

Workbook



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Carbohydrates

Monosaccharides and Disaccharides

Questions

- 1) Which statement is true?
 - A. Carbohydrates are the most abundant biomolecules on Earth.
 - B. Photosynthesis converts less than 1 billion metric tons of CO₂ and H₂O into cellulose [a polysaccharide] and other plant.
 - C. Sugar and starch are a dietary staple in few parts of the world.
 - D. The oxidation of carbohydrates is the central energy-yielding pathway in most photosynthetic cells.
 - E. Soluble carbohydrate polymers serve as structural and protective elements. Other carbohydrate polymers lubricate skeletal joints and participate in recognition and adhesion between cells.
- 2) What are **glycoconjugates**?
- 3) Monosaccharides, the simplest carbohydrates, are classified into 2 main types, name and describe these.
- 4) There are 3 major size classes of carbohydrates, list and describe these.
- 5) Which statement is true?
 - A. The simplest of the carbohydrates are monosaccharides.
 - B. Monosaccharides are either aldehydes or ketones with two or more hydroxyl groups.
 - C. The six-carbon monosaccharides glucose and fructose have five hydroxyl groups.
 - D. Many of the carbon atoms to which hydroxyl groups are attached are chiral centers.
 - E. All of the above.

6) Part I:

Complete these statements:

- i. If the carbonyl group is at an end of the carbon chain the monosaccharide is __?
- ii. If the carbonyl group is at any other position the monosaccharide is __?

Part II:

Give an example of each type of the monosaccharides mentioned in Part I.

7) Part I:

What are the **simplest** monosaccharides in nature?

Part II:

What are the **most common** monosaccharides in nature?

8) What is a Fischer projection formulas?

9) What are the 2 groups that stereoisomers of monosaccharides can be divided into, and how do they differ?

10) If an aldohexose has four chiral centers, how many stereoisomers does it have?

11) Which statement is false?

- A. Of the 16 possible aldohexoses, 8 are D forms and 8 are L
- B. Most of the hexoses of living organisms are L isomers.
- C. The structures of the D stereoisomers have 3-6 carbon atoms.
- D. The carbons of a sugar are numbered beginning at the end of the chain nearest the carbonyl group.
- E. Each of the eight D-aldohexoses, has its own name.

12) What are **epimers**?

13) Define hemiacetals and hemiketals.

14) Define and differentiate between pyranoses and furanoses.

15) Define the terms anomer, anomeric carbon, and mutarotation.

16) Part I:

There are a number of sugar derivatives in which a hydroxyl group in the parent compound is replaced with another substituent, or a carbon atom is oxidized to a carboxyl group, list these.

Part II:

Elaborate on each one of the sugar derivatives mentioned in part I.

17) List at least 2 examples of hexose derivatives found in nature.

Answer Key

- 1) **A.** is the true statement.
- 2) **Glycoconjugates** are carbohydrate polymers that are covalently attached to proteins or lipids.
- 3) Carbohydrates are either aldehydes or ketones.
 - Polyhydroxy aldehydes or ketones are molecules with more than one hydroxyl group ($-\text{OH}$), and a carbonyl group ($\text{C}=\text{O}$) either at the terminal carbon atom - **aldose** or at the second carbon atom - **ketose**.
 - **Polyhydroxy aldehydes** are organic compounds containing many hydroxyl groups ($-\text{OH}$) and an aldehyde group ($-\text{C}(=\text{O})\text{H}$).
 - **Polyhydroxy ketones** are organic compounds containing many hydroxyl groups and a ketone group ($-\text{C}(=\text{O})-$).

4) The 3 major size classes of carbohydrates are:

1. **Monosaccharides**, simple sugars, consist of a single polyhydroxy aldehyde or ketone unit.
 - a. The most abundant monosaccharide in nature is the six-carbon sugar D-glucose.
 - b. Monosaccharides of more than four carbons tend to have cyclic structures.
2. **Oligosaccharides** consist of short chains of monosaccharide units, or residues, joined by glycosidic bonds.
 - The most abundant are the disaccharides, with two monosaccharide units.
 - Sucrose (cane sugar), which consists of the six-carbon sugars D-glucose dextrose and D-fructose.
 - In cells, oligosaccharides consisting of three or more units [do not occur as free entities but] are joined to nonsugar molecules (lipids or proteins) in glycoconjugates.
3. The **polysaccharides** are sugar polymers containing more than 20 or so monosaccharide units, and some have hundreds or thousands of units.
 - Some polysaccharides, such as cellulose, are linear chains;
 - others, are branched.

5) E

6) **Part I:**

- i. If the carbonyl group is at an end of the carbon chain (that is, in an aldehyde group) the monosaccharide is an **aldose**.
- ii. If the carbonyl group is at any other position (in a ketone group) the monosaccharide is a **ketose**.

Part II:

Glucose is an aldose

Fructose is a ketose

7) **Part I:**

The simplest monosaccharides are the two 3-carbon trioses:

1. glyceraldehyde, an aldotriose,
2. dihydroxyacetone, a ketotriose.

Part II:

The hexoses, which include the aldohexose D-glucose and the ketohexose D-fructose, are the most common monosaccharides in nature.

8) Fischer projection formulas are used to represent 3-dimensional sugar structures .

9) The stereoisomers of monosaccharides can be divided into 2 groups that differ in the configuration about the chiral center most distant from the carbonyl carbon. The 2 groups are:

1. D isomers –those in which the configuration is the same as that of D-glyceraldehyde (*dextro*).
2. L isomers those with the same configuration as L-glyceraldehyde (*levo*).

10) A molecule with n chiral centers can have 2^n stereoisomers, so:

An aldohexoses, with four chiral centers, has:

$$2^4 = 16 \text{ stereoisomers}$$

11) The correct statement is:

B. Most of the hexoses of living organisms are **D isomers**.

12) Epimers are sugars that differ only in the configuration around one carbon atom.

13) In aqueous solution, all monosaccharides with five or more carbon atoms in the backbone occur.

- The formation of cyclic structures is the result of a reaction between alcohols and aldehydes or ketones to form **hemiacetals** or **hemiketals**.
 - Alcohols react with the carbonyl groups of aldehydes to form **hemiacetals**.
 - Alcohols react with the carbonyl groups of ketones to form **hemiketals**.

14) If the --OH and carbonyl groups are on the same molecule, a 5- or 6-membered ring results.

- These 6-membered ring compounds are called **pyranoses**.
- Aldohexoses exist in cyclic forms having 5-membered rings, called **furanoses**.
- The 6-membered aldopyranose ring is much more stable than the aldofuranose ring
- Only aldoses having 5 or more carbon atoms can form pyranose rings.

15)

- Isomeric forms of monosaccharides that differ only in their configuration about the hemiacetal or hemiketal carbon atom are called **anomers**.
- The hemiacetal (or carbonyl) carbon atom is called the **anomeric carbon**.
- The process by which α and β anomers of D-glucose interconvert in aqueous solution is called **mutarotation**.

16) Part I:

1. **Aldonic acid** such as gluconic acid.
2. **Uronic acids**.
3. **Alditols**.
4. **Deoxy sugars**.
5. **Amino sugar**.

Part II:

1. Oxidation of an aldose converts its aldehyde group to a carboxylic acid group, yielding and **aldonic acid** such as gluconic acid.
2. Oxidation of the primary alcohol group of aldoses yields **uronic acids**.
 - Named by appending *-uronic acid* to the root name.
 - Uronic acids can assume the pyranose, furanose, and linear forms
3. Aldoses and ketoses can be reduced to yield polyhydroxyl alcohols know as **alditols**.
 - Named by appending the suffix *-itol* to the root name.
4. Monosaccharide units in which an OH group is replaced by H are known as **deoxy sugars**.
 - The most important biologically is **β -D-2-deoxyribose** - component of DNA's sugar phosphate backbone.
5. **Amino sugar** is formed when one or more OH groups are replaced by an amino group.
 - **D-glucosamine** and **D-galactosamine** are the most common amino sugars.

17)

1. Oxidation of the carbonyl (aldehyde) carbon of glucose to the carboxyl level produces gluconic acid; other aldoses yield other aldonic acids.
2. Oxidation of the carbon at the other end of the carbon chain—C-6 of glucose, galactose, or mannose—forms the corresponding uronic acid: glucuronic, galacturonic, or mannuronic acid.
3. The most important deoxy sugar biologically is **β -D-2-deoxyribose** - the sugar component of DNA's sugar phosphate backbone.
4. L-fucose or L-rhamnose are deoxy sugars found in plant polysaccharides and in the complex oligosaccharide components of glycoproteins and glycolipids.
5. **D-glucosamine** and **D-galactosamine** are the most common amino sugars - a glucosamine derivative is part of many structural polymers, including those of the bacterial cell wall.

Polysaccharides

Questions

- 1) What are **glycans**?
- 2) List at least 3 things that differentiate between various types of glycans.
- 3) Name and define what glycans are classified into?
- 4) What are the 3 types of polysaccharides mentioned in the lesson and where are they found (in what organisms)?
- 5) Fill in the blanks:
 1. The most important storage polysaccharides are _____ in plant cells and _____ in animal cells.
 2. Both polysaccharides occur _____ (location) as large clusters or _____.
 3. Starch and glycogen molecules are heavily _____.
- 6) Define the term **Starch**.
- 7) Which statement is false?
 - A. The primary structure of glycogen is similar to amylopectin, yet is more highly branched.
 - B. The storage of glucose as starch greatly reduces the large intracellular osmotic pressure that would result from its storage in monomeric form.
 - C. Glycogen's highly branched structure, which has many reducing ends, permits the rapid utilization of glucose in time of metabolic need.
 - D. Glycogen granules contain in tightly bound form, the enzymes responsible for the synthesis and degradation of glycogen.
 - E. Dental plaque is rich in dextrans, which are adhesive and allow the bacteria to stick to teeth and to each other.
 - F. Glycogen is present in all cells, abundant in the liver, and in skeletal muscle.

- 8) Mention and define the 2 types of polymers in starch.
- 9) Name the types of structural polysaccharides mentioned in the lesson and in what organisms are they found?
- 10) Which statement is false about Cellulose?
- A. It is a tough, fibrous, water-insoluble substance.
 - B. It is a core component in the cell walls of all plants.
 - C. It can withstand great osmotic pressure differences between the extracellular and intracellular spaces.
 - D. It accounts for over half of the carbon in the biosphere.
 - E. It is a linear, branched homopolysaccharide.
- 11) Define the terms **Hemicellulose**, **Pectin**, and **cellulase**.
- 12) Define **Chitin** and mention a number of its characteristics.
- 13) Which statement refers to Cellulose and which to Chitin?
- 1. It provides the organism with an excellent biomaterial for building a strong body frame by virtue of hydrogen-bonding contacts within and between polysaccharide strands.
 - 2. It is a linear, unbranched homopolysaccharide, consisting of nearly a thousand repeating units of a disaccharide called cellbiose.
 - 3. Herbivores and termites can utilize this as a form of fuel since they harbor symbiotic organisms in their digestive tract that secrete enzymes which can break it down.
 - 4. It is a component of the cell walls in most types of fungi and many algae.
 - 5. It is the most abundant carbohydrate on earth.
 - 6. It is the second most abundant polysaccharide on earth.

14) Which statement is false:

- A. The rigid component of bacterial cell walls is a heteropolymer.
- B. The linear polymers are crosslinked by short peptides which weld the polysaccharide chains into a strong cover.
- C. Penicillin kill bacteria by preventing synthesis of the cross-links of their cell walls.
- D. Certain marine red algae have cell walls that contain agar, a heteropolysaccharide.
- E. Agarose is the agar component with the most charged groups.

15)Part I:

What is the enzyme mentioned that kills bacteria and how does it do so?

Part II:

Give an example of where the enzyme is present.

16)What is the extracellular matrix and what is it composed of?

17)What is the general name for the heteropolysaccharides that make up the extracellular matrix? Mention its characteristics.

18)Define hyaluronan?

19)Name and characterize the other 4 glycosaminoglycans that differ from hyaluronate.

Answer Key

1) Most carbohydrates found in nature occur as polysaccharides, which are polymers of medium to high molecular weight, and are termed – **glycans**

2) Glycans differ from each other in:

1. The identity of their recurring monosaccharide units.
2. The length of their chains.
3. The types of bonds linking the units.
4. [and in] The degree of branching.

3) Glycans are classified into either **homopolysaccharides** or **heteropolysaccharides**.

homopolysaccharides consist of 1 type of monosaccharides.

heteropolysaccharides consist of different types of monosaccharides.

4)

1. Glycogen - the main storage polysaccharide of animal cells. It is present in all cells, yet is especially abundant in the liver where it may constitute as much as 7% of the wet weight and in skeletal muscle.
2. Starch - is the principal energy reserve in most plant cells, and is especially abundant in tubers (underground stems), such as potatoes, and in seeds.
3. Dextran - are bacterial and yeast polysaccharides – they provide a source of glucose for bacterial metabolism.

5)

1. The most important storage polysaccharides are **starch** in plant cells and **glycogen** in animal cells.
2. Both polysaccharides occur **intracellularly** as large clusters or **granules**.
3. Starch and glycogen molecules are heavily **hydrated**.

6) Starch - is an important storage polysaccharide in plant cells, composed of a mixture of glycans that plants synthesize as their principal energy reserve which is especially abundant in tubers (underground stems).

- It contains two types of glucose polymers: amylose and amylopectin

7) C - is the false statement.

To make it correct: Glycogen's highly branched structure, which has many **nonreducing** ends, permits the rapid utilization of glucose in time of metabolic need.

8) Starch contains two types of glucose polymers: amylose and amylopectin:

- **Amylose** is a linear polymer that consists of long, unbranched chains of D-glucose residues connected by α -1,4 linkages.
 - It is made of several thousand glucose residues.
 - Such chains vary in molecular weight from a few thousand to more than a million.
- **Amylopectin** is a highly branched molecule with α -1,4 glycosidic linkages joining successive glucose residues in its chains, with branch points occurring every 24 to 30 residues that are α -1,6 linkages.
 - It has a high molecular weight of up to 200 million, making these some of the largest molecules in nature.

9) Chitin and cellulose.

Cellulose, a fibrous, tough, substance, is found as the core component in the cell walls of all plants.

Chitin forms extended fibers similar to those of cellulose, and is the main component of invertebrate exoskeletons of arthropods— insects and crustaceans.

- It is also a component of the cell walls in most fungi and algae.

10)E - the correct statement would be:

Cellulose is a linear, **unbranched** homopolysaccharide.

11) Cellulose consists of hydrogen-bonded cellulose fibrils which are held together by hemicellulose and **pectin** polymers:

- **Hemicellulose** is a branched heteropolymer containing up to 6 different sugars.
- **Pectin** is a homopolymer of galacturonic acid.
- **Cellulase** is an enzyme that is able to hydrolyze the (β 1→ 4) linkages.
 - Most animals cannot use cellulose as a fuel source, because they lack this enzyme.

12) Chitin is a linear homopolysaccharide composed of N- acetylglucosamine residues linked together.

- Chitin is the main structural component of invertebrate exoskeletons of nearly a million species of arthropods—insects and crustaceans.
- It is also a component of the cell walls in most types of fungi and many types of algae,
- It is the 2nd most abundant polysaccharide in nature.

13) 1- Chitin

2 - Cellulose

3 - Cellulose

4 - Chitin

5 - Cellulose

6 - Chitin

14) E - Agarose is the agar component with the fewest charged groups.

15)Part I:

The enzyme **lysozyme** kills bacteria, and it does this by hydrolyzing the β -1,4 glycosidic bond between *N*-acetylglucosamine and *N*-acetylmuramic acid.

Part II:

- Lysozyme is present in human tears, presumably as a defense against bacterial infections of the eye.
- Lysozyme is produced by certain bacterial viruses to allow them entry and infection.

16)The extracellular matrix is a gel-like material that fills the extracellular space in the tissues of multicellular animals.

- It holds the cells together and allows the diffusion of nutrients and oxygen to individual cells.
- The extracellular matrix is composed of interlinked heteropolysaccharides and fibrous proteins such as collagen, elastin, and fibronectin, and laminin.

17)The heteropolysaccharides that make up the extracellular matrix are called – **glycosaminoglycans**

- They are a family of linear polymers composed of repeating disaccharide units
- One of the two monosaccharides is always either *N*-acetylglucosamine or *N*-acetylgalactosamine; the other is in most cases a uronic acid.
- The combination of sulfate groups and the carboxylate groups of the uronic acid residues gives glycosaminoglycans a very high density of negative charge.

18) Hyaluronan is the glycosaminoglycan hyaluronic acid

hyaluronic acid comes from The Greek word: *hyalos* - "glass"

- It contains alternating residues of D-glucuronic acid and N-acetylglucosamine.
- Hyaluronates have molecular weights greater than 1 million.
- It forms a clear, highly viscous solution that serve as lubricant in joints.
- Hyaluronate is also an essential component of the ECM of cartilage and tendons.

19)

1. **Chondroitin sulfate** (Greek *chondros*, "cartilage") contributes to the malleable strength of cartilage, tendons, ligaments, and the walls of the aorta.
2. **Dermatan sulfate** (Greek *derma*, "skin") contributes to the pliability of skin and is also present in blood vessels and heart valves.
3. **Keratan sulfate** (Greek *keras*, "horn") have no uronic acid and their sulfate content is variable. They are present in cornea, cartilage, bone, and a variety of horny structures formed of dead cells: horn, hair, hoofs, nails, and claws.
4. **Heparan Sulfate** (Greek *hepar*, "liver") is produced by all animal cells and contains variable arrangements of sulfated and nonsulfated sugars.

Glycoconjugates

Questions

- 1) What are Glycoconjugates and what are the main 3 types mentioned?
- 2) What roles to glycoconjugates play in organisms?
- 3) Fill in the blank with the correct glycoconjugate.
 - I. _____ are major components of connective tissue such as cartilage, in which their many noncovalent interactions provide strength and flexibility.
 - II. _____ have one or several oligosaccharides of varying complexity joined covalently to a protein - the oligosaccharide portions of these are not very repetitive and are rich in information, forming highly specific sites for recognition and high-affinity binding by other proteins.
 - III. _____ are membrane lipids in which the hydrophilic head groups are oligosaccharides, which act as specific sites for recognition by carbohydrate-binding proteins.
- 4) Fill in the blanks for the following sentences.
 - I. Matrix Mammalian cells can produce at least _____ types of molecules that are members of the _____ superfamily.
 - II. These molecules act as tissue _____, influence the development of specialized tissues, mediate the activities of various growth factors, and regulate the extracellular assembly of collagen fibrils.
 - III. The basic proteoglycan unit consists of a _____ protein with covalently attached _____.
- 5) Define a proteoglycan.
- 6) How do Proteoglycans attract water to the ECM for compression resistance?

7) Part I:

What are S domains?

Part II:

How do S domains induce their effect, their function – what is the mechanism?

8) What are proteoglycan aggregates?

9) What are Glycoproteins, and how are they different from proteoglycans?

10) Define Glycophorin A and what was it given as an example for?

11) Which statement is false?

- A. The same protein produced in two types of tissues can result in different the oligosaccharide chains represent a tissue-specific marker.
- B. Glycoproteins have multiple oligosaccharide chains.
- C. Oligosaccharide chains that are attached to newly synthesized proteins may influence the sequence of polypeptide-folding events.
- D. The bulkiness and negative charge of oligosaccharide chains protect some proteins from attack by proteolytic enzymes.

12) Part I:

Which statement is false?

- A. Glycolipids and Lipopolysaccharides are cell Membrane Components
- B. Like the oligosaccharide moieties of glycoproteins, those of membrane lipids are found on the outer face of the plasma membrane.
- C. Lipopolysaccharides are the dominant surface feature of the outer membrane of gram-negative bacteria.
- D. These molecules are prime targets of antibodies and are therefore important determinants of the serotype of bacterial.
- E. Lipopolysaccharides of bacteria are toxic to humans and other animals.

Part II:

Correct the false statement.

13) What are gangliosides?

Answer Key

- 1) Glycoconjugates are molecules composed of carbohydrates covalently joined to another type of molecule - a protein or a lipid.

There are Proteoglycans, Glycoproteins, and Glycolipids – the first 2 of glycoconjugates made of proteins bound to carbohydrates, while glycolipids are glycoconjugates of carbohydrates and lipids.

2)

- Glyconjugates play important roles as stored fuels (starch, glycogen, dextran).
- As structural materials (cellulose, chitin, peptidoglycans).
- As information carriers: they serve as destination labels for some proteins and as mediators of specific cell-cell interactions and interactions between cells and the extracellular matrix.

3)

- I. **Proteoglycans** are major components of connective tissue such as cartilage, in which their many noncovalent interactions provide strength and flexibility.
- II. **Glycoproteins** have one or several oligosaccharides of varying complexity joined covalently to a protein - the oligosaccharide portions of these are not very repetitive and are rich in information, forming highly specific sites for recognition and high-affinity binding by other proteins.
- III. **Glycolipids** are membrane lipids in which the hydrophilic head groups are oligosaccharides, which act as specific sites for recognition by carbohydrate-binding proteins.

4)

- I. Matrix Mammalian cells can produce at least **30** types of molecules that are members of the **proteoglycan** superfamily.
- II. These molecules act as tissue **organizers**, influence the development of specialized tissues, mediate the activities of various growth factors, and regulate the extracellular assembly of collagen fibrils.
- III. The basic proteoglycan unit consists of a “**core** protein” with covalently attached **glycosaminoglycan(s)**.

5) Proteoglycans are proteins that are heavily glycosylated. The basic proteoglycan unit consists of a "core protein" with one or more covalently attached glycosaminoglycan (GAG) chain(s).

6) The basic proteoglycan unit consists of a “core protein” with covalently attached glycosaminoglycan(s).

Glycosaminoglycans (GAGs) are highly negatively charged because of sulfate or carboxyl groups on most of their sugars. Their highly negative charge attracts osmotically active cations like sodium ions, which causes large amounts of water to be incorporated into the matrix. This results in porous hydrated gels and is responsible for the turgor that enables the matrix to withstand compressive force.

7) **Part I:**

The S domains are regions in the proteoglycans that are rich in sulfated sugars, and these bind specifically to extracellular proteins and signaling molecules to alter their activities.

Part II:

The S domains are suggested to alter activities of molecules they bind using inducing these mechanisms:

- I. The change in activity may result from a conformational change in the protein that is induced by the binding.
- II. or it may be due to the ability of adjacent domains of heparan sulfate to bind to two different proteins, bringing them into close proximity and enhancing protein-protein interactions.
- III. A third general mechanism of action is the binding of extracellular signal molecules (growth factors, for example) to heparan sulfate, which increases their local concentrations and enhances their interaction with growth factor receptors in the cell surface; in this case, the heparan sulfate acts as a coreceptor.
- IV. The S domains interact electrostatically and otherwise with a variety of soluble molecules outside the cell, maintaining high local concentrations at the cell surface.

8) Some proteoglycans can form **proteoglycan aggregates** - enormous supramolecular assemblies of many core proteins all bound to a single molecule of hyaluronate.

- The proteoglycan aggregate is the major structural component of the extracellular matrix of the cartilage, composed of aggrecan, hyaluronan (HA) and link protein (LP).
- The aggregates provide cartilage with unique gel-like property and resistance to distortion through water absorption.

- 9) Glycoproteins are carbohydrate-protein conjugates in which the carbohydrate moieties are smaller and more structurally diverse than the glycosaminoglycans of proteoglycans.
- The carbohydrate is attached at its anomeric carbon through a glycosidic link to the -OH of a Ser or Thr residue (*O*-linked), or through an *N*-glycosyl link to the amide nitrogen of an Asn residue (*N*-linked).
- 10) Glycophorin A is one of the best-characterized membrane glycoproteins of the erythrocyte membrane. It was given as an example of an external surface of the plasma membrane glycoproteins.
- It contains 60% carbohydrate by mass, in the form of 16 oligosaccharide chains (totaling 60 to 70 monosaccharide residues) covalently attached to amino acid residues near the amino terminus of the polypeptide chain.
 - Fifteen of the oligosaccharide chains are *O*-linked to Ser or Thr residues, and one is *N*-linked to an Asn residue.

11)B – this statement is inaccurate.

The correct statement is: **Some** glycoproteins have **a single oligosaccharide chain**, but **many** have **more** than one.

12)Part I:

E – false statement

Part II:

Correct statement: The lipopolysaccharides of **some** bacteria are toxic to humans and other animals; for example, they are responsible for the dangerously lowered blood pressure that occurs in toxic shock syndrome resulting from gram negative bacterial infections.

13) Gangliosides are membrane lipids of eukaryotic cells in which the polar head group, is a complex oligosaccharide containing sialic acid and other monosaccharide residues.

- Some of the oligosaccharide moieties of gangliosides, are identical with those found in certain glycoproteins.
- The oligosaccharide moieties of membrane lipids are found on the outer face of the plasma membrane.

