

chapter

7

Carbohydrates and Glycobiology

- 1. Sugar Alcohols** In the monosaccharide derivatives known as sugar alcohols, the carbonyl oxygen is reduced to a hydroxyl group. For example, D-glyceraldehyde can be reduced to glycerol. However, this sugar alcohol is no longer designated D or L. Why?

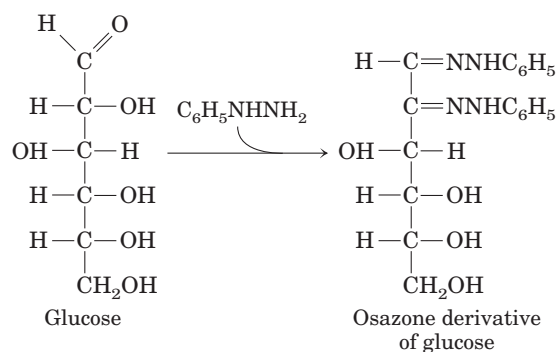
Answer With reduction of the carbonyl oxygen to a hydroxyl group, the stereochemistry at C-1 and C-3 is the same; the glycerol molecule is not chiral.

- 2. Recognizing Epimers** Using Figure 7-3, identify the epimers of **(a)** D-allose, **(b)** D-gulose, and **(c)** D-ribose at C-2, C-3, and C-4.

Answer Epimers differ by the configuration about only *one* carbon.

- (a)** D-altrose (C-2), D-glucose (C-3), D-gulose (C-4)
(b) D-idose (C-2), D-galactose (C-3), D-allose (C-4)
(c) D-arabinose (C-2), D-xylose (C-3)

- 3. Melting Points of Monosaccharide Osazone Derivatives** Many carbohydrates react with phenylhydrazine ($C_6H_5NHNH_2$) to form bright yellow crystalline derivatives known as osazones:



The melting temperatures of these derivatives are easily determined and are characteristic for each osazone. This information was used to help identify monosaccharides before the development of HPLC or gas-liquid chromatography. Listed below are the melting points (MPs) of some aldose-osazone derivatives:

Monosaccharide	MP of anhydrous monosaccharide (°C)	MP of osazone derivative (°C)
Glucose	146	205
Mannose	132	205
Galactose	165-168	201
Talose	128-130	201

As the table shows, certain pairs of derivatives have the same melting points, although the underivatized monosaccharides do not. Why do glucose and mannose, and similarly galactose and talose, form osazone derivatives with the same melting points?

Answer The configuration at C-2 of an aldose is lost in its osazone derivative, so aldoses differing only at the C-2 configuration (C-2 epimers) give the same derivative, with the same melting point. Glucose and mannose are C-2 epimers and thus form the same osazone; the same is true for galactose and talose (see Fig. 7-3).

- 4. Interconversion of D-Glucose Forms** A solution of one enantiomer of a given monosaccharide rotates plane-polarized light to the left (counterclockwise) and is called the levorotatory isomer, designated (−); the other enantiomer rotates plane-polarized light to the same extent but to the right (clockwise) and is called the dextrorotatory isomer, designated (+). An equimolar mixture of the (+) and (−) forms does not rotate plane-polarized light.

The optical activity of a stereoisomer is expressed quantitatively by its *optical rotation*, the number of degrees by which plane-polarized light is rotated on passage through a given path length of a solution of the compound at a given concentration. The *specific rotation* $[\alpha]_{\lambda}^t$ of an optically active compound is defined thus:

$$[\alpha]_{\lambda}^t = \frac{\text{observed optical rotation } (^{\circ})}{\text{optical path length (dm)} \times \text{concentration (g/mL)}}$$

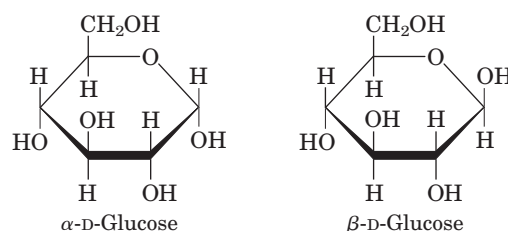
The temperature (t) and the wavelength of the light (λ) employed (usually, as here, the D line of sodium, 589 nm) must be specified.

A freshly prepared solution of α -D-glucose shows a specific rotation of $+112^{\circ}$. Over time, the rotation of the solution gradually decreases and reaches an equilibrium value corresponding to $[\alpha]_{\text{D}}^{25^{\circ}\text{C}} = +52.5^{\circ}$. In contrast, a freshly prepared solution of β -D-glucose has a specific rotation of $+19^{\circ}$. The rotation of this solution increases over time to the same equilibrium value as that shown by the α anomer.

- Draw the Haworth perspective formulas of the α and β forms of D-glucose. What feature distinguishes the two forms?
- Why does the specific rotation of a freshly prepared solution of the α form gradually decrease with time? Why do solutions of the α and β forms reach the same specific rotation at equilibrium?
- Calculate the percentage of each of the two forms of D-glucose present at equilibrium.

Answer

(a)



The α and β forms of D-glucose differ only at the hemiacetal carbon (C-1; the anomeric carbon).

- A fresh solution of the α form of glucose undergoes mutarotation to an equilibrium mixture containing both the α and β forms. The same applies to a fresh solution of the β form.
- The change in specific rotation of a solution in changing from 100% α form ($[\alpha]_{\text{D}}^{25^{\circ}\text{C}} 112^{\circ}$) to 100% β form ($[\alpha]_{\text{D}}^{25^{\circ}\text{C}} 19^{\circ}$) is 93° . For an equilibrium mixture having $[\alpha]_{\text{D}}^{25^{\circ}\text{C}} 52.5^{\circ}$, the fraction of D-glucose in the α form is

$$\frac{52.5^{\circ} - 19^{\circ}}{112^{\circ} - 19^{\circ}} = \frac{33.5^{\circ}}{93^{\circ}} = 0.36 = 36\%$$

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Thus, ignoring the small portions of furanose forms (~0.5% each), the mixture contains about 36% α -D-glucose and 64% β -D-glucose.

- 5. Configuration and Conformation** Which bond(s) in α -D-glucose must be broken to change its configuration to β -D-glucose? Which bond(s) to convert D-glucose to D-mannose? Which bond(s) to convert one “chair” form of D-glucose to the other?

Answer To convert α -D-glucose to β -D-glucose, the bond between C-1 and the hydroxyl on C-5 must be broken and reformed in the opposite configuration (as in Fig. 7-6). To convert D-glucose to D-mannose, either the —H or the —OH on C-2 must be broken and reformed in the opposite configuration. Conversion between chair conformations does not require bond breakage; this is the critical distinction between configuration and conformation.

- 6. Deoxysugars** Is D-2-deoxygalactose the same chemical as D-2-deoxyglucose? Explain.

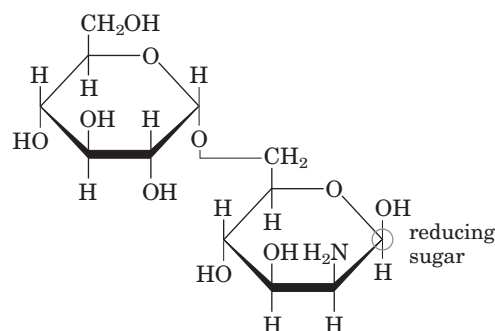
Answer No; glucose and galactose differ in their configuration at C-4.

- 7. Sugar Structures** Describe the common structural features and the differences for each pair: (a) cellulose and glycogen; (b) D-glucose and D-fructose; (c) maltose and sucrose.

Answer (a) Both are polymers of D-glucose, but they differ in the glycosidic linkage: (β 1 \rightarrow 4) for cellulose, (α 1 \rightarrow 4) for glycogen. **(b)** Both are hexoses, but glucose is an aldohexose, fructose a ketohexose. **(c)** Both are disaccharides, but maltose has two (α 1 \rightarrow 4)-linked D-glucose units; sucrose has (α 1 \leftrightarrow 2 β)-linked D-glucose and D-fructose.

- 8. Reducing Sugars** Draw the structural formula for α -D-glucosyl-(1 \rightarrow 6)-D-mannosamine and circle the part of this structure that makes the compound a reducing sugar.

Answer



- 9. Hemiacetal and Glycosidic Linkages** Explain the difference between a hemiacetal and a glycoside.

Answer A hemiacetal is formed when an aldose or ketose condenses with an alcohol; a glycoside is formed when a hemiacetal condenses with an alcohol (see Fig. 7-5, p. 238).

- 10. A Taste of Honey** The fructose in honey is mainly in the β -D-pyranose form. This is one of the sweetest carbohydrates known, about twice as sweet as glucose; the β -D-furanose form of fructose is much less sweet. The sweetness of honey gradually decreases at a high temperature. Also, high-fructose corn syrup (a commercial product in which much of the glucose in corn syrup is converted to fructose) is used for sweetening *cold* but not *hot* drinks. What chemical property of fructose could account for both these observations?

Answer Straight-chain fructose can cyclize to yield either the pyranose or the furanose structure. Increasing the temperature shifts the equilibrium in the direction of the furanose form, reducing the sweetness of the solution. The higher the temperature, the less sweet is the fructose solution.

- 11. Reducing Disaccharide** A disaccharide, which you know to be either maltose or sucrose, is treated with Fehling's solution, and a red color is formed. Which sugar is it, and how do you know?

Answer Maltose; sucrose has no reducing (oxidizable) group, as the anomeric carbons of both monosaccharides are involved in the glycosidic bond.

- 12. Glucose Oxidase in Determination of Blood Glucose** The enzyme glucose oxidase isolated from the mold *Penicillium notatum* catalyzes the oxidation of β -D-glucose to D-glucono- δ -lactone. This enzyme is highly specific for the β anomer of glucose and does not affect the α anomer. In spite of this specificity, the reaction catalyzed by glucose oxidase is commonly used in a clinical assay for total blood glucose—that is, for solutions consisting of a mixture of β - and α -D-glucose. What are the circumstances required to make this possible? Aside from allowing the detection of smaller quantities of glucose, what advantage does glucose oxidase offer over Fehling's reagent for the determination of blood glucose?

Answer The rate of mutarotation (interconversion of the α and β anomers) is sufficiently high that, as the enzyme consumes β -D-glucose, more α -D-glucose is converted to the β form, and, eventually, all the glucose is oxidized. Glucose oxidase is specific for glucose and does not detect other reducing sugars (such as galactose). Fehling's reagent reacts with any reducing sugar.

- 13. Invertase “Inverts” Sucrose** The hydrolysis of sucrose (specific rotation $+66.5^\circ$) yields an equimolar mixture of D-glucose (specific rotation $+52.5^\circ$) and D-fructose (specific rotation -92°). (See Problem 4 for details of specific rotation.)
- Suggest a convenient way to determine the rate of hydrolysis of sucrose by an enzyme preparation extracted from the lining of the small intestine.
 - Explain why, in the food industry, an equimolar mixture of D-glucose and D-fructose formed by hydrolysis of sucrose is called invert sugar.
 - The enzyme invertase (now commonly called sucrase) is allowed to act on a 10% (0.1 g/mL) solution of sucrose until hydrolysis is complete. What will be the observed optical rotation of the solution in a 10 cm cell? (Ignore a possible small contribution from the enzyme.)

Answer

- An equimolar mixture of D-glucose and D-fructose, such as that formed from sucrose hydrolysis, has optical rotation $= 52.5^\circ + (-92.0^\circ) = -39.5^\circ$. Enzyme (sucrase) activity can be assayed by observing the change in optical rotation of a solution of 100% sucrose (specific rotation $= +66.5^\circ$) as it is converted to a 1:1 mixture of D-glucose and D-fructose.
- The optical rotation of the hydrolysis mixture is negative (inverted) relative to that of the unhydrolyzed sucrose solution.
- The addition of 1 mol of water (M_r 18) in the hydrolysis of 1 mol of sucrose (M_r 342) gives the products an increase in weight of $(18/342)(100\%) = 5.26\%$ with respect to the starting sugar. Accordingly, a 10% sucrose solution yields a $[10 + (0.053 \times 10)]\% = 10.5\%$ solution of invert sugar. Of this 10.5%, 5.25% (0.0525 g/mL) is D-glucose and 5.25% is D-fructose. By rearranging the equation in Problem 4,

$$[\alpha]_D^{25^\circ\text{C}} = \frac{\text{observed optical rotation } (^\circ)}{\text{optical path length (dm)} \times \text{concentration (g/mL)}}$$

we can determine the optical rotation of each sugar in the mixture in a 10 cm cell:

$$\text{Optical rotation of glucose} = (52.5^\circ)(1 \text{ dm})(0.0525 \text{ g/mL}) = 2.76^\circ$$

$$\text{Optical rotation of fructose} = (92^\circ)(1 \text{ dm})(0.0525 \text{ g/mL}) = -4.8^\circ$$

$$\text{The observed optical rotation of the solution is } 2.76^\circ + (-4.8^\circ) = -2.0^\circ$$

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- 14. Manufacture of Liquid-Filled Chocolates** The manufacture of chocolates containing a liquid center is an interesting application of enzyme engineering. The flavored liquid center consists largely of an aqueous solution of sugars rich in fructose to provide sweetness. The technical dilemma is the following: the chocolate coating must be prepared by pouring hot melted chocolate over a solid (or almost solid) core, yet the final product must have a liquid, fructose-rich center. Suggest a way to solve this problem. (Hint: Sucrose is much less soluble than a mixture of glucose and fructose.)

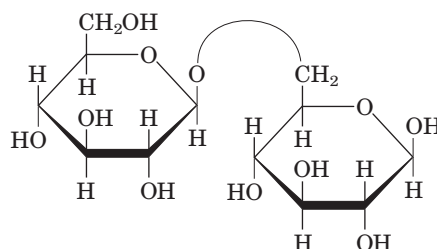
Answer Prepare the core as a semisolid slurry of sucrose and water. Add a small amount of sucrase (invertase), and quickly coat the semisolid mixture with chocolate. After the chocolate coat has cooled and hardened, the sucrase hydrolyzes enough of the sucrose to form a more liquid center: a mixture of fructose, glucose, and sucrose.

- 15. Anomers of Sucrose?** Lactose exists in two anomeric forms, but no anomeric forms of sucrose have been reported. Why?

Answer Lactose ($\text{Gal}(\beta 1 \rightarrow 4)\text{Glc}$) has a free anomeric carbon (on the glucose residue). In sucrose ($\text{Glc}(\alpha 1 \leftrightarrow 2\beta)\text{Fru}$), the anomeric carbons of both monosaccharide units are involved in the glycosidic bond, and the disaccharide has no free anomeric carbon to undergo mutarotation.

- 16. Gentiobiose** Gentiobiose ($\text{D-Glc}(\beta 1 \rightarrow 6)\text{D-Glc}$) is a disaccharide found in some plant glycosides. Draw the structure of gentiobiose based on its abbreviated name. Is it a reducing sugar? Does it undergo mutarotation?

Answer



It is a reducing sugar; it undergoes mutarotation.

- 17. Identifying Reducing Sugars** Is *N*-acetyl- β -D-glucosamine (Fig. 7-9) a reducing sugar? What about D-gluconate? Is the disaccharide $\text{GlcN}(\alpha 1 \leftrightarrow 1\alpha)\text{Glc}$ a reducing sugar?

Answer *N*-Acetyl- β -D-glucosamine is a reducing sugar; its C-1 can be oxidized (see Fig. 7-10, p. 241). D-Gluconate is not a reducing sugar; its C-1 is already at the oxidation state of a carboxylic acid. $\text{GlcN}(\alpha 1 \leftrightarrow 1\alpha)\text{Glc}$ is not a reducing sugar; the anomeric carbons of both monosaccharides are involved in the glycosidic bond.

- 18. Cellulose Digestion** Cellulose could provide a widely available and cheap form of glucose, but humans cannot digest it. Why not? If you were offered a procedure that allowed you to acquire this ability, would you accept? Why or why not?

Answer Humans cannot break down cellulose to its monosaccharides because they lack cellulases, a family of enzymes, produced chiefly by fungi, bacteria, and protozoans, that catalyze the hydrolysis of cellulose to glucose. In ruminant animals (such as cows and sheep), the rumen (one of four stomach compartments) acts as an anaerobic fermenter in which bacteria and protozoa degrade cellulose, making its glucose available as a nutrient to the animal. If cellulase were present in the human digestive tract, we could use foods rich in cellulose as nutrients. This would greatly increase the forms of biomass that could be used for human nutrition. This change might require some changes in the teeth that would allow cellulosic materials to be ground into small pieces to serve as cellulase substrates.

- 19. Physical Properties of Cellulose and Glycogen** The almost pure cellulose obtained from the seed threads of *Gossypium* (cotton) is tough, fibrous, and completely insoluble in water. In contrast, glycogen obtained from muscle or liver disperses readily in hot water to make a turbid solution. Despite their markedly different physical properties, both substances are (1→4)-linked D-glucose polymers of comparable molecular weight. What structural features of these two polysaccharides underlie their different physical properties? Explain the biological advantages of their respective properties.

Answer Native cellulose consists of glucose units linked by (β 1→4) glycosidic bonds. The β linkages force the polymer chain into an extended conformation. Parallel series of these extended chains can form *intermolecular* hydrogen bonds, thus aggregating into long, tough, insoluble fibers. Glycogen consists of glucose units linked by (α 1→4) glycosidic bonds. The α linkages cause bends in the chain, and glycogen forms helical structures with *intramolecular* hydrogen bonding; it cannot form long fibers. In addition, glycogen is highly branched and, because many of its hydroxyl groups are exposed to water, is highly hydrated and therefore very water-soluble. It can be extracted as a dispersion in hot water.

The physical properties of the two polymers are well suited to their biological roles. Cellulose serves as a structural material in plants, consistent with the side-by-side aggregation of long molecules into tough, insoluble fibers. Glycogen is a storage fuel in animals. The highly hydrated glycogen granules, with their abundance of free, nonreducing ends, can be rapidly hydrolyzed by glycogen phosphorylase to release glucose 1-phosphate, available for oxidation and energy production.

- 20. Dimensions of a Polysaccharide** Compare the dimensions of a molecule of cellulose and a molecule of amylose, each of M_r 200,000.

Answer Cellulose is several times longer; it assumes an extended conformation, whereas amylose has a helical structure.

- 21. Growth Rate of Bamboo** The stems of bamboo, a tropical grass, can grow at the phenomenal rate of 0.3 m/day under optimal conditions. Given that the stems are composed almost entirely of cellulose fibers oriented in the direction of growth, calculate the number of sugar residues per second that must be added enzymatically to growing cellulose chains to account for the growth rate. Each D-glucose unit contributes ~0.5 nm to the length of a cellulose molecule.

Answer First, calculate the growth per second:

$$\frac{0.3 \text{ m/day}}{(24 \text{ h/day})(60 \text{ min/h})(60 \text{ s/min})} = 3 \times 10^{-6} \text{ m/s}$$

Given that each glucose residue increases the length of the cellulose chain by 0.5 nm (5×10^{-10} m), the number of residues added per second is

$$\frac{3 \times 10^{-6} \text{ m/s}}{5 \times 10^{-10} \text{ m/residue}} = 6,000 \text{ residues/s}$$

- 22. Glycogen as Energy Storage: How Long Can a Game Bird Fly?** Since ancient times it has been observed that certain game birds, such as grouse, quail, and pheasants, are easily fatigued. The Greek historian Xenophon wrote, “The bustards . . . can be caught if one is quick in starting them up, for they will fly only a short distance, like partridges, and soon tire; and their flesh is delicious.” The flight muscles of game birds rely almost entirely on the use of glucose 1-phosphate for energy, in the form of ATP (Chapter 14). The glucose 1-phosphate is formed by the breakdown of stored muscle glycogen, catalyzed by the enzyme glycogen phosphorylase. The rate of ATP production is limited by the rate at which glycogen can be broken down. During a “panic flight,” the game bird’s rate of glycogen breakdown is quite high, approximately 120 $\mu\text{mol/min}$ of glucose 1-phosphate produced per gram of fresh tissue. Given that the flight muscles usually contain about 0.35% glycogen by weight, calculate how long a game bird can fly. (Assume the average molecular weight of a glucose residue in glycogen is 162 g/mol.)

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Answer Given the average molecular weight of a glucose residue = 162, the amount of usable glucose (as glycogen) in 1 g of tissue is

$$\frac{3.5 \times 10^{-3} \text{ g}}{162 \text{ g/mol}} = 2.2 \times 10^{-5} \text{ mol}$$

In 1 min, 120 μmol of glucose 1-phosphate is produced, so 120 μmol of glucose is hydrolyzed. Thus, depletion of the glycogen would occur in

$$\frac{(2.2 \times 10^{-5} \text{ mol})(60 \text{ s/min})}{120 \times 10^{-6} \text{ mol/min}} = 11 \text{ s}$$

- 23. Relative Stability of Two Conformers** Explain why the two structures shown in Figure 7–19 are so different in energy (stability). Hint: See Figure 1–21.

Answer The ball-and-stick model of the disaccharide in Figure 7–19 shows no steric interactions, but a space-filling model, showing atoms with their real relative sizes, would show several strong steric hindrances in the $-170^\circ, -170^\circ$ conformer that are not present in the $30^\circ, -40^\circ$ conformer.

- 24. Volume of Chondroitin Sulfate in Solution** One critical function of chondroitin sulfate is to act as a lubricant in skeletal joints by creating a gel-like medium that is resilient to friction and shock. This function seems to be related to a distinctive property of chondroitin sulfate: the volume occupied by the molecule is much greater in solution than in the dehydrated solid. Why is the volume so much larger in solution?

Answer In solution, the negative charges on chondroitin sulfate repel each other and force the molecule into an extended conformation. The polar molecule also attracts many water molecules (water of hydration), further increasing the molecular volume. In the dehydrated solid, each negative charge is counterbalanced by a counterion, such as Na^+ , and the molecule collapses into its condensed form.

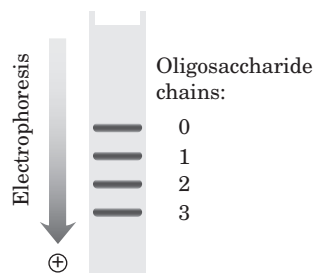
- 25. Heparin Interactions** Heparin, a highly negatively charged glycosaminoglycan, is used clinically as an anticoagulant. It acts by binding several plasma proteins, including antithrombin III, an inhibitor of blood clotting. The 1:1 binding of heparin to antithrombin III seems to cause a conformational change in the protein that greatly increases its ability to inhibit clotting. What amino acid residues of antithrombin III are likely to interact with heparin?

Answer Positively charged amino acid residues would be the best candidates to bind to the highly negatively charged groups on heparin. In fact, Lys residues of antithrombin III interact with heparin.

- 26. Permutations of a Trisaccharide** Think about how one might estimate the number of possible trisaccharides composed of *N*-acetylglucosamine 4-sulfate (GlcNAc4S) and glucuronic acid (GlcA), and draw 10 of them.

Answer If GlcNAc4S is represented as A, and GlcA as B, the trimer could have any of these sequences: AAA, AAB, ABB, ABA, BBB, BBA, BAA, or BAB (8 possible sequences). The connections between each pair of monosaccharides could be 1 \rightarrow 6, 1 \rightarrow 4, 1 \rightarrow 3, or 1 \rightarrow 1 (4 possibilities for each of two bonds, or $4 \times 4 = 16$ possible linkages in all), and each linkage could involve either the α or the β anomer of each sugar (2 possibilities for each of two bonds, so $2 \times 2 = 4$ stereochemical possibilities). Therefore there are $8 \times 16 \times 4 = 512$ possible permutations!

- 27. Effect of Sialic Acid on SDS Polyacrylamide Gel Electrophoresis** Suppose you have four forms of a protein, all with identical amino acid sequence but containing zero, one, two, or three oligosaccharide chains, each ending in a single sialic acid residue. Draw the gel pattern you would expect when a mixture of these four glycoproteins is subjected to SDS polyacrylamide gel electrophoresis (see Fig. 3–18) and stained for protein. Identify any bands in your drawing.

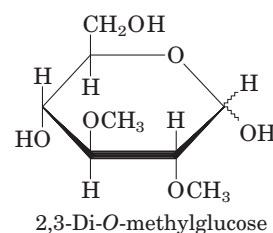
Answer

The significant feature of sialic acids is the negative charge of their carboxyl group. The four glycoproteins would have the same charge except for the additional 1, 2, or 3 negative charges of the sialic acid residues. In SDS gel electrophoresis, the proteins are coated uniformly with a layer of sodium dodecyl sulfate (which is negatively charged, p. 89) and thus move toward the positive electrode. The glycoproteins with 1, 2, or 3 extra negative charges will move progressively faster than the form without sialic acid.

- 28. Information Content of Oligosaccharides** The carbohydrate portion of some glycoproteins may serve as a cellular recognition site. In order to perform this function, the oligosaccharide moiety of glycoproteins must have the potential to exist in a large variety of forms. Which can produce a greater variety of structures: oligopeptides composed of five different amino acid residues or oligosaccharides composed of five different monosaccharide residues? Explain.

Answer Oligosaccharides; their monosaccharide residues can be combined in more ways than the amino acid residues of oligopeptides. Each of the several hydroxyl groups of each monosaccharide can participate in glycosidic bonds, and the configuration of each glycosidic bond can be either α or β . Furthermore, the polymer can be linear or branched. Oligopeptides are unbranched polymers, with all amino acid residues linked through identical peptide bonds.

- 29. Determination of the Extent of Branching in Amylopectin** The amount of branching (number of $(\alpha 1 \rightarrow 6)$ glycosidic bonds) in amylopectin can be determined by the following procedure. A sample of amylopectin is exhaustively methylated—treated with a methylating agent (methyl iodide) that replaces the hydrogen of every sugar hydroxyl with a methyl group, converting $-\text{OH}$ to $-\text{OCH}_3$. All the glycosidic bonds in the treated sample are then hydrolyzed in aqueous acid, and the amount of 2,3-di-*O*-methylglucose so formed is determined.



- (a) Explain the basis of this procedure for determining the number of $(\alpha 1 \rightarrow 6)$ branch points in amylopectin. What happens to the unbranched glucose residues in amylopectin during the methylation and hydrolysis procedure?
- (b) A 258 mg sample of amylopectin treated as described above yielded 12.4 mg of 2,3-di-*O*-methylglucose. Determine what percentage of the glucose residues in amylopectin contain an $(\alpha 1 \rightarrow 6)$ branch. (Assume the average molecular weight of a glucose residue in amylopectin is 162 g/mol.)

Answer

- (a) In glucose residues at branch points, the hydroxyl of C-6 is protected from methylation because it is involved in a glycosidic linkage. During complete methylation and subsequent hydrolysis, the branch-point residues yield 2,3-di-*O*-methylglucose and the unbranched residues yield 2,3,6-tri-*O*-methylglucose.

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- (b) Given the average molecular weight of a glucose residue = 162, then 258 mg of amylopectin contains

$$\frac{258 \times 10^{-3} \text{ g}}{162 \text{ g/mol}} = 1.59 \times 10^{-3} \text{ mol of glucose}$$

The 12.4 mg yield of 2,3-di-*O*-methylglucose (M_r 208) is equivalent to

$$\frac{12.4 \times 10^{-3} \text{ g}}{208 \text{ g/mol}} = 5.96 \times 10^{-5} \text{ mol of glucose}$$

Thus, the percentage of glucose residues in amylopectin that yield 2,3-di-*O*-methylglucose is

$$\frac{(5.96 \times 10^{-5} \text{ mol})(100\%)}{1.59 \times 10^{-3} \text{ mol}} = 3.75\%$$

- 30. Structural Analysis of a Polysaccharide** A polysaccharide of unknown structure was isolated, subjected to exhaustive methylation, and hydrolyzed. Analysis of the products revealed three methylated sugars: 2,3,4-tri-*O*-methyl-D-glucose, 2,4-di-*O*-methyl-D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-glucose, in the ratio 20:1:1. What is the structure of the polysaccharide?

Answer The polysaccharide is a branched glucose polymer. Because the predominant product is 2,3,4-tri-*O*-methyl-D-glucose, the predominant glycosidic linkage must be (1→6). The formation of 2,4-di-*O*-methyl-D-glucose indicates that branch points occur through C-3. The ratio of these two methylated sugars indicates that a branch occurs at an average frequency of once every 20 residues. The 2,3,4,6-tetra-*O*-methyl-D-glucose is derived from nonreducing chain ends, which compose about $\frac{1}{20}$, or 5%, of the residues, consistent with a high degree of branching. Thus, the polysaccharide has chains of (1→6)-linked D-glucose residues with (1→3)-linked branches, about one branch every 20 residues.

Data Analysis Problem

- 31. Determining the Structure of ABO Blood Group Antigens** The human ABO blood group system was first discovered in 1901, and in 1924 this trait was shown to be inherited at a single gene locus with three alleles. In 1960, W. T. J. Morgan published a paper summarizing what was known at that time about the structure of the ABO antigen molecules. When the paper was published, the complete structures of the A, B, and O antigens were not yet known; this paper is an example of what scientific knowledge looks like “in the making.”

In any attempt to determine the structure of an unknown biological compound, researchers must deal with two fundamental problems: (1) If you don't know what *it* is, how do you know if *it* is pure? (2) If you don't know what *it* is, how do you know that your extraction and purification conditions have not changed *its* structure? Morgan addressed problem 1 through several methods. One method is described in his paper as observing “constant analytical values after fractional solubility tests” (p. 312). In this case, “analytical values” are measurements of chemical composition, melting point, and so forth.

- (a) Based on your understanding of chemical techniques, what could Morgan mean by “fractional solubility tests”?
- (b) Why would the analytical values obtained from fractional solubility tests of a *pure* substance be constant, and those of an *impure* substance not be constant?

Morgan addressed problem 2 by using an assay to measure the immunological activity of the substance present in different samples.

- (c) Why was it important for Morgan's studies, and especially for addressing problem 2, that this activity assay be quantitative (measuring a level of activity) rather than simply qualitative (measuring only the presence or absence of a substance)?

The structure of the blood group antigens is shown in Figure 10–15. In his paper (p. 314), Morgan listed several properties of the three antigens, A, B, and O, that were known at that time:

1. Type B antigen has a higher content of galactose than A or O.
2. Type A antigen contains more total amino sugars than B or O.
3. The glucosamine/galactosamine ratio for the A antigen is roughly 1.2; for B, it is roughly 2.5.

- (d) Which of these findings is (are) consistent with the known structures of the blood group antigens?
 (e) How do you explain the discrepancies between Morgan's data and the known structures?

In later work, Morgan and his colleagues used a clever technique to obtain structural information about the blood group antigens. Enzymes had been found that would specifically degrade the antigens. However, these were available only as crude enzyme preparations, perhaps containing more than one enzyme of unknown specificity. Degradation of the blood type antigens by these crude enzymes could be inhibited by the addition of particular sugar molecules to the reaction. Only sugars found in the blood type antigens would cause this inhibition. One enzyme preparation, isolated from the protozoan *Trichomonas foetus*, would degrade all three antigens and was inhibited by the addition of particular sugars. The results of these studies are summarized in the table below, showing the percentage of substrate remaining unchanged when the *T. foetus* enzyme acted on the blood group antigens in the presence of sugars.

Sugar added	Unchanged substrate (%)		
	A antigen	B antigen	O antigen
Control—no sugar	3	1	1
L-Fucose	3	1	100
D-Fucose	3	1	1
L-Galactose	3	1	3
D-Galactose	6	100	1
<i>N</i> -Acetylglucosamine	3	1	1
<i>N</i> -Acetylgalactosamine	100	6	1

For the O antigen, a comparison of the control and L-fucose results shows that L-fucose inhibits the degradation of the antigen. This is an example of product inhibition, in which an excess of reaction product shifts the equilibrium of the reaction, preventing further breakdown of substrate.

- (f) Although the O antigen contains galactose, *N*-acetylglucosamine, and *N*-acetylgalactosamine, none of these sugars inhibited the degradation of this antigen. Based on these data, is the enzyme preparation from *T. foetus* an endo- or exoglycosidase? (Endoglycosidases cut bonds between interior residues; exoglycosidases remove one residue at a time from the end of a polymer.) Explain your reasoning.
 (g) Fucose is also present in the A and B antigens. Based on the structure of these antigens, why does fucose fail to prevent their degradation by the *T. foetus* enzyme? What structure would be produced?
 (h) Which of the results in (f) and (g) are consistent with the structures shown in Figure 10–15? Explain your reasoning.

Answer

- (a) The tests involve trying to dissolve only part of the sample in a variety of solvents, then analyzing both dissolved and undissolved materials to see whether their compositions differ.
 (b) For a pure substance, all molecules are the same and any dissolved fraction will have the same composition as any undissolved fraction. An impure substance is a mixture of more than one compound. When treated with a particular solvent, more of one component may dissolve, leaving more of the other component(s) behind. As a result, the dissolved and undissolved fractions have different compositions.

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- (c) A quantitative assay allows researchers to be sure that none of the activity has been lost through degradation. When determining the structure of a molecule, it is important that the sample under analysis consist only of intact (undegraded) molecules. If the sample is contaminated with degraded material, this will give confusing and perhaps uninterpretable structural results. A qualitative assay would detect the presence of activity, even if it had become significantly degraded.
- (d) Results 1 and 2. Result 1 is consistent with the known structure, because type B antigen has three molecules of galactose; types A and O each have only two. Result 2 is also consistent, because type A has two amino sugars (*N*-acetylgalactosamine and *N*-acetylglucosamine); types B and O have only one (*N*-acetylglucosamine). Result 3 is *not* consistent with the known structure: for type A, the glucosamine:galactosamine ratio is 1:1; for type B, it is 1:0.
- (e) The samples were probably impure and/or partly degraded. The first two results were correct possibly because the method was only roughly quantitative and thus not as sensitive to inaccuracies in measurement. The third result is more quantitative and thus more likely to differ from predicted values, because of impure or degraded samples.
- (f) An exoglycosidase. If it were an endoglycosidase, one of the products of its action on O antigen would include galactose, *N*-acetylglucosamine, or *N*-acetylgalactosamine, and at least one of those sugars would be able to inhibit the degradation. Given that the enzyme is not inhibited by any of these sugars, it must be an exoglycosidase, removing only the terminal sugar from the chain. The terminal sugar of O antigen is fucose, so fucose is the only sugar that could inhibit the degradation of O antigen.
- (g) The exoglycosidase removes *N*-acetylgalactosamine from A antigen and galactose from B antigen. Because fucose is not a product of either reaction, it will not prevent removal of these sugars, and the resulting substances will no longer be active as A or B antigen. However, the products should be active as O antigen, because degradation stops at fucose.
- (h) All the results are consistent with Figure 10–15. (1) D-Fucose and L-galactose, which would protect against degradation, are not present in any of the antigens. (2) The terminal sugar of A antigen is *N*-acetylgalactosamine, and this sugar alone protects this antigen from degradation. (3) The terminal sugar of B antigen is galactose, which is the only sugar capable of protecting this antigen.

Reference

Morgan, W.T. (1960) The Croonian Lecture: a contribution to human biochemical genetics; the chemical basis of blood-group specificity. *Proc. R. Soc. Lond. B Biol. Sci.* **151**, 308–347.