Indira Gandhi National
Open University
School of Sciences

BBCCT-101
MOLECULES OF LIFE

Block

2

CARBOHYDRATES

BLOCK 2 : CARBOHYDRATES

In Block 1, we dealt with the history and scope of biochemistry followed by the detailed explanation on structure and functions of water.The unique properties of water which make it a universal solvent and role of buffers in the biological system were also discussed. You also studied about amino acids, peptides and proteins. While studying amino acids and peptides, you came across various types of amino acids and learnt about their properties under physiological conditions and how these amino acids joins together to form peptides. In addition to these aspects, you also got an idea of organization of amino acids at levels of primary, secondary, tertiary and quaternary structure of protein. The present block consists of four units (Unit 5 to Unit 8) beginning from simplest carbohydrates to more complex classes of carbohydrates.

Carbohydrates commonly known as sugars are one of the important biomolecules which perform diversity of biological functions. This block deals with the structural and functional aspects of these biomolecules.

In Unit 5, we shall give overview of carbohydrates and discuss structure and properties of monosaccharides which form basic unit for complex carbohydrates. Bio-chemical reactions of significance of some important monosaccharides which result in formation of biologically important derivatives are also described.

Unit 6 deals with oligosaccharides, which are chains of 2-10 monosaccharides linked by glycosidic bonds. We shall learn to differentiate between reducing and non-reducing sugars. We shall also give examples of different oligosaccharides and discuss their properties and biological importance.

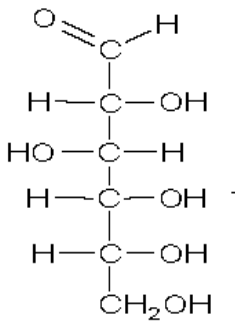
In Unit 7, you will study about types of polysaccharides and their significance as structural and storage sugars. The unit also deals with conjugate carbohydrates such as proteoglycans, glycorproteins and glycolipids.

In Unit 8, you will learn about glycobiology and how the information of glycoconjugates decoded in the cells. We shall also discuss different methods to study carbohydrates and the difficulties scientist face during this task.

Objectives

After studying this block you will be able to:

- describe classification, structures and biological significance of carbohydrates;
- draw structures of monosaccharides and explain their optical and chemical properties;
- describe the glycosidic bond and its importance in formation of carbohydrates;
- classify oligosaccharides into reducing and non-reducing sugars and write their structures and importance;
- describe role of polysaccharides in context with their chemical structures; and
- recognise how sugar code plays a role in communication between cell and its surrounding.



UNIT 5

MONOSACCHARIDES

Structure

5.1 INTRODUCTION

Carbohydrates constitute the most abundant organic molecules found in nature and are widely distributed in all living organisms. These are synthesized in nature by green plants, algae and some bacteria by photosynthesis. They also form major part of our diet and provide us energy required for the life sustaining activities such as growth, metabolism and reproduction. At microscopic level, these constitute the structural components of the cell such as cell membrane and cell wall.

In this unit, we shall begin with general overview of the chemical nature of carbohydrates and their classification. The unit focuses mainly on the simplest carbohydrates known as monosaccharides. We shall learn about the chemical structures of different monosaccharides and how to draw them. We shall also discuss their stereochemistry in detail which would help to understand how change in orientation of same substituents results in different molecules with same chemical formula but different properties. We shall also discuss about some of the chemical reactions of monosaccharides resulting in formation of important derivatives and their biological importance.

Objectives

After studying this unit you should be able to:

- define and classify carbohydrates;
- classify monosaccharides into aldoses and ketoses and give their examples;
- draw structures of carbohydrates as Fischer and Haworth projections;
- Identify stereo isomeric relations of monosaccharides;
- describe chemical reactions of monosaccharides; and
- write about the biological importance of monosaccharides and their derivatives.

5.2 OVERVIEW OF CARBOHYDRATES

Carbohydrates are diverse group of compounds that are ubiquitous in nature. Most of them are made of carbon, hydrogen and oxygen. Earlier they were believed to be hydrates of carbon with an empirical formula $C_n(H_2O)_n$ where $n \geq 3$. Therefore, the name carbohydrate was given. It was, however, soon recognised that this definition is unsatisfactory as many exceptions began to accumulate. There are carbohydrates that do not satisfy the above formula such as deoxy sugars and those that contain elements other than C, H and O. In addition, many non carbohydrate compounds can be represented by the same empirical formula as carbohydrates, for example, lactic acid has an empirical formula $C_3(H_2O)_3$.

The terms "carbohydrate," "saccharide," and "sugar" are often used interchangeably.

"Saccharide" comes from the word for "sugar" in several early languages (*sarkara* in Sanskrit, *sakcharon* in Greek, and *saccharum* in Latin).

With better understanding of their structures, carbohydrates are now defined as **polyhydroxy aldehydes or ketones**, although the original name "carbohydrates" is still retained. They also include the compounds which give polyhydroxy aldehydes or ketones on hydrolysis.

Carbohydrates are polymeric molecules that are classified into three classes:

1) **Monosaccharides** (*mono – single*)**:** They are the simplest carbohydrates and consist of one polyhydroxy aldehyde or ketone unit. These are the monomeric units for other classes of carbohydrates. Most of the naturally occurring monosaccharides are unbranched chain of 3-7 carbon atoms. Glucose is the most abundant monosaccharide in nature. The monosaccharides join through glycosidic bonds to form oligo and polysaccharides.

Oligosaccharides and polysaccharides can be broken down to their constituent monosaccharide units by hydrolysis, for example, a disaccharide yields two and a tetrasaccharide yields four monosaccharide units upon hydrolysis.

2) **Oligosaccharides** *(oligo – few)***:** They are made of 2-20 covalently linked monosaccharide units. Oligosaccharides are named based on the number of monosaccharide units they are made of. For example, oligosaccharides which consist of two, three, four or five monosaccharide units are termed as disaccharides, trisaccharides, tetrasaccharides, and pentasaccharides, respectively. Disaccharides are the most abundant oligosaccharides. Sucrose, commonly known as table sugar is a disaccharide.

3) **Polysaccharides** *(poly–many)***:** Carbohydrates with more than 20 monosaccharide units are classified as polysaccharides. The monosaccharides present in a polysaccharide chain may be similar or different and arranged in linear or branched chain. Polysaccharides also include complex carbohydrates in which carbohydrates link covalently to other biomolecules such lipids and proteins. Glycogen and starch, the storage form of glucose in animals and plants, respectively are examples of storage polysaccharides.

Carbohydrates constitute a versatile group of molecules. Their major functions are:

i) Biochemical fuel: Carbohydrates are used as primary source of energy through metabolic reactions. Glucose, fructose and galactose are used to derive energy by most of living organisms.

ii) Food reserve: Carbohydrates are stored in the animals and plants and mobilized as instant source of energy as and when required. Glycogen and starch are storage carbohydrates in animals and plants, respectively.

Our brain and RBCs are mainly dependent on glucose for energy.

iii) Structural support: Carbohydrates constitute component of cell membrane and cell wall helping in protection against the external environment and exchange of substances between cells or cell and environment. For example cellulose and xylose are involved in formation of woody material of trees.

iv) Information molecules: Carbohydrates are also involved in specialized functions of carrying codes of specific information which when decoded helps cell to perform functions like communication.

In this unit we shall focus on the structural, chemical and functional aspects of monosaccharides.

5.3 MONOSACCHARIDES

Monosaccharides are white crystalline, colorless solids which are soluble in water and insoluble in non polar solvents. These are classified in two ways:

1) Based on the functional group: Monosaccharides having aldehyde group (HC=O) are classified as ***aldoses*** and those having ketone (C=O) group *as* ***ketoses.***

2) Based on the number of carbon atoms: Monosaccharides are given generic names such as trioses (3 carbon), tetroses (4 carbon), pentoses (5 carbon), hexoses (6 carbon) and heptoses (7 carbon) (Table 5.1).

To avoid confusion, detailed generic names which describe both, the important functional group as well as the number of carbon atoms are used. For example, a monosaccharide with four carbon chain and aldehyde group is termed as **aldotetrose** (aldose + tetrose) and that with ketone group as **ketotetrose** (ketose + tetrose).

Monosaccharides exist in linear chain (open) structure, however, pentoses and hexoses also form cyclic (ring) structures. Let us discuss about these two structural forms.

Table 5.1

Chain length	Type of monosaccharide
3	Triose
4	Tetrose
5	Pentose
6	Hexose
7	Heptose

5.3.1 Linear Structure

In general, linear structures of aldoses and ketoses of chain length up to seven carbon atoms are represented as shown in the Fig. 5.1.

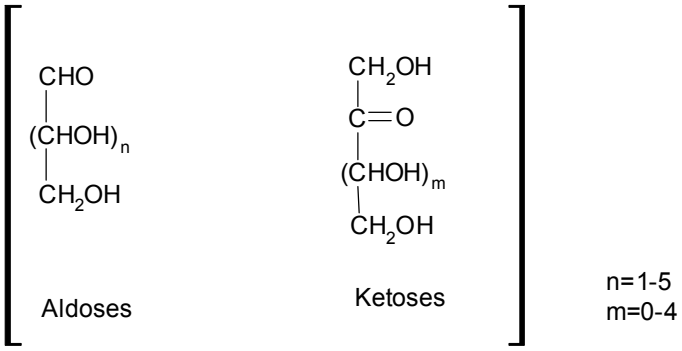


Fig. 5.1: General structures of aldoses and ketoses.

Functional group in aldoses is CHO while in ketoses, it is C=O and the remaining carbon atoms carry hydroxyl (OH) groups which vary in number from 1-5 in aldoses and 0-4 in ketoses.

Structures of naturally occurring aldoses are shown in Fig. 5.2 and 5.3 and ketoses in Fig. 5.4 and 5.5. Let us discuss some important points related to their structure.

1) Linear structure of a monosaccahride is drawn as an unbranched chain. Numbering of the chain begins from the end having most oxidized carbon or the carbon nearest to it.

2) CHO group is the most oxidized carbon in aldoses, it is numbered at position 1. In ketoses, C=O group is the most oxidized group and the numbering begins from the carbon nearest to it. Therefore, it is numbered at position 2.

3) Rest of the carbon atoms in a monosaccharide chain carry –OH groups.

4) Glyceraldehyde, an aldotriose and dihydroxyacetone, a ketotriose are the smallest monosaccharides.

Chiral or asymmetric carbon is the one in which a tetrahedral carbon atom is attached to four different substituents or groups.

5) All the aldoses and ketoses shown in the Fig. 5.2 and 5.4 are given notation D-before their name which represents that –OH group at penultimate (last but one) carbon, is at right side. There is another family of similar aldoses and ketoses which are notated as L-form because –OH group at penultimate carbon in these molecules is oriented on the left side (Fig. 5.3 and 5.5). D and L forms are mirror images and form pair of stereoisomers. Stereoisomers are molecules which have same molecular and structural formula but differ in arrangement of substituents around chiral centers in space. We shall discuss more about this aspect in the section 5.3.4.

5) All monosaccharides have one or more chiral carbon atoms which are indicated by * in all the structures shown in the Fig. 5.2- 5.5. Only exception is dihydroxyacetone.

SAQ 1

a) General empirical formula of carbohydrates is ..... .

b) Two major functions of carbohydrates are ..... .

c) Two types of functional groups present in monosaccharides are ..... and .....

d) The number of carbon atoms present in the smallest carbohydrate is .....

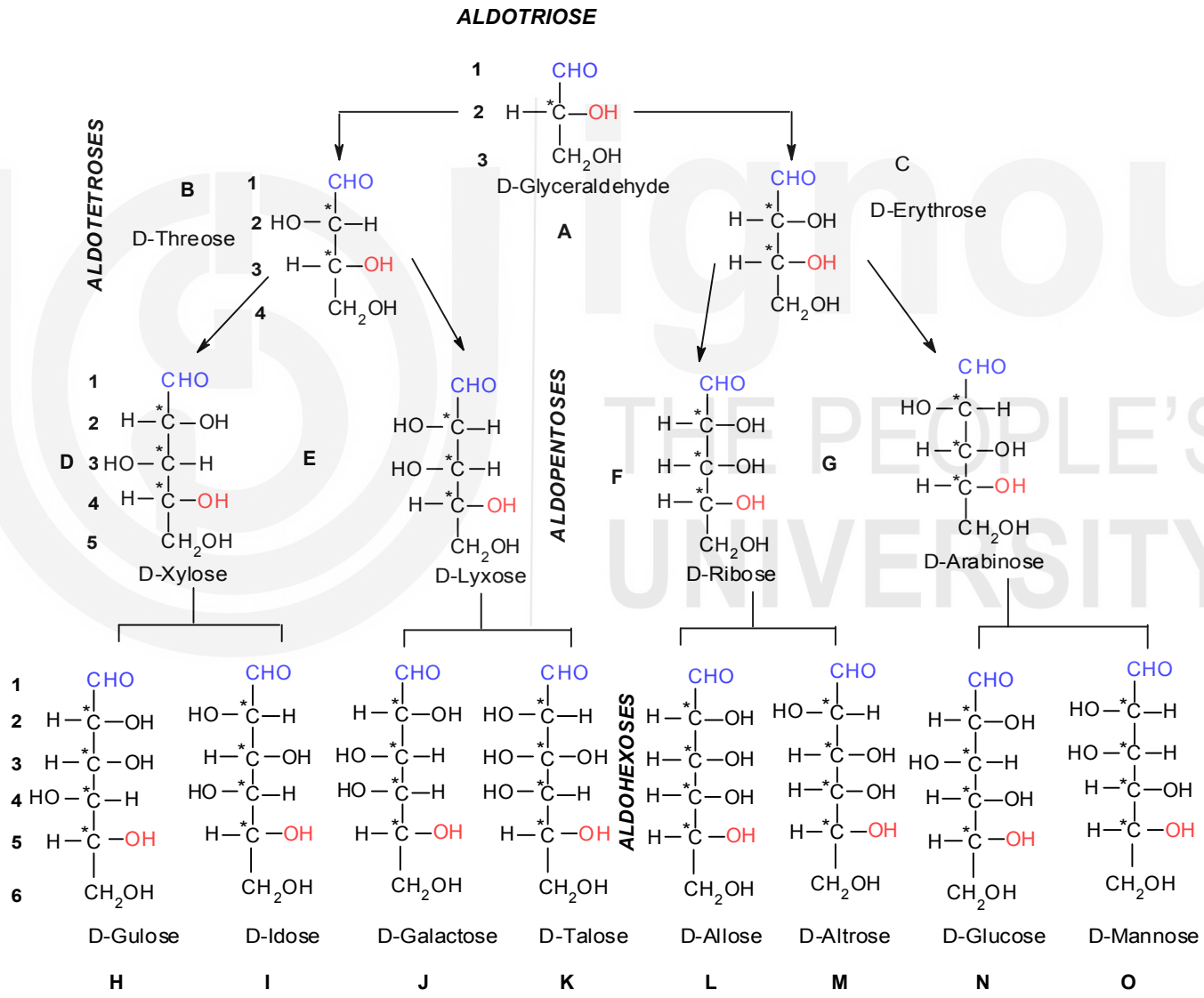


Fig. 5.2: Structures of D- aldoses. The figure presents D-aldoses in terms of increasing chain length for ease of understanding. Naturally occurring sugars have 3-7 carbon atoms. Each aldose is numbered beginning from –CHO group (shown in blue), the most oxidized carbon. Except for the first and last carbons, intermediary carbons carry –OH groups and are chiral centers marked by *. –OH group present on the right side at the penultimate carbon is marked in red. It is this group which determines the D/L notation of any sugar.

Sometimes ketoses are named simply by inserting –ul- in their generic names such as tetruloses, pentuloses, hexuloses and so on. For example note the names ribose and ribulose, erythrose and erythrulose, xylose and xylulose are aldoses and ketoses with same number of carbons.

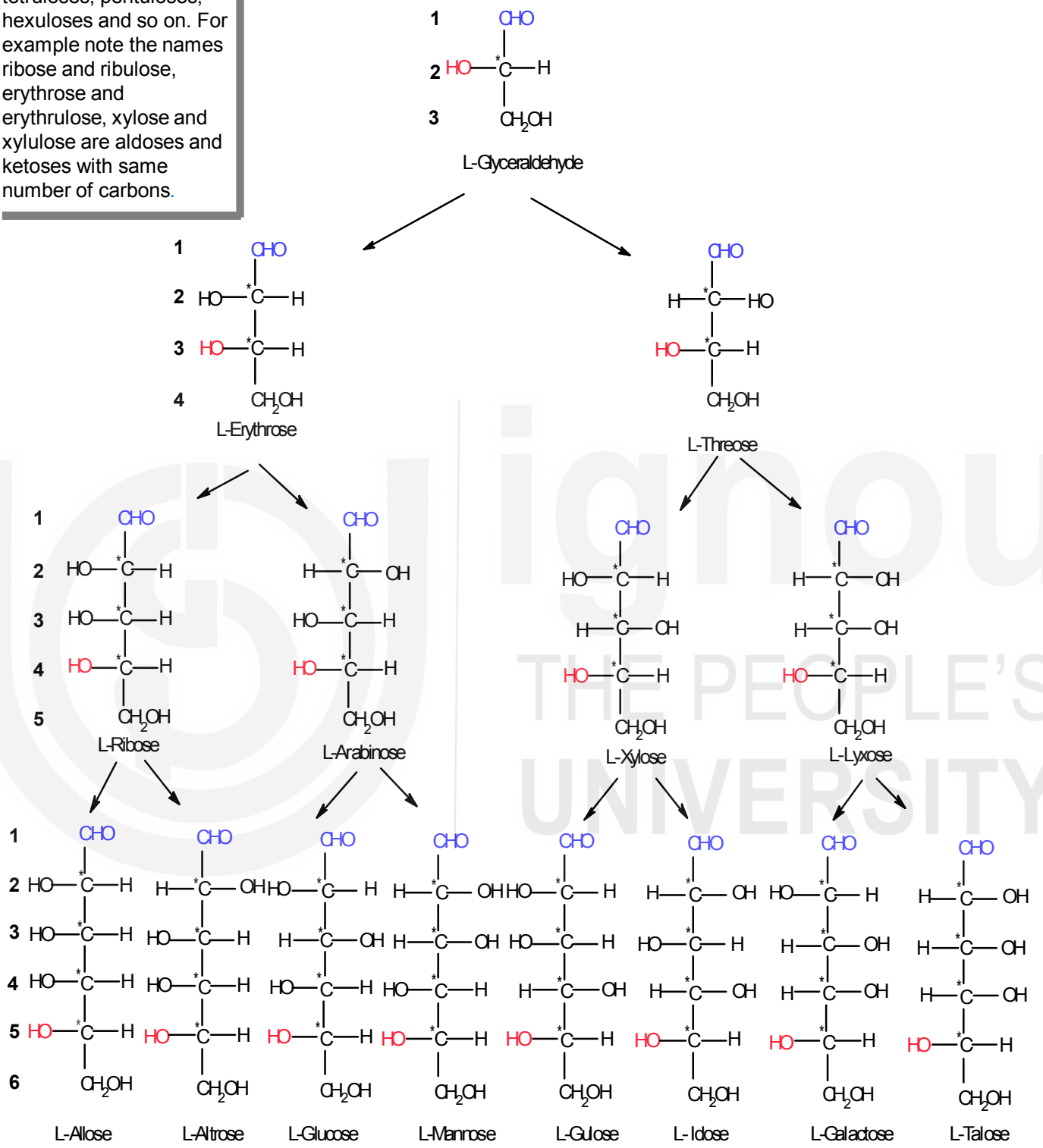


Fig. 5.3: Structures of L-aldoses. The figure presents L-aldoses in terms of increasing chain length for ease of understanding. Naturally occurring sugars have 3-7 carbon atoms. Each aldose is numbered beginning from –CHO group (shown in blue), the most oxidized carbon. Except for the first and last carbons, intermediary carbons carry –OH groups and are chiral centers and indicated with *. – OH group present on the left side at the penultimate carbon is marked in red. It is this group which determines the D/L notation of any sugar.

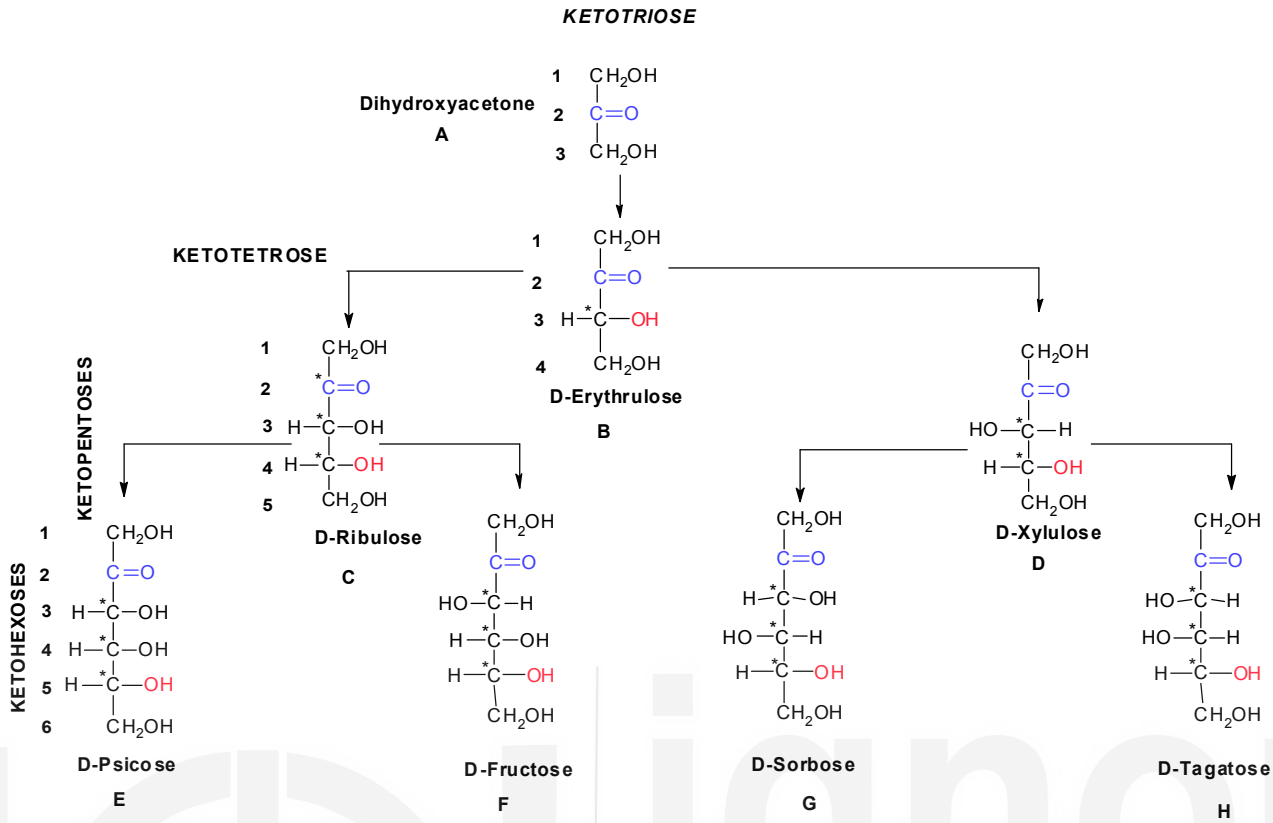


Fig. 5.4: Structures of D- ketoses. The figure presents D-ketoses in terms of increasing chain length for ease of understanding. Naturally occurring sugars have 3-7 carbon. The most oxidized carbon in ketoses is C=O (shown in blue) and is present in between the chain, therefore, numbering begins from the carbon nearest to it and it is numbered at position 2.

Chiral centers are marked with *. –OH group present on the penultimate carbon (marked in red) is on the right side in all D-ketoses.

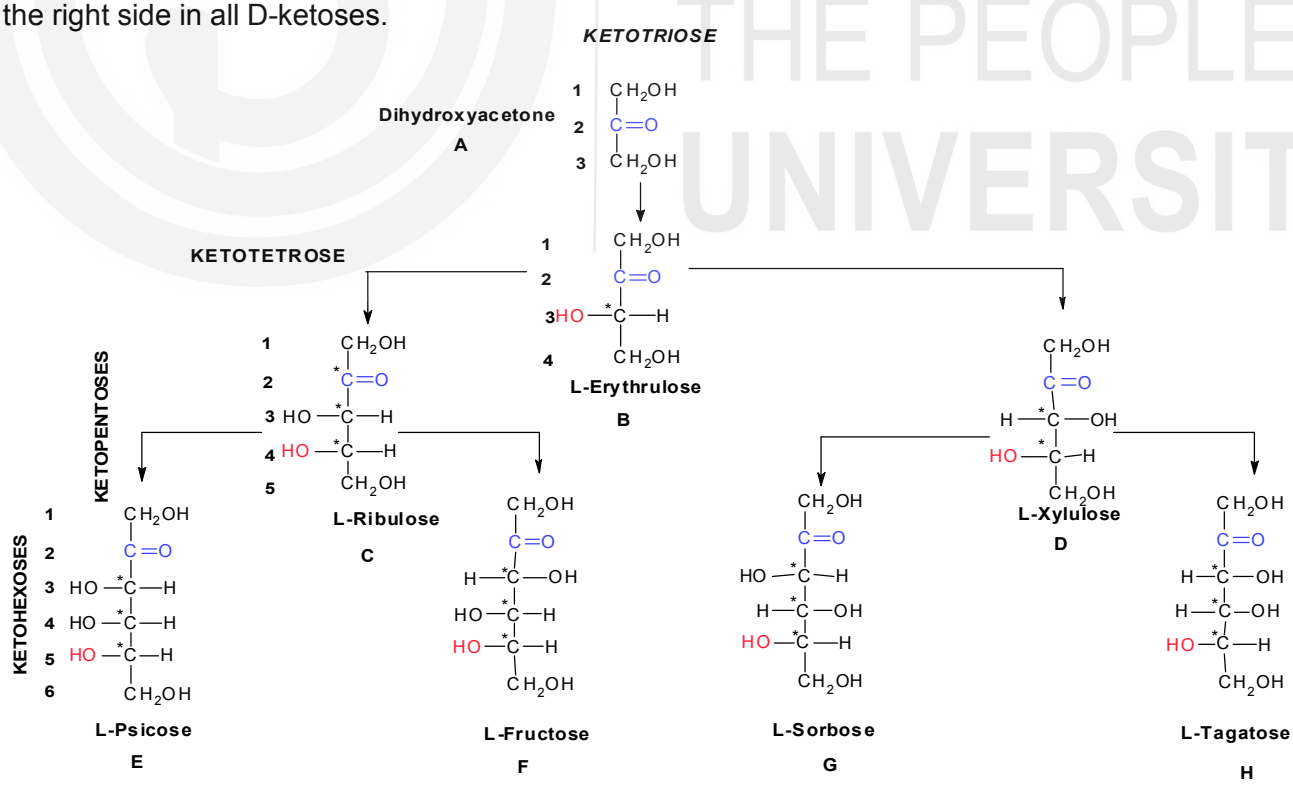
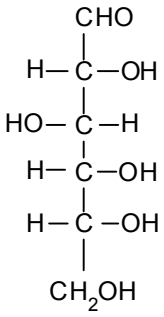


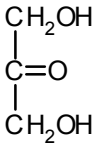
Fig. 5.5: Structures of L-ketoses. The figure presents L-ketoses in terms of increasing chain length for ease of understanding. Naturally occurring sugars have 3-7 carbon. The most oxidized carbon in ketoses is C=O (shown in blue) and is present in between the chain, therefore, numbering begins from the carbon nearest to it and it is numbered at position 2. Chiral centers are marked with *. –OH group present on the penultimate carbon (marked in red) is on the left side in all L-ketoses.

SAQ 2

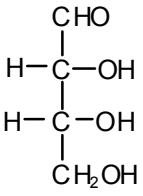
A) Identify the given structures, and mention the type of aldose or ketose and number of chiral centers present:



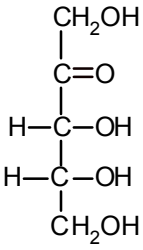
a)



b)



c)



d)

B) Match the following:

a)	Glucose	i)	5 monosaccharides
b)	Pentasaccharide	ii)	Ketohexose
c)	Pentose	iii)	Aldohexose
d)	Fructose	iv)	5-carbon chain
e)	Polysaccharide	v)	> 20 monosaccharides

C) Name two examples of the following; one each from aldoses and ketoses:

a) Triose

b) Tetrose

c) Pentose

d) Hexose

You know that molecules are three dimensional, while the structures given in Fig. 5.2-5.5 are all two dimensional. To address this issue, Emil Fischer developed Fischer projections. These are an easy and quick way to show three dimensional structures of sugars on a paper and give useful information about the orientation of various groups by following certain convention (See box 5.1). These projections are generally used for molecules which have one or more chiral centers.

Let's see how do Fischer projections for D-glucose, and D-fructose look like (Fig 5.6)

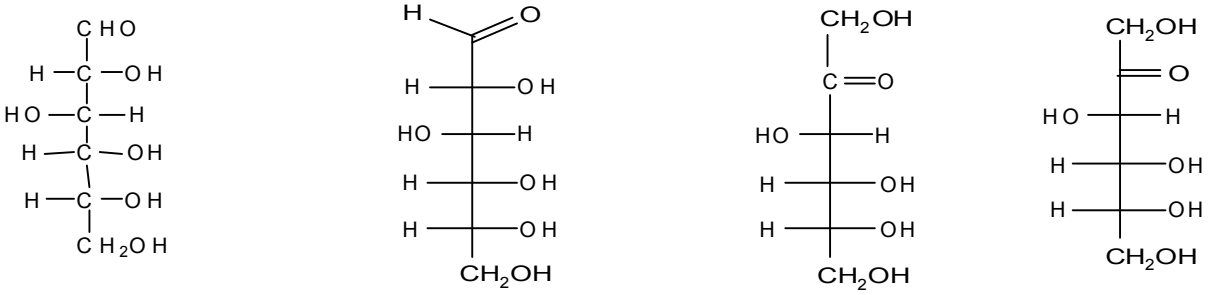


Fig. 5.6: Fischer projections for D-glucose and D-fructose.

Box 5.1: Following steps explain how to draw Fischer projection and the information that can be interpreted using this projection.

1) Place the principle functional group at the top and write the carbon chain straight down.

2) Chiral centers are represented as cross lines with their attached groups on the ends of the horizontal line.

3) The horizontal line represents bonds extending out of the plane of the paper (you may imagine as two arms coming to hug you), whereas the vertical line represents bonds extending into the plane of the paper away from the viewer.

4) Fischer projections can be rotated by 180° without changing their meaning, but not by 90° or 270°.

5) Carbohydrates with more than one chiral center are shown by stacking the centers on top of one another, with the carbonyl carbon again placed at or near the top.

5.3.2 Ring Structure

In aqueous solutions, monosaccharides with five or more carbon atoms occur predominantly in cyclic (ring) structures. In fact, when dissolved in water, their linear structures are present in extremely low proportions (1-2%). Cyclic structures of sugars are formed due to their ability to form intramolecular hemiacetal or hemiketal.

Let us understand what a hemiacetal or hemiketal is. Reaction between carbonyl group (C=O) of aldehyde or ketone and hydroxyl group (OH) of an alcohol results in formation of addition product called hemiacetal and hemiketal, respectively **(**Fig. 5.7**).** This reaction is readily reversible and catalyzed by an acid or base.

Formation of ring structure of sugars is observed only in aqueous solution as water provides hydrogen ions for formation of ring. Non polar solvents do not provide any ions, therefore, ring structures of sugars are not formed and they exist as rigid linear structures.

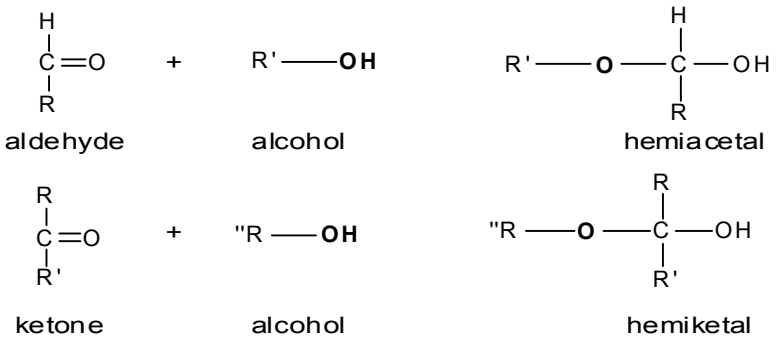


Fig. 5.7: Formation of hemiacetal and hemiketal.

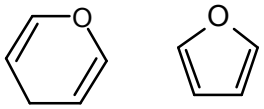


Fig. 5.8: Structures of pyran and furan.

Monosaccharides form two types of oxygen containing rings:

Pyranose- six member cyclic structure of a monosaccharide is called pyranose due to it resemblance to pyran ring (Fig. 5.8). Aldoses having five or more carbon atoms predominantly exist as pyranoses.

Furanose-Five member cyclic structure is called furanose due to its resemblance to furan (Fig. 5.8). Aldotetroses and ketoses having five or more carbon atoms exist predominantly as furanoses.

Cyclic structures of sugars with less than five members or more than six members are generally not formed as these are too strained and unstable.

We shall now understand the formation of these structures by taking glucose and fructose as examples. Fig. 5.9 explains the formation of pyranose of D-glucose from its linear form. The -OH group at C5 in D-glucose attacks HC=O at C1 resulting in hemiacetal formation. Formation of the internal hemiacetal leads to cyclization of the glucose molecule forming two forms of D-glucopyranose.

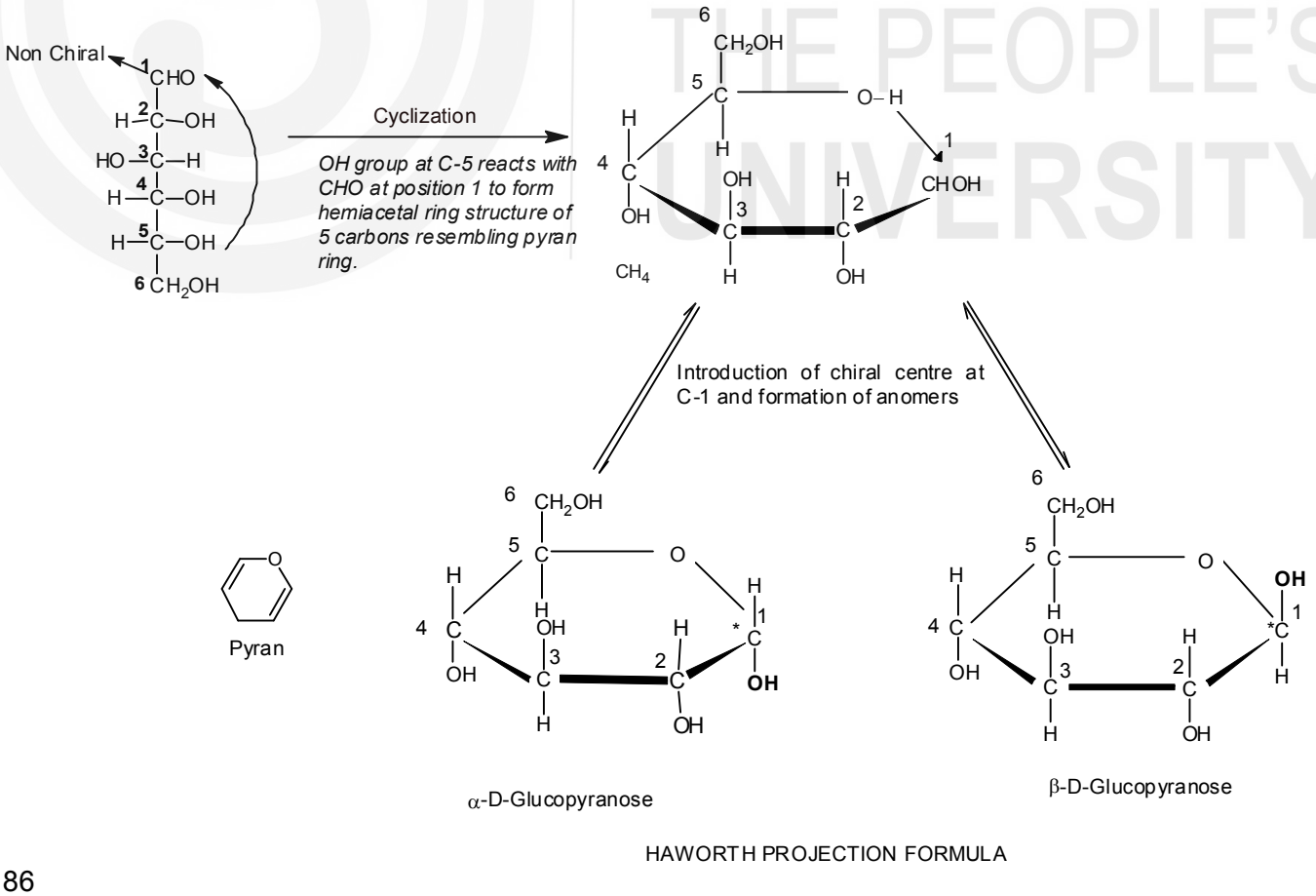


Fig. 5.9: Formation of ring structure of D-glucose from its linear form.

You must be wondering how cyclization of one molecule of D-glucose results in two structures. Note in the Fig. 5.9; when hemiacetal is formed, C1 which was earlier non chiral becomes a chiral centre. It is now referred to as anomeric carbon. -OH group attached to the anomeric carbon can assume any of the two positions, either below or above the plane of the ring structure. If it is above the plane of the ring, it is referred as β-**form** and if it is below the plane of the ring, it is referred as α-**form** of the sugar. Just like D/L forms, α and β forms are pair stereoisomers.

D-Fructose undergoes similar reaction as –OH at C5 attacks C=O at position 2 resulting in formation of cyclic hemiketal, D-fructofuranose (Fig. 5.10). As explained for glucopyranose, formation of a hemiketal also introduces a new chiral center at C2, which is now referred to as anomeric carbon. Thus, fructofuranose exists in two stereo isomeric forms; α and β-D-fructose.

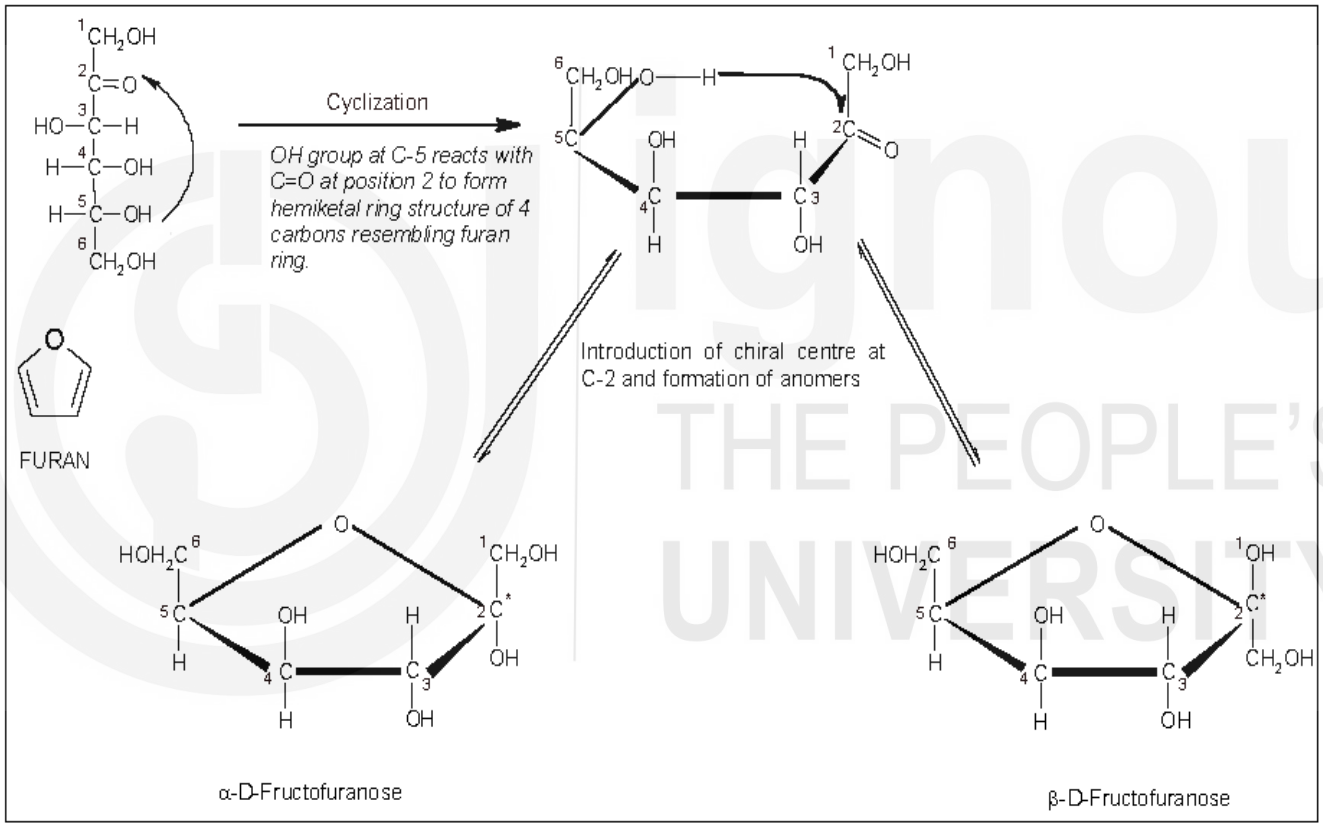


Fig. 5.10: Formation of ring structure of D-fructose from its linear form.

The ring structures shown in Fig. 5.9 and 5.10 are called **Haworth projections**. These projections represent pyranose as hexagonal ring and furanose as pentagonal ring lying perpendicular to the plane of the paper with thickened lines indicating the side of the ring closest to the reader. The oxygen atom in the pyranose ring is usually drawn in the upper right-hand position of the hexagon, with the hemiacetal carbon to the right of this position. The bonds to the groups attached to the ring carbons are drawn using vertical lines to indicate whether a group is oriented to the top-face or to the bottom-face of the ring. Box 5.2 outlines the steps to draw Haworth projection of D-glucose from it linear structure.

Box 5.2: You can easily draw Haworth projections corresponding to Fischer projections by keeping following rules in mind: a) OH groups on right side of linear structure are placed below the plane of the ring and those on the left side are drawn above the plane of the ring structure; C6 becomes a substituent on C5 and is above the plane of the ring in D hexoses (Fig. 5.11).

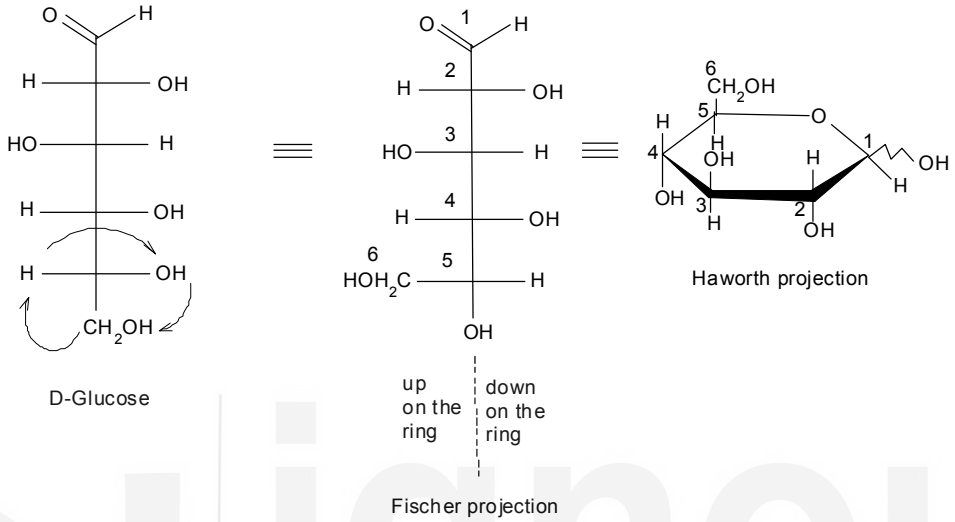


Fig. 5.11: Drawing Haworth projection of D-glucose. –OH group at anomeric C1 is shown with a kinked line which means, it may be placed below or above the ring depending on whether it is α and β form of D-glucose, respectively.

Just like Fischer projections, Haworth projections convenicently represent configurations of the stereoisomers of ring form of monosaccharide; however, they do not provide a realistic depiction of conformation in three dimensions. The structures of pyranose and furanose are not planar as suggested by Haworth projection, but tend to assume different conformations. Let us learn about some of possible conformations for sugars.

5.3.3 Conformations

In compounds with C-C single bonds, there is complete freedom of rotation and the substituents attached to the carbon atom can assume different positions in space known as conformations. A given compound can theoretically have many conformations termed as conformers but only few are energetically favoured. The different conformers are inter-convertible **without bond breakage** and minimal energy expenditure. Both pyranoses and furanoses are known to exist in different conformations because the C-O-C bond angle is 111° and C-C-C is 109°, therefore, neither pyranose nor furanose form planar rings, instead they are puckered. Here we shall briefly discuss about only two favoured conformations of pyranose rings; boat and chair (Fig. 5.12). In these forms, substituents occupy axial (parallel to the axis of the ring) and equatorial (along the plane of the ring) positions.

Conformations are interconvertible without breaking the covalent bond, whereas two configurations can be inter- converted only by breaking the covalent bonds.

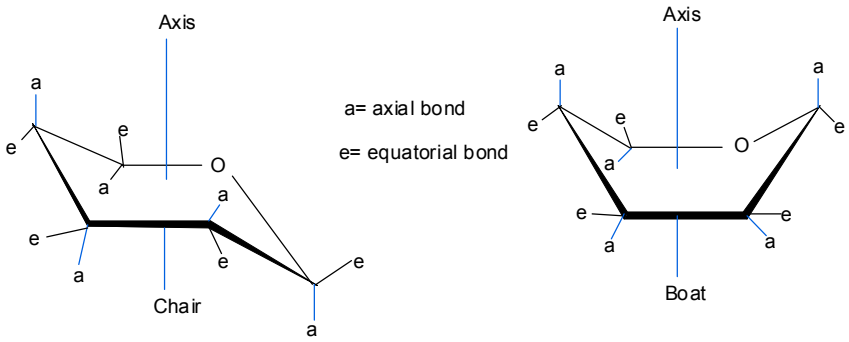


Fig. 5.12: Chair and boat conformations of pyranose ring.

Both α-D-glucopyranose *and* β-*D*-glucopyranose can exist in chair form however, β D-glucopyranose is more stable than α anomer. It is because all the bulky groups (OH and CH_2OH occupy equatorial positions and H are axial resulting in less steric hindrance. Fig. 5.13 shows the chair conformations of both α and β-*D*-glucopyranose. The structures are shown along with the Fischer and Haworth projection so as to give you an idea of positioning of different substituents.

The word 'chiral' originates from the Greek word 'cheir' meaning hand.

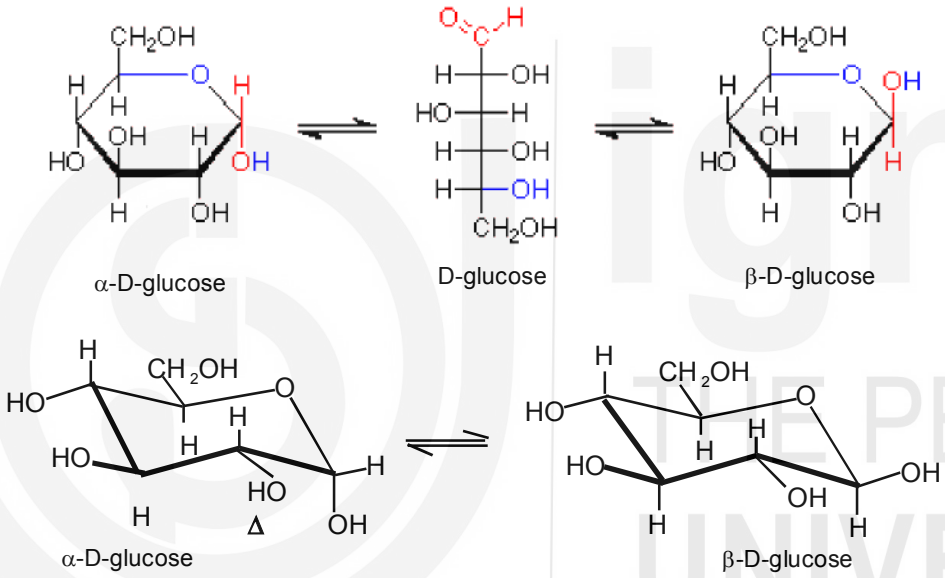


Fig. 5.13: Chair conformations of α and β D-glucopyranose.

SAQ 3

A) Circle the correct option:

a) Formation of hemiacetal or hemiketal results in formation of ring structure of sugars. (True/False)

b) Haworth projections are suitable for drawing the ring structures of sugars. (True/False)

c) β–D-glucose is more stable than α-D-glucose as the arrangement of its bulky groups in chair conformation have less steric hindrance. (True/False)

d) Aldoses form hemiketals and ketoses form hemiketals. (True/False)

B) What type of structural forms (linear or ring) do the following monosaccharides form:

a) Glycerldehyde b) Ribose c) Glucose d) Erythrulose

So far you learnt about linear and cyclic structures of monosaccharides and how to draw these according to certain conventions. These conventions not only make it easy to draw structures on paper but also give some idea about arrangement of different atoms in space. You must have noticed that except for dihydroxyacetone, all monosaccharides have one or more chiral carbons. As a result, monosaccharides exist as isomers and of which stereoisomerism plays an important role in their structure and function. Before we go further lets discuss about this feature.

5.3.4 Stereoisomers

Stereoisomers are those isomers which have same molecular and structural formula but differ in the arrangement of substituents around chiral carbon in space. Study of properties of stereoisomers is known as stereochemistry. If you look at Fig. 5.3, you would find that all aldohexoses have the same molecular formula ($C_6H_{12}O_6$) and same connectivity given by the structural formula (CHO $(CHOH)_4$ CH_2OH) but they differ in the orientation of substituents attached to the chiral carbon atoms resulting in different configurations. These configurations represent different stereoisomers.

Let us look at the structures of stereoisomers of glyceraldehyde. As it is the smallest monosaccharide with only one chiral centre, so it will be easy to understand. Fig. 5.14 shows two possible arrangements of the substituents around chiral carbon (indicated by *). In order to differentiate these stereoisomers, Rosanoff in 1906 arbitrarily assigned D/L notation. Glyceraldehyde having hydroxyl (-OH) group on chiral centre to the right is denoted as D-glyceraldehyde while the other form having hydroxyl (-OH) group to the left is designated L-glyceraldehyde. **D/L forms represent the pair of stereoisomers that are mirror images and cannot be superimposed on each other. Such stereoisomers are known as enantiomers.**

Structural isomers, like stereoisomers have same molecular formula but different structural formula as their atoms are linked in different order. D-glucose and D-fructose are structural isomer while D-glucose and D- galactose are stereoisomers

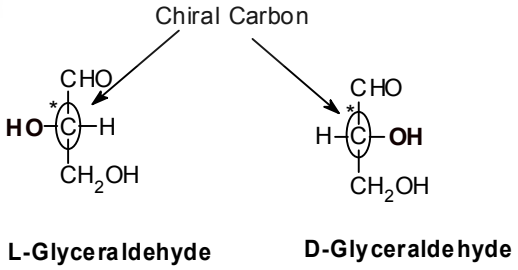


Fig. 5.14: Stereoisomers of Glyceraldehyde.

D and L labels were derived from the Latin words *dexter* (on the right) and *laevus* (on the left)

How do we designate D/L form for monosaccharides having more than one chiral center? Rosanoff proposed glyceraldehyde as a standard to which the configuration of all sugars could be related. He extended the designation of D/L configuration to all aldoses and ketoses by applying same logic to their last chiral carbon atom. All aldoses and ketoses drawn in Fig 5.2 and 5.3 respectively are by the above logic D sugars and those shown in Fig, 5.4 and 5.5 are L- sugars. As a quick reference, D and L forms of both glucose and fructose are shown in Fig. 5.15.

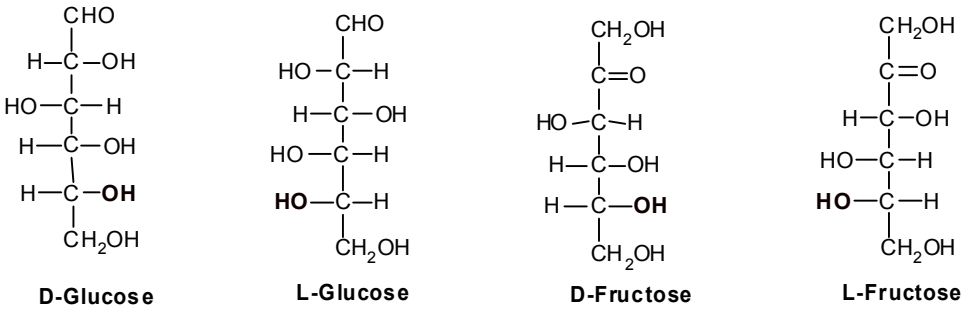


Fig. 5.15: Structures of enantiomers of glucose and fructose.

Remember, one configuration cannot be converted to another without breaking one or more covalent bonds. Therefore, stereoisomers are not easily interconvertible.

In nature, D-form of monosaccharides is more abundant than L-form. L-form of monosaccharides do exist, for example L-arabinose is a constituent of bacterial cell wall and L-galactose is found in some polysaccharides.

By looking at the structure of glyceraldehyde, one may understand that one chiral center results in two possible configurations. Therefore, one can easily predict the number of possible stereoisomers of a compound simply by counting the number of chiral centres it has. ***For a compound having n number of chiral centres, there are 2^n different stereoisomers possible. It is called Van't Hoff rule.***

An aldotetrose, for example, and has 2 chiral centres. Therefore, by Van't Hoff rule, $2^2 = 4$ stereoisomers are possible. These are D- threose and D- erythrose, L- threose and L- erythrose. Now you know that pair of D/L erythrose or threose are related as enantiomers and have opposite configurations. Is there any relation between other stereoisomers which do not form a pair of mirror images?

The stereoisomers which differ in configuration at one or more chiral centers but are not mirror images of each other are known as **diastereoisomers**, for example, D-glucose, D-talose, D-altrose, D-idose and D-gulose are all diastereoisomers.

Diastereoisomers that differ in configuration at only one chiral carbon other than the penultimate carbon are called **epimers**. For example, D-galactose and D-mannose are 4-epimer and 2-epimer of D-glucose, respectively as they differ from D-glucose at these positions only. Diastereoisomers differ in both physical and optical properties. You may explore these relationships among other monosaccharides.

Similarly, α and β anomers are also diastereoisomers as they differ in the orientation only at anomeric carbon.

5.3.5 Optical Properties

All monosaccharides except dihydroxyacetone are optically active. What does this mean? When a plane polarized light is passed though solution containing a monosaccharide, it rotates the plane of light either towards left or right. If plane of polarised light is rotated to the right, the molecule is said to be dextrorotatory and is notated by + or d sign. On the other hand, if plane of light rotates towards left, the molecule is said to be levorotatory. Levorotation is notated by – or l sign. For example, D-glucose is dextrorotatory while D-fructose is levorotatory. Thus ***optical activity is the ability of a solution to rotate the plane polarized light.*** The degree of rotation of light by a solution can be determined by an instrument known as polarimeter.

You may see many examples of pair of chiral objects in daily life which are non superimposable mirror images such as hands, feet, gloves,

Achiral objects- a T-shirt or a ball.

For any molecule to be optically active, it must possess two structural features;

1) One or more chiral centers

2) Lack of plane of symmetry

Monosaccharides possess both the features. Such molecules are also known as chiral. The molecules which do not possess one or both of these features are known as achiral**,** for example, dihydroxyacetone.

Enantiomers are optical isomers

Optically active compounds exist as stereoisomeric pair of enantiomers. The pair consists of molecules which are non superimposable mirror image of each other and rotate the plane of polarized light to the same degree but in opposite directions. They have almost identical physical (melting point, boiling point and solubility) and chemical properties but can be distinguished based on their optical activity. In addition some of them may smell and taste differently.

Optically active substances when prepared in lab generally exist as a 50:50 mixture of the two enantiomers called ***racemic mixture***. It does not rotate the plane polarised light.

An important point which you must remember that D/L notation is a structural feature it has no correlation with the +/- or d/l notation associated with optical activity. The direction and extent to which sugars rotate the plane of polarised light has to be experimentally determined. Table 5.2 which gives experimentally determined values of D/L forms of glucose and fructose demonstrates that D-glucose is dextrorotatory (d/+), while D-fructose is levorotatory (l/-)

Table 5.2: Experimentally determined values of optical rotation for D/L forms of glucose and fructose

Glucose and fructose are often called dextrose and levulose, respectively.

Name of the sugar	Optical rotation
D-Glucose	$+52.7^0$
L-Glucose	-52.7^0
D-Fructose	-92^0
L-Fructose	$+92^0$

As we have seen that during formation of cyclic structures that linear and cyclic structures of a monosaccharides are interconvertible which gives rise to an interesting phenomenon related to optical activity known as mutarotation.

Mutarotation: If you take a solution of α-D-glucose, its optical activity is +112.2 the beginning, however with time its optical activity starts changing and stabilizes at +52.7°. Similarly, if you allow a solution of β-D-glucose having optical activity +18.7° to stand in a test tube for some time, you would still end up having a solution of glucose with optical activity of +52.7°. The process involves interconversion of α-D-glucose and β-D-glucose via linear D-glucose establishing as equilibrium mixture consisting of 63.6% of β-D-glucose and 36.4% of α-D-glucose. This process of **change in optical rotation of a sugar solution upon standing is called mutarotation (Fig. 5.16). It is a property of hemiacetal/ hemiketal linkages.**

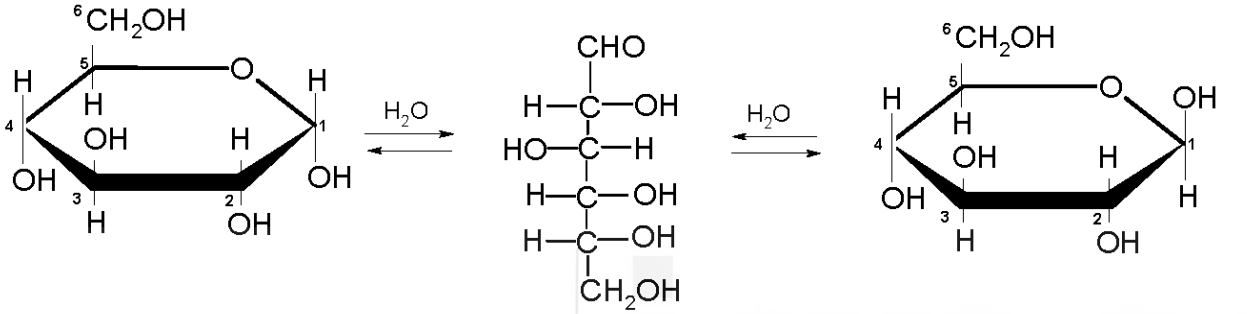


Fig. 5.16: Phenomenon of mutarotation of glucose.

SAQ 4

a) Choose the correct option:

i) Two sugars which differ in their configuartion at anomeric carbon are:

i. anomers ii. epimers iii. optical isomers

ii) In glucose, orientation of –OH group at penultimate carbon determines:

i. epimerism ii. anomerism iii. optical isomerism iv. D/L series

b) Do solutions of following sugars show mutarotation?

i) Dihydroxyacetone; ii) Ribose; iii) Glucose; iv) Fructose; v) Erythrose; vi) Erythrulose; and vii) Ribulose

c) Fill in the blanks:

i) An aldopentose has ..... stereoisomers.

ii) Most of the monosaccharides in nature are ..... form.

iii) Epimers of D-glucose are ..... and .....

iv) Anomerism is observed only for sugars in ..... solution.

d) Draw D and L forms of glyceraldehyde, ribose, fructose, glucose and galactose.

With all this understanding, you now know that monosaccharides have multiple functional groups. These groups undergo different chemical reactions to yield a number of useful monosaccharide derivatives. We shall discuss some of these sugar derivatives and how they are formed.

5.4 BIOLOGICALLY IMPORTANT SUGAR DERIVATIVES

5.4.1 Sugar Acids

Monosaccharides are good reducing agents because of presence of free ano meric carbon and give different derivatives under different conditions:

Reducing property of glucose is used to determine its increased levels in blood and urine for the diagnosis of diabetes mellitus.

Molecule with single chiral centre is always asymmetric. Some molecules having two or more chiral centers are achiral because a plane of symmetry divides it into similar halves. In such a case, each half rotates the light to the same degree but in opposite direction. Such molecules are known as *meso*. They lack optical activity.

1) They reduce mild oxidizing agents such as hydrogen peroxide, ferricyanide, certain metals (Cu^{2+} or Ag^{+}) and in turn anomeric carbon itself is oxidized to carboxylic acid. Such acid derivatives are called **aldonic acids**, for example, mild oxidation of D-glucose and D-mannose produces D-gluconic acid and D-mannonic acid, respectively.

   Carbohydrates that can reduce oxidizing agents are referred to as **reducing sugars.** All monosaccharides are reducing sugars.

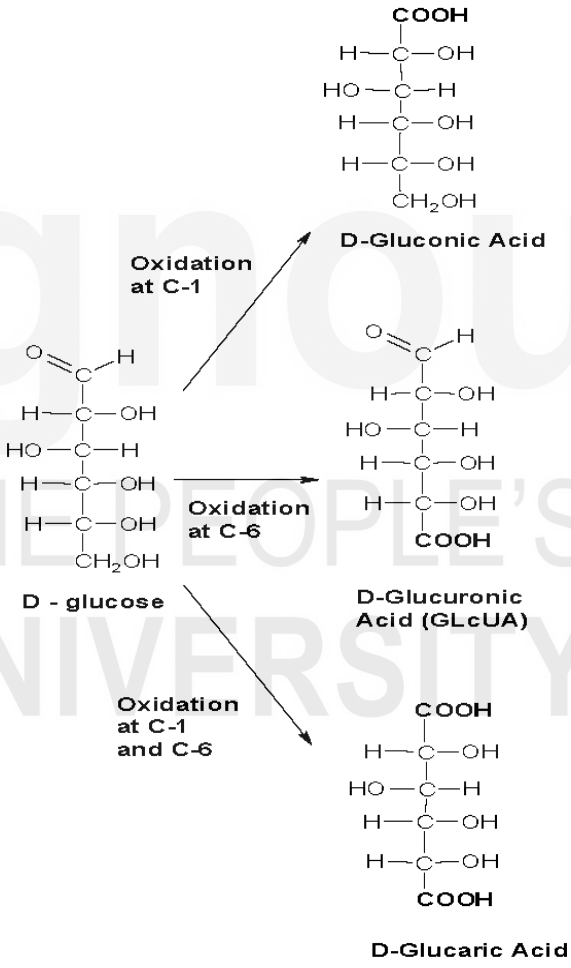


Fig. 5.17: Oxidized forms of D- Glucose.

2) In the presence of strong oxidising agents such as nitric acid, both the carbonyl and primary hydroxyl group are oxidised to yield dicarboxylic acids or **aldaric acids,** for example, glucose yields glucaric acid. Aldaric acids are meso compounds and do not possess optical activity like their parent sugars.

3) Some enzymes catalyze specific oxidation of the primary hydroxyl group only yielding **uronic acid** such as D-glucuronic acid from D-glucose. It is an important derivative as it acts as a carrier and helps on excretion of drugs and other toxic compounds from our body.

The structures of three types of acid derivatives of glucose are given in Fig. 5.17.

Ascorbic acid also known as vitamin C is also derived from glucose after its oxidation. It is synthesized by many plants and animals but humans and other primates like guinea pigs lack the enzyme for it synthesis. Therefore, they have to take vitamin C in diet and its deficiency leads to scurvy.

Sugar solutions are stored under acidic or neutral conditions to prevent their isomerization and preserve identity.

As compared to aldoses, ketoses are not oxidized easily. However, under alkaline conditions, they undergo enediol isomerization to form isomeric aldoses, which in the presence of oxidizing agents form aldonic acid (Fig. 5.18).

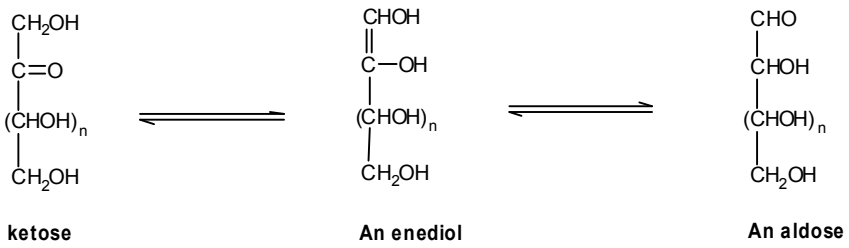


Fig. 5.18: Enediol isomerization reaction in ketoses.

5.4.2 Sugar Alcohols

The carbonyl group of monosaccharides reduces to hydroxyl group in the presence of stronger reducing agents such as sodium borohydride ($NaBH_4$) to form sugar alcohols or alditols. Alditols are linear molecules which do not cyclize like their parent monosaccharide and are sweet tasting. Reduction of D-glucose, D-mannose and D-xylose results in formation of D-glucitol or sorbitol, D-mannitol (Fig. 5.19 a & b) and D- xylitol, respectively. These three sugar alcohols are widely used as sweetener for sugarless gum or mints. Sorbitol is clinically important as its accumulation in eyes of diabetic patients has been shown to result in cataract.

Alditols lack -CHO group and can't form hemiacetals. Therefore exist as linear structures only.

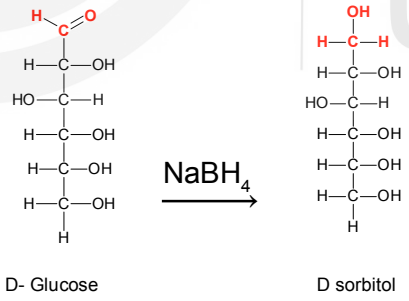


Fig. 5.19(a): Reduction reaction of D-glucose to D-sorbitol.

Mannitol is a meso molecule; therefore it loses its optical activity

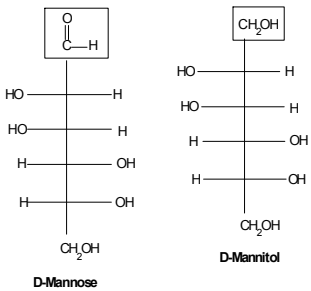


Fig. 5.19(b): Formation of mannitol form mannose

5.4.3 Amino Sugars

Amino sugars are formed when one of the hydroxyl (–OH) of sugar is replaced by amino group (–NH_2), e.g. β-D-glucosamine has amino group instead of hydroxyl group at C2 (Fig. 5.20). Along with its acetylated derivative β-D- N- acetyl glucosamine, β-D-glucosamine is found in many oligosaccharides and polysaccharides, for example, chitin, which forms exoskeleton of insects and crustaceans.

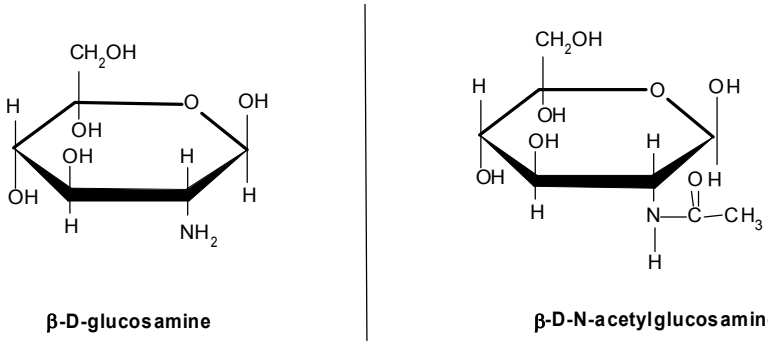


Fig. 5.20: Structures of Amino derivatives of β-D-glucose: β-D-glucosamine and β-D- N- acetyl glucosamine.

Muramic acid and N- acetylneuraminic acid (NANA also known has sialic acid), two important examples of amino sugars are components of polysaccharides of bacterial cell walls and cell membranes in higher organisms. Lets us look at their structures (Fig. 5.21). Muramic acid is a 9 carbon sugar that has hydroxyl group of lactic acid linked by ether linkage to C3 of glucosamine. NANA has C-C bond between C3 of pyruvic acid and C1 of N-acetylmannosamine. N-acetyl and N-glycosyl derivatives of neuraminic acid are collectively known as sialic acids and NANA is the most common member of this family.

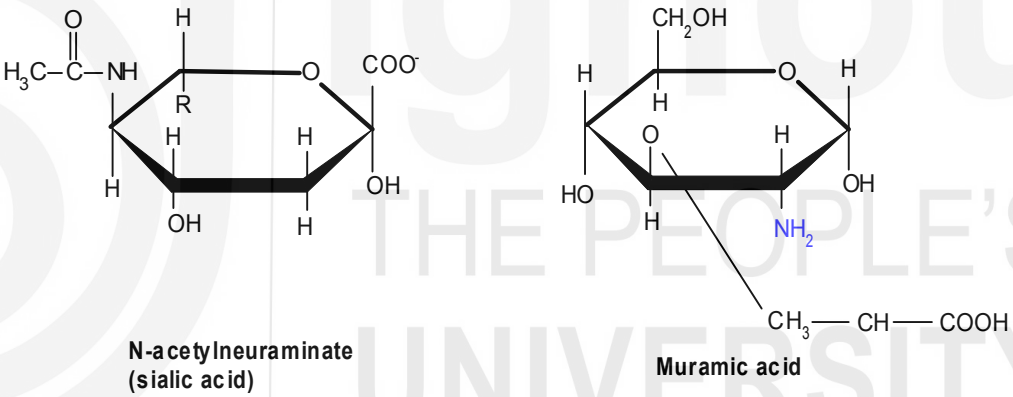


Fig. 5.21: Structure of Sialic acid and Muramic acid

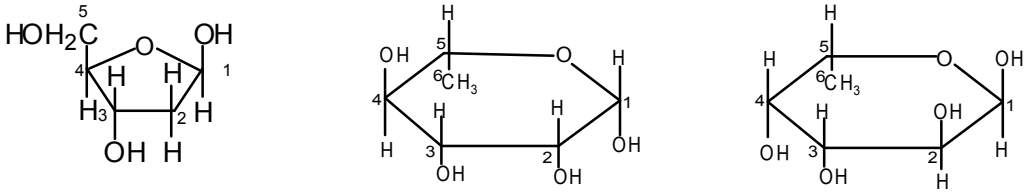
5.4.4 Deoxy Sugars

When one or more hydroxyl groups of a monosaccharide is replaced by hydrogen, it is called deoxy sugar. (Fig. 5.22)

2-deoxy ribose is one such derivative at C2 and is a constituent of DNA strand, the genetic material in living organisms.

Fucose is deoxy derivative of glucose in which CH_2OH becomes CH_3. It is secreted in mother's milk and helps in development of immune system of new born babies. It is also involved in nerve transmission. It is a common constituent of mammalian insects and plant cell surface.

Rhamnose is deoxy derivative of mannose which occurs naturally as L-sugar. It is more commonly found in plants and bacteria than animals.



2-Deoxyribose **L- Rhamnose** **L-Fucose**

Fig. 5.22: Structures of deoxysugars

5.4.5 Sugar Esters

One or more phosphate or sulfate groups may be attached to hydroxyl group of monosaccharides by ester bond formation. Phosphate esters of monosaccharides such as glucose, fructose, glyceraldehyde are important intermediates of metabolic pathways which generate energy. Ribose-5-phosphate which is a component of nucleotides such as ATP, GTP is also phosphate ester of ribose (Fig. 5.23).

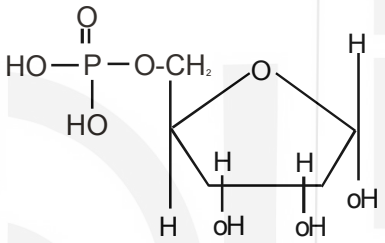


Fig. 5.23: Structure of α–D-Ribose-5-phosphate.

5.4.6 Glycosides

Glycosides are acetals and ketals which are formed by reaction between hemiacetal (pyranose) or hemiketal (furanose) ring form of monosaccharides and an alcohol. It is a condensation reaction with loss of water. The bond formed between anomeric carbon of monosaccharide and oxygen atom of the alcohol (R-OH) is called O-glycosidic bond (Fig 5.24). In this reaction, equilibrium mixture of methyl-α-D glucoside and methyl-β-D glucoside is formed in the ratio 66% to 34%. We have studied earlier that β-D glucose is more stable than α-D glucose. This unusual effect is known as **anomeric effect**.

Amygdalin was the first glycoside identified by French chemists Pierre Robiquet and Antoine Boutron-Charlard in 1830

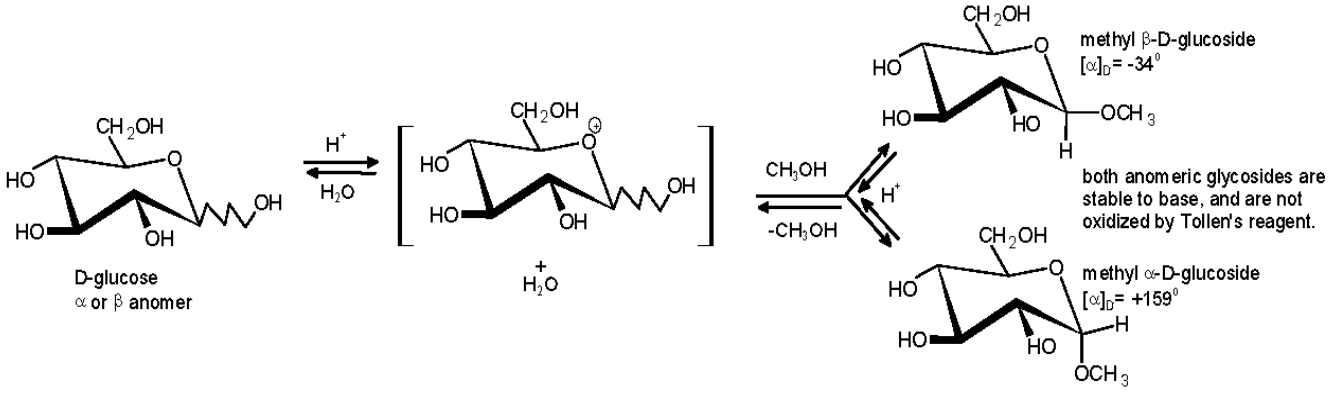


Fig 5.24: Reaction showing the formation of methyl-β-D glucoside in a reaction between β-D glucose and methanol.

In addition to -OH group, sugars also react in a similar way with thiol (-SH) group to from thioglycosides. Glycosides thus consist of two parts: **glycone**, the sugar part and **aglycone,** the non sugar part.

Many medicinally important compounds from plants are glycosides. Their aglycan part has been used for medicinal purpose. For example, digitalis used in medicines for heart failure is a glycoside which causes heart to pump more forcefully.

Glycosides are classified based on their glycone part. Glycosides having glucose as glycone are classified as **glucosides**. Likewise, glycosides of fructose and glucuronic acid are classified as **fructosides** and **glucuronides**, respectively.

Sugars, when attached to non sugar molecules as glycosides, increase their solubility. In humans, many toxic substances are often bonded to glucuronic acid to form glucuronides, which in turn increases their water solubility and they are excreted more easily out of the body.

Glycosidic bond is also formed between different monosaccharides to form long chains of oligosaccharides and polysaccharides. However, they are not considered as glycosides. We shall discuss more about oligosaccharides and polysaccharides in Units 6 and 7.

SAQ 5

Choose ( ) the correct option:

a) Which of the following vitamins is derived from glucose?

i. Vitamin A ii. Vitamin C

iii. Vitamin D iv. Vitamin K

b) Which of the following is used in sugarless gums and candies?

i. xylitol ii. Inositol iii. ribitol

c) Which amino sugar is/ are found in bacterial cell wall:

i. muramic acid ii glucaric acid

iii. N- acetylglucosamine iv. Both i and iii

d) Sugars found in DNA is:

i. 2-deoxyribulose ii. Ribose

iii. 2-deoxyribose iv. 3-deoxyribose

5.5 SUMMARY

1) Carbohydrates more commonly known as sugars are the most abundant biomolecules in nature.

2) They are broadly defined as polyhydroxy aldehydes or ketones and their derivatives.

3) These are large molecules which are made up of repeating units called monosaccharides. Based on the number of monosaccharides present, carbohydrates have been classified into: Monosaccharides- basic monomeric unit of sugars which can not be hydrolyzed to smaller unit.

Oligosaccharides-made up of 2-20 monosaccharide units and polysaccharides-made up of more than 20 monosaccharide units.

4) Monosaccharides are generally sweet in taste, white crystalline, water soluble molecules which have been further classified as triose, tetroses, pentoses, hexoses and heptoses based on the length of the carbon skeleton. The chain length found in nature varies from 3-7 carbon atoms.

5) Diferent classes of monosaccharides are categorised as aldoses and ketoses based on nature of carbonyl group. They are given generic names such as aldotrioses, ketotrioses, aldotetroses, ketotetroses and so on to indicate their length of carbon atoms and nature of primary functional group.

6) Monosaccharides are linear unbranched chains of 3-7 carbon atoms. These structures are presented as Fischer projections which indicates 3D orientation of different groups in a sugar. D-Glucose is the most abundant monosccharide in nature.

7) Tetroses and above, also form five or six membered ring structures which predominate in aqueous solution. Such cyclic structures are known as furanoses and pyranoses, respectively.

8) Furanoses and pyranoses are result of internal hemiketal and hemiacetal reaction in ketoses and aldoses, respectively. Cyclic strutures are presented by Haworth projections.

9) One of the characteristic features of monosaccharides is the presence of one or more asymmetric carbons with exception of dihydroxyacetone. This results in phenomenon of stereoisomerism of different kind- enantiomerism, anomerism, epimerism.

10) Monosaccharides except dihydroxyacetone are optically active molecules. When a plane polarized light is passed through their solution, they rotate it either towards right side (dextrorotatory) or left side (levorotatory).

11) Monosaccharides are chemically reactive because of multiple functional groups and undergo oxidation and reduction, addition and substitution reactions to form biologically important derivatives.

5.6 TERMINAL QUESTIONS

1) Define carbohydrates. Give their general formula.

2) Draw structure of L-glucose; mark its chiral carbon atoms. Calculate the number of stereoisomers it can form. Draw structure of its enantiomer.

3) What is mutarotation? Do all sugars possess this property?

4) Explain why D-mannose is optically active but its alcohol derivative, D-mannitol is not?

5) What are glycosides? Explain their formation and importance.

6) Describe oxidation reactions of monosaccharides.

7) Explain the formation of furanose ring of fructose.

8) What are different stereoisomeric relations of glucose to other

5.7 ANSWERS

1) Carbohydrates are defined as aldehydes or ketones with multiple hydroxyl groups

2) Refer to fig 5.6 for structure of L-glucose. As it has 4 Achiral carbons, therefore according to Von't Hoff rule, 2^4= 16 stereosiomers are possible. D-glucose is the enantiomer of L-glucose.

3) The change in optical rotation of a sugar solution upon standing is called mutarotation. It is a property of hemiacetal/ hemiketal linkages. Thus, monosaccharides with five or more carbon atoms which can form cyclic (ring) structures possess this property.

4) Mannose has four chiral carbons and the molecule itself is chiral.Therefore it is optically active. However its alcohol derivative mannitol is a chiral maolecule as it may be divided into two similar halves by a line passing between its third and fourth carbon. Therefore, it is not optically active. Refer Fig. 5.19b

5) Ring forms of monosaccharides react with alcohols to form glycosides. This is a condensation reaction between anomeric carbon and –OH group of an alcohol which proceeds with loss of water. In addition to –OH group, sugars may react with –SH group of a thiol compound to form thioglycosides. Glycosides thus consist of two parts: **glycone**, the
sugar part and **aglycone,** the non sugar part. Refer to section 5.4.6

Glycoside formation is important in two ways:

i) Sugars, when attached to non sugar molecules as glycosides, increase their solubility. In humans, many toxic substances are often bonded to glucuronic acid to form glucuronides, which inturnincreases their water solubility and they are excreted moreeasilyout of the body.

ii) Ability to form glycosidic bond helps in polymerization of monosaccharides resulting in formation of wide variety of oligosaccharides and polysaccharides.

6) Refer to section 5.4.1 to show the formation of sugar acids by oxidation of their aldehyde and hydroxyl groups.

7) Refer to Fig. 5.10 to explain the formation of furanose ring of fructose.

8) Glucose is related to other aldohexoses in the following ways:

   i) D-glucose and L-glucose are enantiomers

   ii) D-glucose and D-galactose are epimers

   iii) D-glucose and D-mannose are also epimers.

   iv) D-glucose and other five D- hexoses (D- talose, D- altrose, D- idose and D-gulose) are all diastereoisomers.

   v) Similar kind of relationships can be observed between L-glucose and L forms of all other aldohexoses.

Self-Assessment Questions

1. a) $C_n(H_2O)_n$ where $n \geq 3$ b) Structure and energy
   c) keto (aldehyde or ketone) and hydroxyl d) three

2. A. a) Glucose- aldohexose- 4 chiral centers
   b) Dihydroxyacetone-ketotriose- none
   c) Erythrose- aldotetrose- 2 chiral centers
   d) Ribulose-ketopentose-2 chiral centers

   B) a–iii, b– i, c–iv, d– iv, e– v

		Aldose	**Ketose**
C) a)	Triose	Glyceraldehyde	Dihydroxyacetone
b)	Tetrose	Erythrose	Erythrulose
c)	Pentose	Ribose	Ribulose
d)	Hexose	Glucose	Fructose

3. A) a) True b) True c) False d) True

   B) a) Linear b) Furanose c) Both pyranose and furanose d) Linear

4. a) i) Anomers ii) D/L series

   b) i. Yes ii. Yes iii. Yes iv. Yes v. No vi. No vii. Yes

   c) i. Eight ii. L iii. D-galactose and D- mannose iv. aqueous

   d) Refer to Fig. 5.2 - 5.5

5. a) ii b) i c) iv d) iii

5.8 FURTHER READINGS

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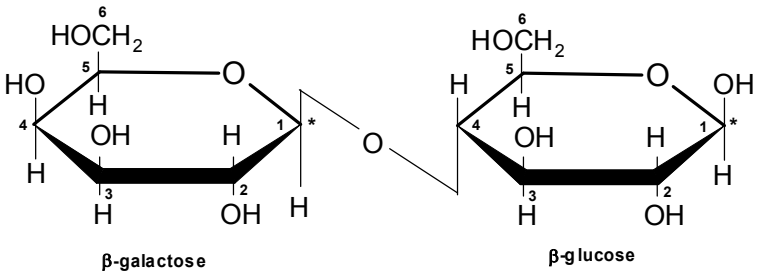


UNIT 6

OLIGOSACCHARIDES

Structure

6.1 INTRODUCTION

In the previous unit, you learnt about the structure and classification of carbohydrates. You also studied about monosaccharides, the simplest of carbohydrates in detail. You learnt about their structures, stereochemistry, how to draw and name different stereoisomers. In this unit we shall discuss about oligosaccharides; the carbohydrates consisting chain of two to ten monosaccharide units. Like monosaccharides, they also play important role in providing energy as they are hydrolyzed by digestive enzymes to their constituent monosaccharides. Smaller oligosaccharides like disaccharides and trisaccharides are commonly present in human diet. Higher oligosaccharides are more common in plants. Oligosaccharides are also found linked to other biomolecules such as proteins and lipids to perform special functions like signal transduction and biomarkers for recognition. These functions will be dealt in Unit 8.

In this unit we shall begin with discussion of glycosidic bond and how monosaccharides of similar or different kind link covalently to form oligosaccharides. You would also learn about the structures of some of the disaccharides as well as other oligosaccharides, their properties and biological importance.

Objectives

After studying this unit you should be able to:

- define glycosidic bond;
- identify different types of glycosidic bonds;
- draw structures of common disaccharides: lactose, maltose, sucrose, isomaltose and trehalose;
- describe the importance of disaccharides; and
- draw structures of higher oligosaccharides such as raffinose, stachyose that have dietary relevance.

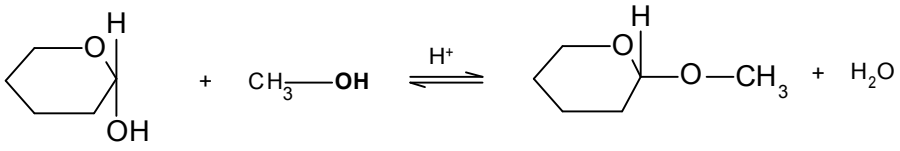
Let us begin with the glycosidic bonds.

6.2 GLYCOSIDIC BONDS

Oligosaccharides are made up of 2-20 monosaccharide units and are named based on the number of monosaccharides present in it. For example, oligosaccharides consisting of 2, 3 and 4 monosaccharides are called disaccharides, trisaccharides and tetrasaccharides, respectively. The constituent monosaccharides of an oligosaccharide may be same or different kind. If they all are same, it is referred to as homo-oligosaccharide and if different type, it is referred to as hetero-oligosaccharide.

Do you know how monosaccharides link together to form oligosaccharides and polysaccharides? They are linked together by the covalent bond known as glycosidic bond. It is the same bond we discussed about in the formation of glycosides in Unit 5.

Glycosidic bond is a covalent bond which is formed by condensation reaction between hydroxyl group present on the anomeric carbon of a monosaccharide and hydroxyl group of an alcohol or another monosaccharide. Thus, hemiacetal is converted to acetal during formation of a glycosidic bond (Fig. 6.1). This type of bond is more specifically known as O-glycosidic bond. The reaction occurs under mild acidic conditions and proceeds with release of a water molecule. As monosaccharides have multiple hydroxyl groups, they can form glycosidic bonds with more than one monosaccharide to form long polymeric chains.



Hemiacetal cyclic sugar **Cyclic acetal**

Fig. 6.1: Formation of acetal sugar.

In addition to the O-glycosidic bond, monosaccharides also form **N-glycosidic bonds in which hydroxyl group present on anomeric carbon condenses with amino group of another molecule.** This type of glycosidic bonds are present in DNA, the genetic material. 2-Deoxyribose sugar of DNA condenses with $–NH_2$ group of nitrogenous bases (A, T, G, C) to form backbone of this nucleic acid (Fig. 6.2). (You may see unit 13 in block 4 for more details).

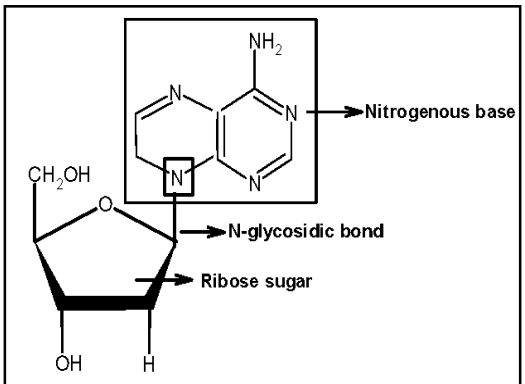


Fig. 6.2: Presence of N-glycosidic bond in genetic material.

Based on the configuration of the anomeric carbon involved, glycosidic bonds are further categorized as:

i) α-glycosidic bond; and

ii) β-glycosidic bond

Let us understand difference between these two types with the help of structures of maltose and cellobiose (Fig. 6.3). Both are disaccharides of glucose but have different glycosidic bond. Glycosidic bond in maltose is termed α -glycosidic bond as the anomeric carbon participating in the bond formation belongs to α- D-glucose. In cellobiose, anomeric carbon forming the glycosidic bond with –OH of 4th carbon of second glucose molecule belongs to β- D-glucose, therefore it is classified as β-glycosidic bond. Both α and β glycosidic bonds are further specified by indicating the numbers of carbons in both sugars forming glycoside bond in parenthesis.

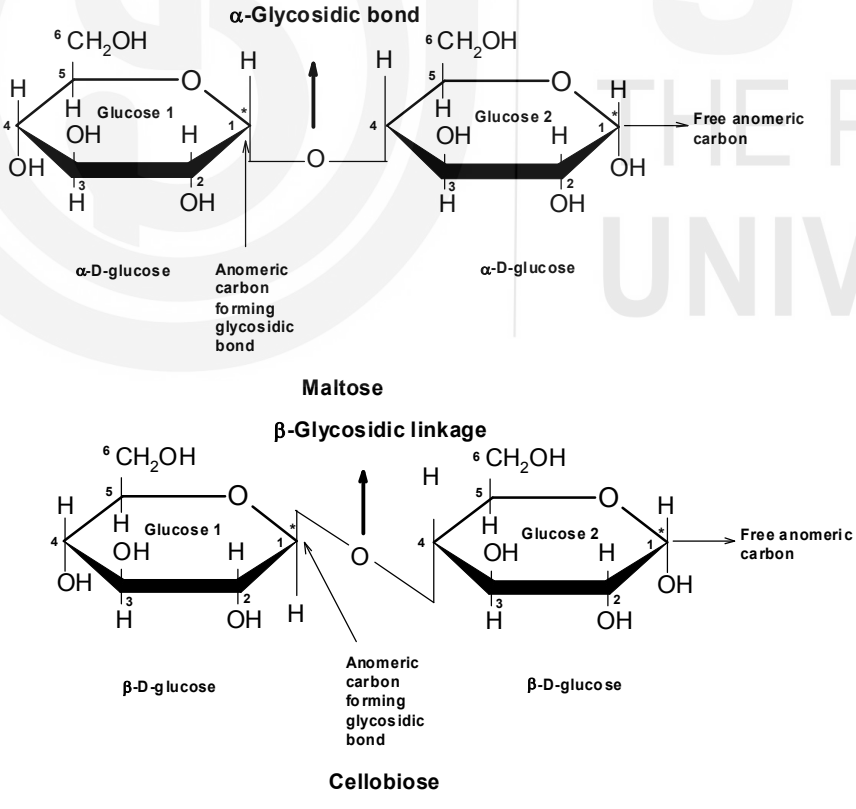


Fig. 6.3: Types of glycosidic bonds. A) Maltose contains α (1 → 4) glycosidic bond as anomeric carbon of glucose 1 forming glycosidic bond with –OH present at C4 of glucose 2 has α configuration; B) Cellobiose represents sugar containing β (1→ 4) glycosidic bond as anomeric carbon of glucose 1 forming glycosidic bond with –OH present at C4 of glucose 2 has β configuration.

O-Glycosidic bond results in formation of ether group where oxygen attached to two carbons is relatively unreactive. Therefore, glycosides tend to be more stable than free sugar. However, these can be hydrolyzed under acidic conditions or by the enzyme glycoside hydrolase (glycosidase). Glycosidases are specific for a bond type; they act either on α- or β-glycosidic bond but not on both. On hydrolysis, oligosaccharides yield their constituent monosaccharides, for example maltose on hydrolysis yields two molecules of α-D glucose and cellobiose yields two molecules of β-D glucose.

Glycosidic bonds are critical as they not only help in linking one monosaccharide to other monosaccharides to form longer chains of oligosaccharides and polysaccharides, but also help attach carbohydrates to other biomolecules like proteins and lipids to form complex molecules like glycoproteins and lipopolysaccharides. You would learn about these molecules as you go through the course.

Systematic Naming of Oligosaccharides

All oligosaccharides have a generic name as well as systematic name. Systematic name gives specific information about the exact configuration of the constituent sugars as well as the type of glycosidic bond they form. These names are assigned following a sequence in which monosaccharides are joined together.

The name begins with the type of glycoside bond (O/N), name of the first monosaccharide unit (α/β) followed by D/L form, and pyranose/ furanose form. It is followed by a parenthesis indicating the numbers of carbons in both sugars forming glycoside bond, followed by the name of the second sugar in the same format as the first one. For example, systematic name of maltose (Fig. 6.4) is O-α-D-glucopyranosyl (1$\rightarrow$ 4) α- D-glucopyranose and cellobiose (Fig. 6.5) is O-β-D-glucopyranosyl (1$\rightarrow$ 4) β-D-glucopyranose. You would see more examples in the next section.

SAQ 1

a) Name the functional groups involved in the formation of O- and ..... N-glycosidic bonds?

b) Identify the type of O-glycosidic bond (α or β) in the following oligosaccharides and give reason:

   i) α- D-glucose (1$\rightarrow$ 6) β- D-galactose

   ii) β- D-glucose (1$\rightarrow$ 4) α- D-galactose

6.3 DISACCHARIDES

Disaccharides are made up of two monosaccharides joined by O-glycosidic bond. They are the most abundant oligosaccharides and are classified as reducing and non-reducing disaccharides.

Reducing disaccharides: A disaccharide is said to be reducing if at least one of the anomeric carbons of the constituent monosaccharides has free -OH group. Such disaccharides show reducing properties like monosaccharides, i.e. give positive Benedict's test and undergo mutarotation, for example, maltose.

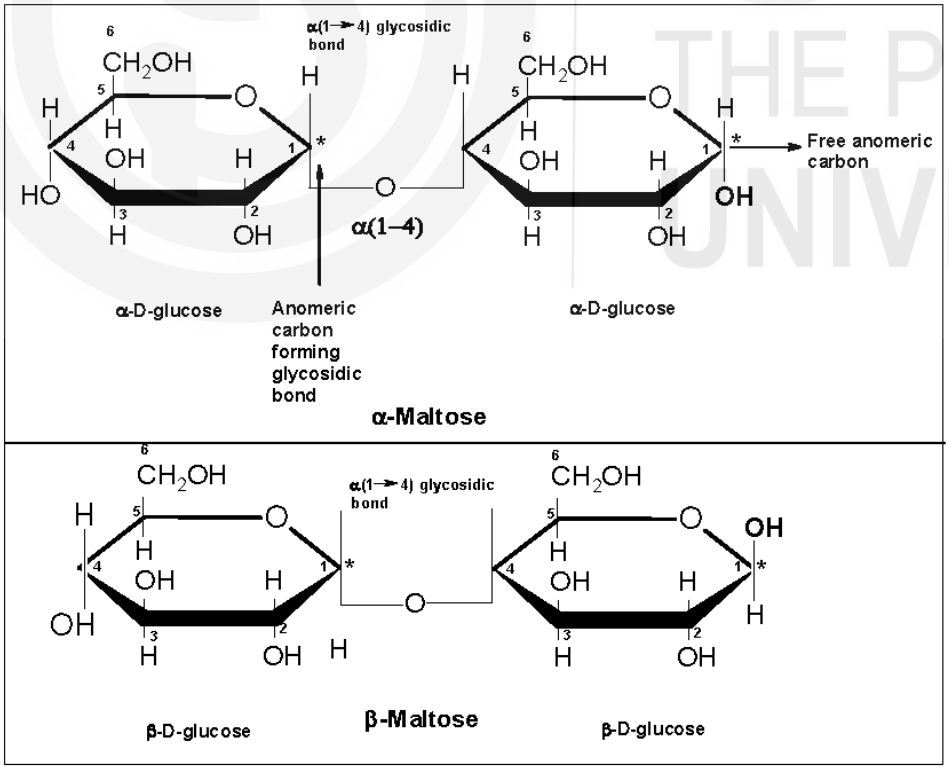
Benedict's test is performed in laboratory to confirm the presence of reducing sugars in a solution. It is based on reduction of (Cu^{2+}) in alkaline $CuSO_4$ solution to (Cu^{+}) in the form of Cu_2O by the reducing sugar and appearance of reddish brown colour precipitate

Non reducing disaccharides: A disaccharide is said to be non- reducing if none of the anomeric carbons of the constituent monosaccharides has free -OH group. Such disaccharides neither show mutarotation nor give positive Benedict's test, for example, sucrose.

Similar criteria of reducing and non-reducing nature apply to other oligosaccharides depending on whether or not one of the terminal anomeric carbons has free -OH. Let us discuss about the structure, properties and importance of some common disaccharides.

6.3.1 Maltose

It is a homo disaccharide as it is made of two D glucose units joined by (1→ 4) *O*-glycosidic bond. It means that C-1 of D glucose forms a glycoside bond with C-4 of another D-glucose (Fig. 6.4). Since anomeric carbon of one of the D-glucose in maltose is free, it acts as a **reducing sugar** in solution. It exists in both, α and β forms (Fig. 6.4), shows mutarotation and gives a positive Benedicts test. Systematic name of maltose is O-α-D-glucopyranosyl-(1→ 4) D-glucopyranose.



Configuration of a disaccharide (α and β) depends on the configuration of the free anomeric carbon.

Malt is prepared by allowing grains, particularly barley to soak in water and germinate. An enzyme diastase produced by the seed itself during germination, hydrolyses starch to malt.

Fig. 6.4: Structures of α- maltose and β- maltose.

Maltose is found in malt which is used in beverages such as malted milk. In humans, it is formed by partial hydrolysis of starch, a polysaccharide, during

the process of digestion. Maltose can be further hydrolyzed to its constituent glucose molecules by the enzyme maltase present in the small intestine.

Other homo disaccharides of glucose are **isomaltose** and **cellobiose**. They are formed by digestion of polysaccharide, cellulose. While isomaltose is α (1→6) isomer of maltose, cellobiose is β (1→ 4) isomer of maltose. Their systematic names are α-D-glucopyranosyl (1→ 6) D-glucopyranose and β-D-glucopyranosyl (1→ 4) D-glucopyranose, respectively (Fig. 6.5). The enzyme maltase catalyzes the hydrolysis of the glycosidic bond in maltose, but is not able to cleave the β isomer; as a result humans are not capable of digesting cellobiose.

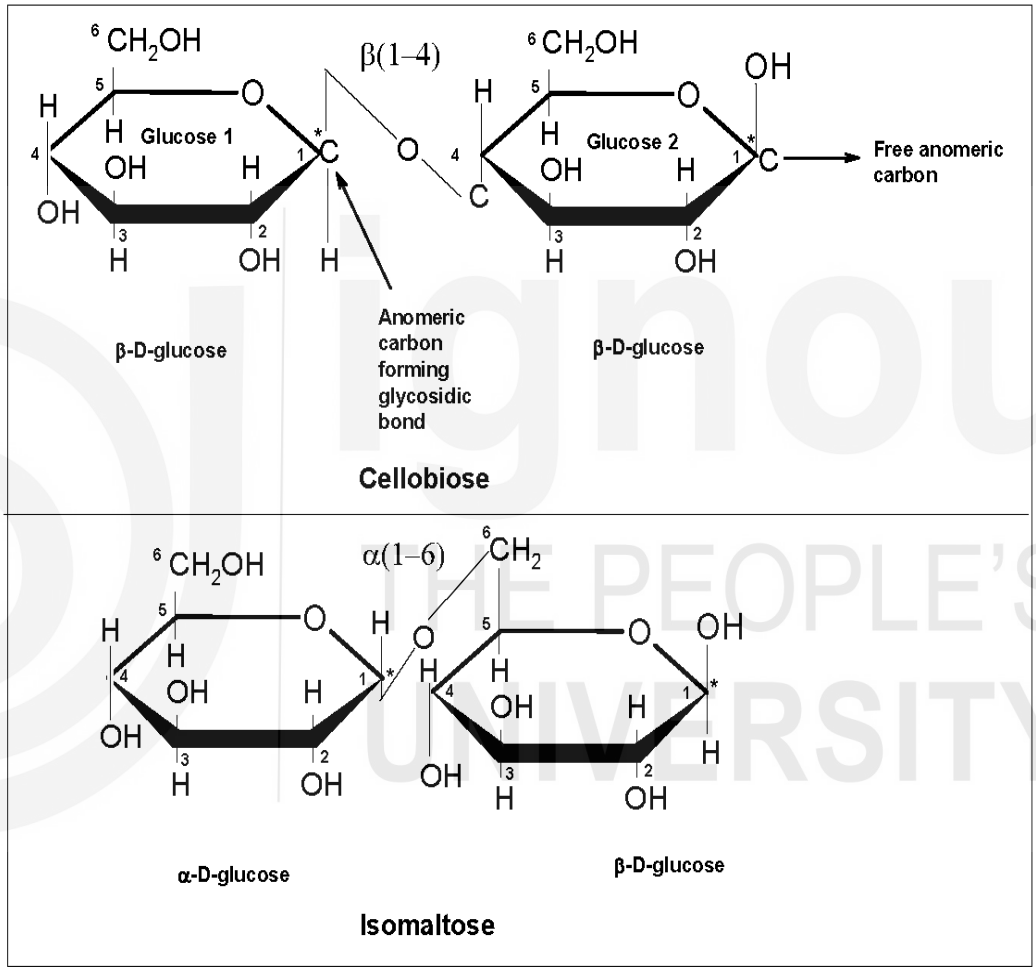


Fig. 6.5: Structures of isomaltose and cellobiose.

6.3.2 Lactose

Lactose occurs naturally in milk, hence it is also known as milk sugar. It is a hetero disaccharide of β- D-galactose and D-glucose joined by (1→ 4) O-glycosidic bond (Fig. 6.6). Since, hydroxyl group present at the anomeric carbon of D-glucose is free, lactose is a reducing sugar. Hence, it gives positive Benedict's test. Lactose also exists in anomeric α and β forms and exhibit mutarotation. Systematic name of β –lactose shown in Fig. 6.6 is O-β-D-galactopyranosyl-(1→ 4)-D-glucopyranose.

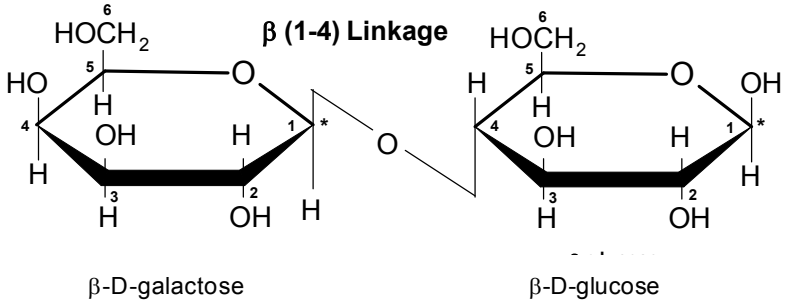


Fig. 6.6: Structure of Lactose, the milk sugar

You would be amused to know that in populations which continued to use dairy products throughout their life, levels of lactase decrease only mildly with age and hence do not cause much trouble.

Lactose has a dietary and clinical significance. Lactose in milk cannot be absorbed directly. It is hydrolyzed to its constituent sugars by the enzyme β- D-galactosidase commonly known as lactase present in the small intestine. This enzyme is present abundantly in the early stages of development in mammals as milk is the main diet. However, its levels decrease in most mammals including humans when they shift to other forms of diet. Most Africans and almost all Asians have low levels of this enzyme. Deficiency of this enzyme causes a disorder known as lactose intolerance.

For reducing sugars, their anomers (α and β forms) exist in equilibrium.

Lactose intolerance: When milk is ingested it moves through intestine undigested due to very low levels of lactase and enters in colon where bacteria converts it into CO_2, H_2 and some organic acids which irritate the digestive tract and result in painful condition known as ***lactose intolerance***. This condition is found in some infants due to deficiency of enzyme β- D-galactosidase/ lactase in the intestine. In such cases, milk and its products are not well tolerated.

6.3.3 Sucrose

Sucrose, the most abundant disaccharide is commonly known as table sugar. It consists of α-D-glucose and β- D- fructose joined by (1 → 2) glycosidic linkage (Fig. 6.7). Its systematic name is *O-α-D-Glucopyranosyl - (1→ 2) -β-D-fructofuranoside*. As anomeric carbons of both monosaccharides are involved in glycoside bond formation, sucrose is a non reducing sugar. Hence, it does not show mutarotation or positive Benedicts test.

Many non carbohydrates including artificial sweeteners such as saccharine, aspartame and sucralose mimic the taste of sucrose; give comparatively less number of calories and are cheaper. Thus these are being used as sweetening agent in food and beverage industry, management of diabetes and weight loss. You would see them in market with commercial names such as Sweet and low, splenda, equal and sugar free.

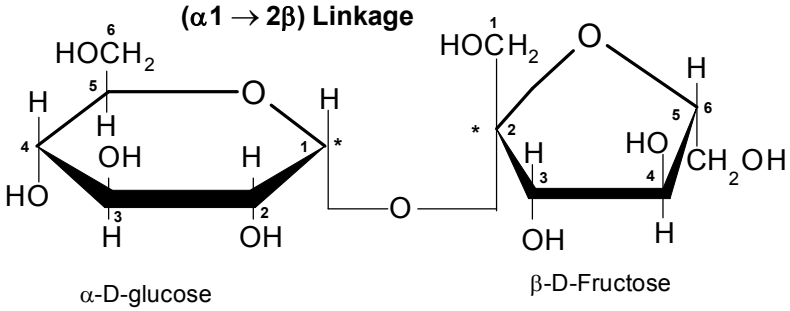


Fig. 6.7: Structure of Sucrose, the table sugar

It is the major form in which carbohydrates are transported in plants. Due to its sweet taste, sucrose is used mainly as sweetener. Like maltose, sucrose cannot be absorbed directly in the body; it is first hydrolyzed to glucose and fructose by the enzyme, sucrase or invertase in human intestine.

Hydrolysis of sucrose by the enzyme sucrase changes its optical rotation from *dextro* to *levo*, hence the name invertase. Inversion occurs because dextrorotatory sucrose (+66.5°) is converted to a mixture of glucose (+52.5°) and fructose (-92°). This mixture is known as invert sugar and is levorotatory mainly because of fructose which overrides the dextrorotatory effect of D-glucose (Fig. 6.8).

Liquefied mixture of sucrose, glucose and fructose is also called invert sugar. Honey is a natural source of invert sugar.

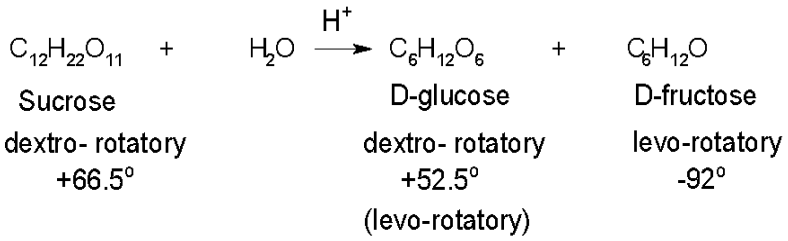


Fig. 6.8: Formation of invert sugar

6.3.4 Trehalose

Trehalose is a homodisaccharide in which two glucose units are linked by α-1, 1-glycosidic linkage. Its systematic name is O-α-D-glucopyranosyl-(1→ ***1)-***α-D-glucopyranoside (Fig. 6.9). As anomeric carbons of both glucose molecules are involved in glycosidic bond formation, therefore it is a **non-reducing sugar**. Trehalose is important component of hemolymph (insect blood). It protects the organisms against temperature variations. Hence, it is found in organisms that are naturally subjected to variations in temperature and other environmental stress such as bacterial spores, yeasts and insects.

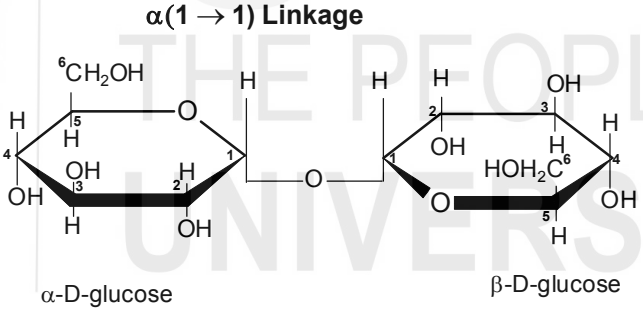


Fig. 6.9: Structure of Trehalose: Note the inverted glucose

6.4 HIGHER OLIGOSACCHARIDES

So far we have studied about disaccharides, now let us know about some biologically important tri or higher oligosaccharides.

Trisaccharides or higher oligosaccharides are not as abundant in nature as disaccharides. They are normally found in plants and part of some antibiotics. Let us discuss about some of oligosaccharides which have nutritional relevance.

6.4.1 Raffinose

Raffinose, also called melitose, is a non-reducing trisaccharide that is widely found in legumes and cruciferous vegetables, including beans, peas, cabbage, brussels sprouts, and broccoli. It consists of galactose connected to sucrose via α (1→6) glycosidic linkage (Fig. 6.10). Humans lack the enzyme

α-galactosidase (α-GAL) which hydrolyses this linkage. As a result, raffinose passes undigested through small intestine and subjected to fermentation by the bacteria present in the large intestine releasing CO_2, hydrogen and methane. That's why beans and these vegetable are sometimes called flatulence food.

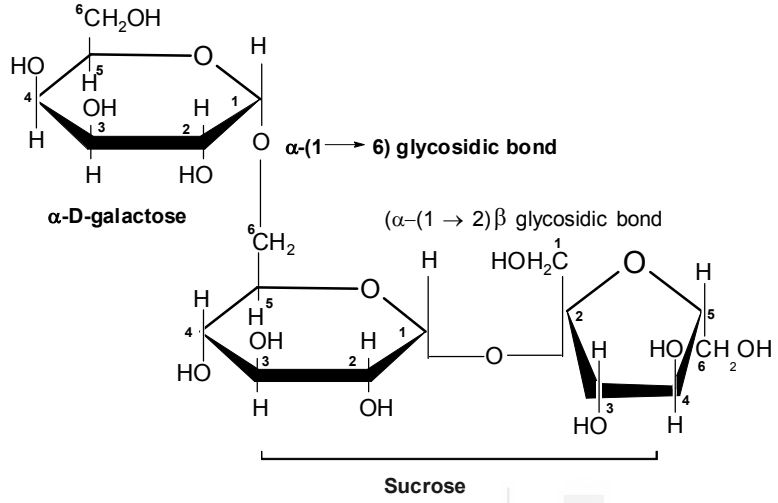


Fig. 6.10: Structure of Raffinose.

6.4.2 Stachyose

Stachyose is a non-reducing tetrasaccharide which is found together with raffinose in beans and other plants. It consists of two α-D-galactose units, one α-D-glucose unit, and one β-D-fructose unit sequentially linked as galactose (α1→6)galactose(α1→ 6) glucose(α1 ↔2β) fructose. Just like raffinose (Fig. 6.11), it cannot be digested in human intestine and hence causes flatulence.

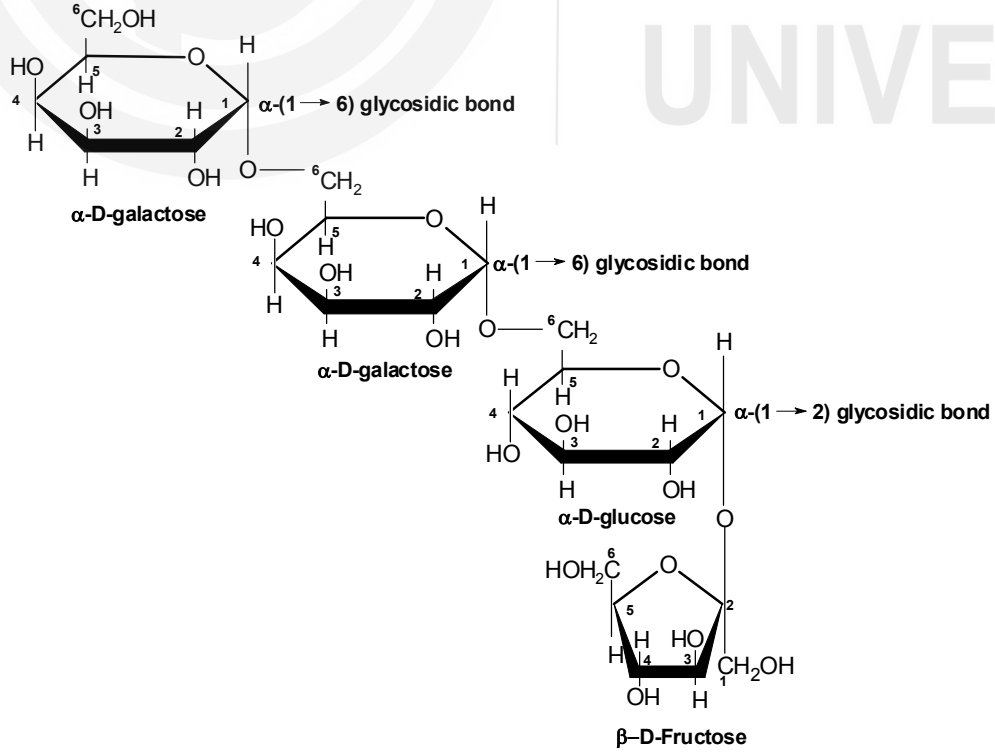


Fig.: 6.11: Structure of Stachyose.

6.4.3 Verbascose

It is a pentasaccharide consisting of three units of galactose, one of glucose and one of fructose. Three galactose units are linked by α $(1 \rightarrow 6)$ linkage to each other and to glucose which is then linked to fructose by α $(1 \rightarrow 5)$ linkage (Fig. 6.12). Thus it has one reducing end and another non reducing end.

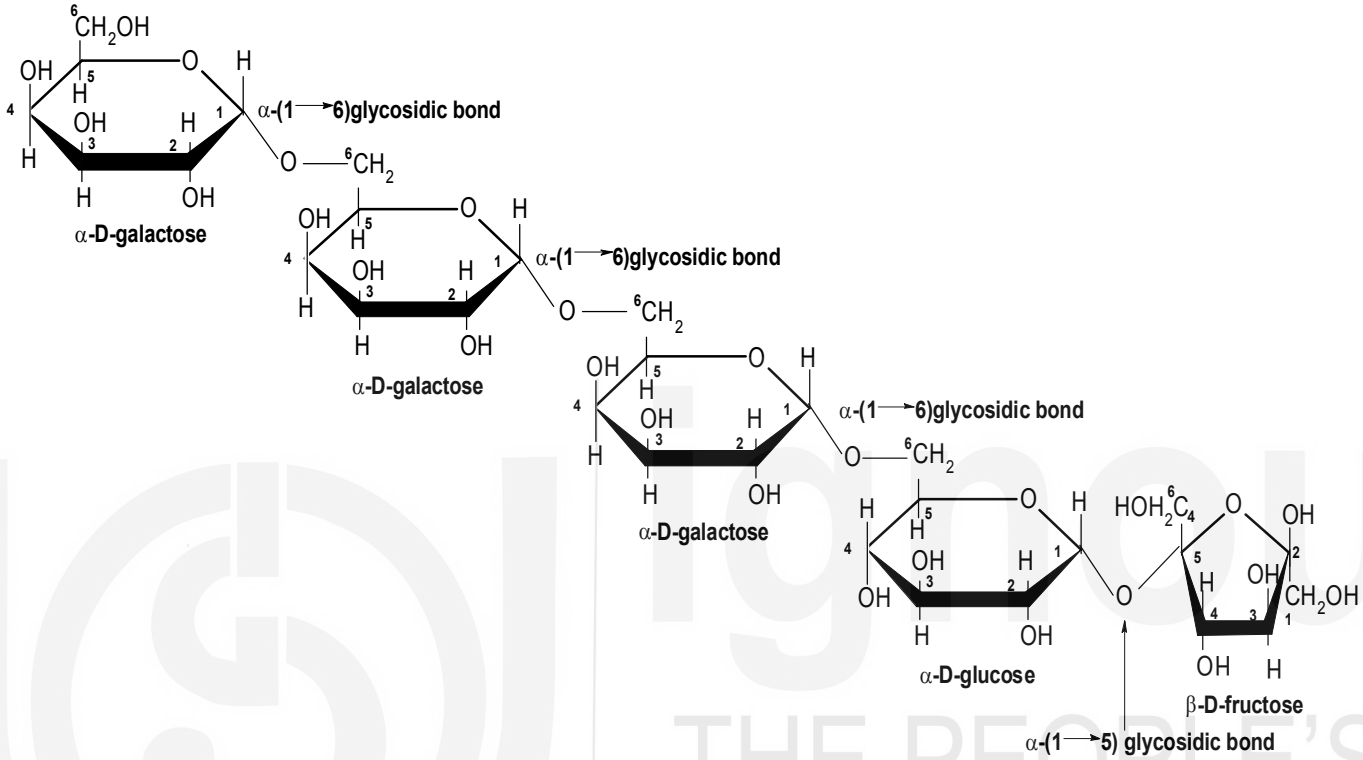


Fig. 6.12: Structure of Verbascose.

All these poorly digested oligosaccharides act as soluble fibers and may help in excretion. However, when consumed in excess can trigger abdominal bloating, excessive gas and diarrhoea.

SAQ 2

a) Match the columns A and B

i)	Sucrose	a)	α	(1–1)
ii)	Maltose	b)	β	(1–4)
iii)	Isomaltose	c)	α	(1–6)
iv)	Lactose	d)	α	(1–4)
v)	Trehalose	e)	α	(1–2)

b) Circle the odd one out and give reason:

i) i. Lactose ii. Sucrose iii. Trehalose

ii) i. Trehalose ii. Raffinose iii. Sucrose iv. Lactose

6.5 SUMMARY

1) Oligosaccharides are made up of 2-20 monosaccharide units and classified as disaccharides, trisaccharides, tetrasaccharides and so on based on number of constituent monosaccharides.

2) Based on the identity of constituent monosaccharides, they are termed homo oligosaccharides, if all of them are same and hetero oligosaccharides if they are different.

3) Monosaccharides present in an oligosaccharide are joined by glycosidic bond. Glycosidic bond is a covalent bond which a monosaccharide forms by condensation of hydroxyl group present on its anomeric carbon with hydroxyl group of an alcohol. This type of glycosidic bond is more specifically known as O-glycosidic bond.

4) Another type of glycosidic bond which monosaccharides make is N-glycosidic bond, in which they react with $-NH_2$ group of an amine instead of –OH of an alcohol

5) Glycosidic bonds are of two types depending upon the configuration of the anomeric carbon involved in formation of bond:

   i) α-glycosidic bond

   ii) β-glycosidic bond

6) Disaccharides are the most abundant of oligosaccharides. They are of two types: reducing and non reducing based on whether or not these contain anomeric carbon with free –OH group.

7) Lactose, maltose and sucrose are common examples of naturally occurring disaccharides.

8) Lactose is milk sugar and is a reducing disaccharide of galactose and glucose joined by $\beta(1\rightarrow 4)$ linkage. An enzyme, β-galactosidase (lactase) present in human intestine hydrolyzes lactose and helps in degestion of milk. Deficiency of this enzyme results in a painful condition known as lactose intolerance as the persons suffering cannot digest lactose containing milk and milk products.

9) Maltose which is found in malt and germinating seeds is also a reducing disaccharide of two glucose residues joined by $\alpha\ (1\rightarrow 4)$ O-glycosidic linkage. Two related structures are isomaltose which is $\alpha\ (1\rightarrow 6)$ isomer and cellobiose which is $\beta(1\rightarrow 4)$ isomer of maltose

10) Sucrose, the table sugar is made up of fructose and glucose joined by $\alpha\ (1\rightarrow 2)$ linkage. Sucrose, the table sugar, is a non reducing sugar as both the anomeric carbons are occupied in glycosidic bond formation.

11) Trisaccharides or higher oligosaccharides are not as abundant in nature as disaccharides. They are normally found in plants and part of some antibiotics.

6.6 TERMINAL QUESTIONS

1. What do you understand by reducing and non reducing sugars? Give examples.
2. Define O- and N-glycosidic bonds. How do you classify a glycosidic bond into α or β type?
3. Draw structures of lactose and sucrose. Label the anomeric carbons in these and and show what type of glycosic linkages (α or β) they have.
4. Write systematic names for isomaltose, maltose and raffinose. Draw their structures.
5. What is the biochemical basis of lactose intolerance?
6. What are flatulence foods? Give some examples.

6.7 ANSWERS

Self-Assessment Questions

1. a) O-Glycosidic bond is a covalent bond which a monosaccharide forms by condensation of hydroxyl group present on its anomeric carbon with hydroxyl group of an alcohol or another sugar where as in N-glycosidic bond hydroxyl group present on its anomeric carbon condenses with amino group.

   b) (i) α (ii) β

2. a) i-e, ii-d, iii-c, iv-b, v-a

3. b) i) Lactose as the other two sugars are non reducing.

   ii) Raffinose as other three sugars are disaccharides.

Terminal Questions

1) **Reducing disaccharides:** They have free -OH group at the anomeric carbon of one of the constituent monosaccharides. They give positive Benedict's test and can undergo mutarotation, for example, maltose and lactose.

   Non reducing disaccharides: None of the anomeric carbons of the constituent monosaccharides has free -OH group. These neither show mutarotation nor give positive Benedict's test, for example, sucrose and trehalose.

2) O-Glycosidic bond is a covalent bond which a monosaccharide forms by condensation of the hydroxyl group present on its anomeric carbon with hydroxyl group of an alcohol.

   N-glycosidic bonds are those in which hydroxyl group present on its anomeric carbon condenses with amino group.

   Glycosidic bond is designated as α -glycosidic bond if anomeric carbon participating in the bond formation belongs to sugar of α configuration.

Similarly, if anomeric carbon participating in the glycosidic bond formation belongs to sugar of β configuration, the glycosidic bond is classified as β -glycosidic bond.

3) Refer to Figures 6.6 and 6.7 in the text for structures of lactose and sucrose, respectively. It is evident from the strctures that in lactose, anomeric carbon belongs to β galactose, therefore, it is β glycosidic bond. In sucrose, anomeric carbon of first sugar, i.e. D-glucose is having β configuration, it is β glycosidic bond.

4) Systematic names are: isomaltose- β -D-glucopyranosyl (1 → 4) D-glucopyranose), maltose- O-α -D-glucopyranosyl-(1 → 4) - α - D-glucopyranose and raffinose- O-α -D-galactopyranosyl-(1 → 6)

-α-D-glucopyranosyl-(1→2)-β -D-fructofuranoside. Refer to the unit for structures.

5) Deficiency or absence of enzyme β - D-galactosidase commonly known as lactase in small intestine results in indigestion of milk or milk products. These undigested products enter in colon where bacteria convert them into CO_2, H_2 and some organic acids which irritate the digestive tract and result in painful condition known as lactose intolerance.

6) Foods like legumes and cruciferous vegetables, such as beans, peas, cabbage, brussels, sprouts, and broccoli are known as flatulence foods. These are rich in oligosaccharides such as raffinose, stachyose and verbascose. Humans lack the enzymes which can hydrolyze these sugars completely. As a result, these undigested sugars are subjected to fermentation by the bacteria present in the large intestine releasing gases like CO_2, hydrogen and methane.

6.8 FURTHER READINGS

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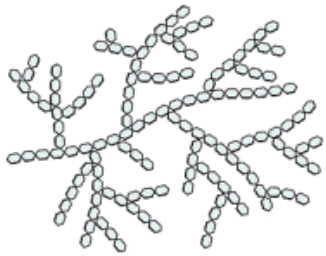


Glycogen

UNIT 7

POLYSACCHARIDES

Structure

7.1 INTRODUCTION

In the previous units, we discussed about structures and properties of monosaccharides and oligosaccharides. We also learnt how nature of the glycosidic bonds affects the properties of the oligosaccharides and their digestion by enzymes in human body. In this unit, we shall discuss about polysaccharides. Most of the carbohydrates in nature are polysaccharides. Some of them are simply polymers of monosaccharides like starch, glycogen and cellulose while others in addition to being polymeric saccharides are also linked covalently to other biomolecules like amino acids, peptides, proteins and lipids. These show not only structural diversity but also perform diverse functions. Let us now look at these aspects by studying different examples of polysaccharides.

Objectives

After going through this unit, you should be able to:

- classify polysaccharides;
- draw structures of energy storing forms of sugars; starch, glycogen, and discuss their properties;
- draw structures of structurally important polysaccharides; cellulose, chitin, and glycosaminoglycans;
- explain the type of structural support provided by structural polysaccharides; and
- describe types of complex polysaccharides and their functions.

7.2 CLASSIFICATION OF POLYSACCHARIDES

Polysaccharides, also known as **glycans,** are large molecules consisting of more than twenty monosaccharides or their derivatives joined by O-glycosidic bonds in linear or branched fashion *(poly- many; saccharide- sugar).* The ability to form branched structure distinguishes them from proteins and nucleic acids that form linear polymers. This is because each monosaccharide has one keto (aldehyde or ketone) group and multiple –OH groups which may act as reaction points for other monosaccharides to form branched chain structures. Polysaccharides are divided into two classes.

Homopolysaccharides- Polysaccharides formed by repetition of the same monosaccharide are known as homopolysaccharides or homoglycans. Based on the identity of constituent monosaccharide units, they may be further classified. For example, polysaccharides consisting of only glucose are known as **glucans**. Similarly, polymers of fructose and galactose are referred as **fructans** and **galactans**, respectively.

Heteropolysaccharides- These polysaccharides are made of different types of monosaccharides. However, in most heterosaccharides, not more than two kinds of monosaccharides are involved. Fig. 7.1 shows different possible arrangements of monosaccharides in homo and hetero polysaccharides.

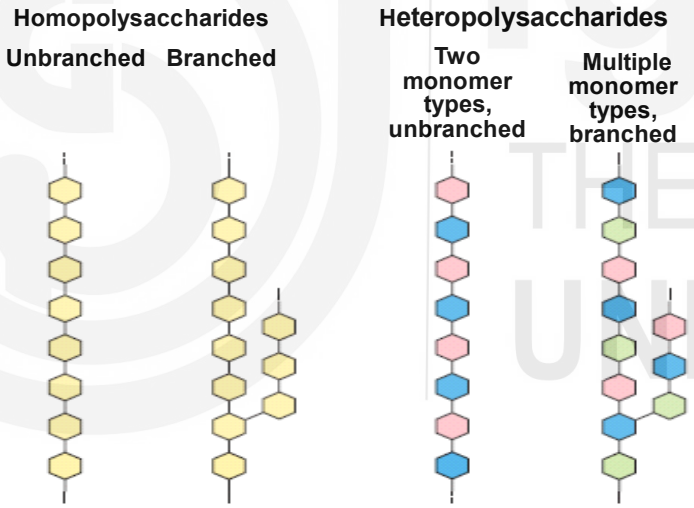


Fig. 7.1: Homo- and heteropolysaccharides.

Diversity at the structural level of polysaccharides reflects in their functions which fall into three major categories: energy reserve, structural support and information code. We shall now take examples in each category and discuss about their structure, properties and function in details.

7.3 STORAGE POLYSACCHARIDES

Organisms store energy in the form of polysaccharides instead of monosaccharides as they have lower osmotic pressure. Starch and glycogen fall into this category of functions. While starch serves as energy reserve in plants; glycogen is the storage polysaccharide in animals and microbial cells.

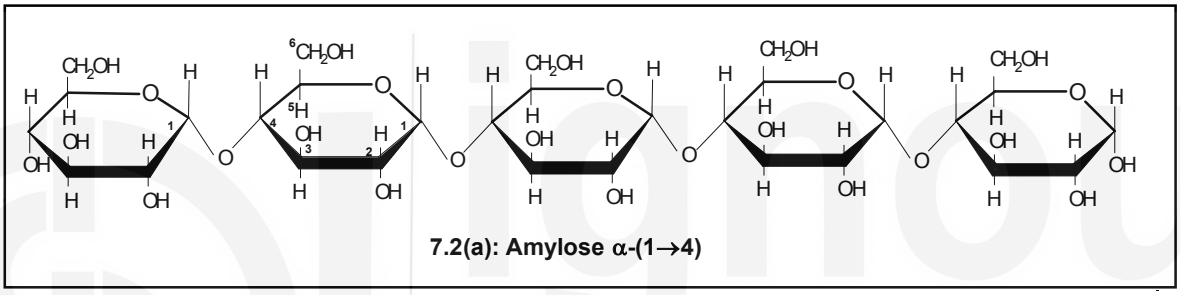
Osmotic pressure is a colligative property which depends on number of molecules. Therefore, one molecule of polysaccharide which is made up of thousands or even more number of monosaccharides has less osmotic pressure than equivalent number of monosaccharides

7.3.1 Starch

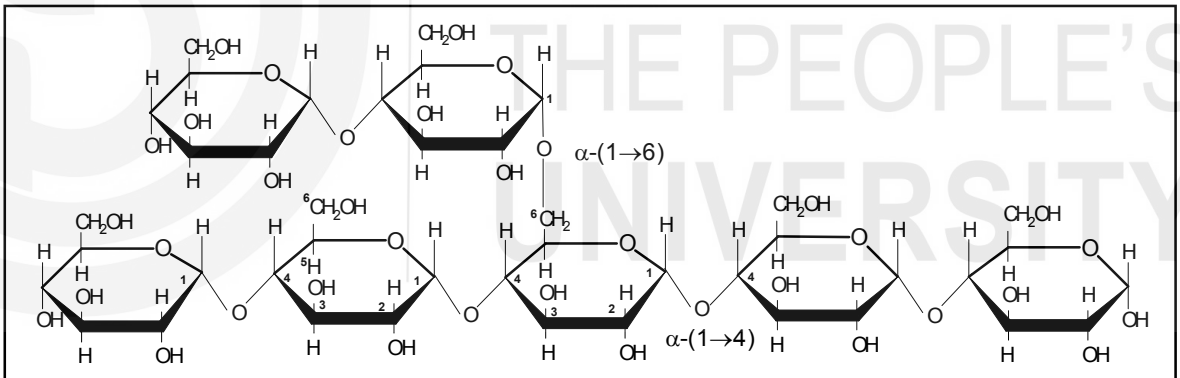
Cooked starch is more digestible than raw starch. This is because raw starch is not very susceptible to salivary amylase. While cooking, exposure to heat results in swelling and absorption of water which increases its susceptibility to amylase.

It is the principal energy reserve in plants and stored as granules in the chloroplast of the plant cell. It is found in almost every kind of plant cell, but grain seeds, tubers and unripe fruits are especially rich in it. It is Starch is a glucan as it is a homopolymer of α-D glucose and consists of two types of polymers: **α- amylose** which constitutes 10% to 30% and **amylopectin** forming remaining 70% to 90% of starch.

α- Amylose is linear polymer of several thousand to half a million α-D glucose units joined by (1→ 4) glycosidic bond (Fig. 7.2a). Each glucose residue is angled with respect to the preceding glucose due to α(1→4) linkage in α- amylose resulting in formation of coil or helical structure as in a telephone wire. Amylopectin also consists of linear chains of α-D glucose units joined by (1→ 4) glycoside bond; however they branch out at every 24-30 glucose residues by forming (1→ 6) glycosidic bonds (Fig. 7.2b) which prevents formation of coil.



7.2(a): Amylose α-(1→4)



7.2(b): Amylopectin

Fig. 7.2: Arrangement of α- D-glucose in amylose: a) and amylopectin; b) components of starch. A. Linear structure of amylose having α- D-glucose joined by α (1→ 4) glycosidic bonds. B. Branched chain structure of amylopectin; α- D-glucose joined by α (1→ 4) glycosidic bonds form linear strands which branch out by forming α (1→ 6) glycosidic bonds at intervals of 24-30 glucose molecules. Also note that one end of these strands is reducing while the other end is non- reducing.

When a solution of starch is mixed with iodine or potassium iodide, the space in the middle of the coil formed by amylose adsorbs iodine to form starch-iodine complex and gives intense blue black color. This property has been used as a qualitative test for the detection of starch.

Starch is present in many foods such as potatoes, bread etc. When taken in food, it is broken down in human body with the help of an enzyme α- amylase present in the saliva. Partial breakdown of starch by enzymes, heat or acids result in a sticky mixture known as dextrin which consists of smaller polysaccharides, monosaccharides and oligosaccharides. When you heat bread, golden brown crust is due to formation of dextrin.

Dextrans are the bacterial and yeast polysaccharides, which are glucan in nature. Bacteria present in our mouth secrete these polysaccharides which get deposited on surface of our teeth as dental plaques. These are different from dextins.

7.3.2 Glycogen

Glycogen, an equivalent of starch in animals, is also a homopolysaccharide of α-D glucose and serves as storage form of glucose in animals. It is present as granules in all cells but is abundant in liver and skeleton muscles. Structurally, it is similar to amylopectin, however it is more branched; almost every 8-14 glucose units. It also shows positive iodine test. When energy is needed, glycogen is hydrolyzed one residue at time from its non reducing end and is converted to glucose-1-phosphate with the help of enzyme glycogen phosphorylase in liver and muscle to provide energy.

It is interesting to note that most of the enzymes hydrolyzing glycogen and starch attack at the non reducing ends, releasing one glucose at a time. You can imagine if these enzymes were attacking anywhere in between these polysaccharides, it would have led to continuous breakdown of the long polysaccharide chains into small fragments and their complete solubilization. Instead, nature has designed branched chain structures of both amylopectin and glycogen in such a way that many non- reducing ends can be simultaneously attacked by the enzymes, allowing rapid mobilization of glucose when needed. α- amylose on the other hand has only one non reducing end and is therefore used mainly for long term storage of glucose.

7.4 STRUCTURAL POLYSACCHARIDES

Structural polysaccharides are abundantly present in plants, animals and microorganisms. Composition of structural polysaccharides is very much similar to storage polysaccharides, however they behave very differently. Their functions range from include providing rigidity, mechanical strength, support in between the cells and tissues to lubrication. Let us look at some of the members of this class.

7.4.1 Cellulose

Cellulose is the most abundant natural polysaccharide as it is found in cell wall in nearly all plants. Due to its tough and fibrous nature, it is one of the major components of woody parts of plants such as stem, trunk and bark of tree. It is also a homopolysaccharide of the class called glucans. It exists as a linear polymer of D-glucose linked by β (1$\rightarrow$ 4) glycoside linkage (Fig. 7.3). So you may notice that cellulose differs from α- amylose only in the type of glycoside bond i.e. it has β (1$\rightarrow$ 4) linkage instead of α (1$\rightarrow$ 4) linkage. However, this minor difference gives a totally different structure and properties to cellulose.

Fibers of cellulose and its derivatives can be spun into different types of fabrics. While cotton is almost pure cellulose, its acetate derivatives give silky material used in dresses, lining etc.

The α (1$\rightarrow$ 4) linkages present in starch are naturally bent giving it a coiled structure, however β (1$\rightarrow$ 4) linkages found in cellulose are more stable when alternating glucose units are flipped at 180^{O} making cellulose chains fully extended. This is known as extended ribbon conformation. In this conformation cellulose chains form large number of inter-chain hydrogen bonds. As a result, cellulose exists as linear, tightly packed rigid structure capable of resisting large pressures.

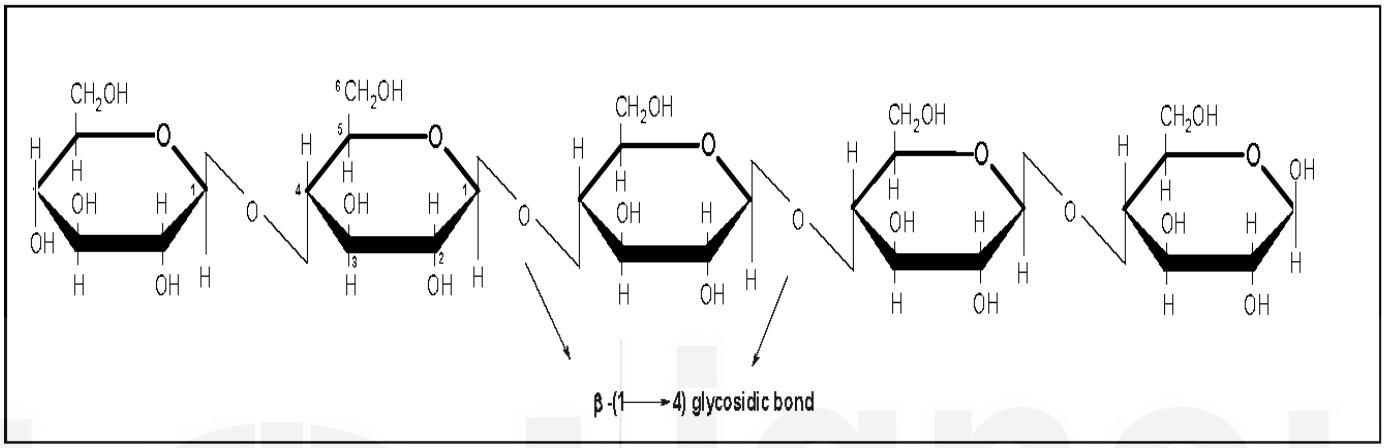


Fig. 7.3: Linkage of D-glucose residues by β (1$\rightarrow$) 4) glycosidic bonds in cellulose.

Cellulose is the most abundant carbohydrate; however, vertebrates cannot use it as food because they do not possess enzymes capable of hydrolyzing β (1$\rightarrow$ 4) linkage. Therefore, humans cannot digest fibrous food. Such foods, however, help in bowel movement and excretion. Digestive tracts of herbivores such as cow, giraffes, and deer contain the enzyme **cellulase** which can hydrolyze β (1$\rightarrow$ 4) linkages and helps in digestion of the plants.

Cruciferous vegetables, such as brussels sprouts, cabbage and kale are high in cellulose.

A comparison of starch, glycogen and cellulose shows how homopolymers of glucose when joined by different type of glycosidic bonds (α or β) or branched to different extent can assume different three dimensional structures with different properties suited to their functions is shown in the Fig. 7.4(a) and 7.4(b).

In addition to cellulose, cell wall of the plants also contains other polysaccharides such as xylan and glucomannans, xyloglucan, arabinoxylan and glucuronoxylan collectively termed as hemicelluloses.

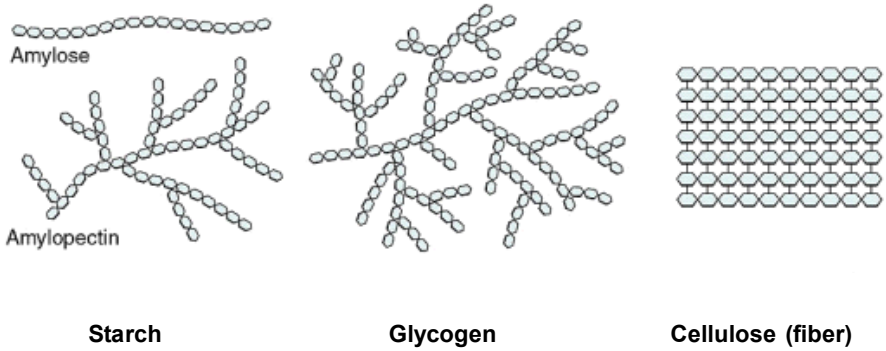


Fig. 7.4(a): Three dimensional structural arrangements of glucose molecules in starch, glycogen and cellulose. (*Source*: https://commons.wikimedia.org/wiki/ File:219 _Three_Important_Polysaccharides-01.jpg)

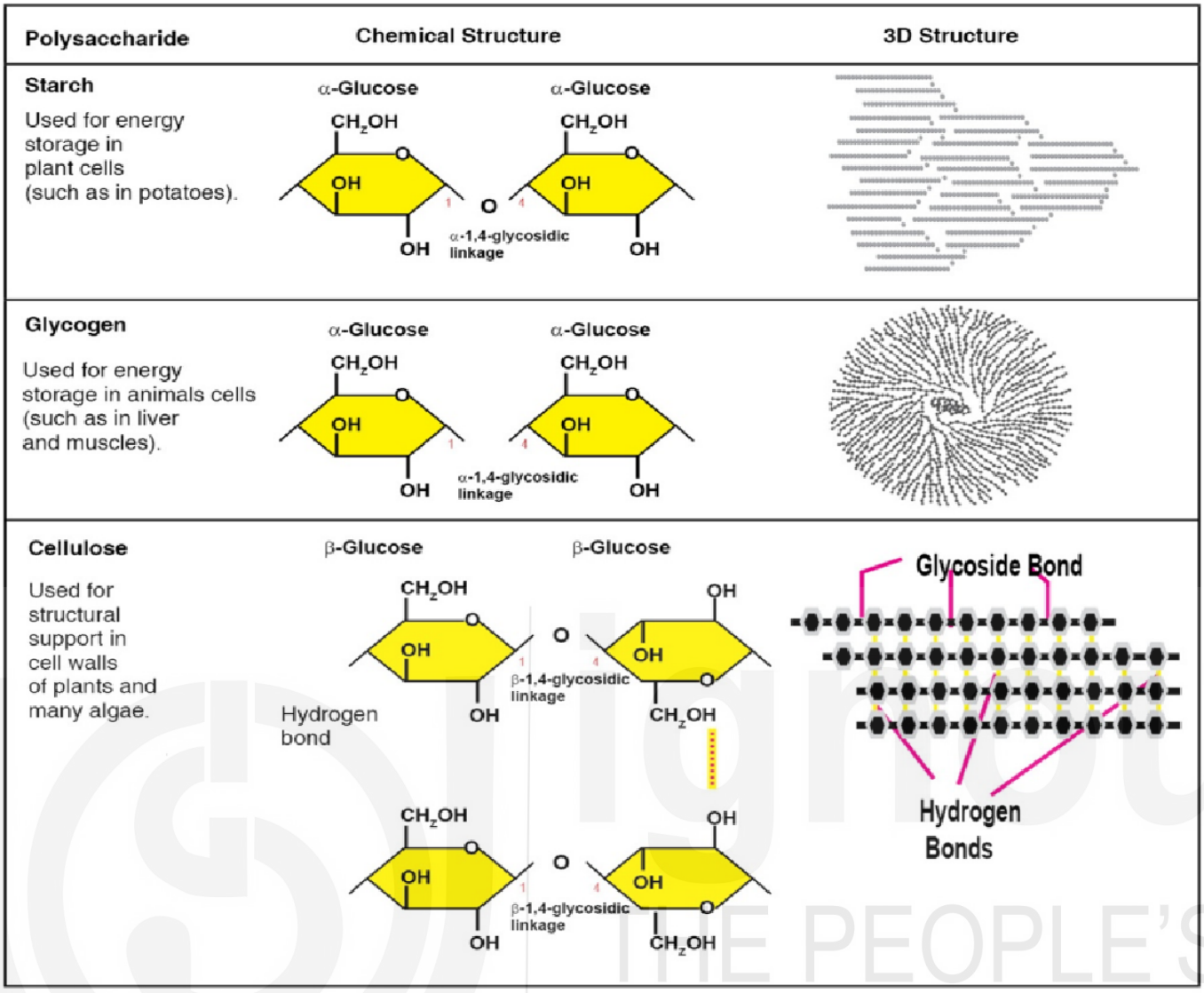


Fig. 7.4(b): Comparison of structure and functions of different homopolysaccharides

7.4.2 Chitin

Chitin, after cellulose, is probably the second most abundant polysaccharide in nature. It is a homopolysaccharide of N- acetyl- β- D-glucosamine linked by β (1→ 4) linkages (Fig. 7.5). Chitin forms hard exoskeleton of arthropods and mollusks like insects, and crabs as well as cell wall constituent of most fungi and many algae. In many of these exoskeletons, chitin provides the matrix for mineralization to occur. This kind of analogy is seen in vertebrate bones where collagen acts as matrix for mineral deposition.

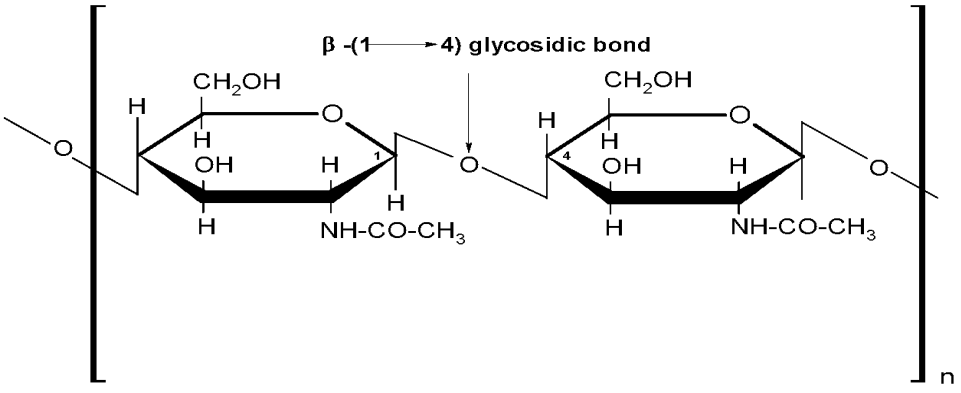


Fig. 7.5: N- acetyl glucosamine residues are linked by β (1→4) bonds.

7.4.3 Glycosaminoglycans (GAGs)

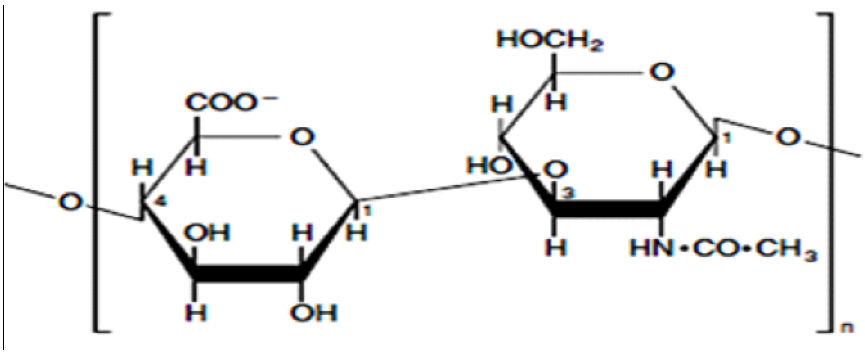
These are family of linear heteropolysaccharides which constitute major component of extracellular matrix (ECM) that surrounds connective tissues such as cartilage, skin, tendon and blood vessels. GAGs are found only in animals and bacteria and are absent in plants. These unbranched polysaccharides are composed of **repeating disaccharide** units. One of the sugars in the repeating disaccharide is amino sugar such as N- acetyl glucosamine or N-acetyl galactosamine and the other is a uronic acid in most of the cases. Some GAGs may also contain esterified sulfate groups. Presence of sulfate and carboxylate groups in GAGs make them highly negative charged and they assume extended rod like helix structures in solution to avoid repulsion between neighboring groups. GAGs also form fundamental unit of proteoglycans which we shall discuss in the next section.

Uronic acids are aldose derivatives in which their -CHO is oxidized to -COOH

GAGs have the ability to absorb lot of water and form viscous jelly which helps in reducing friction; that's why these are also called **mucopolysaccharides**. They can resist compression like a sponge which compresses to a certain extent on applying pressure and then regains its shape.

Let us discuss structures and functions of some physiologically important GAGs.

Hyaluronic acid: It consists of as many as 50,000 disaccharide units of D-glucoronic acid linked by ($\beta 1 \rightarrow 3$) glycosidic bond to N-acetyl-D glucosamine (Fig.7.6). It is an important component of synovial fluid (fluid which lubricates joints) and vitrous humor of eye as it forms highly viscous solution. It also forms part of ECM of cartilage and tendons where it contributes to their elasticity and high tensile strength.

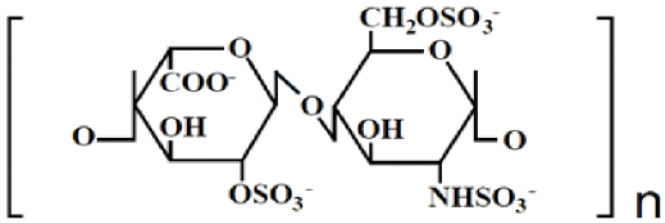


β-Glucuronic acid *N*-Acetylglucosamine

Fig. 7.6: Structure of repeating dimer in hyaluronic acid.

Heparin: It is a natural anticoagulant and is routinely added to blood samples for clinical analysis to prevent its clotting. It is also used as a therapeutic agent to inhibit clotting in blood vessels. Heparin is a mixture of highly sulfated heteropolysaccharides. One of its common repeating disaccharide; L-iduronate-2-sulfate: ($\alpha 1 \rightarrow 4$): N-sulfo-D-glucosamine-6-sulfate is shown in Fig. 7.7. Heparin like other GAGs is not present extracellularly but is found in mast cells which line the arterial walls, especially lungs, liver and skin.

Heparin has the highest negative charge density of any known biological macromolecules.

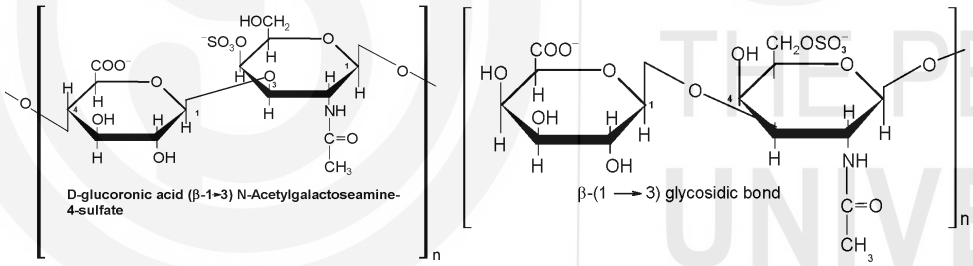


Iduronic acid is C- 5 epimer of glucuronic acid.

Fig. 7.7: Repeating disaccharide in heparin.

Chondroitin sulfates: There are two members in this category; **chondroitin-4- sulfate** which is polymer of disaccharide [D-glucoronic acid: ($\beta 1 \rightarrow 3$): N-acetyl-D galactosamine- 4-sulfate] (Fig 7.8a). Another is **chondroitin-6- sulfate** which has N-acetyl-D galactosamine- 6-sulfate in place of N-acetyl-D galactosamine- 4-sulfate. Both provide tensile strenth to cartilage, tendons, ligaments and walls of aorta. Recently, these are being recommended as dietary supplement for osteoarthritis patients (Fig 7.8b).

Chondroitin comes from Greek word "*chondros*" meaning cartilage



a) N-Acetylgalactosamine-4-sulfate **b) N-Acetylgalactosamine-6-sulfate**

Chondroitin-4-Sulfate **Chondroitin-6-Sulfate**

Fig. 7.8: Repeating disaccharide in (a) chondroitin-4- sulfate and (b) chondroitin-6- sulfate.

Karatan sulfate: It is present in cornea, cartilage, bone and horny structures like hoof, nails, claws. It lacks uronic acid. Repeating disaccharides are D-galactose: ($\beta 1 \rightarrow 4$): N- acetyl-D-glucosamine-6-sulfate (Fig 7.9).

Keratan comes from Greek word *keras* meaning horn

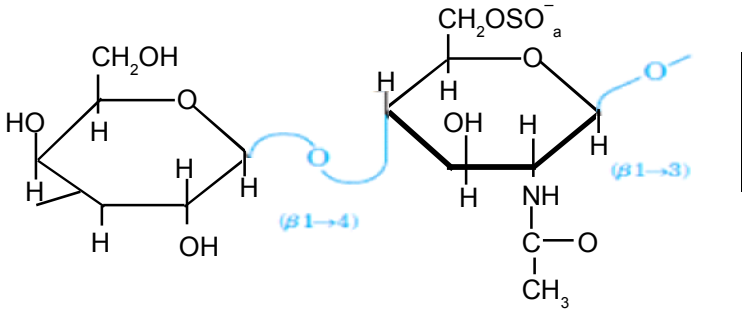


Fig. 7.9: Repeating disachharide of keratin sulfate.

Dermatan Sulfate: It is found in skin, blood vessels and heart valves. They consist of repeating disaccharide N- acety-D-galactosamine-4-sulfate and L- iduronic acid with variable amounts of glucuronic acids (Fig, 7.10).

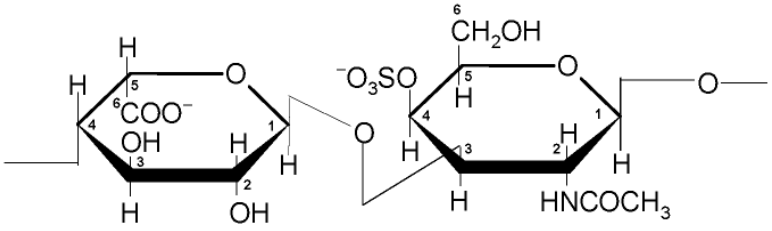


Fig. 7.10: Repeating disaccharide found in dermatan sulfate

SAQ 1

a) **Choose the correct option:**

1) Which of the following polysaccharides acts a plant food reserve?
(i) Glycogen,(ii) Hyaluronic acid, (iii) cellulose, (iv) starch

2) Branch points in starch and glycogen are due to formation of..... glycosidic bonds between glucose residues.
(i) α (1→4), (ii) α (1→2), (iii) α (1→6), (iv) α(1→6)

3) In amylopectin, branching is observed at the interval of every ..... glucose residues.
(i) 8-14, (ii) 12-20, (iii) 24- 30, (iv) 40-50

4) Which of the following disaccharides is obtained by digestion of amylose by the enzyme amylase?
(i) Maltose, (ii) sucrose, (iii) lactose, (iv) trehalose

5) Which of the following polysaccharides is found in plant fibres?
(i) Cellulose, (ii) Starch, (iii) Chitin (iv) Glycogen

6) Which of the following mucopolysaccharides is not sulfated?
(i) Heparin (ii) Hyaluronic acid (iii) Keratans (iv) Dermatans

7) Which of the following does not have nutritional value?
(i) Chitin (ii) Starch (iii) Glycogen

b) **Match the following:**

1)	Chitin	a)	Extracellular Matrix
2)	Keratan sulfate	b)	Insects
3)	Iodine	c)	Starch and glycogen
4)	Heparin	d)	cartilage and tendons
5)	Hyaluronic acid	e)	Iduronic acid
6)	Chondroitin sulfates	f)	D-Galactose: (β1→ 4): N- acetyl-D-glucosamine-6-sulfate

7.5 GLYCOCONJUGATES

Oligosaccharides and polysaccharides also exist in covalent linkage with lipids and proteins to form complexes known as glycoconjugates. Glycoconjugates are believed to be more dynamic and information rich than proteins and nucleic acids. These carry the information for communication between cells and their surroundings, labeling and targeting proteins for transport from their site of synthesis to action, cell- cell recognition, blood clotting, wound healing, immune response and many more.

Glycoconjugates are divided into three broad classes:

1) Proteoglycans
2) Glycoproteins
3) Glycolipids and lipopolysaccharides

Let us discuss the structure and role of these glycoconjugates.

7.5.1 Proteoglycans

These include diverse group of molecules which are composed of proteins and glycosaminoglycans. The two components may be linked covalently or non-covalently. These form the ground substance in extracellular matrix.

Proteoglycans have structure like a bottle brush. Fig. 7.11 shows the structure of a proteoglycan, where the backbone is made up of hyaluronic acid and core protein to which GAGs such as keratan sulfate and chondroitin sulfate are covalently attached like bristles. The covalent linkage to core protein is through –OH group of serine present in the sequence Ser-Gly-X-Gly where X is any amino acid. The monosaccharides in GAGs attached to protein remain extended because they are negatively charged and repel each other. The extended brush like structure and negatively charged GAGs allow the proteoglycans to retain lot of water and hence make them resistant to compression. This property also makes them suitable as a component of cartilage.

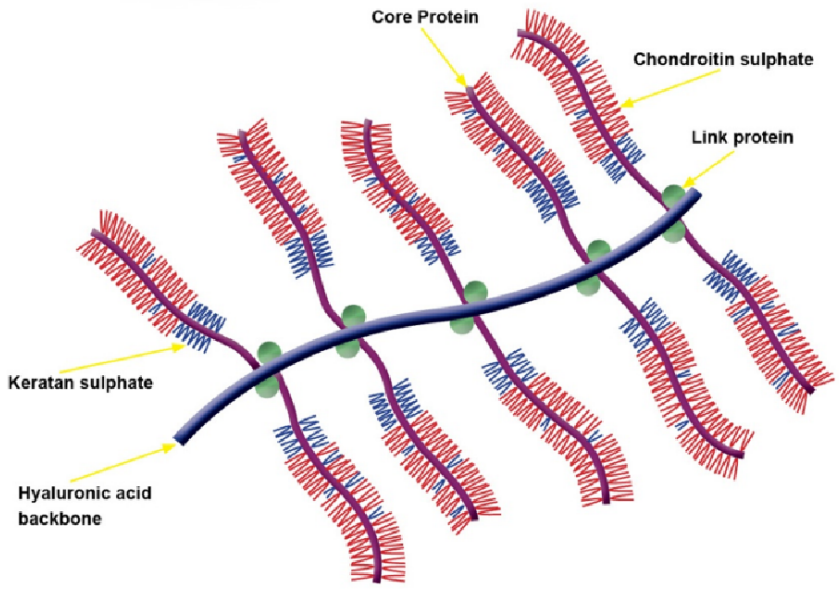


Fig. 7.11: Bottle brush like structure of proteoglycan (Source: themedicalbiochemistrypage.org/glycans.php)

In bacteria, the cell wall consists of framework of polysaccharides which are covalently linked to polypeptide chains. It is known as **peptidoglycan or murein** and provides protection to bacteria against environment. Antibiotic penicillin inhibits the enzymes which crosslink the peptidoglycan fibres in cell wall. As a result the cell wall can not form and bacteria can not multiply.

7.5.2 Glycoproteins

As the name suggest these molecules consist of both carbohydrates and proteins. More than 50% of the eukaryotic proteins carry covalently attached oligosaccharides or polysaccharides which vary in content from $\leq$ 1% to $\geq$ 90%. These complexes are known as glycoproteins. The carbohydrates present in glycoproteins are smaller, more branched and structurally more diverse than GAGs in proteoglycans. The predominant sugars found in glycoproteins are glucose, galactose, mannose, fucose, *N*-acetylgalactosamine, *N*-acetylglucosamine and *N*-acetylneuraminic acid (NANA).

Carbohydrates in glycoproteins form glycosidic bond with amino acid residues of the proteins by O- or N-glycosidic bonds and are therefore divided into two types: O- linked and N- linked.

N- linked glycoproteins contain glycosidic bond which is usually formed by N- acetylglucosamine or sometimes N- acety galactosamine of an oligosaccharide with the side chain amide nitrogen of asparagine in protein. Asparagine is generally present in a sequence –Asp-X-Ser/Thr, where X may be any amino acid. For example; immunoglobulins which make our antibodies and ovalbumin in egg white are N- linked glycoproteins.

Antarctic fish contains antifreeze, which is O-linked glycoprotein and prevents freezing of body fluids even in extremely cold water.

O- linked glycoproteins contain oligosaccharides linked by O-glycosidic bond by N-acetyl glucosamine with hydroxyl (–OH) group of serine or threonine present in the protein. Mucins, the glycoproteins present in saliva belong to this category. There are examples where other amino acids may be involved, for example, in collagen, this type of bond is formed with hydroxylysine or hydroxyproline.

Many glycoproteins contain both N-linked and O- linked glycoproteins, for example, erythropoietin. It is a hormone synthesized in kidney and stimulated formation of erythrocytes and is used in cancer chemotherapy.

So we can say that glycoproteins not only have very diverse structure but also perform many specialized functions such as part of cell membrane, enzymes, hormones, immunoglobulins (the molecules that fight against infection) and cell markers (they help in the identification of cell or recognition of one cell by the other).

Blood group antigens (A, B, O) are oligosaccharides which determine the blood group types in humans. Interestingly, these oligosaccharides exist as both glycoproteins as well as glycolipids. We shall discuss about these antigens in the next unit.

7.5.3 Glycolipids and Lipopolysaccharides

Lectins are the carbohydrate binding proteins which are found in all organisms. They are highly specific in their binding, though the affinity varies from moderate to high.

Glycolipids are the membrane lipids found in animals and plants to which oligosaccharides are attached. These oligosaccharides act as cell surface markers by providing specific sites for recognition by carbohydrate-binding proteins. Gangliosides are example are the lipids found on the outer surface of eukaryotic cell membranes and are associated with oligosaccharides containing sialic acid and other monosaccharides. These oligosaccharides act as cell markers helping in cell recognition and cell to cell communication. We shall discuss about glycolipids in greater detail under lipids in Block 3.

Lipopolysaccharides are oligosaccharides attached to outer cell membranes of gram negative bacteria such as *Salmonella typhimurium* (typhoid causing bacteria). It consists of lipids anchored in the outer cell membrane joined to a polysaccharide long chain which is made of characteristic and repeating oligosaccharides. Some of these oligosaccharides give antigenicity to the bacteria. Whenever bacteria attack our body, our immune system produces antibodies against these components. Hence, these are used as important markers to identify the bacterial strains (Fig. 7.12).

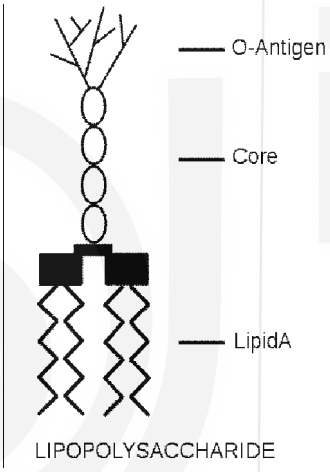


Fig. 7.12: Graphic presentation of bacterial lipopolysaccharides. Lipid part is embedded in the bacterial outer cell membrane and is attached to long polysaccharide chain. Core part of this polysaccharide is same while the antigen part varies with type of bacteria and determines its antigenicity.

Glycoproteins and glycolipids are considered as information carrying molecules like proteins and nucleic acids. We shall discuss about these molecules in greater details in the next unit.

SAQ 2

Fill in the appropriate word or phrase:

a) Proteoglycans are made of.....

b) Oligosaccharides in N- linked glycoproteins are linked to proteins via .....

c) Informational carbohydrates include.....

d) Polypeptide and carbohydrate framework of bacterial cell wall is known.....

e) ..... part of the lipopolysaccharide determines antigene city of a gram negative bacteria.

7.6 SUMMARY

1) Polysaccharides, based on their structure, are classified into **homopolysaccharides** when they are made of one kind of monosaccharide and **heteropolysaccharides** which are made of two or more differnt monosaccharides.

2) They are also classified based on their function into **storage polysaccharides** and **structural polysaccharides**. Examples of stotage polysaccharides are starch and glycogen which serve as food reserves in plants and animals, respectively.

3) Starch and glycogen are branched homopolysaccharides of α- D glucose linked by α (1$\rightarrow$4) glycosidic linkages and branching is by α (1$\rightarrow$6) glycosidic linkages.

4) Cellulose is a structural homopolysaccharide which forms the woody part of plants and provides them strength and rigidity. It is also a linear polymer of D-Glucose but linked by β (1$\rightarrow$4) glycoside linkage

5) Glycosaminoglycans (GAGs) also provide structural support and consist of family of linear heteropolysaccharides such as hyaluronic acid, chondroitin sulfate, heparin and keratan sulfate

6) GAGs constitute major component of extracellular matrix (ECM) and are found only in animals and bacteria and absent in plants.

7) Oligosaccharides and polysaccharides also exist in covalent linkage with lipids and proteins to form complexes known as glycoconjugates.

8) Glycoconjugates are of three types: proteoglycans, glycoproteins and glycolipids.

9) Proteoglycans are composed of proteins and GAGs, an important component of cartilage and can resist compression to certain extent.

10) Glycoproteins consist of both carbohydrates and proteins. The carbohydrate content of these molecules may vary from $\leq$ 1% to $\geq$ 90%. Blood group substances (A,B, O) are examples of glycoprotein.

11) Glycolipids are the membrane lipids bound to oligosaccharides. They function as markers for recognition by carbohydrate binding protein.

7.7 TERMINAL QUESTIONS

1) What are storage polysaccharides? Explain with suitable examples.

2) Which property of proteoglycans makes them suitable for ground substance of extra cellular matrix?

3) Draw structure of cellulose and write its biological importance.

4) What are glycosaminoglycans? Describe structure and functions of different glycosaminoglycans.

5) Write structure and functions of chitin.

6) Explain different types of glycoproteins. What type of functions do they perform?

7) What are polysaccharides and how are they classified?

7.8 ANSWERS

Self-Assessment Questions

1. a) 1. Starch 2. α (1$\rightarrow$6) 3. 24-30 4. Maltose
   5. Cellulose 6. Hyaluronic acid 7. Chitin

   b) 1:b, 2:f, 3:c, 4: e, 5:a, 6:d

2. a) Proteins, glycosaminoglycans

   b) Asparagine

   c) Glycoproteins and glycolipids

   d) Peptidoglycan or murein

   e) O-Antigen

Terminal questions

1. Polysaccharides also known as **glycans** are large molecules made up of more than ten units of monosaccharides or their derivatives joined by O-glycosidic bonds *(Poly- many; saccharide- sugar.* They may be linear or branched. Polysaccharides are classified based on their constituent monosaccharides as well as functions. Based on their constituent monosaccharides, they are divided into two classes:

   Homopolysaccharides- Polysaccharides in which repeating monosaccharides are of same type are known as homopolysaccharides or homoglycans. Based on the identity of constituent monosaccharide units, they may be further classified. For example, **glucans** are the polysaccharides consisting of only glucose, while **fructans** and **galactans** are polymers of fructose and galactose, respectively.

   Heteropolysaccharides- These polysaccharides are made of different types of monosaccharides.

   Functionally, polysaccharides fall into two major categories; **storage polysaccharides** and **structural polysaccharides**. However, there are molecules where polysaccharides are linked to amino acids, peptides, proteins, lipids and other structures. These are known as **glycoconjugates.**

2) Starch and glycogen are known as storage polysaccharides as they act as food reserves in plants and animals, respectively. See section 7.2 for their structure and properties.

3) Cellulose is a linear polymer of D-Glucose linked by β (1→ 4) glycoside linkage. It is found cell wall in nearly all plants. Due to its tough and fibrous nature, it is one of the major components of woody parts of plants such as stem, trunk and bark of tree. It cannot be digested by human intestine due to lack of suitable enzyme. Therefore, it acts as roughage and helps in bowel movement and excretion.

4) Chitin, after cellulose, is probably the second most abundant polysaccharide in nature. It is a homopolysaccharide of N- acetylglucosamine linked by β (1→ 4) linkages. Chitin forms hard exoskeleton of arthropods like insects, and crabs as well as cell wall constituent of most fungi and many algae.

5) Glycosaminoglycans are family of linear heteropolysaccharides composed of **repeating disaccharide** units. One sugar in the repeating disaccharide is amino sugar such as N- acetyl glucosamine or N- acetyl galactosamine and the other is uronic acid in most of the cases. They constitute major component of extracellular matrix (ECM) that surrounds connective tissues such as cartilage, skin, tendon and blood vessels. GAGs are found only in animals and bacteria and are absent in plants. Hyaluronic acid, heparin, keratans, dermatans and chondroitin sulphates are some of the examples of GAGs. Refer to section 7.4.2 for their structures.

6) Proteoglycans are the diverse group of molecules which are composed of proteins and glycosaminoglycans (GAGs). GAGs present in proteoglycans are negatively charged, therefore, they form extended brush like structure and allow proteoglycans to retain lot of water and hence make them resistant to compression. This property also makes them suitable for ground substance of extra cellular matrix?

7) Glycoproteins consist of both carbohydrates and proteins. The carbohydrate content of these molecules may vary from $\leq$ 1% to $\geq$ 90%. Glycoproteins are of two types: O- linked and N- linked.

   In **N- linked glycoproteins**, an oligosaccharide forms N-glycosidic bond between one of its anomeric carbon and amide nitrogen of asparagine in protein.

   In **O- linked glycoproteins**, oligosaccharide and proteins are linked by O-glycosidic bond between one of anomeric carbon of oligosaccharide and –OH group of serine or threonine on protein.

   Glycoproteins perform many specialized functions such as part of cell membrane, enzymes, hormones, immunoglobulins (the molecules that fight against infection) and cell markers (they help in the identification of cell or recognition of one cell by the other). Blood group antigens (A, B, O) are glycoproteins in nature.

7.9 FURTHER READINGS

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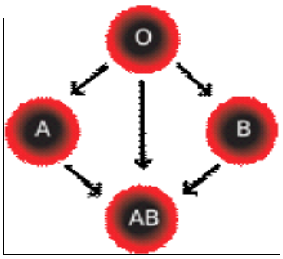


Blood group compatibility

UNIT 8

GLYCOBIOLOGY

Structure

8.1 INTRODUCTION

You have learnt the previous units that monosaccharides can form endless number of oligosaccharides and polysaccharides by varying the type and number of glycosidic bonds, type and orientation of substituents as well as extent of branching. In fact, recent studies on carbohydrates prove that they are far more dynamic and information rich molecules than proteins and nucleic acids. Such information carrying function is performed by glycoconjugates. We have already discussed different classes and general features of these glycoconjugates with few examples. You may understand the importance of these information molecules from the fact that a whole new branch glycobiology is emerging which deals with study of these oligosaccharides and polysaccharides and the information coded by them.

In this unit you will learn what glycobiology deals with and how the information of glycoconjugates decoded in the cells. We shall also discuss different methods to study carbohydrates and the difficulties scientist face during this task

Objectives

After studying this unit you should be able to:

- define glycobiology and sugar code;
- write about different types of information carried by oligosaccharides in glycoconjugates;
- explain the role of different proteins involved in reading sugar code;
- distinguish different blood groups based on their oligosaccharide markers; and
- explain different techniques and methods involve in analysis of carbohydrates.

8.2 CARBOHYDRATES AS INFORMATION MOLECULES

Based on learning from the previous units, we can say that carbohydrates are very dynamic molecules performing functions like biochemical fuel, food reserves, structural support and information carrying. Oligosaccharides present in glycoconjugates carry diverse kind of information. Each oligosaccharide codes for a word and its monosaccharide units act as alphabets. The branch of biology which deals with the study of structure and functions of glycoconjugates is called **glycobiology** and is emerging as an important area of research. The information encoded in an oligosaccharide sequence of a glycoconjugate is referred to as **sugar code**. Let us look at different cell activities encoded in sugar code:

1) Communication between cells and their extracellular surroundings;
2) Labeling proteins for transport and localization in specific organelles;
3) Degradation of non functional protein;
4) Recognition sites for extracellular signal molecules (growth factors, for example) or parasites (bacteria and viruses).
5) In eukaryotic cells, specific oligosaccharide chains attached to components of the plasma membrane form a carbohydrate layer known as glycocalyx. This layer serves as an information-rich surface by which the cell interacts with its surroundings. These oligosaccharides play important role in cell-cell recognition and adhesion, migration of cells during development, blood clotting, immune response, wound healing and other cellular processes.

What makes carbohydrates suitable for carrying such diverse information? It is because of their following structural features:

1) They are chiral molecules; therefore, they form different stereoisomers and exhibit optical activity.
2) They can form different types of glycosidic bonds.
3) They have multiple functional groups which allow them to undergo different modifications, add different substituents, form linear chains of varying lengths and have extensive branching

You may understand the extent of variation possible in carbohydrates by the following example. 20 different monosaccharides can form as many as 1.44×10^{15} different hexameric oligosaccharides. On the other hand, 20 common amino acids can form 6.4×10^{7} (20^{6}) different hexapeptides and 4 nucleotides can form 4,096 (4^{6}) different hexanucleotides.

First lectin, named concanavalin A was discovered in plants. Later on, lectins were found to be present in all organisms.

Let us now try to understand how cell decodes the sugar code? Cells are able to do this by using proteins named **lectins**, which can read the sugar code. Lectins are present in all organisms and bind carbohydrates with very high specificity and moderate to high affinity. However, it is important to keep in mind

that there are different types of lectins which bind to their specific oligosaccharide substrates. As they are decoding sugar code, therefore they mediate all the biological functions encoded in the respective oligosaccharide to which they bind. Let us look at some specific examples of functions mediated by lectins.

Degradation of glycoproteins: Many glycoproteins have N- acetyl neuraminic acid, a sialic acid residue at the end of their oligosaccharide chain which protects them from degradation. These sialic acid residues can be removed by the enzyme sialidase or neuraminidase. The glycoprotein after removal of sialic acid is known as asialoglycoprotein which is recognized by hepatic asialoglycoprotein receptor. This receptor is a lectin which binds to oligosaccharide chains with galactose and degrades them. Ceruloplasmin, a copper binding protein undergoes degradation by this process.

Antiviral drugs like tamiflu bind viral sialidase and prevent release of virus from infected cells.

Even old red blood cells are also thought to be removed from circulation by this process. Influenza virus uses a lectin hemagglutinin (HA) to bind glycoproteins on surface of host membrane and gain entry into host cell. Once inside host, it multiplies and secretes enzyme sialidase. Sialidase removes terminal sialic acid from host oligosaccharides resulting in their degradation and releasing viruses free.

Human selectins are involved in inflammatory responses in asthma, rheumatoid arthritis, multiple sclerosis and rejection of transplant rejection. Thus researchers are looking for drugs which can inhibit selectin mediated cell adhesion.

Cell- cell recognition and adhesion: Immune responses such as fighting infections or inflammation need movement of circulating white blood cells/ leukocytes from blood to tissues at the site of infection, injury, inflammation or tissue repair. **Selectins** are the family of plasma membrane lectins which mediate this process. These lectins are present at the endothelial (inner) wall of blood capillaries and can recognize specific glycoproteins on the surface of leukocytes. Binding to selectins slows down the circulating cells and allows them to attach to capillary wall at the site of infection with help another set of molecules known as adhesins and finally exit through capillary wall (Fig. 8.1).

Adhesins are a family of animal proteins involved in the adhesion of cells to each other and to their substrate.

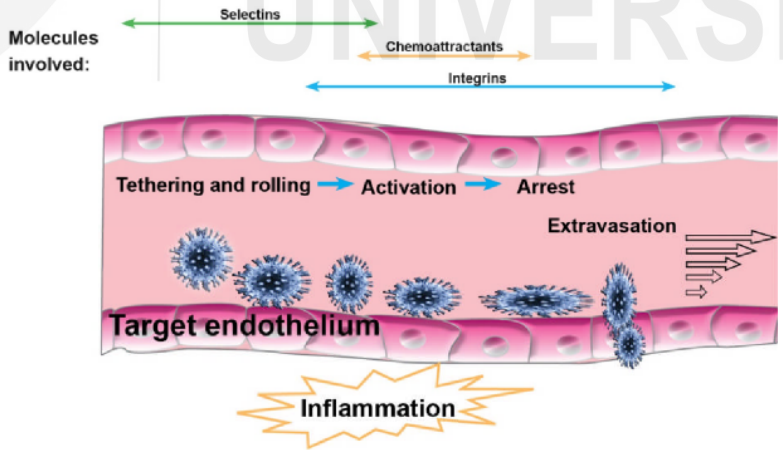


Fig. 8.1: Selectin mediated inflammatory response

Protein sorting and targeting: You know that synthesis of new proteins takes place in rough endoplasmic reticulum. From the very beginning, the protein is marked with a complex oligosaccharide. This oligosaccharide acts like address of the protein. Different lectins bind sequentially to it reading information for its proper folding and delivery to its specific target organelle inside cell or secretion outside the cell.

SAQ 1

Define the following terms:

a) Glycobiology .....

b) Sugar code.....

c) Lectins.....

d) Asialoglycoprotein .....

So far we studied the role of glycoconjugates as informational molecules and understood how this information at the cellular level to perform different functions with the help of carbohydrate binding proteins, lectins. Let us discuss role of oligosaccharides in determination of human blood groups.

8.3 BLOOD GROUP ANTIGENS

You may be aware that there are four types of blood groups in humans: A, B, AB and O. These types are based on three types of oligosaccharide chains on the surface of red blood cells (RBCs). These chains are attached to proteins and lipids that lie in the RBC membrane and act as markers called antigens as they can cause an immune response. These antigen markers are of three types- A, B, and O

Mixing of two different types of blood groups results in fatal immune response and aggregation of RBCs

Karl Landsteiner recognized these markers and explained four blood groups: A, B, AB and O. The most basic oligosaccharide is known as O antigen made up of one unit of N- acetyl glucose, two units of galactose (one on either side of N-acetyl glucose) and one of Fucose. In case of antigen B, this oligosaccharide has an additional unit of Galactose while antigen A carries unit of N- Acetyl galactose (Fig. 8.2).

Landsteiner got Nobel prize for discovery of ABO blood group system.

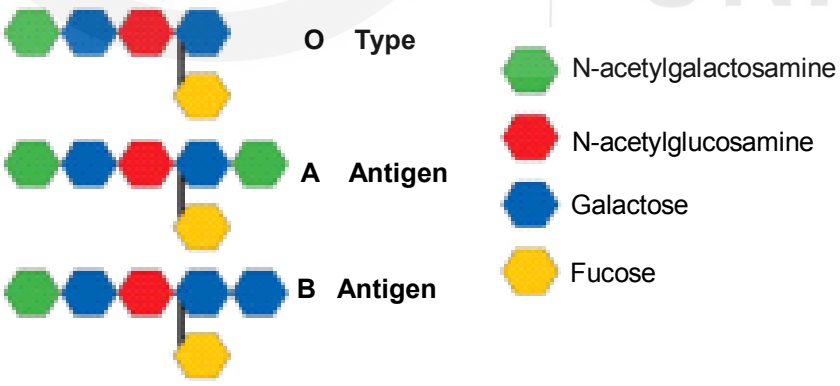


Fig. 8.2: Oligosaccharide composition of A, B and O antigens

Functions of these antigens are not clear, however they have clinical significance. Person with particular blood group antigen has antibodies against other blood antigens. When a person is transfused with blood group of antigen not similar to his own antigen, antibodies present in his blood react with antigen of foreign blood antigens leading to aggregation of blood. This may lead to death in severe cases. Fig. 8.3(a) shows the type of antigen and antibodies present in different blood groups.

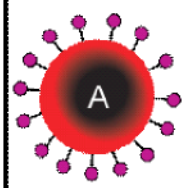
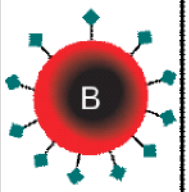
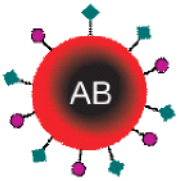
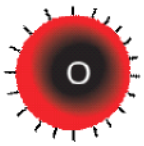
	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in Plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in Red Blood Cell	 A antigen	 B antigen	 A and B antigens	None

Fig. 8.3(a): Type of antigen and antibodies present in blood groups A, B, AB and O.

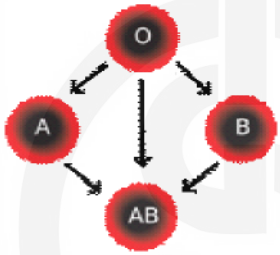


Fig. 8.3(b): Blood group compatibility

From the Fig. 8.3, it is clear that persons with blood group A may be given blood group A and AB, those with blood group B can receive B and AB, group AB can receive any of the four blood groups as it has no antibodies and those with group O can receive only blood group O as they have both antibodies A and B. Thus, person with blood group AB is known as universal receiver and blood group O as universal donor Fig. 8.3(b).

SAQ 2

Match the following:

1)	Blood group AB	a)	Universal receiver
2)	Blood group O	b)	Anti A
3)	Blood group A	c)	Universal donor
4)	Blood group B	d)	Anti B

We have studied about diversified roles of carbohydrates. Hence they are very rich in information. To understand this information, it is important to develop techniques and methods which can establish complete structure of oligosaccharides and polysaccharides. However, it is not an easy task; firstly because of diversity in structure of these molecules. Secondly, techniques for studying carbohydrates are still being developed.

In this section we shall briefly discuss different approaches being used for carbohydrate analysis.

8.4 WORKING WITH CARBOHYDRATES

In order to establish complete structure of oligosaccharides and polysaccharides, one needs to determine linear sequence of

monosaccharides, points of branching, configuration of each monosaccharide as well as type and position of glycosidic bonds joining them. This poses a challenging task which requires systematic approach and application of different techniques (Fig. 8.4). We shall discuss about different methods used to study carbohydrates and what type of information they provide.

1) **Release of oligosaccharides from glycoconjugates:** Oligosaccharides need to be released from their protein or lipid partners and purified before they are further analyzed. It is accomplished using enzymes glycosidases which specifically cleave N- or O- linked oligosaccharides and lipases that remove lipids. O- linked oligosaccharides can also be released from glycoproteins by treatment with hydrazine.

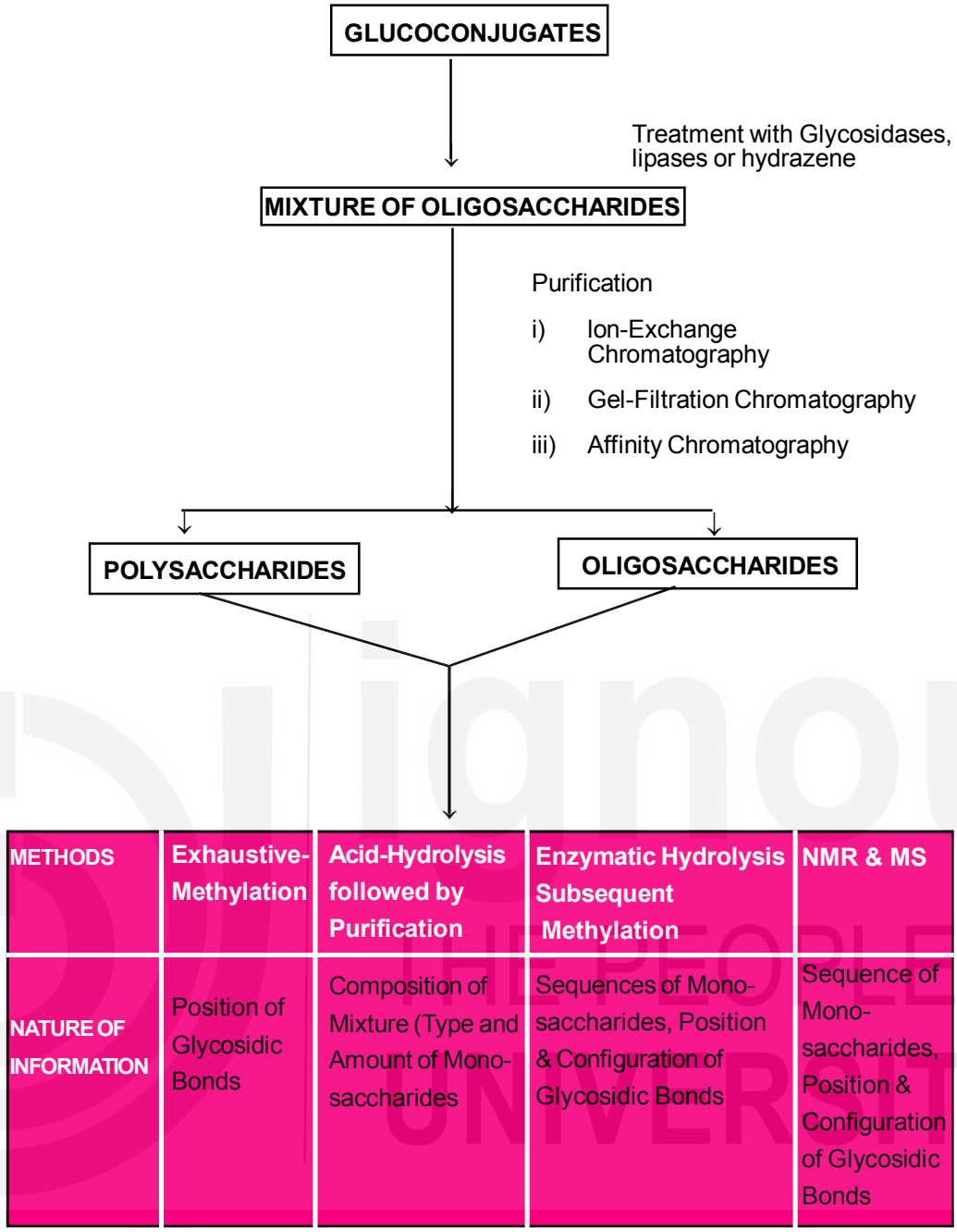
2) **Purification of oligosaccharides and polysaccharides**: Glycan can be purified using chromatographic techniques such as ion exchange, size exclusion and affinity chromatography. These techniques use a column of gel or solid matrix which separates oligosaccharides and polysaccharides based on their charge, size or ability to bind specific ligands. In affinity chromatography, carbohydrates are purified using columns of lectins.

3) **Determination of position of glycosidic bonds:** In a linear polysaccharide chain, position of glycosidic bonds can be determined using exhaustive methylation method. Methyl iodide in basic medium converts all free hydroxyl groups to methyl ether (OCH3) groups. These methyl ether groups, except for those involved in formation of glycosidic bond, are resistant to hydrolysis in the presence of acid. Thus, acid hydrolysis results in hydroxyl groups in methylated derivatives only at carbons involved in glycosidic bond formation.

4) **Determination of sequence of monosaccharides and branching points in polysaccharide**: Exoglycosidases are the enzymes which remove monosaccharide units one at a time starting from the non-reducing end of polysaccharide chain. Enzymes of different specificity are used to deduce position and type of glycosidic bonds, for example, α glycosidase acts only on α glycosidic bonds.

5) **Identification of monosaccharides and oligosaccharides**: Hydrolysis of oligosaccharides under strong acidic conditions yields monosaccharides which can be identified by various chromatographic techniques such as high performance liquid chromatography and gas liquid chromatography. Oligosaccharides as such may also be analyzed using mass spectroscopy and NMR spectroscopy. All these technique are highly sensitive.

6) **Chemical synthesis of oligosaccharides**: Scientists are trying to make oligosaccharides in the laboratory similar to those found in nature as it is difficult to purify them from natural sources. These synthetic oligosaccharides are then used to study their interaction for different lectins.



METHODS	Exhaustive-Methylation	Acid-Hydrolysis followed by Purification	Enzymatic Hydrolysis Subsequent Methylation	NMR & MS
NATURE OF INFORMATION	Position of Glycosidic Bonds	Composition of Mixture (Type and Amount of Mono-saccharides	Sequences of Mono-saccharides, Position & Configuration of Glycosidic Bonds	Sequence of Mono-saccharides, Position & Configuration of Glycosidic Bonds

Fig. 8.4: gives an overview of different methods to analyze carbohydrates.

8.5 SUMMARY

1) Oligosaccharides present in glycoconjugates carry diverse kind of information. The branch of biology which deals with the study of structure and functions of glycoconjugates is called **glycobiology.**

2) The information encoded in an oligosaccharide sequence of a glycoconjugate is referred to as **sugar code**.

3) Carbohydrates can make endless number of molecules carrying different kind of information. It is possible because these have structural features such as chirality, optical activity, ability to form stereoisomer, different types of glycosidic bonds, linear and branched polymeric chains of variable lengths and add different substituents.

4) Information coded within structure of carbohydrates is read by proteins named **lectins.** Lectins are generally present on outer surface of the cells in all organisms and have highly specific carbohydrate binding domain.

5) Lectins mediate all the biological functions encoded in the respective oligosaccharides to which they bind. These functions include communication between cells and their extracellular surroundings, protein sorting and degradation, cell-cell recognition and adhesion, migration of cells during development, blood clotting, immune response and wound healing.

5) Some bacteria and viruses secrete lectins which bind to surface oligosaccharides on their animal cell target.

8.6 TERMINAL QUESTIONS

1) What are the different ways in which glycans can mediate or modulate biological functions?

2) What are the possible benefits for pathogens that are able to mimic host glycans?

3) Which factors make it difficult to study glycans as compared to proteins and nucleic acids?

4) What makes carbohydrates suitable for carrying such diverse information?

5) What is the basis of classification of blood groups?

6) What are lectins and how do they help in decoding the sugar code?

8.7 ANSWERS

Self-Assessment Questions

1. a) The branch of biology which deals with the study of structure and functions of glycoconjugates is called glycobiology

   b) The information encoded in an oligosaccharide sequence of a glycoconjugate is referred to as sugar code

   c) Lectins are the proteins which bind carbohydrates with very high specificity and moderate to high affinity.

   d) The glycoprotein after removal of sialic acid is known as asialoglycoprotein

2. 1- a; 2- c; 3- d; 4- b

Terminal Questions

1) Cell communication, protein targeting, degradation of non functional proteins and cell-cell recognition and adhesion, migration of cells during development, blood clotting, immune response, wound healing and other cellular processes.

2) Pathogens which are able to mimic host glycans can penetrate the host and gain entry leading to infections.

3) First; diversity in structure of these molecules. Secondly, techniques for studying carbohydrates are still being developed

4) i) chirality and ability to form different stereoisomers

   ii) They can form different types of glycosidic bonds.

   iii) multiple functional groups, form linear chains of varying lengths and have extensive branching

5) `Answer: Oligosaccharide chains on the surface of red blood cells (RBCs). Refer to section 8.3

6) Lectins are the proteins which specifically bind carbohydrates with moderate to high affinity. Different lectins bind different types of oligosaccharides and help in reading the information encoded by these sequences. Refer to section 8.2.

8.8 FURTHER READINGS

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