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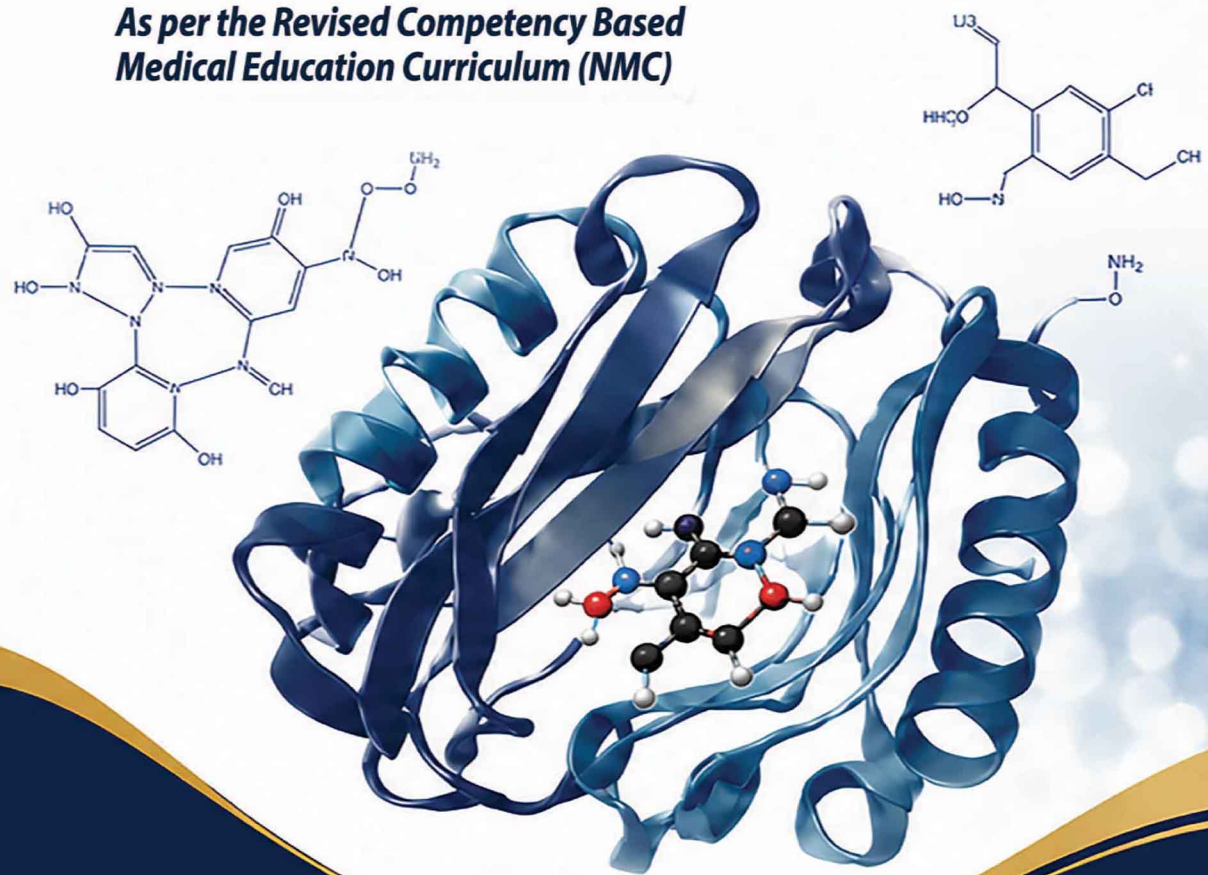
5th Edition

Essentials of Biochemistry

(for Medical Students)

As per the Revised Competency Based Medical Education Curriculum (NMC)



Shivananda Nayak B

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Content

1. Cell 1

Structural and Functional Aspects of Cell Organelles *1*

- Plasma Membrane *1*
- Prokaryotic and Eukaryotic Cells *4*
- Transport Across Membranes *5*
- Fluid Mosaic Model *5*
- Transport Mechanisms in the Membranes *6*
- Cell Fractionation *8*

2. Bioenergetics 14

- Free Energy *14*
- High-energy Compounds *15*

3. Enzymes 17

- Chemical Nature of Enzymes *17*
- Ribozymes *18*
- Zymogens or Proenzymes *18*
- Coenzymes *18*
- Energy of Activation of Catalyzed and Uncatalyzed Reactions *19*
- List and Explain the Factors that Affect the Enzyme Activity *19*
- Classification of Enzymes *21*
- Mechanism of Enzyme Catalysis *21*
- Enzyme Kinetics *22*
- Michaelis–Menten Equation *23*
- Enzyme Inhibition *24*
- Therapeutic use of Competitive Inhibition *25*
- Allosteric Enzymes *28*
- Isoenzymes *28*
- Diagnostic Enzymes *28*
- Lactate Dehydrogenase *29*

4. Chemistry of Amino Acids and Proteins 38

- Essential and Nonessential Amino Acids *38*
- Glucogenic and Ketogenic Amino Acids *41*
- Ninhydrin Reaction *41*
- Peptides and Peptide Bond *42*
- Classification of Proteins *43*
- Important Functions of Proteins *44*
- Structure of Proteins *45*
- Denaturation of Proteins *48*
- Plasma Proteins *48*
- Multiple Myeloma *52*

5. Metabolism of Amino Acids 58

- Digestion and Absorption of Proteins in the Gastrointestinal Tract *58*
- Amino Acid Pool *59*
- Metabolism of Amino Acids *60*
- Urea Cycle (Krebs–Henseleit Cycle) *61*
- Metabolism of Important Amino Acids *64*
- One-carbon Metabolism with Examples *66*
- Metabolism of Glycine Including the Products Formed from it *66*
- Importance of Specialized Products Formed from Glycine *67*
- Metabolism of Phenylalanine and Tyrosine *69*
- Metabolism of Tyrosine *70*
- Specialized Products Formed from Tyrosine *71*
- Synthesis of Catecholamines *72*
- Metabolism of Tryptophan *74*
- Metabolic Fate of Tryptophan *74*
- Disorders *74*
- Histidine Metabolism *75*
- Metabolism of Branched-chain Amino Acid *76*
- Metabolic Disorders *78*
- Inborn Errors of Metabolism *79*

6. Chemistry of Carbohydrates 87

- Classification of Carbohydrates *87*
- Heteropolysaccharides with Examples and their Biomedical Importance *89*
- Isomerism in Carbohydrates *89*
- Optical Activity of Carbohydrates, Including Stereoisomerism with Specific Examples *90*
- Invert Sugar with Example *91*
- Chemical Properties of Monosaccharides 92
- Action of Phenylhydrazine *92*
- Glycosidic Bond *93*
- Tests for Carbohydrates *94*
- Special Carbohydrates of Clinical Importance *94*

7. Metabolism of Carbohydrates 99

- Digestion and Absorption of Carbohydrates *99*
- Metabolism of Carbohydrate *102*
- Glycolysis *104*
- Compounds Formed from Pyruvate *109*
- Compounds Formed from Acetyl-CoA *110*
- TCA Cycle *111*
- Gluconeogenesis and its Importance in Regulating the Blood Glucose *113*
- Cori Cycle and its Clinical Importance *117*
- Metabolism of Glycogen *117*

- Synthesis of Glycogen from Glucose *118*
- Oxidative and Nonoxidative Phases of Hexose Monophosphate Pathway and its Importance *122*
- Galactose Metabolism *125*
- Fructose Metabolism *125*
- Sorbitol Pathway and its Clinical Significance *126*
- Lactose Intolerance *127*
- Regulation of Blood Glucose *128*
- Role of Hormones in Regulating the Blood Glucose *129*
- Insulin *129*
- Mechanism of Action of Insulin in Regulating the Blood Glucose *130*
- Hyperglycemic Hormones and their Role in Regulating the Blood Glucose *131*
- Criteria used to Diagnose the Diabetes Mellitus *133*
- Clinical Complications of Diabetes Mellitus *133*
- Glucose Tolerance Test and its Clinical Significance *134*
- Case Study *147*

8. Chemistry of Lipids 149

- Functions of Lipids *149*
- Classification of Lipids with Specific Examples *149*
- Simple Lipids *150*
- Complex or Compound Lipids *151*
- Apoproteins *153*
- Derived Lipids *155*
- Properties of Lipids *157*

9. Metabolism of Lipids 163

- Digestion and Absorption of Lipids *163*
- Metabolism of Lipids *167*
- Regulation of Cholesterol Biosynthesis and Significance of Biochemical Basis of use of Hypolipidemic Drugs *174*
- Ketone Body Metabolism *177*
- Conditions in Which Ketone Body Formation Occurs *178*
- Phospholipid Metabolism *179*
- Lipoprotein Metabolism *181*
- Fatty Liver *185*
- Regulation of Lipid Metabolism *187*
- Alcohol Metabolism *188*
- Synthesis of Prostaglandins *189*
- Biochemical Actions of Prostaglandins *190*
- Leukotrienes *191*
- Lipid Storage Disorders *191*
- Risk Factors for Cardiovascular Disease *195*

10. Integration of Metabolism 205

- Pathways of Metabolism *206*
- Hormonal Regulation of Metabolism during Well-Fed State *208*
- Hormonal Regulation of Metabolism during Fasting/Starvation *209*
- Adipose Tissue Hormones *211*
- Diabetes Mellitus: Types, Metabolic Changes, and Complications *213*
- Atherosclerosis *214*
- Laboratory Tests in Myocardial Infarction *215*

11. Hemoglobin 220

- Structure of Hemoglobin *220*
- Comparison of Myoglobin with Hemoglobin *221*
- Cooperative Binding of Oxygen by Hemoglobin *223*
- Clinical Aspects of Heme Metabolism *226*
- Disorders of Hemoglobin Catabolism *228*
- Role of Hemoglobin in Disease *230*
- Abnormal Hemoglobin *231*
- Hemoglobinopathy *231*
- Thalassemia and its Important Types *231*
- Sickle Cell Hemoglobin and its Causes and Symptoms *232*
- Test for the Detection of Abnormal Hemoglobin *233*
- Derived Hemoglobin Compounds *234*
- Case Study *237*

12. Hormones 238

- Classification of Hormones *238*
- Mechanism of Hormonal Action *239*
- Hypothalamic and Pituitary Hormones *240*
- Hypothalamic Hormones *240*
- Pituitary Hormones *241*
- Thyroid Hormones *244*
- Parathyroid Hormones *245*
- Adrenal Gland Hormones *245*
- Pancreatic Hormones *250*
- Female Sex Hormones *250*
- Case Studies *255*

13. Acid–Base Balance 259

- Regulation of Acid–Base Balance *260*
- Blood Buffer System *260*
- Role of Respiratory and Renal Mechanism in Regulating the Blood pH *262*
- Acid–Base Disorders *264*
- Assessment of Acid–Base Analysis *267*
- Case Studies *271*

14. Biological Oxidation 273
- Electron Transport Chain, its Organization and Components *274*
- Uncouplers *278*

15. Mineral Metabolism 281
- Macrominerals or Bulk Elements *281*
- Micro Minerals or Trace Elements *291*

16. Vitamins 302
- Fat-Soluble Vitamins *302*
- Water-Soluble Vitamins *307*
- B-complex Vitamins *311*

17. Nutrition 323
- Caloric Values of Macronutrients *324*
- Respiratory Quotient of Food Stuffs *324*
- Nutritional Importance of Carbohydrates, Proteins, and Fats *325*
- Dietary Fiber *327*
- Metabolic Rate/Energy Expenditure *329*
- Calculation of Basal Metabolic Rate *331*
- Methods of Determining Caloric Needs *331*
- Nitrogen Balance *333*
- Balanced Diet *333*
- Assessing Nutritional Status *334*
- Methods Available to Measure the Nutritional Status *336*
- Nutrition-related Diseases *340*
- Causes, Signs, and Symptoms of Different Types of Protein-energy Malnutrition *341*

18. Chemistry of Nucleic Acid 346
- Nucleosides and Nucleotides *346*
- Synthetic Analogs of Nucleotides or Antimetabolites *350*
- The Structure of DNA *353*
- Types of RNA *354*

19. Nucleic Acid Metabolism 359
- Purine Metabolism *359*
- Pyrimidine Metabolism *363*

20. Molecular Biology 369
- Deoxyribonucleic Acid *369*
- Genomics *370*
- Proteomics and its Applications *370*
- Difference between Genomics and Proteomics *371*
- Metabolomics *371*
- Replication *371*
- Telomere and Telomerase *374*

- Deoxyribonucleic Acid Repair *374*
- Mutation *376*
- Ribonucleic Acid *380*
- Transcription *380*
- Genetic Code *382*
- Translation *382*
- Recombinant DNA Technology *392*
- Polymerase Chain Reaction *395*
- Southern, Northern, and Western Blotting and their Applications *397*
- Restriction Enzymes *400*
- Plasmid *400*
- Restriction Fragment Length Polymorphism *401*
- Deoxyribonucleic Acid Libraries *402*

21. Organ Function Tests 409

- Liver Function Tests *409*
- Renal Function Tests *413*
- Thyroid Function Tests *416*
- Adrenal Gland *421*
- Pathophysiology *422*
- Case Studies *427*

22. Extracellular Matrix 430

- Biomolecules in Extracellular Matrix *430*
- Collagen *430*
- Elastin *432*
- Fibrillin *433*
- Fibronectin *433*
- Laminin *433*
- Glycosaminoglycans *433*
- Proteoglycans *435*

23. Biochemistry of Muscle Structure and Function 440

- Types of Muscles *440*
- Activation of Skeletal Muscle *442*
- Type I Versus Type II Fibers *445*
- Cardiac Muscle *446*
- Smooth Muscle *449*
- Adenosine Triphosphate Hydrolysis for Muscle Contraction *450*
- Muscle Diseases *450*

24. Water and Electrolyte Balance 457

- Water Balance *457*
- Causes and Symptoms of Hypovolemia and Hypervolemia *460*
- Electrolyte Balance *462*
- Causes and Symptoms of Different types of Hyponatremia and Hypernatremia *464*

25. Biochemistry of AIDS 470
- Factors Influencing Transmission of AIDS *470*
- Symptoms of HIV Infection *470*
- Routes of Transmission *472*
- Pathophysiology *473*
- Molecular Basis of HIV *474*
- Diagnosis of AIDS *475*
- Prevention of AIDS *475*
- Treatment of AIDS *476*

26. Biochemistry of Cancer 478
- Cell Cycle *481*
- Tumor Markers *492*
- Oncofetal Antigens *495*
- Biochemical Basis of Cancer Therapy *496*

27. Radioisotopes in Medicine 501
- Types of Radiation *502*
- Handling Radioactive Material *503*
- Applications of Radioactive Isotopes *504*
- Radiation Effects *506*

28. Metabolism of Xenobiotics (Detoxification) 508
- Phase I Detoxification *508*
- Phase II Detoxification (Conjugation) *510*

29. Biochemistry of Free Radicals and Antioxidants 513
- Reactive Oxygen Species *514*
- Antioxidants *515*
- Oxidative Stress in Disease *518*

30. Immunology 521
- Innate Immunity *521*
- The Immune System *522*
- Immunoglobulins *523*
- Hypergammaglobulinemia *527*

31. Biochemistry of Vision 531
- Structure of the Eye *532*
- Phototransduction *533*
- Visual Cycle *535*
- Color Vision *535*
- Human Disorders of Phototransduction *535*
- Sorbitol Pathway and Diabetes Mellitus *536*

32. Clinical Chemistry 538
- Purpose of the Laboratory Medicine *538*
- Critical Alerts *542*

- Legal and Ethical Regulations *543*
- Laboratory Values *544*

33. Instrumentation and Techniques 549

- Spectrophotometry/Colorimetry *549*
- Centrifugation *551*
- Electrophoresis *553*
- Chromatography *554*
- Radioimmunoassay *556*
- Enzyme-linked Immunosorbent Assay *557*

34. Buffers and Biochemical Tests 560

- Buffer *560*
- Acids and Bases *561*
- Chemical Components of Normal Urine *562*
- Urine Analysis: Abnormal Constituents of Urine *564*
- Determination of Creatinine Content in Urine, Calculation of Creatinine Clearance *566*
- Estimation of Plasma Proteins *566*

***Index* 569**

CHAPTER

6 Chemistry of Carbohydrates

Learning Objectives

At the end of this chapter, students should be able to:

- Classify the carbohydrates with examples.
- Explain the structure and function of starch and glycogen.
- Understand the isomerism property of carbohydrates.

INTRODUCTION

Carbohydrates are organic substances containing carbon (C), hydrogen (H), and oxygen (O) atoms usually in the ratio of 1:2:1. They are polyhydroxy aldehyde or ketone derivatives.

CLASSIFICATION OF CARBOHYDRATES

Carbohydrates are classified into four major groups, as follows:

Monosaccharides

Monosaccharides are simple sugars that cannot be hydrolyzed further into simpler forms **(Table 6.1)**.

Monosaccharides are further classified on the basis of number of carbon atoms present as well as on the presence of functional groups.

TABLE 6.1: Monosaccharides.

Number of carbon atoms	***Examples***	***Functional groups present***
Trioses (three carbons)	Glyceraldehyde	Aldehyde (aldotriose)
	Dihydroxy acetone	Ketone (ketotriose)
Tetroses (four carbons)	Erythrose	Aldehyde (aldotetrose)
Pentoses (five carbons)	Ribose	Aldehyde (aldopentose)
	Xylose	Aldehyde (aldopentose)
	Xylulose	Ketone (ketopentose)
Hexoses (six carbons)	Glucose	Aldehyde (aldohexose)
	Galactose	Aldehyde (aldohexose)
	Fructose	Ketone (ketohexose)

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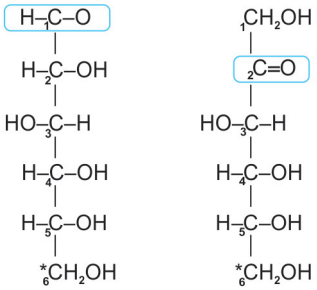

Disaccharides

Disaccharides contain two molecules of the same or different monosaccharide units that get separated on hydrolysis. These units are joined by glycosidic bond **(Table 6.2)**.

Polysaccharides

Polysaccharides consists of repeat units of monosaccharides or their derivatives, held together by glycosidic bonds. They are primarily concerned with two important functions-structural, and storage of energy. Polysaccharides are linear as well as branched polymers. They are further classified into homopolysaccharides and heteropolysaccharides.

Homopolysaccharides

Homopolysaccharides are polymer of same monosaccharide units **(Table 6.3)**.

Starch: It is a mixture of two polysaccharides—amylose and amylopectin **(Fig. 6.1)**. The major differences between amylose and amylopectin are shown in **Table 6.4**.

TABLE 6.3: Examples of homopolysaccharides.

Examples	*Monosaccharide unit*	*Sources*
Starch	Glucose	Plants, rice
Dextrin	Glucose	From starch hydrolysis
Glycogen	Glucose	Liver, muscle
Cellulose	Glucose	Plant fibers
Inulin	Fructose	Dahlia roots
Chitin	*N*-acetyl glucosamine	Arthropod

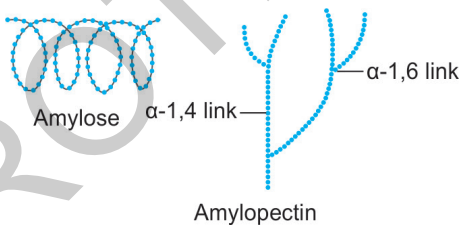

Fig. 6.1: Structure of starch.

Glycogen: It is stored in liver and muscle. It is a polymer of glucose units. It is also called animal starch. It is similar to the amylopectin component of starch. But it has more branches than starch. There are 11–18 glucose residues between any branch points.

Dextrin: These are partially hydrolyzed product of starch.

Cellulose: It is made up of β-D-glucose joined by β-1,4-glycosidic bonds. Cellulose is digested by *cellulase* enzyme, which is not present in human body. However, cellulose acts as dietary fiber, adds bulk to the food, and helps in peristalsis.

TABLE 6.2: Examples of disaccharides.

Examples	*Product formed upon hydrolysis*	*Glycosidic linkage*	*Sources*
Maltose	Glucose + glucose	α-1,4	Malt
Lactose	Galactose + glucose	β-1,4	Milk
Sucrose	Glucose + fructose	β-1,2	Sugarcane
Isomaltose	Glucose + glucose	α-1,6	Digestion of amylopectin

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TABLE 6.4: Differences between amylose and amylopectin.

Characteristics	*Amylose*	*Amylopectin*
Amount present in starch	15–20%	80–85%
Structure	Unbranched, linear	Highly branched; branch point appears after every 24–30 glucose in straight chain form
Molecular weight	60 kDa	500 kDa
Linkage	250–300 glucose residues are joined by α-1,4-glycosidic linkage	Mainly formed by α-1,4 linkages between glucose residues; branch point occurs by forming α-1,6 glycosidic linkage
Reaction with iodine solution	Blue color forms because the iodine molecules are trapped inside the helical structure. Color disappears upon heating and reappears upon cooling	Reddishviolet color

Inulin: It consists of a small number of β-D-fructose joined by β-2,1-glycosidic linkages. It is used to measure glomerular filtration rate, a test to assess the function of kidney.

Difference between Amylose and Amylopectin

Differences between amylose and amylopectin are shown in **Table 6.4**.

HETEROPOLYSACCHARIDES WITH EXAMPLES AND THEIR BIOMEDICAL IMPORTANCE

Heteropolysaccharides are polymer of different monosaccharide units or their derivatives, e.g., mucopolysaccharides (MPS) and blood group substances.

Mucopolysaccharides

Hyaluronic acid, chondroitin sulfate, heparin, keratan sulfate, heparan sulfate, and dermatan sulfate are the examples for mucopolysaccharides **(Table 6.5)**. Mucopolysaccharides are heteropolysaccharides, which remain when protein is removed from proteoglycan. Mucopolysaccharides are also known as glycosaminoglycans.

Biomedical importance of mucopolysaccharides

1. Mucopolysaccharides are acidic in nature because of their polyanionic property. They are the components of ground substances throughout the extracellular space. They are attached to proteins and form proteoglycans.
2. Hyaluronic acid acts as a barrier in tissues against the penetration of bacteria. Hyaluronidase present in bacteria can digest hyaluronic acid and acts as "spreading factor." Hyaluronidase present in testicular secretions helps in fertilization by favoring the entry of spermatozoa into the ovum.
3. Heparin acts as anticoagulant in vitro as well as in vivo. It inhibits thrombin.

ISOMERISM IN CARBOHYDRATES

The presence of asymmetric carbon atoms (a carbon atom to which four different atoms or groups attached is known as asymmetric carbon) in a compound produces the following effects:

- Formation of the stereoisomerism of the compound
- Confers optical activity to the compound.

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TABLE 6.5: Mucopolysaccharides.

Mucopolysaccharide	*Composition*	*Occurrence*
Chondroitin sulfate	Glucuronic acid and *N*-acetyl galactosamine	Cartilage
Dermatan sulfate	Glucuronic acid or iduronic acid and *N*-acetylgalactosamine	Animal tissue
Keratan sulfate	Galactose and *N*-acetyl glucosamine	Cornea and helps in corneal transparency
Heparin (highly sulfate)	Glucuronic acid and *N*acetyl glucosamine	Liver, lungs, arterial wall, and blood anticoagulant
Hyaluronic acid (sulfate free)	Glucuronic acid and *N*acetylgalactosamine	Synovial fluid of joint, vitreous humor of eye, umbilical cord, cell membrane, and skin

OPTICAL ACTIVITY OF CARBOHYDRATES, INCLUDING STEREOISOMERISM WITH SPECIFIC EXAMPLES

Optical Activity

The compounds having asymmetric carbon atoms can rotate the beam of plane-polarized light and are said to be optically active.

1. An isomer, which rotate the plane of polarized light to the right is called dextrorotatory and is designated as (*d*) or+, for example, D-(*d*)-glucose or it is also known as dextrose.
2. While the isomer, which rotates the plane of polarized light to the left is known as levorotatory and is identified as (*l*) or (–), for example, D-(*l*)-fructose.
3. A compound with D-configuration can be dextrorotatory (D+) or levorotatory (D–), for example, D+ glucose and D– fructose.

Mutarotation

Ordinary crystalline glucose (a form of glucose) is dissolved in water. Then, the plane of polarized light is passed through this solution. The optical rotation of plane-polarized light gradually changes to a constant fixed rotation. This change in rotation is called mutarotation. The mechanism behind this is a slow change α form of sugar to its β form to an equilibrium mixture.

Sugar

α-D-glucose→Equilibrium→β-D-glucose mixture

Optical Rotation

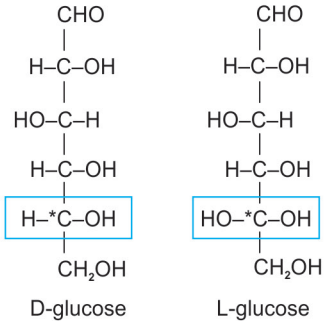
+112→+52.2→+19

Stereoisomerism

Compounds, which are identical in composition and structural formula but differ in spatial configuration, are called stereo-isomers. These include the following:

Enantiomers

Dextrose (D)- and Levo (L)-sugars are referred to as enantiomers. Their structures are the mirror images of each other. Only D-glucose or D-sugars are utilized by humans. The D- and L-glucose are termed D, and L form depending on the arrangement of –H and –OH on the penultimate carbon atom. When the sugar has –OH group on the right side, then it is D-isomer. If the OH group is on left side, then it is L-isomer.



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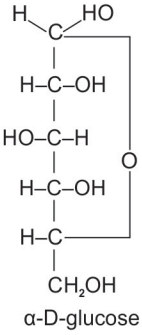
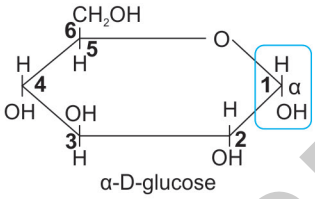
Anomerism

Sugars in a solution exist in a ring form and not in a straight chain form. Aldo sugar forms mainly pyranose ring and keto-sugar form furanose ring structure. Carbon 1, after ring formation, becomes asymmetric, and it is called anomeric carbon atom. The two sugars, which differ in the configuration at only C1 in case of aldoses and C2 in ketoses, are known as anomers and represented as α- and β-sugars. For example:

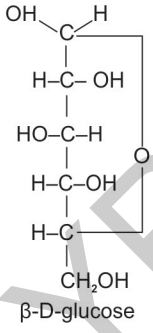
- α-D-glucose and β-D-glucose
- α-D-fructose and β-D-fructose.



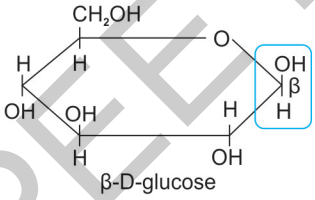
α-D-glucose



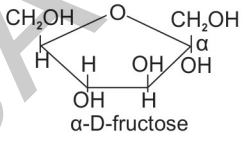
α-D-glucose



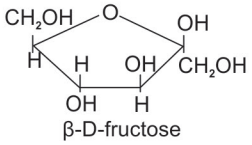
β-D-glucose



β-D-glucose



α-D-fructose

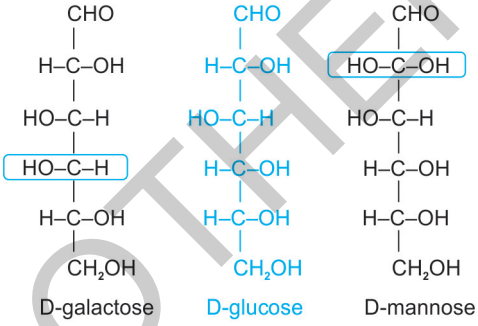


β-D-fructose

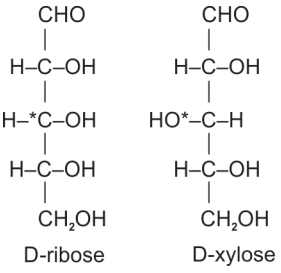
Epimerism

The isomers formed due to variations in the configuration of –H and –OH around a single-carbon atom in a sugar molecule is called epimers. For example:

- Mannose is an epimer of glucose because these two have different configurations only around C2.
- Similarly, galactose is an epimer of glucose because these two have different configurations only around at C4.



- Ribose and xylose are epimers differing in configuration of groups around carbon:



INVERT SUGAR WITH EXAMPLE

Inversion

- Sucrose shows this phenomenon.
- Sucrose is dextrorotatory in nature.
- After hydrolysis, it gives the mixture of glucose and fructose.
- The hydrolyzed mixture shows levorotatory activity. This phenomenon is called inversion. This is because optical activity of fructose is –92° and glucose is 52.5°. The sum is negative.
- The enzyme that digests sucrose is sucrase, it is also known as invertase.
- Sucrose is the example for invert sugar.

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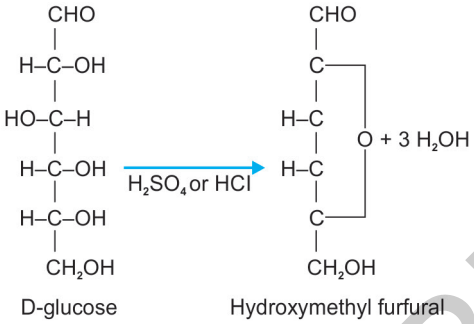
CHEMICAL PROPERTIES OF MONOSACCHARIDES

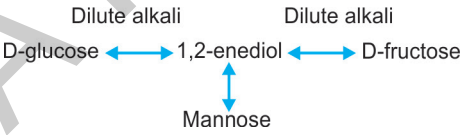
Action of Strong Acids

When monosaccharide reacts with strong acids such as sulfuric acid or hydrochloric acid, it loses a water molecule and forms furfural derivatives. These derivatives may combine with α-naphthol or resorcinol to form colored complex:



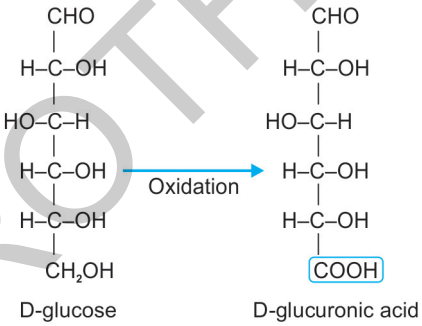
Action of Alkali

Monosaccharides when reacted with alkali are converted to enediols (involves either free aldehyde or keto group in the molecule). This enediol is the enol form of sugar because two hydroxyl groups (–OH) are attached to the double-bonded carbon. Enediols are the good reducing agents and which form on the basis of the Benedict's test. The alkali enolizes the sugar and causes them to be a strong reducing agent:



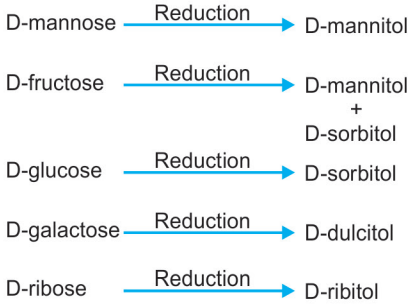
Oxidation of Monosaccharides

1. Glucose undergoes oxidation in the presence of hypobromous acid to form gluconic acid. Similarly, mannose and galactose form mannonic and galactonic acid, respectively.
2. Oxidation of aldoses with nitric acid under proper conditions converts both aldehyde and terminal primary alcohol groups, forming dibasic saccharic or aldaric acids.
3. Uronic acid (D-glucuronic acid) is formed when aldose (D-glucose) is oxidized.
4. This glucuronic acid is involved in the process of detoxification of benzoic acid, bilirubin, and certain drugs. It is also a component of heteropolysaccharides and glycoproteins.



Reduction Reaction

Aldoses and ketoses may be reduced (enzymatically or nonenzymatically) to corresponding polyhydroxy alcohols:



Sorbitol concentration increases in the lens in diabetic cataract.

ACTION OF PHENYLHYDRAZINE

1. The presence of a free aldehyde or ketone group in the molecule is essential for the osazone formation **(Fig. 6.2)**.

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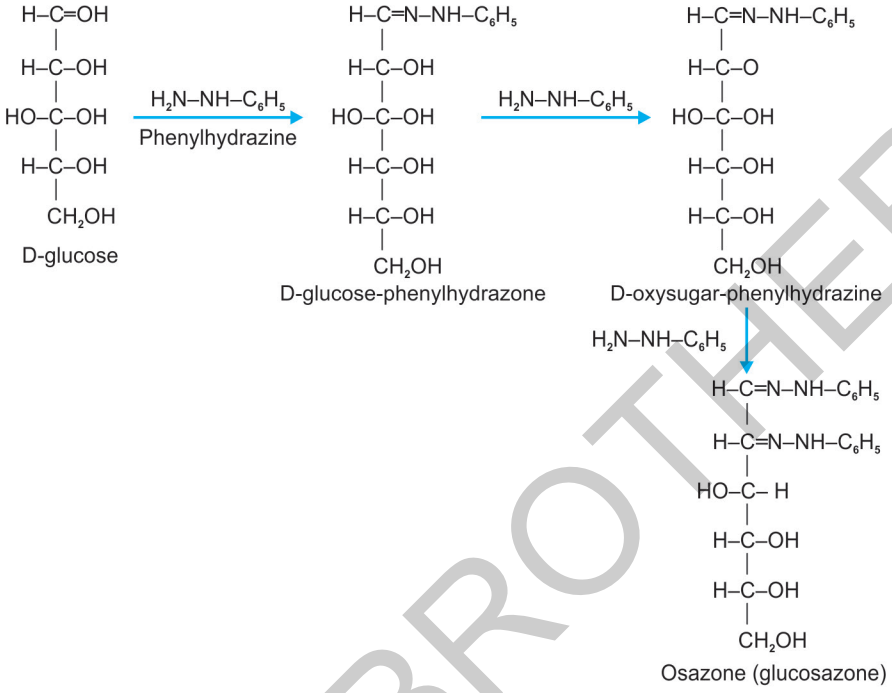

Fig. 6.2: Action of phenylhydrazine and osazone formation.

2. This osazone formation is helpful in the characterization and identification of different sugars having closely related properties.
3. Osazones are yellow or orange crystalline derivatives of reducing sugars with phenylhydrazine.
4. Phenylhydrazine in acetic acid on heating to 100°C reacts with the carbonyl carbon of reducing sugar and forms glucose phenylhydrazine with the elimination of a water molecule.
5. Then, the second molecule of phenylhydrazine reacts with the second carbon of D-glucose-phenylhydrazine and creates carbonyl carbon group on it.
6. The third molecule of the phenylhydrazine reagent reacts with the newly formed carbonyl carbon with the elimination of water molecule.
7. The osazone formed from glucose (glucosazone), mannose (mannosazone), and fructose (fructosazone) are identical because they are similar in the carbon 3–6:
   - Maltose forms maltosazone crystals, which are sunflower shaped.
   - Lactose forms lactosazone crystals, which are powder puff or tennis ball shaped.
   - Glucosazone is needle shaped.
   - Therefore, the osazone crystal formation helps to identify glucose, lactose, and maltose.
8. Sucrose will not form osazone due to the absence of a free carbonyl (aldehyde or keto) group.

GLYCOSIDIC BOND

Glycosidic bond is the linkage formed between –OH group of anomeric carbon of one sugar with any –OH group of another sugar (or alcohol), which results in the loss of a water molecule. This linkage is

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involved in the formation of disaccharide and polysaccharides.

TESTS FOR CARBOHYDRATES

Reduction Tests

Due to the presence of a free aldehyde or ketone group, carbohydrates are readily oxidized and behave as the reducing agents. These sugars have the capacity to reduce cupric ion (Cu^{2+}) to cuprous ion (Cu^{+}). Therefore, only the reducing sugars such as glucose will give positive reactions. Nonreducing sugars, i.e., sucrose will respond to these tests provided it is first hydrolyzed into its reducing components—glucose and fructose.

Benedict's Test

When 0.5-mL solution containing reducing sugar is boiled with 5.0-mL Benedict's reagent (blue color) for 5 minutes, brick red-, green-, or yellow-colored precipitate appears. This indicates the presence of reducing sugar in the given sample. This test is applied for the detection of reducing sugars in urine in the case of diabetes and galactosemia. (Benedict's reagent contains sodium citrate, sodium carbonate, and copper sulfate.)

SPECIAL CARBOHYDRATES OF CLINICAL IMPORTANCE

Amino Sugars

Sugars containing an amino (NH_2) group in their structure are called amino sugars, e.g., D-glucosamine. *N*-acetyl derivative of D-glucosamine and D-galactosamine occurs as a constituent of mucopolysaccharides. It is also present in some antibiotics.

Glycosides

Glycosides are compounds containing a carbohydrate and a noncarbohydrate residue in a same molecule linked together by a glycosidic bond.

Cardiac glycosides:

- Digitoxin or digoxin—stimulates muscular contraction (cardiac stimulant)
- Streptomycin—antibiotic used in treatment of tuberculosis
- Cardiac glycosides—used in cardiac insufficiency
- Ouabain—a sodium pump inhibitor.

Sialic Acid

Sialic acid is *N*-acetylneuraminic acid (NANA). It is present in mucopolysaccharide, glycolipid, and ganglioside.

Sugar Alcohols

D-Sorbitol and **D**-dulcitol, when accumulated in large amounts, cause strong osmotic effects leading to swelling of cells, including cataract, peripheral neuropathy, and nephropathy.

D-Mannitol: Is useful in reducing intracranial tension by forced diuresis.

Xylitol—sweetener.

Deoxy Sugar

Deoxy sugar lacks one oxygen atom in carbon and two or three of a carbohydrate.

For example:

- **Deoxyribose:** It is present in nucleic acid DNA.
- **6-deoxy-L-galactose or fructose:** It is present on cell membrane.

Glycoproteins

Glycoproteins are proteins to which carbohydrates are covalently attached, e.g., immunoglobulin and egg albumin.

Proteoglycans

Proteoglycans are also proteins to which carbohydrates are covalently attached, but the carbohydrates differ chemically from those attached to glycoproteins. The carbohydrates may be glucosamine or galactosamine and/or their acetyl derivatives, uronic acids, and sulfate groups.

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SUMMARY

- Carbohydrates are organic substances of polyhydroxy aldehyde or ketone derivatives.
- Carbohydrates are classified into monosaccharides (glucose), disaccharides (sucrose), and polysaccharides.
- Polysaccharides are of two types—homo (starch, glycogen, cellulose, and inulin) and heteropolysaccharides (hyaluronic acid, dermatan sulfate, keratan sulfate, and heparin).
- Carbohydrates form stereoisomers, such as D- and L-sugars, and they are referred to as enantiomers (mirror images of each other). Carbon 1 of sugars is anomeric carbon atom (α-D-glucose and β-D-glucose). The isomers formed due to variations in the configuration of –H and –OH around a single carbon atom in a sugar molecule is called epimers (mannose and galactose are two and four epimers of glucose, respectively). Sucrose, also called invert sugar, is nonreducing.

SELF-ASSESSMENT QUESTIONS

1. Define carbohydrates.
2. Classify the carbohydrates with a suitable example for each class.
3. Explain the stereoisomerism of carbohydrates.
4. How monosaccharides are further classified?
5. What are the two types of polysaccharides?
6. Define the asymmetric carbon atom.
7. Explain the term "mutarotation."
8. What are disaccharides? Give example and composition.
9. What are proteoglycans and mucopolysaccharides?
10. Write briefly on mucopolysaccharide. Write their biomedical importance.
11. Discuss on the structure of starch and glycogen.
12. Differentiate between amylose and amylopectin.
13. If the two monosaccharides differ in the configuration around a single carbon atom, then what are they called as?
14. Name the nonreducing disaccharide.
15. Name the α- and β-cyclic forms of D-glucose?
16. Name the noncarbohydrate moiety present in glycoside.
17. Give an example for a glycoside.
18. Name the polysaccharide employed for the assessment of kidney function tests.
19. Name the glycosaminoglycan that serves as a lubricant and shock absorbent of joints.

MULTIPLE CHOICE QUESTIONS

1. Which of the following is not an aldose sugar?

a. Glucose
b. Galactose
c. Mannos
d. Fructose

2. One of the following serves as an anticoagulant:

a. Heparin
b. Hyaluronic acid
c. Chondroitin sulfate
d. Keratan sulfate

3. The polysaccharide containing β-glycosidic linkage is:

a. Starch
b. Glycogen
c. Dextrin
d. Cellulose

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4. In general, the carbon atoms involved in reducing action are:
a. 1 and 2
b. 2 and 3
c. 3 and 4
d. 5 and 6

5. Concerning glucose, all of the following statements are true, *except*:
a. It is an aldohexose
b. It is a reducing sugar
c. It is present in starch and cellulose
d. It is an epimer of fructose

6. The glycosaminoglycan, which does not contain uronic acid, is:
a. Heparin
b. Hyaluronic acid
c. Chondroitin sulfate
d. Keratan sulfate

7. Which of the following is a deoxy sugar?
a. Ribose
b. Fucose
c. Glucosamine
d. Xylulose

8. _____ is not found in glycosaminoglycan.
a. L-Iduronic acid
b. D-glucuronic acid
c. Ribose
d. *N*-Acetylgalactosamine

9. _____ is a heteropolysaccharide.
a. Dextrin
b. Chitin
c. Inulin
d. Heparin

10. Concerning starch, one of the following statements is incorrect:
a. Starch is made of both linear and branched polysaccharide
b. Starch gives red color with I_2 reactions
c. Starch can be hydrolyzed to glucose in the body
d. Glycogen is known as animal starch

11. One of the following is an example of invert sugar:
a. Glucose
b. Fructose
c. Sucrose
d. Maltose

12. All the following are the examples of monosaccharide, *except*:
a. Glucose
b. Fructose
c. Sucrose
d. Galactose

13. The bond, which links the monosaccharide units, is:
a. Ester bond
b. Phosphate bond
c. Disulfide bond
d. Glycosidic bond

14. All of the following polysaccharides contain glucose as the monosaccharide units *except*:
a. Starch
b. Inulin
c. Glycogen
d. Dextrin

15. Regarding amylopectin component of starch, the following statements are true, *except*:
a. It is highly branched
b. It is unbranched
c. It gives reddish-violet color with iodine
d. Its amount present in starch is 80–85%

16. Concerning glycogen, one of the following statements is incorrect:
a. A polymer of glucose
b. Stored in liver and muscle
c. Stored in liver and brain
d. Having 11–18 glucose residues between any branching points

17. Concerning cellulose, one of the following statements is incorrect:
a. Contains β-D-glucose and β-1,4-glycosidic bonds
b. Digested by sucrase enzyme
c. Acts as a dietary fiber
d. Digested by cellulase enzyme

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18. Regarding the heteropolysaccharides, the following statements are correct, *except*:
a. Polymer of different monosaccharide units
b. Heparin and chondroitin sulfate are the examples
c. Components of ground substances throughout the extracellular space
d. Glycogen and starch are the examples

19. All the following are the functions of heteropolysaccharides, *except*:
a. Hyaluronic acid acts as a barrier in tissues against the penetration of bacteria
b. Hyaluronidase present in testicular secretions helps in fertilization by favoring the entry of spermatozoa into the ovum
c. Chondroitin sulfate acts as anticoagulant
d. Heparin acts as anticoagulant in vitro as well as in vivo. It inhibits thrombin

20. Concerning stereoisomerism of carbohydrates, all the following statements are true, *except*:
a. D- and L-sugars are referred to as enantiomers
b. D-Sugars are utilized by humans
c. When the sugar has –OH group on right, it is D-isomer
d. When the sugar has –OH group on left, it is D-isomer

21. Anomerism means:
a. Carbon 1, after ring formation becomes asymmetric and it is called anomeric carbon atom
b. Carbon 6, after ring formation becomes asymmetric and it is called anomeric carbon atom
c. Carbon 2, after ring formation becomes asymmetric and it is called anomeric carbon atom
d. Mirror images

22. Regarding epimerism, all of the following statements are true, *except*:
a. The isomers formed due to variations in the configuration of –H and –OH around a single carbon atom in a sugar molecule is called epimers
b. Mannose is 2-epimer of glucose
c. Galactose is 4-epimer of glucose
d. Fructose is α-4-epimer of glucose

23. Which of the following is not a reducing sugar?
a. Glucose
b. Sucrose
c. Fructose
d. Mannose

24. Concerning carbohydrates, one of the following statements is incorrect:
a. Are most abundant dietary source of energy for all organisms
b. Are precursors for many organic compounds such as fats and amino acids
c. Participate in the structure of cell membrane and cellular functions
d. Build the muscle mass

25. The monosaccharide, which is present in DNA, is:
a. Glucose
b. Ribose
c. Fructose
d. Mannose

26. The 2-epimer of glucose is:
a. Mannose
b. Galactose
c. Ribose
d. Gibulose

27. Glucose is an:
a. Ketohexose
b. Aldohexose
c. Aldotriose
d. Aldopentose

28. Polysaccharide, which has fructose as their monosaccharide unit, is:
a. Starch
b. Dextrin
c. Inulin
d. Cellulose

29. Concerning the structure of starch, one of the following statements is false:
a. It has 85% of amylopectin
b. Amylose is highly branched
c. Blue color appears when amylose reacts with iodine
d. The glucose units in amylose held by α-1,4- glycosidic linkage

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30. Concerning cellulose, all the following statements are true, *except*:
a. It has β-D-glucose joined by β-1,4-glycosidic bonds
b. It is digested by cellulase enzyme in the humans
c. It acts as dietary fiber
d. It acts as a storage form of glucose

31. Which of the following is not a heteropoly-saccharide?
a. Hyaluronic acid
b. Heparin
c. Dextrin
d. Chondroitin sulfate

32. 4-Epimer of glucose is:
a. Galactose
b. Mannose
c. Fructose
d. Maltose

33. All the following include answers of Benedict's test, *except*:
a. Glucose
b. Ascorbic acid
c. Sucrose
d. Fructose

34. Concerning stereoisomerism, all the following statements are true, *except*:
a. D- and L-sugars are referred to as enantiomers
b. Sugars in solution exist in ring form and not in the straight chain form
c. Carbon 1 in aldo sugars is the anomeric carbon atom.
d. Keto sugar forms pyranose ring

35. A 16-year-old male patient complains that lately after the ingestion of dairy products, he experiences bloating, cramps, flatulence, and sometimes diarrhea. Therefore the patient is intolerant to:
a. Lactose
b. Mannose
c. Sucrose
d. Maltose

36. This heteropolysaccharide has multiple uses in medicine that include its use in blood transfusions to prevent the blood from coagulating before administration, as anticoagulant therapy in prophylaxis and treatment of venous thrombosis and its extension in pulmonary embolisms, and in other similar situations:
a. Hyaluronic acid
b. Chondroitin sulfate
c. Heparin
d. Dermatan sulfate

37. Human beings do not have the enzymes necessary for the hydrolysis of the β-1,4-*O*-glycosidic linkages between molecules of glucose. That is why humans cannot digest this compound and it is part of some laxatives:
a. Glycogen
b. Cellulose
c. Amylopectin
d. Sucrose

Answers

1. d	2. a	3. d	4. a	5. d
6. d	7. b	8. c	9. d	10. b
11. c	12. c	13. d	14. b	15. b
16. c	17. b	18. d	19. c	20. d
21. a	22. d	23. b	24. d	25. b
26. a	27. b	28. b	29. b	30. c
31. c	32. a	33. c	34. d	35. a
36. c	37. b			

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