosomes intact and providing DNA fragments of one nucleosome (200bp) to five nucleosomes (1000bp) in length.

Thereafter, methods similar to XChIP are used for clearing the cell debris, immunoprecipitating the protein of interest, removing protein from the immunoprecipated complex, and purifying and analyzing the complex-associated DNA.

Comparison of XChIP and NChIP

The major advantage for NChIP is antibody specificity. It is important to note that most antibodies to modified histones are raised against unfixed, synthetic peptide antigens and that the epitopes they need to recognize in the XChIP may be disrupted or destroyed by formaldehyde cross-linking, particularly as the cross-links are likely to involve lysine e-amino groups in the N-terminals, disrupting the epitopes. This is likely to explain the consistently low efficiency of XChIP protocols compare to NChIP.

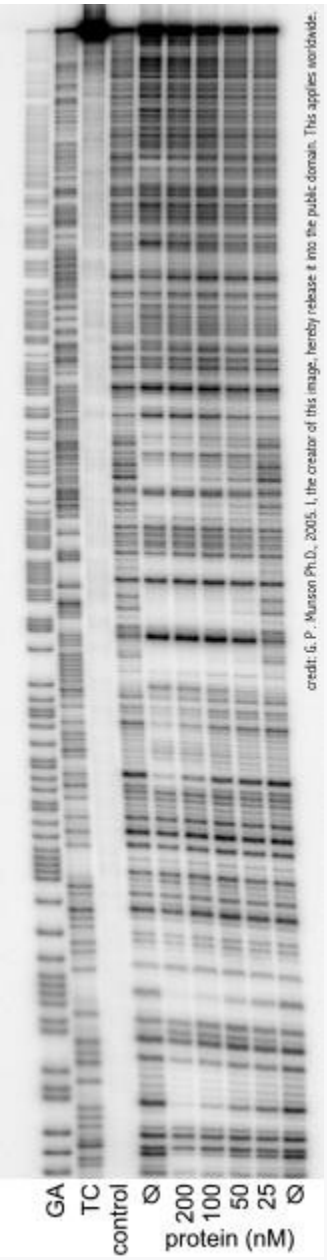
But XChIP and NChIP have different aims and advantages relative to each other. XChIP is for mapping target sites of transcription factors and other chromatin associated proteins; NChIP is for mapping target sites of histone modifiers (see Table 1).

Table 1 Advantages and disadvantages of NChIP and XChIP

	XChIP	NChIP
Advantages	Suitable for transcriptional factors, or any other weakly binding chromatin associated proteins. Applicable to any organisms where native protein is hard to prepare	Testable antibody specificity Better antibody specificity as target protein naturally intact Better chromatin and protein recovery efficiency due to better antibody specificity
Disadvantages	Inefficient chromatin recovery due to antibody target protein epitope disruption	Usually not suitable for non-histone proteins Nucleosomes may rearrange during

	May cause false positive result due digestion to fixation of transient proteins to chromatin Wide range of chromatin shearing size due to random cut by sonication.	

DNase I footprinting



DNaseI footprint of a protein binding to a radiolabelled DNA fragment. Lanes "GA" and "TC" are

Maxam-Gilbert chemical sequencing lanes, see DNA Sequencing. The lane labelled "control" is for quality control purposes and contains the DNA fragment but not treated with DNaseI.

A DNase footprinting **assay** is a DNA footprinting technique from molecular biology/biochemistry that detects DNA-protein interaction using the fact that a protein bound to DNA will often protect that DNA from enzymatic cleavage. This makes it possible to locate a protein binding site on a particular DNA molecule. The method uses an enzyme, deoxyribonuclease (DNase, for short), to cut the radioactively end-labeled DNA, followed by gel electrophoresis to detect the resulting cleavage pattern.

For example, the DNA fragment of interest may be PCR amplified using a ^{32}P 5' labeled primer, with the result being many DNA molecules with a radioactive label on one end of one strand of each double stranded molecule. Cleavage by DNase will produce fragments. The fragments which are smaller with respect to the ^{32}P-labelled end will appear further on the gel than the longer fragments. The gel is then used to expose a special photographic film.

The cleavage pattern of the DNA in the absence of a DNA binding protein, typically referred to as free DNA, is compared to the cleavage pattern of DNA in the presence of a DNA binding protein. If the protein binds DNA, the binding site is protected from enzymatic cleavage. This protection will result in a clear area on the gel which is referred to as the "footprint".

By varying the concentration of the DNA-binding protein, the binding affinity of the protein can be estimated according to the minimum concentration of protein at which a footprint is observed.

POSSIBLE QUESTIONS

UNIT-V

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Explain in detail about the leucine zipper
2. Discuss about DNase I footprinting
3. Explain the basic helix loop helix (bHLH)
4. Discuss helix-Loop helix

PART-C (8 MARKS)

` 1. Write notes on chromatin immunoprecipitation assay
2. Discuss in detail about RNA binding motifs in proteins
3. Explain about zinc finger
4. Discuss about DNase I footprinting
5. Write notes on chromatin immunoprecipitation assay
6. Discuss in detail about RNA binding motifs in proteins

31	The proof reading activity of the newly synthesized DNA is present on the enzyme	DNA helicase	DNA polymerase I	DNA polymerase II	DNA polymerase III	DNA polymerase I
32	The problem of supercoils during DNA replication is overcome by a group of enzymes called	DNA topoisomerases	DNA ligases	DNA polymerases	DNA helicases	DNA topoisomerases
33	The enzyme responsible for the replication of mitochondrial DNA	DNA polymerase	DNA polymerase	DNA polymerase	DNA polymerase	DNA polymerase+G3
34	Okazaki fragments are initiated with	DNA primer	RNA primer	DNA template	RNA template	RNA primer
35	Watson and Crick elucidated ds DNA structure by using	NMR spectroscopy	X-ray diffraction	Circular dichroism	IR and Raman spectroscopy	X-ray diffraction
36	Single strand binding protein binds to single strand DNA	to prevent replication	to repair base pairs	to initiate transcription	to prevent reformation of duplex state	to prevent reformation of duplex state
37	Rolling circle replication is otherwise called as	□ replication	□ replication	D-loop replication	L-loop replication	□ replication
38	The DNA replication is discontinuous was proved by	Messelson-Stahl	Reigi Okazaki	Albert Lehninger	Arthur Kornberg	Reigi Okazaki
39	RNA primers are removed by	DNA polymerase I	DNA polymerase II	DNA polymerase III	DNA topoisomera se	DNA polymerase I
40	Primase initiates the following activities except	leading strand synthesis	replication	Okazaki fragments	transcription	transcription
41	The Klenow fragment exhibits the activity of	5’-3’ exonuclease	polymerase and 3’-5’	polymerase and 5’-3’	an endo nuclease	polymerase and 3’-5’ exonuclease
42	Which of the following reactions is required for proofreading during DNA replication by DNA polymerase III?	3' - 5' exonuclease activity	5' - 3' exonuclease activity	3' - 5' endonuclease activity	5' - 3' endonucleas e activity	3' - 5' endonucleas e activity
43	All of the following are differences between eukaryotic and prokaryotic DNA replication except	the type and number of polymerases involved in DNA	multiple vs. single replication origins	the rate of DNA synthesis	the ability to form a replication fork	the ability to form a replication fork
44	In the rolling circle method of replication	the 5' tail of DNA is nicked	RNA is nicked	one strand of DNA in the circle is nicked	both strands of DNA in the circle are nicked	one strand of DNA in the circle is nicked
45	In the Meselson -Stahl experiment, which mode of replication can be eliminated based on data derived after	Dispersive	Semiconserva tive	Conservative	all three modes	Conservative
46	The discovery of Okazaki fragments suggested that DNA synthesis is	discontinuous	continuous	3 ' to 5'	semiconserv ative	discontinuous
47	A replicating prokaryotic chromosome has -------- replication forks	One	Many	Three	Two	Two
48	A replicating eukaryotic chromosome has ---------- replication forks	One	Many	Three	Two	Many
49	Which molecule serves to destabilize the DNA helix in order to open it up, creating a replication fork?	DNA helicase	DNA ligase	DNA polymerase	SSBPs	DNA helicase
50	For DNA Replication, unwinding of DNA is done by	Helicase	Ligase	Hexonuclease	Topoisomera se	Helicase
51	In vivo synthesis of DNA is	3' to 5'	5' to 3'	both 3' to 5' and 5' to 3'	neither 3' to 5' nor 5' to 3'	5' to 3‘
52	All of the following are differences between eukaryotic and prokaryotic DNA replication except	the type and number of	multiple vs. single	the rate of DNA synthesis	the ability to form a replication fork	the ability to form a replication fork
53	A replicating prokaryotic chromosome has -------- replication forks	One	Many	Three	Two	Two
54	A replicating eukaryotic chromosome has ---------- replication forks	One	Many	Three	Two	Many
55	Which molecule serves to destabilize the DNA helix in order to open it up, creating a replication fork?	DNA helicase	DNA ligase	DNA polymerase	SSBPs	DNA helicase
56	For DNA Replication, unwinding of DNA is done by	Helicase	Ligase	Hexonuclease	Topoisomera Se	Helicase
57	In vivo synthesis of DNA is	3' to 5'	5' to 3'	both 3' to 5' and 5' to 3'	neither 3' to 5' nor 5' to 3'	5' to 3‘
58	Which of the following forms of DNA can serve as a template for DNA polymerase	Partially double stranded DNA	Circular double stranded DNA	Intact double stranded DNA	Circular single stranded DNA	Partially double stranded DNA
59	Ribozymes are	Enzymes with cata	RNAs with catal	Proteins with catalytic activity	Nucleic acids with catalytic	RNAs with catalytic activity