1.2 General Properties, Occurrence, and Preparation of Carbohydrates

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Abstract

D-Glucose and its derivatives and analogues, *N*-acetyl-D-glucosamine, *N*-acetyl-D-muramic acid, D-glucopyranosyl uronic acid, and D-glucitol represent 99.9% of the carbohydrates on the earth. D-Glucose is found in the free state in human blood and in the combined state in disaccharides, sucrose, lactose, and α,α-trehalose, in cyclic dextrins, and in polysaccharides, starch, glycogen, cellulose, dextrans; *N*-acetyl-D-glucosamine and an analogue *N*-acetyl-D-muramic acid are found in bacterial cell wall polysaccharide, murein, along with teichoic acids made up of poly-glycerol or -ribitol phosphodiesters. Other carbohydrates, D-mannose, D-mannuronic acid, D-galactose, *N*-acetyl-D-galactosamine, D-galacturonic acid, L-iduronic acid, L-guluronic acid, L-rhamnose, L-fucose, D-xylose, and *N*-acetyl-D-neuraminic acid are found in glycoproteins, hemicelluloses, glycosaminoglycans, and polysaccharides of plant exudates, bacterial capsules, alginates, and heparin. D-Ribofuranose-5-phosphate is found in many coenzymes and is the backbone of RNAs (ribonucleic acid), and 2-deoxy-D-ribofuranose-5-phosphate is the backbone of DNA (deoxyribonucleic acid). D-Fructofuranose is found in sucrose, inulin, and levan. The general properties and occurrence of these carbohydrates and general methods of isolation and preparation of carbohydrates are presented.

Keywords

D-Glucose; D-Fructose; Sucrose; Lactose; α,α-Trehalose; Starch; Glycogen; Cyclodextrins; Dextrans; Alternan

Abbreviations

ADPGlc	adenosine-diphospho-glucose
ATP	adenosine triphosphate
CGTase	cyclomaltodextrin glucanyltransferase
d.s.	degree of substitution
DNA	deoxyribonucleic acid
FACE	fluorophore-assisted capillary electrophoresis
FAD	oxidized flavin adenine dinucleotide
HPLC	high pressure liquid chromatography
MALDI-TOF MS	matrix-assisted laser desorption ionization–time of flight mass spectrometry
NAD^+	oxidized nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NADPH	reduced nicotinamide adenine dinucleotide phosphate
ORD	optical rotatory dispersion
RNA	ribonucleic acid
NADPH	nicotinamide adenine dinucleotide-phosphate
NAG	*N*-acetyl-D-glucosamine
NAM	*N*-acetyl-D-muramic acid
TLC	thin-layer chromatography
TFMS	trifluoromethane sulfonic acid
UDPGlc	uridine-diphospho-glucose

1 General Properties and Occurrence of Carbohydrates

Carbohydrates have the following major properties: (1) they are polyhydroxy aldehydes or ketones; (2) they have chiral or asymmetric carbons that are generally manifested by the rotation of plane polarized light; (3) they have the ability to form multiple hydrogen bonds, generally giving them the property of being water-soluble, but they also can be water-insoluble when they form intermolecular hydrogen bonds with each other to give crystals or large, high molecular weight, insoluble crystalline aggregates, granules, or fibers; (4) many have reactivities of aldehydes that can be oxidized to acids by reagents that are thereby reduced (e. g., reducing an oxidizing agent such as an alkaline solution of copper(II) or ferricyanide/cyanide), and they, hence, are considered to be reducing sugars, or they can themselves be reduced by reducing reagents, such as $NaBH_4$, to give sugar alcohols; (5) the aldehyde or ketone groups in carbohydrates with five or more carbons will react with intramolecular alcohol groups to form cyclic structures with hemiacetal and hemiketal hydroxyl groups; (6) the hemiacetal or hemiketal hydroxyls are more reactive than the alcohols and can react intermolecularly with alcohols and amines to give acetals or ketals (glycosidic bonds) that are fairly stable; (7) they have two kinds of alcohol groups, secondary and primary, that can undergo the usual reactions of alcohols to give esters and ethers and can be replaced, for example, by hydrogen, halogens (F, Cl, Br, and I), amino groups, *N*-acetyl amino groups, and sulfhydryl groups; (8) they are generally, although not all of them, sweet-tasting (for example, D-glucose, D-glucitol, D-fructose, D-xylose, D-xylitol and sucrose are sweet-tasting) by forming specific hydrogen and hydrophobic bonds with the sweet-taste receptors on the tongue; and (9) when attached to proteins or cell surfaces, the structural diversity of oligosaccharides mediate a large number of biochemical and biological processes.

In the 19th century, several naturally occurring carbohydrates were known, such as glucose (then called dextrose), fructose (then called levulose), mannose, galactose, sucrose, lactose, starch, and cellulose. Some of these had been known for thousands of years, for example, sucrose, starch, and cellulose. Also in the 19th century, the empirical formula for all of these materials was found to be $C_n(H_2O)_n$ and they were originally thought to be hydrates of carbon, hence the name carbohydrates.

Carbohydrates are now more completely defined as polyhydroxy aldehydes or ketones and compounds that can be derived from them by reduction to give sugar alcohols, oxidation to give sugar acids, substitution of hydroxyl group(s) by hydrogen to give deoxy sugars or by amino or *N*-acetyl amino groups to give deoxy-amino sugars, derivatization of a hydroxyl group by phosphate or sulfate to give sugar phosphates or sugar sulfates, and by condensation reactions of a hydroxyl group of one sugar with the hemiacetal group of another sugar to give disaccharides, trisaccharides, oligosaccharides, and polysaccharides.

2 Carbohydrate Property of Optical Rotation of Plane Polarized Light

An important property of carbohydrates that was recognized in the 19th century was that they generally, but not always, rotated plane polarized light and that this was specific for each carbohydrate. This property is due to the presence of asymmetric or chiral carbons that have four different groups attached to the carbons. Those carbohydrates that rotate plane polarized light

are said to be optically active. It was also recognized that the optical rotation was dependent on several factors: (1) the structure of the substance; (2) the length of the cell; (3) the concentration of the substance; (4) the wavelength of the plane polarized light; and (5) the temperature. The following relationship was derived to encompass these variables:

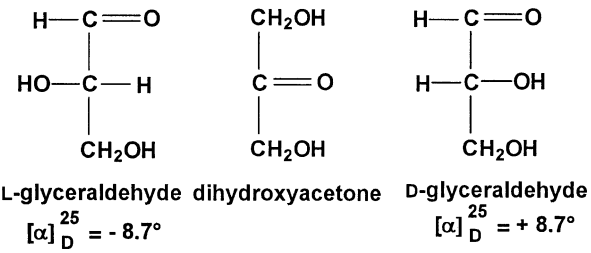
$$\alpha_{obs} = [\alpha]^t_\lambda lc$$

where α_{obs} = the observed optical rotation in degrees, l = the length of the cell holding the compound in dm (decimeter), c = the concentration of the sample in g mL^{-1}, usually in water, $[\alpha]_\lambda{}^t$ = the specific optical rotation constant of the substance at temperature, t, and wavelength, λ. Most polarimetric measurements are made with the D-line from a sodium lamp and each carbohydrate has a characteristic $[\alpha]^t_D$, although the optical rotation can also be measured continuously as a as a function of the wavelength (i. e., optical rotatory dispersion, ORD). Carbohydrate molecules with two-fold symmetry about a central point or plane do not rotate plane polarized light and are said to have a meso-structure.

3 The Structures of Carbohydrates

3.1 The Simplest Carbohydrates

There are three carbohydrates that are the simplest carbohydrates that fulfill the definition given above. They are the following:



Scheme 1
The simplest carbohydrates

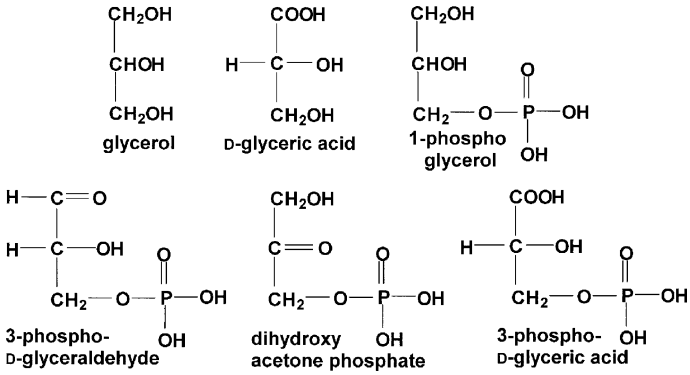
There are two forms for glyceraldehyde that are distinct and cannot be superimposed onto each other. Prof. Fischer defined the one with the chiral hydroxyl group to the right as D-glyceraldehyde, where the "D" indicates that the hydroxyl group is to the right or *dextro* and the one with the chiral hydroxyl group to the left as L-glyceraldehyde where the "L" indicates that the hydroxyl group is to the left or *levo*. It just so happened that for D-glyceraldehyde, plane polarized light was rotated to the right and L-glyceraldehyde rotated plane polarized light to the left. This is not always the case. Some carbohydrates with the D-configuration rotate plane polarized light to the left and some carbohydrates with the L-configuration rotate plane polarized light to the right.

A large majority of the carbohydrates found on the earth belong to the D-family of structural isomers. In the course of evolution, the reason that the D-family of structural isomers was

selected over those of the L-family of structural isomers is not clear. It, however, is not likely that it was a matter of chance. It has been known for many years that irradiation of a racemic mixture of D- and L- isomers with circularly polarized light will selectively destroy one of the two isomers, leaving the other more or less intact [1]. Circularly polarized light has been observed when there is high sunspot activity. High levels of circularly polarized light have also been observed coming from the *Orion nebula* [2]. The selection of D-carbohydrates could have occurred by this type of irradiation when carbohydrates were first being formed on the earth.

3.2 Analogues of D-Glyceraldehyde

D-Glyceraldehyde has some derived analogues, such as the reduced sugar alcohol, glycerol, its oxidized product, D-glyceric acid, and their phosphorylated analogues, 1-phospho-D-glycerol, 3-phospho-D-glyceraldehyde, and 3-phospho-D-glyceric acid, whose structures are shown as:



Scheme 2
Analogues and derivatives of the naturally occurring three-carbon carbohydrates

Glycerol is found as the backbone compound that is esterified by fatty acids to give a class of lipids known as triacyl glycerols (glycerides), and glycerol-1-phosphate is the backbone of a major class of phospholipids. 3-Phospho-D-glyceraldehyde, dihydroxy acetone phosphate, and 3-phospho-D-glyceric acid are all found in both the reactions of photosynthesis and in the degradative reactions of glycolysis.

3.3 The Formation of Carbohydrates Containing More than Three Carbons

From a theoretical stand point, Professor Emil Fischer showed that by adding a new chiral carbon between the aldehyde group and the asymmetric carbon of D-glyceraldehyde, a chiral pair of 4-carbon D-tetraoses would be obtained, namely, D-erythrose and D-threose. Adding another set of similar chiral carbons to each of the two D-tetraoses, gives four 5-carbon D-pentoses, and likewise adding a similar set of chiral carbons to each of the four D-pentoses, gives eight 6-carbon D-hexoses. It should be noted that it is only the configuration of the last asymmetric

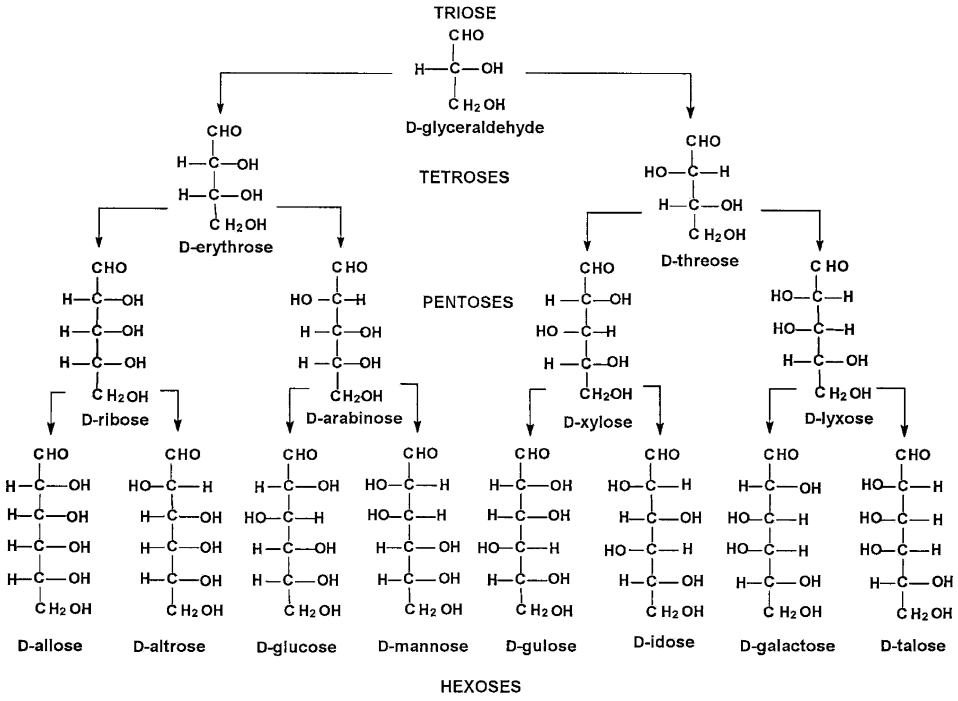


Figure 1
Structural family of D-carbohydrates, from triose to hexoses, with their names

carbon with the hydroxyl group to the right that makes it a "D" carbohydrate and the entire mirror image of the D-carbohydrates gives the L-carbohydrate. See *Fig. 1* for the names and structures of the family of D-carbohydrates.

3.4 Special Properties of Pentoses and Hexoses

The pentoses and hexoses have a propensity for forming six-membered rings in which one of their hydroxyl groups reacts intramolecularly with the aldehyde group to form a cyclic hemiacetal. This reaction creates a new asymmetric center on the aldehyde carbon to give two isomers, called alpha (α) and beta (β). Two five-membered rings are also formed, but in much smaller amounts, as the six-membered cyclic structures are much more thermodynamically stable (i. e., less strained) than are the five-membered rings. The six-membered rings are called pyranoses and the five-membered rings are called furanoses. For D-glucose at 20 °C and equilibrium, there are five compounds: 0.004% the open aldehyde chain, 66% β-D-glucopyranose, 33% α-D-glucopyranose, and 0.498% each of β-D-glucofuranose and α-D-glucofuranose. See *Fig. 2* for the structures of the five forms of D-glucose in equilibrium.
If one starts with α-D-glucopyranose, $[\alpha\text{-}]_D^{25} = +112°$, the optical rotation drops to +52° and if one starts with β-D-glucopyranose, $[\alpha\text{-}]_D^{25} = +19°$, the optical rotation increases and becomes constant at +52°, which is the optical rotation for an equilibrium mixture of the five structural

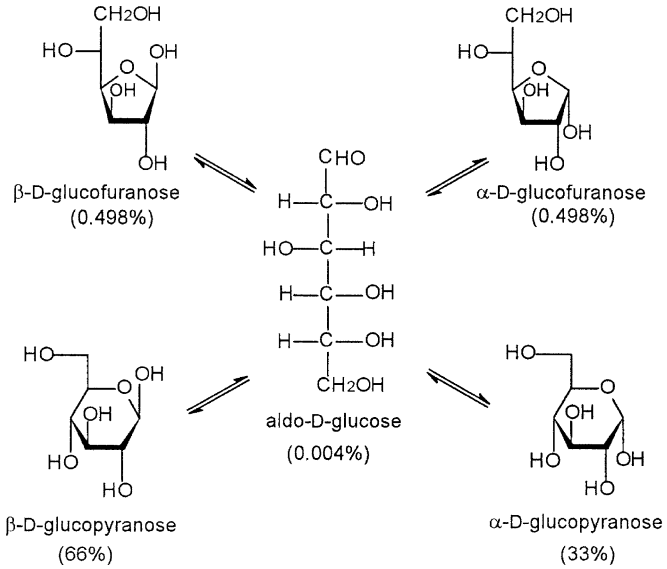


Figure 2
The five structural forms of D-glucose at equilibrium in aqueous solution at 20 °C

forms of D-glucose. The process is known as mutarotation and is relatively slow at pH 7 and 20 °C. It can be accelerated by catalysis with either acid or base or by adding an enzyme, known as mutarotase. Dilute base (pH 10) is a better catalyst by a factor of 5,000 than dilute acid (pH 4). Mutarotase acts as an acid–base catalyst and catalyzes the reactions 4–5 orders of magnitude faster than base.

3.5 D-Glucose: the Most Prominent Carbohydrate on the Earth

Of the 15 possible D-carbohydrates in *Fig. 1*, only a handful occurs in nature to any extent. By far, D-glucose and its analogues are the most prominent and represent 99.9% of the carbohydrates on the earth. Why is this? While D-glucose forms the six-membered cyclic structure and has the Haworth structure as shown in *Fig. 3*, the ring actually has a three-dimensional chair conformation, with two kinds of geometric bonds around the carbons, those that are within the plane of the ring (called equatorial bonds) and those that are perpendicular to the ring (called axial bonds). D-Glucose can exist in two chair conformations, the C1 or $^{4}C_{1}$ chair and the 1C or $_{4}C^{1}$ chair (see *Fig. 3* for the structures). In the C1 or $^{4}C_{1}$ conformation, all of the hydroxyl or bulkiest groups for β-D-glucopyranose are attached to the ring by equatorial bonds that put the hydroxyl or bulkiest groups as far apart as possible from each other, giving the most thermodynamically stable structure possible. If β-D-glucopyranose is in the other chair conformation, 1C or $_{4}C^{1}$, all of the hydroxyl groups are axial and are placed as close together as possible, giving the most thermodynamically unstable structure possible. Thus, β-D-glucopyranose exists primarily in the C1-conformation. α-D-Glucopyranose also exists

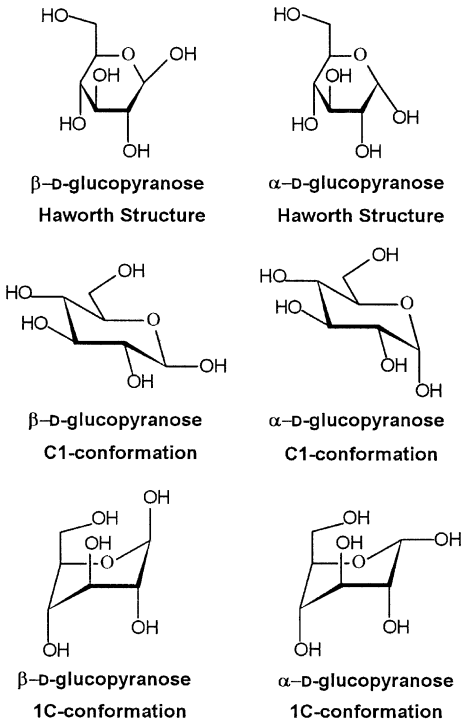


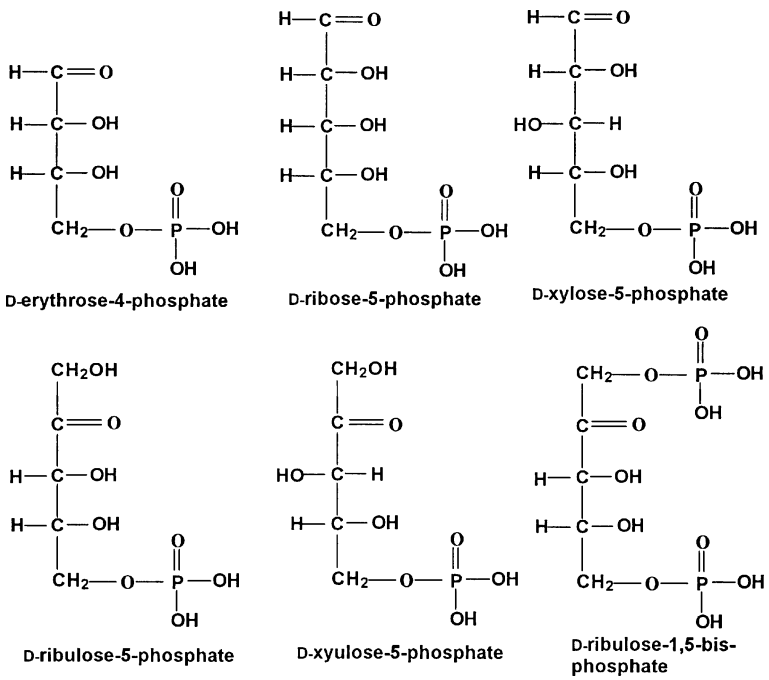
Figure 3
Haworth structures for α- and β-D-glucopyranose and their C1 and 1C conformations

in the C1-conformation, with only the hemiacetal hydroxyl group in the axial position. This is the most likely reason that D-glucose is the predominant carbohydrate on the earth, as it is the only D-hexose that can have all of its hydroxyl groups (exclusive of the hemiacetal hydroxyl group) equatorial, and at equilibrium in solution β-D-glucopyranose has a ratio of $\sim$2:1 to α-D-glucopyranose, which has its hemiacetal hydroxyl group axial. D-Xylose, a pentose, can also form a six-membered ring by its terminal hydroxyl group reacting with the aldehyde group and form a C1 conformation and place all of its bulky hydroxyl groups equatorial, but because it has five carbons, it would have to be split into a 2-carbon fragment and a 3-carbon fragment for metabolism and would require two separate pathways for further metabolism. D-Glucose has 6-carbons and is split into two 3-carbon fragments, D-glyceraldehyde-3-phosphate and dihydroxy acetone-phosphate that are interconvertible and requires only a single metabolic pathway for further metabolism. This is an additional plausible reason that D-glucopyranose is the predominant carbohydrate on the earth.

The D-pentoses will also exist in solution as the six-membered ring structure, but both D-xylose and D-ribose often have their C-5 hydroxyl groups phosphorylated and the C-5 hydroxyl group cannot react with the aldehyde group to form the six-membered ring, and therefore they do the next best thing, with the C-4 hydroxyl group reacting with the aldehyde group to form the five-membered, furanose ring, hemiacetal structure.

3.6 Occurrence of D-Erythrose, D-Ribose, and D-Xylose

Of the two D-tetroses, only 4-phospho-D-erythrose is found in any quantity, as an intermediate in the photosynthetic reactions. Of the four pentoses, only D-ribose and D-xylose occur to any extent. The phospho-*aldo*-D-pentoses, D-ribose-5-phosphate and D-xylose-5-phosphate, are found in the photosynthetic reactions. Three phospho-*keto*-D-pentoses are also found as intermediates in the photosynthetic reactions: D-ribulose-5-phosphate, D-xylulose-5-phosphate, and D-ribulose-1,5-bis-phosphate, the latter carbohydrate being directly involved in the fixing of CO_2in photosynthesis.



Scheme 3
Important naturally occurring four- and five-carbon sugar phosphates

Nicotinamide adenine dinucleotide (NAD^+ and NADH), coenzymes containing D-ribofuranose-diphosphate, are involved in many oxidation and reduction reactions, respectively, of carbohydrate metabolism. D-Ribofuranose is also the main component of the universal energy donor and energy carrier, adenosine triphosphate (ATP), which is one of the primary products of the light reactions of photosynthesis and is responsible for providing the energy for the formation of the carbon–carbon bond in the fixation of CO_2. It is also important in the transfer and utilization of energy in the metabolism of nonphotosynthesizing organisms. Another important D-ribofuranose coenzyme that is formed as a primary product of the light reactions of photosynthesis is the reducing coenzyme, nicotinamide adenine dinucleotide-phosphate,

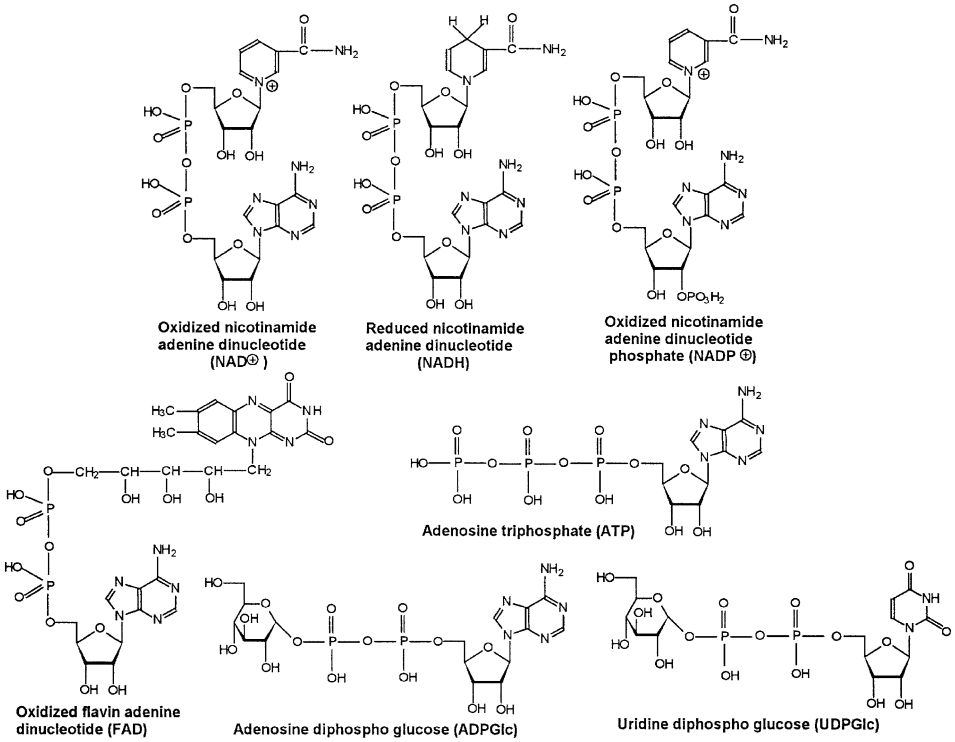


Figure 4
Structures of the D-ribofuranose-5-phosphate nucleotide coenzymes that are important in biochemical metabolism

(NADPH), which is similar to the coenzymes, NAD^+ and NADH, mentioned above, but with an additional phosphate attached to the 2-position of the ribose unit. See *Fig. 4* for the structures of these coenzymes. NADPH is responsible for reducing the carbon–carbon bond that is formed in fixing CO_2in photosynthesis.

ATP is the universal energy carrier and source of energy in biochemical systems; NAD^+ and NADH, oxidation and reduction coenzymes; FAD, oxidative coenzyme, containing ribitol-5-phosphate; ADPGlc, high-energy glucose donor, involved in starch biosynthesis; and UDPGlc, another high-energy glucose donor, involved in cellulose, glycogen, and sucrose biosyntheses, and in the enzymatic conversions of D-glucopyranose to many other sugars, such as, D-galactopyranose, D-glucopyranouronic acid, D-xylopyranose, and L-arabinopyranose.

D-Ribofuranose-5-phosphate occurs as the backbone component of the ribonucleic acids, RNA, that are involved in the biosynthesis of proteins. There are three kinds of RNA's: a small RNA, transfer-RNA that forms a high-energy, amino-acid covalent compound that transfers individual amino acids to the ribosome to be incorporated into proteins; an intermediate sized RNA, messenger-RNA that carries the codon or genetic information of a protein to the ribosome where the code is read and the peptide bonds of the protein are synthesized; and the largest sized RNA, ribosomal-RNA that composes the ribosome, the organelle where pro-

teins are synthesized and assembled. 2-Deoxy-D-ribofuranose-5-phosphates are the backbone components of deoxyribonucleotides (DNA), which primarily act as the carrier of the genetic information, necessary for the formation of proteins involved in life processes. Like RNA, these molecules have two purines (adenine and guanine) and two pyrimidines (uracil and cytosine for RNA and thymidine and cytosine for DNA) that are linked β to carbon-1 of the D-ribofuranose-phosphate units to give *N*-glycosides.

D-Xylopyranose occurs as one of the major components in the hemicelluloses (see ❯ *Sect. 6* on cellulose and ❯ *Sect. 7* on hemicelluloses and ❯ Chap. 6.3 on cellulose).

3.7 Occurrence of Hexoses

Of the eight D-hexoses in ❯ *Fig. 1*, only three occur to any extent: D-glucose, its 2-isomer, D-mannose, and its 4-isomer, D-galactose. Another hexose that is found is D-fructose, which is a keto-sugar that can be derived from dihydroxy acetone, as the aldehyde carbohydrates were derived from D-glyceraldehyde. D-Fructose is formed when D-glucose or D-mannose are treated with alkali, which isomerizes carbons 1 and 2 of the two D-hexoses [3]. The diphosphate of D-fructose, D-fructose-1,6-bis-phosphate is the first hexose that is formed in the photosynthetic process and it is rapidly converted into D-fructose-6-phosphate, and then into D-glucose-6-phosphate.

4 Properties and Occurrence of D-Glucose

4.1 D-Glucose in the Free State

Free D-glucose occurs primarily in the blood of many higher animals, where it serves as an immediate source of energy and as a stabilizer of the osmotic pressure, and a precursor for the formation of glycogen and fat in muscle tissue. In normal humans, the concentration of blood glucose is 80–120 mg/100 mL^{-1} or 5–7 mM. This can increase to 200–300 mg/100 mL^{-1} or 11–20 mM after a high-carbohydrate meal, but then is relatively rapidly decreased by the action of insulin and often goes below 80 mg/mL^{-1} and then goes slowly up to normal levels. In uncontrolled diabetics the glucose often goes much higher, to 140–1100 mg/100 mL^{-1} or 8–60 mM. In controlled diabetics, the glucose will often be slightly higher than normal, for example, 120–140 mg/100 mL^{-1}.

D-Glucose is also found in the free state in honey, grapes, and raisins. It is produced by the action of glucoamylase (amyloglucosidase) on starch, where glucoamylase hydrolyzes the α-(1→4) linkage of the glucose units at the nonreducing-ends of the starch chains, giving inversion of the configuration, forming β-D-glucose. Glucoamylase will also hydrolyze α-(1→6) branch linkages, although at a rate about 0.1 that of the α-(1→4) linkage. Eventually glucoamylase will completely convert all of the starch into D-glucose.

4.2 D-Glucose in the Combined State

D-Glucose is found in a combined form in which its hemiacetal group has reacted with a hydroxyl on another glucose or another carbohydrate to form an acetal or glucosidic link-

age and form such carbohydrate substances as, starch, dextran, cellulose, sucrose, lactose, α,α-trehalose.

4.2.1 Occurrence of D-Glucose Combined with D-Fructose, D-Galactose, and D-Glucose, and High-Energy D-Glucose Donors

There are three major, naturally occurring disaccharides: sucrose, lactose, and α,α-trehalose and D-glucopyranose is found in all three. Sucrose is a nonreducing disaccharide composed of D-glucopyranose joined to D-fructofuranose (1→2) to give α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside. Sucrose is widely distributed in plants, primarily as 6^{Fru}-phosphosucrose, which is the transport form of carbohydrates in plants. When it reaches certain parts of the plant, the glucose moiety is converted into starch by a series of reactions that give adenosine-diphospho-glucose (ADPGlc) or uridine-diphospho-glucose (UDPGlc), high-energy donors of glucose for the biosynthesis of starch and cellulose, respectively. Relatively large amounts (15–20% by weight) of free sucrose are found in the stems and tubers of sugar cane and the tubers of sugar beets. Free sucrose is also found in many other plants but in lower amounts, such as their fruits. Sucrose is the predominant sugar found in honey, produced by bees, in the sap of maple trees, giving maple syrup, and in sorghum, and in dates. See *Fig. 5* for the structures of sucrose, lactose, and α,α-trehalose and *Fig. 4* for the structures of ADPGlc and UDPGlc.

4.2.2 Properties and Occurrence of Sucrose and Sucrose Oligosaccharides Containing D-Galactose

Sucrose has five important properties: (a) the linkage between D-glucopyranose and D-fructofuranose is of high energy being an acetal–ketal linkage of a six-membered ring attached to a five-membered ring, making sucrose a nonreducing sugar. (b) The acetal–ketal linkage is

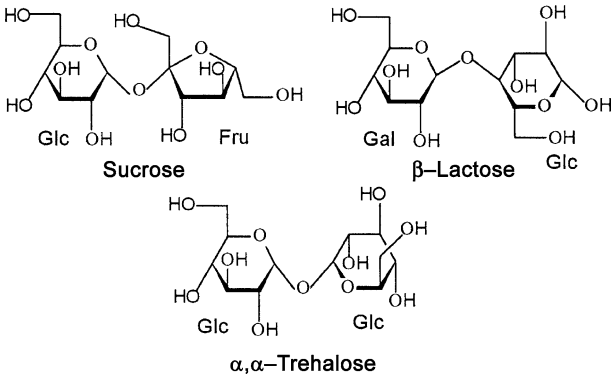


Figure 5
Structures of the three naturally occurring disaccharides: sucrose, lactose, and α-α-trehalose, containing α-D-glucopyranose combined with β-D-fructofuranose, β-D-galactopyranose, and α-D-glucopyranose, respectively

relatively labile and is hydrolyzed by mild acid (pH 4) and is the donor of D-glucopyranose for the biosynthesis of dextrans and related polysaccharides by glucansucrases and the donor of D-fructofruanose for the biosynthesis of levan and inulin (see *Sect. 11*), fructofuranose polysaccharides by levansucrase and inulinsucrase; (c) it is the sugar of commerce because of the ease of obtaining it in large quantities in a pure state from sugar cane and sugar beets; (d) it crystallizes relatively easily; and (e) it has a pleasant sweet taste and has been recognized by humans for over 10,000 years as a sweet food and a natural sweetening agent. See *Fig. 5* for the structure of sucrose.

The origin of sucrose is thought to have been in the Indus Valley, where many woody, wild sugar cane plants that have the fundamental characteristics of the modern cultivated strains can still be found growing today. Sugar cane grows well in a warm, humid, tropical or semi-tropical climate. In the late 18th century on the European continent, the sugar beet was found to be an alternative source of sucrose that did not require a tropical or semi-tropical climate for growth.

Sucrose is hydrolyzed into its component sugars (D-glucose and D-fructose) by the action of the enzyme, invertase, a β-fructofuranosidase, and by mild acid. In this form it is known as invert sugar, due to the fact that the direction of rotation of polarized light is inverted from dextrorotatory to levorotatory on hydrolysis. Honey is usually a mixture of sucrose and invert sugar. Yeasts also have invertase and can hydrolyze sucrose and then ferment the component sugars into ethyl alcohol.

In addition to sucrose, several plants also form a series of sucrose-based oligosaccharides with chains of α-1→6 D-galactopyranose units linked to the D-glucose moiety of sucrose [4]. The first in the series is the trisaccharide, raffinose, in which D-galactopyranose is linked α-(1→6) to sucrose; the second is a tetrasaccharide, stachyose, in which D-galactopyranose is linked α-(1→6) to the D-galactopyranose unit of raffinose. The next is a pentasaccharide, verbascose, with D-galactopyranose linked α-(1→6) to the terminal D-galactose unit of stachyose, and the next is a hexasaccharides, ajugose, with D-galactopyranose linked α-(1→6) to the terminal D-galactose unit of verbascose.

These D-galactopyranosyl sucrose oligosaccharides are particularly found in the tubers and seeds of legumes. Raffinose is found in cottonseeds and in sugar beets. Although sugar beets only contain about 0.05% by weight raffinose as compared with 16–18% sucrose, it has been isolated and crystallized with a purity of better than 99% from sugar beet syrup, where it accumulates during the processing of sucrose.

Soybeans are a good source of stachyose, where it is found to the extent of 2–3% by weight. In general, legume seeds and the mullein root are sources of verbascose. The enzyme invertase and mild acid specifically hydrolyze the oligosaccharides to give D-fructose and the corresponding reducing oligosaccharides that are terminated at the reducing-end with D-glucose. For example, raffinose is hydrolyzed to give D-fructose and the reducing disaccharide, melibiose [α-D-galactopyranosyl-(1→6)-D-glucose].

Another series of galacto-sucrose oligosaccharides involves the attachment to the D-fructofuranose moiety of sucrose [4]. The attachment of α-D-galactopyranose 1→6 to the fructose moiety gives the nonreducing trisaccharide, planteose. It is found primarily in the seeds of the *Plantago* family of plants, for example, the common weed and herb, plantain. Mild acid hydrolysis gives D-glucose and the reducing keto-disaccharide, planteobiose [α-D-galactopyranosyl-(1→6)-D-fructose]. Another nonreducing trisaccharide, melezitose, has α-D-glucopyranosyl linked (1→3) to the D-fructofuranose moiety of sucrose. It is found

in the sweet exudates of many trees, such as larch, Douglas fir, Virginia pine, and poplars. Mild acid hydrolysis of melezitose gives D-glucose and the reducing disaccharide, turanose, [α-D-glucopyranosyl-(1→3)-D-fructose].

Several sucrose analogues have been enzymatically synthesized in the laboratory. Levansucrase can transfer a D-fructofuransoyl unit from raffinose to D-xylose, giving a nonreducing sucrose disaccharide analogue, xylsucrose [α-D-xylopyranosyl-(1→2)-D-fructofuranoside] and the reducing disaccharide, melibiose [α-D-galactopyranosyl-(1→6)-D-glucose [5]. A similar reaction of levansucrase with raffinose and D-galactose gives galactosucrose [α-D-galactopyranosyl-(1→2)-D-fructofuranoside] (also referred to as galsucrose) and melibiose [6]. Reaction of sucrose and lactose with levansucrase gives D-glucose and the nonreducing trisaccharide, lactosucrose [4^{Glc}-β-D-galactopyranosyl sucrose] [7]. Reaction of dextransucrase with sucrose and D-fructose gives an unusual reducing disaccharide, leucrose, containing an α-(1→5) linkage of D-glucopyranosyl linked (1→5) to D-fructopyranose [α-D-glucopyranosyl-(1→5)-D-fructopyranose] [8,9].

A relatively large number of sucrose derivatives have been chemically synthesized [10]. Some notable chloro derivatives have been obtained by the reaction of sucrose with sulfuryl chloride in pyridine/chloroform at low temperatures, for example 4,6,1′,6′-tetrachloro-4,6,1′,6′-tetradeoxy-glactosucrose, 4,6,6′-trichloro-4,6,6′-trideoxy-sucrose, and many others were formed [11,12,13,14,15]. These chloro-compounds of sucrose were 10–100 times sweeter than sucrose. One of them, Sucralose (4,1′,6′-trichloro-4,1′,6′-trideoxy-galactosucrose), was 650-times sweeter than sucrose, with no after-taste, and a sweet-taste identical to sucrose. It is used commercially as a noncariogenic and noncaloric sweetener in soft drinks, candies, cookies, jellies, and many other prepared foods, as well as a general substitute for table sugar. Sucralose is enzymatically inert and passes through the human body without being metabolized or absorbed.

4.2.3 Properties and Occurrence of D-Glucose Combined with D-Galactose to Give Lactose and Higher Oligosaccharides

Lactose is a disaccharide composed of β-D-galactopyranose linked (1→4) to D-glucose and is found in the milk of mammals, where it serves as a source of energy and nourishment for the newborn. Lactose is a reducing disaccharide because the D-glucopyranose residue has a free hemiacetal group at C1. Human milk contains 85 g L^{-1} lactose and cow's milk contains about 50 g L^{-1}.

Human milk also contains lactose oligosaccharides in which various different monosaccharide residues are attached to the D-galactopyranosyl residue. α-L-Fucose (6-deoxy-L-galactose) is attached (1→2) to the galactose moiety [16], α-*N*-acetyl-D-neuraminic acid is attached (2→3) to the galactose moiety [16,17], and the β-*N*-acetyl-D-glucosamine residue is attached either (1→3) or (1→6) to the galactose moiety [18]. The latter serves to produce a core structure that can be further extended by the addition of β-D-galactopyranose residues linked either (1→2) or (1→4) [19]. The β-D-galactopyranosyl-β-*N*-acetyl-D-glucosamine disaccharide is often added in multiples to give a repeated core structure to which α-*N*-acetyl-D-neuraminic acid and α-L-fucose residues are added to the ends of the oligosaccharides. The so called human blood group determinants (see ❯ *Sect. 14*) have structural similarities to the human milk oligosaccharides [19] and it is thought that through this relationship the milk oligosac-

charides impart some form of early immunological protection to the newborn. See *Fig. 5* for the structure of lactose.

4.2.4 Properties and Occurrence of α-D-Glucose Combined with α-D-Glucose to Give α,α-Trehalose

α,α-Trehalose is also one of the three naturally occurring disaccharides. It is a nonreducing disaccharide with two D-glucopyranose residues joined together in an α-1↔α-1 acetal–acetal linkage and is of relatively high energy, like sucrose. Unlike sucrose, however, the acetal–acetal linkage is quite stable and is one of the most difficult linkages to be hydrolyzed by acid. The reason for this is not absolutely clear, but it has been hypothesized to be due to the stabilization of the molecule by intramolecular hydrogen bonds between the D-glucose residues. α,α-Trehalose is found in insect lymph fluid ("insect blood") where it acts as a source of chemical energy [19] and it is also found in mushrooms, honey, yeast, fungi, lobster, and shrimp as a source of energy [20,21] all of which have the enzyme, trehalase, that hydrolyzes α,α-trehalose to give two molecules of D-glucose that can be used for energy.
Besides being used as a source of energy, some plants and animals also use α,α-trehalose as a stabilizing agent during extreme conditions [22]. High concentrations of α,α-trehalose in the tissues of certain insects and in desert plants allows them to survive in a state of suspended animation under conditions of water deficiency. α,α-Trehalose helps frogs to survive in a frozen state and it helps to protect the DNA of salmon sperm from dehydration. α,α-Trehalose has also found applications in the preservation of organs taken for use in organ transplants [23,24]. See *Fig. 5* for the structure of α,α-trehalose.

5 Properties and Occurrence of D-Glucose in Polysaccharides and Cyclodextrins

5.1 Properties and Occurrence of Starch

Starch is an abundant polysaccharide composed of D-glucose residues. It is found in the green leaves, stems, roots, seeds, fruits, tubers, and bulbs of most plants, where it serves as the storage of chemical energy obtained from the energy of the sun light in the process of photosynthesis. Starch also serves as the major source of chemical energy for most nonphotosynthesizing organisms such as bacteria, fungi, insects, and animals. It is found in relatively large amounts in the major food crops of the world. Starch is present 80% by weight in the rice kernel, 78% in the potato tuber, 75% in green bananas, 73% in the maize kernel, 68% in wheat flour, and 60% in rye and lentils. Starch provides about 65% of the dietary calories in the human diet.
Starch occurs in plants as water-insoluble granules produced in plant organelles, plastids (chloroplasts and amyloplasts). The granules have specific shapes and sizes that are characteristic of their botanical source [25]. Most starches are composed of a mixture of two types of polysaccharides, a linear polysaccharide, consisting of α-(1→4) linked D-glucopyranose residues, called amylose, and a branched polysaccharide of α-(1→4) linked D-glucopyranose

residues with 5–6% α-(1→6) branch linkages, called amylopectin. Amylose has an average of 500 to 5,000 D-glucopyranose residues per molecule, depending on the source; amylopectin is much larger and has an average of 100,000 to 1,000,000 D-glucopyranose residues per molecule [26,27]. When at equilibrium with its surroundings, starch granules will contain 10–15% w/w water. The amylose and amylopectin molecules in the granules can be solubilized by heating the granules in water, where they swell and eventually burst, releasing the individual molecules. Starch granules can also be dissolved in 9:1 dimethyl sulfoxide/water solutions [28]. See ❯ Fig. 1 in ❯ Chap. 6.2 for the structures of segments of amylose and amylopectin.

The amounts of amylose and amylopectin differ for starches from different botanical sources. Most so-called normal starches have 20–30% amylose and 80–70% amylopectins, respectively [29,30]. There are mutant varieties, such as waxy maize, waxy rice, and waxy potato, that are composed of 100% amylopectin. There also are the high amylose varieties, such as amylomaize-V that consists of 53% amylose and 47% amylopectin and amylomaize-VII that is 70% amylose and 30% amylopectin, just the reverse of the "normal" starches. Many of the "normal" starches have been found to have an intermediate component that is slightly branched amylose with 0.5–3% α-(1→6) branch linkages [26,27,29,30].

All starches can be completely converted into D-glucose by acid hydrolysis at high temperatures (100 °C) and by the action of the enzyme, glucoamylase, at lower temperatures (20–40 °C), when the granules are solubilized. Humans and other organisms can completely convert solubilized starches into D-glucose by the combined action of several enzymes, such as α-amylases found in saliva and in the small intestine, and α-(1→6)-glucosidase and α-(1→4)-glucosidase that are secreted by special cells in the lining of the small intestines.

Starches have been chemically modified to improve their solution and gelling characteristics for food applications. Common modifications involve the cross linking of the starch chains, formation of esters and ethers, and partial depolymerization. Chemical modifications that have been approved in the United States for food use, involve esterification with acetic anhydride, succinic anhydride, mixed acid anhydrides of acetic and adipic acids, and 1-octenylsuccinic anhydride to give low degrees of substitution (d.s.), such as 0.09 [31]. Phosphate starch esters have been prepared by reaction with phosphorus oxychloride, sodium trimetaphosphate, and sodium tripolyphosphate; the maximum phosphate d.s. permitted in the US is 0.002. Starch ethers, approved for food use, have been prepared by reaction with propylene oxide to give hydroxypropyl derivatives [31].

The solubility of the starch granules has been increased by reaction of starch granules in water with 7% hydrochloric acid for one week at 20 °C to give "Lintner soluble starch". Recent modifications to increase the solubility of starch granules have involved the reaction of the starch granules with hydrochloric acid in anhydrous alcohols, such as methanol, ethanol, 2-propanol, and 1-butanol to give a new class of limit dextrins whose average degree of polymerization can be controlled between 1800 and 30 [32,33,34]. Enzymatic conversions of starches into mixtures of maltodextrins are used in food preparations. Starch is the major source for the commercial preparation of D-glucose and D-fructose. Starches have been modified to give tertiary amino alkyl ethers, quaternary ammonium ethers, amino ethylated ethers, cyanamide ethers, starch anthranilates, cationic dialdehyde starch, carboxymethyl ethers, and carboxy starch for various applications in the sizing of paper, formation of coatings, sizing of textiles, flocculation, and emulsification technologies [35].

5.2 Properties and Occurrence of Glycogen

Glycogen is an α-glucan that is widely distributed in mammals in the liver, muscle, and brain and in fish, insects, and some species of bacteria, fungi, protozoa, and yeasts, as a reserve form of chemical energy. It is a high molecular weight polysaccharide (1×10^6 to 2×10^9 Da) composed of D-glucopyranose residues linked together by α-(1→4) glycosidic linkages with 10–12% α-(1→6) branch linkages [36]. It has been compared with amylopectin and called "animal starch." But, it is quite different from amylopectin in that it has over twice as many α-(1→6) branch linkages per molecule, giving the many chains an average chain length of 8 to 10 D-glucopyranose residues compared to 20 for amylopectin. Further, the branch linkages do not occur in clusters, as they do in amylopectin, and are randomly distributed, giving glycogen different chemical and physical properties from amylopectin. Glycogen does occur in particles or granules of about 25 nm, called β-particles [37]. The β-particles are further combined into a larger mass, called α-particles, which consists of approximately 100 β-particles. Nevertheless, in contrast to starch granules, the glycogen particles are quite water-soluble, because of the relatively high percent of branch linkages and the absence of intermolecular bonding, giving the absence of crystallinity. Glycogen reacts poorly with triiodide, giving a light brown color or no color, but never the blue color given by starch granules and amylose nor the maroon color given by amylopectin.

Glycogen has a specific function in the liver of mammals, where its primary role is to maintain the normal concentration of D-glucose in the blood. In humans, it can provide 100–150 mg of glucose per minute over a sustained period of 12 h, if necessary [38]. In skeletal muscle, its primary function is to provide immediate energy for muscle movement by being converted into α-glucopyranose-1-phosphate and in the human brain, where glycogen normally provides about 100 g of α-glucose-1-phosphate per day for energy used by the brain [38].

Blue-green algae, which are photosynthetic bacteria (cyanobacteria) and not eukaryotic algae, synthesize glycogen as a reserve energy storage polysaccharide instead of synthesizing starch [39]. Glycogen is also synthesized by nonphotosynthetic bacteria, such as *Escherichia coli* that synthesizes it intracellularly from UDPGlc [39] and *Neisseria perflava* that synthesizes it extracellularly from sucrose by the enzyme, amylosucrase [40]. The function of glycogen for these bacteria has been postulated to provide reserve energy in times of the absence of nutrients and as a source of energy for the formation of spores [39].

5.3 Properties and Occurrence of Dextrans, Alternan, Mutan, and Pullulan

Dextrans are a large family of bacterial polysaccharides that have a contiguous series of D-glucopyranose residues linked α-(1→6) to each other [41]. Over 100 strains of *Leuconostoc mesenteroides* [42], *Streptococcus mutans*, *S. sobrinus*, and *S. salivarius* produce specific enzymes, dextransucrases that synthesizes dextrans from sucrose [41]. All of the dextrans are branched, primarily by α-(1→3) glycosidic linkages, but also by α-(1→2) and α-(1→4) linkages in specific *L. mesenteroides* strains. Differences in the number and arrangements of these branches, such as having single glucose branches or long α-(1→6) linked branch chains, and the order and frequencies of the branches, impart differences in the structures and properties [41,42]. The classic prototypical dextran is the commercial product synthesized by dex-

transucrase from *L. mesenteroides* NRRL B-512F. It has 95% α-(1→6) linkages with 5% α-(1→3) branch linkages that have branches that are both single glucose residues and long α-(1→6) linked chains and contains ~10^6 to 10^8 D-glucopyranose residues. Other strains make a wide variety of dextrans with various degrees of branching, not only through α-(1→3) linkages, but through α-(1→2) and/or α-(1→4) linkages as well. In some cases, the degree of branching is as low as 3%, and in other instances, virtually every glucose residue in the backbone may be substituted with a branch linkage [41].

Some of these bacteria also elaborate glucansucrases that synthesize polysaccharides that are not considered dextrans because they do not have contiguous α-(1→6) linked main chains. *L. mesenteroides* NRRL B-1355 secretes a dextransucrase that synthesizes a B-512F-type dextran and another enzyme, alternansucrase, that synthesizes an α-glucan from sucrose that has alternating α-(1→6) and α-(1→3) linked glucose residues in the main chains with 7–11% α-(1→3) branch chains of alternating α-(1→6) and α-(1→3) linked glucose residues [41]. This α-glucan is called alternan. Another glucansucrase, mutansucrase, secreted by *Streptococcus mutans*, synthesizes a linear glucan in which the D-glucopyranose residues are linked α-(1→3). It is particularly characterized by being extremely water-insoluble in contrast to the dextrans and alternan that are highly water-soluble [41].

Pullulan is a polysaccharide that is elaborated by several species of the fungus, *Aureobasidium*, particularly *A. pullulans*. This fungus is typified by the presence of black pigments and is sometimes called "black yeast" [43]. Pullulan is a water-soluble, linear polysaccharide of D-glucopyranose residues joined together by a repeating sequence of two α-(1→4) and one α-(1→6) linkages. The structure is that of a polymer of maltotriose units joined together end to end by α-(1→6) linkages [44,45,46]. In addition to maltotriose units, it also has ~5–7% maltotetraose units located in the interior of the polysaccharide chain [47].

These bacterial polysaccharides have been considered to be "slimes"; they are often in reality loose capsules that are produced extracellularly by the bacteria. It was found that low molecular weight *L. mesenteroides* NRRL B-512F dextran could be used as a blood plasma extender and was produced on a relatively large scale during the "cold war", but also found uses as a gel-filtration material when cross-linked by epichlorohydrin to give a family of cross-linked dextrans [41].

5.4 Properties and Occurrence of D-Glucose in Cyclic Dextrins

A number of different kinds of nonreducing cyclic dextrins containing D-glucopyranose residues occur. The first to be observed were the cyclomaltodextrins (sometimes referred to in the older literature as cyclodextrins or Schardinger dextrins), which have been known for over 100 years. They were first found in rotting vegetables and then in the fermentation of starch by a heat-resistant microorganism called *Bacillus macerans*. The compounds were crystallized from alcohol solutions and shown to be α-(1→4) linked, nonreducing, cyclic dextrins composed of six, seven, and eight D-glucopyranose residues, named cyclomaltohexaose, cyclomaltoheptaose, and cyclomaltooctaose or α-CD, β-CD, and γ-CD [48]. These cyclomaltodextrins are formed from starch by the enzyme, cyclomaltodextrin glucanyltransferase (CGTase). *Bac. macerans* CGTase primarily forms α-CD; other bacteria, for example *Bac. circulans* elaborates a CGTase that primarily forms β-CD and *Brevibacterium sp.* elaborates a CGTase that primarily forms γ-CD [49]. Larger cyclomaltodextrins, having 9, 10, 11, and

12 D-glucopyranose residues were later obtained in relatively small quantities, and even later, cyclomaltodextrins having as many as 25 glucose residues were obtained [50]. The internal cavity of the cyclomaltodextrins is relatively hydrophobic, giving them the property of forming complexes with a wide variety of organic molecules [51].

Cycloisomaltodextrins, linked α-(1→6) containing seven, eight, and nine D-glucopyranose residues have been found to be formed by a bacterial cycloisomaltodextrin dextran-glucanyltransferase, acting on B-512F dextran [52]. A cyclic tetrasaccharide, containing four glucose residues with alternating α-(1→6) and α-(1→3) linkages has been obtained from the reaction of a bacterial enzyme on alternan [53]. This enzyme, 3-α-isomaltosyltransferase, is part of a 2-enzyme system that converts starch to the cyclic tetrasaccharide [54,55]. Also, *Bacillus stearothermophilus* starch branching enzyme catalyzed a reaction with amylose to give macrocyclic dextrins with one α-(1→6) linkage at the site of transglycosylation coupling [56].

Another group of cyclic dextrins is the cyclosophorans, which consist of 17–40 D-glucopyranose residues linked β-(1→2). They are produced by *Rhizobium* species involved in nitrogen-fixing nodules on the roots of legumes [59] and are also found in plant crown galls, produced by *Agrobacterium tumefaciens* [58]. The sizes of the cyclosophorans vary depending on the particular species of *Rhizobium*, which are also specific for the particular type of legume that they associate with to form the nodules. There is some evidence that the cyclosophorans play a role in the formation of the nodules and the crown galls [59].

Bradyrhizobium species synthesize a related cyclic dextrin that contains 12 D-glucopyranose residues linked by a repeating sequence of three contiguous β-(1→6) linkages followed by three contiguous β-(1→3) linkages. One of the β-(1→3) sequences has a single branched D-glucopyranose residue substituted β-(1→6) onto the center D-glucopyranose residue [60]. A cyclodextrin containing only β-(1→3) linkages (cyclolaminarinose) has been found to be elaborated by a recombinant strain of *Rhizobium meliloti* TY7 mutant that is deficient in forming cyclosophoran, but carrying the genetic locus of *Bradyrhizobium japonicum* USDA 110. The cyclolaminarinose dextrin has 10 D-glucopyranose residues, with a single laminaribiose disaccharide substituted β-(1→6) onto the ring [61].

5.5 Properties and Occurrence of Cellulose

Cellulose is usually considered the most abundant carbohydrate on the earth, occurring in all plant cell walls to the extent of approximately 50% by weight; 20–40% of the cell wall is made up of hemicelluloses, and the remaining 10–30% is the noncarbohydrate, lignin, which acts as a cross-linking and cementing agent in the plant cell wall, covalently attached to the hemicelluloses [62]. Hemicelluloses are a family of polysaccharides, with a structure similar to cellulose, but besides D-glucopyranose residues, they contain several other monosaccharide residues, such as D-xylopyranose, D-mannopyranose, D-galactopyranose, D-glucopyranose uronic acid, and L-arabinofuranose residues (see *Sect. 6*).

Cellulose is a very large, linear polysaccharide of $\sim 10^6$ to 10^8 D-glucopyranose residues, linked β-(1→4) to each other. Because of its high water-insolubility, its actual size has never been accurately determined. It is a β-glucan with a very tight helical structure in which the individual glucose residues are oriented 180°to each other [63]. Because of this conformation and the β-linkages, cellulose chains readily form intermolecular hydrogen bonds, giving

multiple chains associated together in 3-dimensional bundles that further associate with other bundles to form micelles or fibers [63,64]. These micelles make a very tough and resistant material that gives shape, strength, and water and substance impermeability to the plant cell

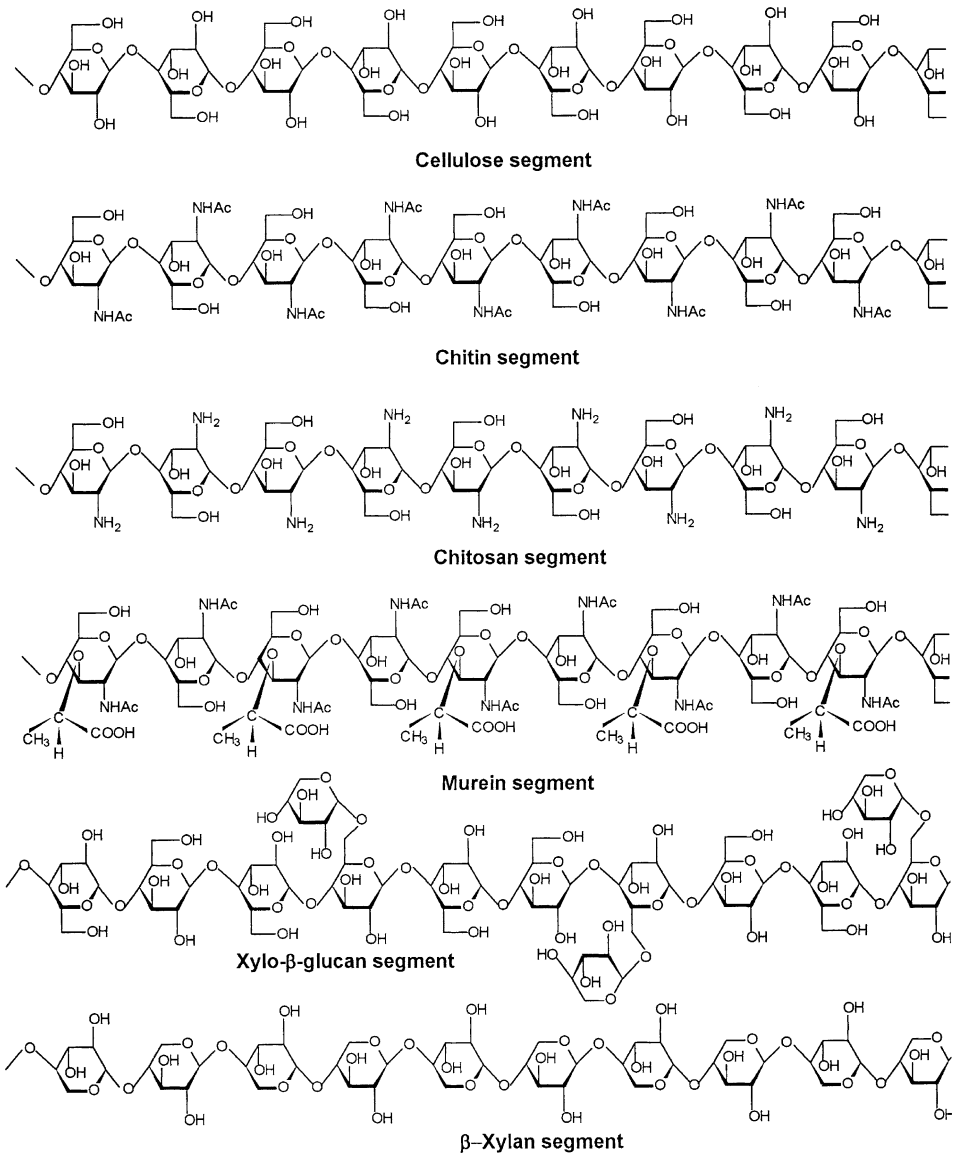


Figure 6
Haworth structures of β-(1→4) linked segments of cellulose, chitin, chitosan, murein, xylo-β-glucan, and xylan

wall. The fibers are also quite resistant to chemical and enzymatic attack. Very pure cellulose is found in the cotton boll and is also synthesized in a relatively pure form by some species of bacteria, such as *Acetobacter xylinum*, *Agrobacterium tumefaciens*, and related bacteria [65]. When one thinks of cellulose, it is usually in connection with trees and wood that come to mind. Cellulose is a major component of flax, comprising 80% (w/w), and jute, comprising 60–70% (w/w). Grasses, such a papyrus and bamboo have been important sources of cellulose going back into ancient times. Papyrus was used as an early form of paper, made from the pith of the papyrus plant, a wetlands sedge that grows to 5 meters (~16 ft) in height and was once abundant in the Nile Delta of Egypt. It was first known to have been used as a writing material in ancient Egypt (at least as far back as the First Dynasty, 3,000 BC) but it was also widely used throughout the Mediterranean, as well as in Europe and Southwest Asia, until about the 11th Century AD. Papyrus was prepared as a thin film from the outer bark that was glued together with starch paste to give it body and the ability to hold ink. Bamboo also served man from very early times, and continues to do so, as a building material to form houses, roofs, furniture, and so forth. Paper today is manufactured from several cellulose sources, such as wood chips and sawdust, the fibers of the sugar cane plant (called bagasse), maize (corn) stalks, and the straws of rye, oats, and rice. The β-(1→4) glycosidic linkage of cellulose is more resistant toward acid hydrolysis than the α-(1→6) linkage of amylopectin. Cellulose is slowly hydrolyzed by 1-M HCl at 100 °C. See ❯ *Fig. 6* for the structure of a segment of the cellulose molecule.

6 Properties and Occurrence of Hemicelluloses

Hemicelluloses are a family of four basic types of polysaccharides, composed of two or more monosaccharide residues. All have structural features similar to cellulose in that they have their main chains that are β-(1→4) linked, with the exception of the arabinoglactans that are β-(1→3) linked. The main chains are homopolysaccharides composed of a single monosaccharide residue, but they are highly branched by one or two different kinds of monosaccharides that are linked for the most part to give single monosaccharide branches.

As previously mentioned, the cell walls of most plants contain 40–60% cellulose. The remaining carbohydrate, representing 40–50% (w/w) of the cell wall is composed of hemicelluloses. The composition of the hemicelluloses varies from one plant type to another [66]. The four basic types are

1. Xyloglucans composed of α-D-xylopyranose linked 1→6 to approximately every third D-glucose residue of cellulose [66,67,68];
2. Xylan composed of D-xylopyranose linked β-(1→4) and glucurono-arabino-xylan, which is composed of β-(1→4) D-xylopyranose chain with 4-*O*-methyl-α-D-glucopyranosyluronic acid linked 1→2 and α-L-arabinofuranosyl linked 1→3 to the xylan chain [66,67,68,69];
3. Mannan is composed of β-(1→4)-D-mannopyranose chains [70]; another type of D-mannan is galactomannan that has D-galactopyranose linked α-(1→6) to the D-mannan chain [71].
4. Arabinogalactan is composed of β-(1→3) linked D-galactopyranose chain with β-(1→6) linked D-galactopyranose branches and to a lesser degree a L-arabinofuranose disaccharide,

β-L-arabinofuranosyl-(1→3)-α-L-arabinopyranosyl linked 1→6 to the D-galactopyranose chain [72,73].

Xyloglucans and xylans are widely distributed, found in most plant cell walls, see *Fig. 6* for the structures of these hemicelluloses. Glucurono-arabino-xylan is also widely distributed, especially in soft-wood trees [74]. Glucuronoxylan is prevalent in hard-wood trees [74]. Hemicellulose composed exclusively of D-mannopyranose is found in palm seed endosperm [70], where it is known as vegetable ivory or ivory nut mannan. Galactomannan are particularly found in soft-wood trees, but also are found in the seedpods of the locust bean. In particular, a galactomannan known as guar gum has been obtained from the seeds of the legume, guar, grown in the semiarid regions of India. It has found widespread use as a thickening agent in food products. Arabinogalactans are prevalent in soft woods such as, larch, black spruce, Douglas fir, cedar, and juniper [74]. Segments of the structures of D-xyloglucan and D-xylan are shown in *Fig. 6*.

6.1 Properties and Occurrence of Pectin

Pectins are related to hemicelluloses and occur in the plant cell wall in low amounts of 1–5% (w/w). They are more prevalent in fruits, for example, apple pulp (10–15%) and in orange and lemon rinds (20–30%). Pectins do have a number of chemical and physical properties that differ from the hemicelluloses. They are composed of D-galactopyranosyl uronic acids linked α-(1→4) and have a relatively wide percentage of the carboxyl groups esterified with methyl groups. There also is a small amount (1 in ~25 uronic acid residues) with L-rhamnopyranosyl (6-deoxy-L-mannopyranosyl) residues linked α-(1→2) to the D-galactopyranosyl uronic acid residues. In addition, pectins also have 2-*O*-acetyl or 3-*O*-acetyl ester groups attached to the uronic acids [75]. The average molecular weight can vary from 20,000 to 400,000 Da, with a typical average molecular weight of 100,000 Da. Pectins act in plants as an intercellular cementing agent that provides body to fruits, and of course, it is used in foods as a gelling agent, especially in the preparation of jellies and confections.

7 Cellulose-like Polysaccharides Containing *N*-Acetyl-D-Glucosamine and D-Glucosamine

There are several polysaccharides containing the β-(1→4) structure, but with monomer residues other than D-glucopyranose, such as *N*-acetyl-D-glucosamine, D-glucosamine, *N*-acetyl-D-muramic acid.

7.1 Properties and Occurrence of Chitin

Chitin is a polysaccharides with the exact same structure as cellulose but containing *N*-acetyl-D-glucosamine or D-glucosamine and are fairly widely distributed [76] (see *Fig. 6* for the structure of a segment of chitin). It is a structural polysaccharide that forms fibers, is water impermeable, and replaces cellulose in the cell walls of many species of lower organisms,

such as fungi, yeasts, green algae, and brown and red seaweeds. It also comprises the major component of the exoskeleton of insects, where it makes a hard shell-like material that is quite strong. Chitin is also found in the cuticles of worms and in the shells of crustaceans, such as mollusks, shrimps, crabs, and lobsters [77].

Chitin, like cellulose, has a highly ordered, crystalline structure in which the chains are intermolecularly hydrogen bonded in an antiparallel arrangement, a parallel arrangement, and a mixed arrangement of two parallel and one antiparallel repeating arrangement [78]. Also like cellulose, it is very insoluble in water and most other solvents. In arthropods, the chitinous shell, or exoskeleton, does not grow, and is periodically cast off or molted. After the old shell is shed, a new, larger shell is produced, providing room for further growth. Chitin is very rigid, except between some body segments and joints, where it is much thinner and allows movement of the various parts.

7.2 Properties and Occurrence of Chitosan

Chitosan is a polysaccharide very similar to chitin, except that the *N*-acetyl-D-glucosamine is replaced by D-glucosamine in which the *N*-acetyl group is removed (see ❯ *Fig. 6* for the structure of a segment of chitosan). Chitosan is found occurring naturally mixed with chitin in the cell walls of some fungi and seaweeds. It is, however, primarily produced chemically by treating chitin with strong alkali to deacetylate the *N*-acetyl-amino group [79]. The degree of deacetylation can range from 60 to 100%, giving a family of chitosans. The free amino group of chitosan has a pK_a value of ~6.5 and it can be protonated in mildly acidic solutions, giving a positive charge to the glucosamine residues. The positive charges on chitosan produce very different physical and chemical properties from chitin. Because of the repulsion of the positive charges, chitosan chains do not line up and associate to form micelles and fibers, as does cellulose and chitin. Chitosan, thus, is water-soluble at acidic pH values.

Because of the positive charges on chitosan, it has found a number of applications. It binds to negatively charged surfaces, such as mucosal membranes, and has been used as a bandage material for wounds that is biocompatible and biodegradable [80,81]. Positively charged chitosan enhances the transport of polar drugs across epithelial tissues and is used to transport drugs in humans [82]. It has been used as an enhancer for plant growth, and as an aid in the defense of plants against fungal infections. Chitosan is used in water purification, as a material in a sand filtration system where it binds fine sediment particles during filtration, greatly aiding the removal of turbidity; it also removes phosphates by ion exchange, heavy metals by chelation, and oils by hydrophobic adsorption from water [81]. Chitosan has also been found useful for the immobilization of enzymes and cells [81,82,83,84].

7.3 Properties and Occurrence of *N*- Acetyl-D-Glucosamine and *N*-Acetyl-D-Muramic Acid in Murein – The Bacterial Cell Wall

The major component of all known bacterial cell walls is a polysaccharide composed of *N*-acetyl-D-glucosamine (NAG) linked together by β-(1→4) glycosidic bonds, as in chitin, but with every other NAG residue substituted at C-3 by an ether linkage to the hydroxyl group of L-lactic acid to give *N*-acetyl-D-muramic acid (NAM) [85,86,87,88]. This results in a nine-

carbon *N*-acetyl-amino-sugar acid, with a repeating β-(1→4)-NAG-NAM sequence of 40–150 residues, giving a polysaccharide, called murein [89].

A pentapeptide is attached to the carboxyl group of the L-lactic acid by an amino group that forms a peptide (amide) bond [90]. Attached to this pentapeptide is a pentaglycine linked to the ε-amino group of an L-lysine by a carboxyl group. The glycine end forms a cross-link to another decapeptide [90]. Using slightly different amino acids in both the pentapeptide and the cross-linking peptide gives different peptides that are genus-dependent.

The murein-peptidoglycan gives rigidity and different specific shapes, such as rods, spheres, or spirals to bacterial cells. Because of the cross-linking of the murein chains, the peptidoglycan is considered one giant, bag-shaped macromolecule [91]. The structures of segments of chitin, chitosan, and murein are shown in *Fig. 6*.

7.4 Properties and Occurrence of Glycosaminoglycans Composed of Amino Sugars and Uronic Acids

Glycosaminoglycans make up a group of polysaccharides that are found in animal tissues. They are composed of repeating disaccharides units of *N*-acetyl-D-glucosamine or *N*-acetyl-D-galactosamine and D-glucopyranosyluronic acid residues. The linkages are primarily β at positions 3 and 4. They are most often attached to protein backbones, forming what is called a proteoglycan [92].

7.4.1 Hyaluronic Acid

Hyaluronic acid consists of repeating disaccharides of β-D-glucopyranosyluronic acid-(1→3)-*N*-acetyl-D-galactosamine linked β-(1→4) to the next disaccharide. This proteoglycan can have between 500 and 50,000 residues per chain [92]. Hyuronic acid is found widely distributed in mammalian cells and tissues, where it is found in synovial fluid that lubricates the joints, in the vitreous humor of the eye, and in connective tissue, such as the umbilical cord, the dermis, and the arterial wall. It also occurs as a capsular polysaccharide around certain bacteria, such as pathogenic streptococci [92].

7.4.2 Chondroitin Sulfate

Chondroitin sulfate also consists of a repeating disaccharide of β-D-glucopyranosyluronic acid-(1→3)-*N*-acetyl-D-galactosamine linked β-(1→4) to the next disaccharide unit, with sulfate groups attached to C-4 or C-6 of the *N*-acetyl-D-galactosamine residue. It occurs as a major component of cartilage found in the cornea of the eye, the aorta, the skin, and lung tissues, where it is located between fibrous protein molecules. Chondroitin sulfate provides a soft and pliable texture to these tissues [92].

7.4.3 Dermatan Sulfate

Dermatan sulfate is derived from chondroitin 4-sulfate by the action of a C-5 epimerase that inverts the carboxyl group of the β-D-glucuronic acid, giving the very rare sugar, α-L-idopy-

ranosyl uronic acid (α-L-iduronic acid). Some of the L-iduronic acids are sulfated at C-2. Dermatan sulfate is found primarily in the skin [93].

7.4.4 Keratan Sulfate

Keratan sulfate consists of the disaccharide, *N*-acetyl-lactosamine, linked β-(1→3) to the D-galactopyranose residue of the next *N*-acetyl-lactosamine unit. Keratan sulfate is the most heterogeneous of the glycosaminoglycans, with variable sulfate content linked to C-4 or C-6 of the D-galactopyranose residue in lactosamine, and small amounts of L-fucose (6-deoxy-L-galactose), D-mannose, and *N*-acetyl-neuraminic acid residues [93] (also see ❯ *Sects. 10.2* and ❯ *14* on the occurrence of *N*-acetyl-D-neuraminic acid and L-fucose in other systems). Keratan sulfate is found in the cornea, on the surfaces of erythrocytes, in cartilage, and in bone.

7.4.5 Heparan Sulfate

Heparan sulfate consists of the repeating disaccharide β-D-glucopyranosyluronic acid-(1→4)-*N*-sulfato-2-amino-2-deoxy-α-D-glucopyranosyl linked (1→4). This polysaccharide is linked to a core protein to give a proteoglycan that is found as a matrix component of arterial wall, lung, heart, liver, and skin [93].

8 Polysaccharides Containing Uronic Acids That Have Some of Their Carboxyl Groups Inverted by a C-5 Epimerase to Give New Polysaccharides with New Properties

8.1 Heparin Sulfate

Heparin sulfate is formed from heparan sulfate by the action of an enzyme, C-5 epimerase that inverts the carboxylate group attached to C-5 of the D-glucopyranosyl uronic acid residues to give the rare and unusual sugar, α-L-idopyranosyl uronic acid. Heparin sulfate is released from the heparin of proteoglycans of mast cells into the blood stream when there is an injury to blood vessels, in the heart, liver, lungs, and skin. The release of heparin near the site of the injury acts as an anti-coagulating agent, preventing massive clotting of the blood and, hence, preventing run-away clot formation [93].

8.2 Alginates

Alginates are found primarily in brown seaweeds in amounts of 18–40% by weight of the plant. The majority is extracellular, being located between the cells [94]. One of the major species of seaweeds that contains alginates is the giant kelp, *Macrocyctis pyrifera*. It grows along the California coast of the US, the northwestern and southwestern coasts of South America, and the southeastern coasts of Australia and New Zealand [95].

Alginates are formed from poly-D-mannopyranosyluronic acid by the action of a C-5 epimerase that inverts the C-5 carboxyl group to give approximately 33% (w/w) of the

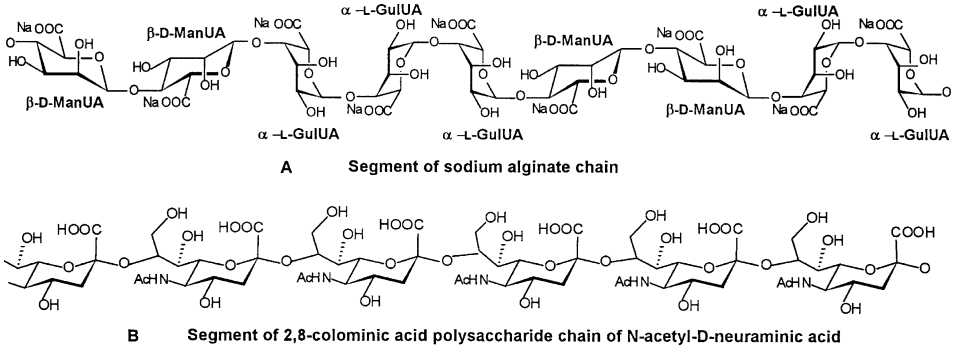


Figure 7
Conformational structures of segments of sodium alginate, containing β-D-mannopyranosyl uronic acid, and α-L-gulopyranosyl uronic acid; and 2,8-α-colominic acid, containing *N*-acetyl-α-D-neuraminic acid, linked (2→8)

rare and unusual sugar α-L-gulopyranosyl uronic acid. The ratio of the two uronic acids varies with different species of seaweed, type of tissue, and age of the plant [96]. The two kinds of uronic acids are combined together in blocks of variable numbers and also as alternating residues. In the brown seaweed, alginates most frequently exists as the calcium salt and are converted to the sodium salt when isolated.

The biological role that alginates play in seaweeds is that of a protective agent against desiccation during low tide. An unusual and useful property of sodium alginate is the ability to instantly form gels when in contact with divalent metal ions, such as calcium, barium, strontium, copper, cobalt, nickel, and so forth [97]. The strength and firmness of the gels are proportional to the amount of α-L-gulopyranosyluronic acid present in the alginate [98]. The strength and firmness is also dependent on the starting concentration of the alginate, the higher the concentration, for example 5% (w/v), gives very strong, firm gels, even though it is only 5% calcium alginate and 95% water.

Sodium alginate is used in food preparations as a thickening agent, a stabilizer, and an emulsifier in ice cream, cream cheese, salad dressings, frozen foods, pharmaceuticals, and so forth. Calcium alginate is the major ingredient in the "pimento" found in stuffed olives. A very important use of calcium alginate is the formation of gels that are used to encapsulate enzymes, hormones, drugs, and whole cells for carrying out various processes while being immobilized [99]. See *Fig. 7* for the conformational structure of a segment of alginate.

Alginates are also produced extracellularly by some bacteria, such as *Pseudomonas aeruginosa* and *Azotobacter vinelandii* [100], where they are believed to play a role in biofilm formation, pathogenesis, and soil aggregation. Their gels, however, are inferior to seaweed alginates, because of the presence of *O*-acetylation, which inhibits gel formation.

9 Occurrence and Properties of Plant Exudate Polysaccharides

Several complex polysaccharides are secreted by plants to seal wounds. Gum arabic is a complex material, containing protein, lipid, and carbohydrate, produced by Acacia trees found in the arid regions of Africa, in Nigeria, Mauritania, Senegal, and the Republic of Sudan. The

structure of the polysaccharide portion has a main chain of D-galactopyranose residues linked β-(1→3) and D-glucopyranosyl uronic acid linked β-(1→6). The main chains have branch chains of two to five residues, consisting of α-L-arabinofuranosyl, α-L-rhamnopyranosyl, β-D-glucopyranosyl uronic acid, and 4-*O*-methyl-β-D-glucopyranosyl uronic acid [101]. The latter two uronic acids occur most frequently at terminal ends of the branched chains.
Another plant exudate is gum ghatti or Indian gum that can be obtained from a large tree grown in the deciduous forests of India and Sri Lanka. Gum ghatti is composed of L-arabinofuranose, D-galactopyranose, D-mannopyranose, D-glucopyranose uronic acid, and D-xylopyranose in approximately the molar ratios of 10:6:2:2:1 [102]. A third exudate gum is gum tragacanth that is primarily obtained from trees growing in Iran, Syria, and Turkey. It is a highly branched arabinogalactan with α-D-xylopyranose and α-L-fucopyranose branch residues [103]. These gums are primarily used to increase viscosity, provide body, stabilize emulsions, and suspend other materials and have been used for thousands of years in confectioneries, cosmetics, textiles, coatings, paints, pastes, and polishes.

10 Occurrence of Carbohydrates in Bacterial Polysaccharides

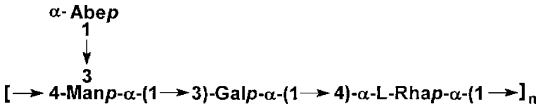
A large number of bacterial polysaccharides are known [104]. The major structural component of the bacterial cell wall is a polysaccharide, known as murein and composed of a repeating unit of one *N*-acetyl-D-glucosamine and an *O*-lactyl substituted *N*-acetyl-D-glucosamine (*N*-acetyl-D-muramic acid) see *Sect. 7.3*.

10.1 Xanthan, a Water-Soluble Bacterial Polysaccharide

In the 1950s, the US Department of Agriculture's Northern Regional Research Laboratories in Peoria, Illinois screened bacterial cultures to obtain a replacement for the plant exudates, which had become rare and expensive. They found that *Xanthomonas campestris*, when grown on D-glucose in an aerobic submerged fermentation, produces xanthan, a water-soluble polysaccharide gum [105,106]. It has a cellulose backbone of β-(1→4) linked D-glucopyranose residues with a trisaccharide of D-mannopyranose linked β-(1→4) to D-glucopyranosyl uronic acid linked β-(1→2) to a D-mannopyranosyl [β-D-Man *p*-(1→3)-β-D-Glc *p*UA-(1→2)-α-D-Man *p*-(1→3)-] attached to every other D-glucose residue in the cellulose chain by an α-(1→3) linkage [107,108]. Some of the nonreducing terminal D-mannopyranose residues of the trisaccharide have a cyclic six-membered pyruvic acid ketal attached to C4 and C6, and some of the inner D-mannopyranose units are acetylated at C6 [109].
The branching of the cellulose chain by the trisaccharide makes the otherwise insoluble cellulose molecule water-soluble. At low concentrations, xanthan produces high viscosities at low temperatures. These properties provide a number of uses as a thickener and bulking agent for prepared foods, such as salad dressings, syrups, toppings, relishes, ice cream, and baked goods. It is also used as a carrier and emulsifying agent in cosmetics and pharmaceuticals [110].
There are other bacterial gel polysaccharides with different properties, composed of D-glucopyranose, L-rhamnopyranose, D-glucuronic acid, and L-mannopyranose that are obtained from *Pseudomonas elodea* (syn. *Sphingomonas elodea*), which produces gellan, and also species of *Alcaligenes* that produce welan and rhamsan [111].

10.2 Pathogenic Bacterial Capsular Polysaccharides

Salmonella species have an O-antigen that is a heteropolysaccharide, imparting pathogenicity to the organism. It is composed of a repeating tetrasaccharide unit, made up of a sequence of D-mannopyranose, L-rhamnopyranose, and D-galactopyranose, with a variable 3,6-dideoxy-D- or L-hexose linked to the D-mannose residue as a branch residue [112]. *Salmonella* easily mutates and over 100 different kinds of capsular polysaccharides have been identified for various species and mutants. The polysaccharides vary according to the linkage positions and the α- or β-configurations [112]. They also vary with the nature of the attachment of four different 3,6-dideoxy carbohydrate residues: D-paratose (3,6-dideoxy-D-glucopyranose), D-tyvelose (3,6-dideoxy-D-mannopyranose), D-abequose (3,6-dideoxy-D-galactopyranose) and L-colitose (3.6-dideoxy-L-galactose) [113]. *Salmonella* readily mutates the structure of this polysaccharide, giving very wide diversity of structures and a basis of avoiding antibody neutralization.



Scheme 4
A typical *O*-antigen *Salmonella* capsule polysaccharide

Streptococcus pneumoniae strains constitute a large group of pathogens, responsible for bacterial pneumonia. All virulent strains have a voluminous capsule that is responsible for their pathogenicity. The capsules are all relatively complex heteropolysaccharides with diverse structures. The monosaccharide residues contain D-glucopyranose, D-glucopyranose uronic acids, L-rhamnopyranose, and *N*-acetyl-D-glucosamine [113]. There are some capsules with unusual carbohydrates, such as sugar alcohols (glycerol, erythritol, D-threitol, and ribitol), amino-sugars (*N*-acetyl-L-fucosamine, *N*-acetyl-D-mannosamine, *N*-acetyl-2-amino-2,6-dideoxy-L-talose [commonly called L-pneumosamine]), as well as D-galactofuranose and phosphodiesters. The structures are repeating tetra-, penta-, and hexa-saccharides. Like *Salmonella O*-antigens, the repeating units have permuted glycosidic linkages at different positions and with either α- or β-configurations to give a wide diversity of structures, with over 120 different known structures [114].

An unusual acidic polysaccharide capsule is produced by the Gram-negative pathogens, *Neisseria meningitides* and *Escherichia coli*. These polysaccharides contain the unusual nine-carbon sugar, *N*-acetyl-D-neuraminic acid, which is formed by an enzyme catalyzed aldo-condensation between the methyl group of pyruvic acid and the aldehyde group of *N*-acetyl-D-mannosamine, followed by the formation of a six-membered ring with a three hydroxy-carbon side chain. The sugar acid is linked α-(2→8) or α-(2→9) with itself to give a linear polysaccharide, called colominic acid [115,116] (see ❯ *Fig. 7* for the structure of α-(2→8) colominic acid). An interesting variation is the colominic acid produced by *E. coli* Bos-2 that has the alternating sequence of α-(2→8) and α-(2→9) linkages [117].

11 Properties and Occurrence of D-Fructose in Polysaccharides

Polysaccharides that exclusively contain D-fructose are known as fructans and there are two known kinds, inulin and levan. Inulin is a polysaccharide containing β-D-fructofuranose linked (2→1) [118]. Inulins are found in the roots and tubers of the family of plants known as the Compositae, which includes asters, dandelions, dahlias, cosmos, burdock, goldenrod, chicory, lettuce, and Jerusalem artichokes. Other sources are from the Liliacae family, which includes lily bulbs, onion, hyacinth, and tulip bulbs. Inulins are also produced by certain species of algae [119]. Several bacterial strains of *Streptococcus mutans* also produce an extracellular inulin from sucrose [120].

Levan is a polysaccharide containing β-D-fructofuranose residues linked (2→6) with (2→1) branch linkages. They are primarily found in grasses [119] and are produced extracellularly by several bacterial strains of *Bacillus subtilis*, *Aerobacter levanicum* (syn. *Erwinia herbicola*) [121], and *Streptococcus salivarius* [122]. They are of higher molecular weight than the inulins, having 100–200 D-fructofuranose residues per molecule. The branch chains are relatively short, containing 2–4 D-fructofuranose residues.

12 Properties and Occurrence of Sugar Alcohols

12.1 Glycerol

When aldoses or ketoses are reduced, sugar alcohols are formed. For example, glycerol is a simple, three carbon sugar alcohol, formed by the reduction of glyceraldehydes. It is found as a major component in two types of lipids, triacylglycerol (triglyceride fats and oils) and phospholipids. In the former, the three hydroxyl groups of glycerol are esterified by fatty acids. In the latter, glycerol is esterified by two fatty acids at the first two carbons and by phosphoric acid at the third carbon. The phosphoric acid is further esterified by the hydroxy groups of ethanolamine, *N*,*N*,*N*-trimethyl ethanolamine (choline), or by the hydroxy group of L-serine. The triglycerides make up the well-known fat deposits found in adipose tissue and the phospholipids are major components found in the lipid bilayers of membranes of cells and organelles and play important roles in nerve transmission. Glycerol is also a common component in the teichoic acids (see *Sect. 12.3*).

Free glycerol is obtained from the saponification of fats and oils. It is a slightly sweet, highly water-soluble liquid. It has the ability to absorb water, making it a valuable humectant and an emollient for skin conditioners. It is also used as a plasticizer in the formation of polymeric materials and is used in the manufacture of pharmaceuticals and the explosive, trinitroglycerine.

12.2 Properties and Occurrence of Free Sugar Alcohols, D-Glucitol, D-Mannitol, Ribitol, Xylitol, and D-Arabinitol

D-glucose can be reduced either chemically or enzymatically to give D-glucitol (frequently called D-sorbitol). It was first obtained from the fresh juice of the berries of the mountain ash [123]. D-Glucitol occurs widely in plants, being found in algae and higher plants. It is especially prevalent in red seaweed, where it occurs to the extent of 10–14% by weight [124]

and is found in relatively large amounts in pears, apples, cherries, prunes, peaches, and apricots, where it imparts a sweet taste to these fruits [125].

D-Mannitol is also widely distributed in plants and was the first crystalline sugar alcohol to be obtained from a natural source, the manna ash [126]. It is also found in large amounts (70–90% w/w) in the exudates of the olive and the plane trees [127]. D-Mannitol is found in relatively large amounts in seaweeds of *Laminaria* and *Mycrocystis* species [128]. Species of the mold *Aspergillus*, produce D-mannitol by fermentation, using D-glucose or acetate as carbon sources [129].

Ribitol, the sugar alcohol from the reduction of D-ribose, is found as a constituent of the vitamin riboflavin (vitamin B_2). It is also a constituent of the teichoic acids, see *Sect. 12.3*.

The reduced product of D-xylose is xylitol, which has a very sweet taste and also imparts an unusual cooling sensation. It is found in several fruits, such as plums, raspberries, and strawberries, where it occurs to the extent of about 1% by weight and gives a distinctive and pleasant taste to these fruits. D-Arabinitol is found in mushrooms in amounts as high as 9–10% by weight [130], in lichens [131], and in avocado seeds [132]. D-Arabinitol is produced by some species of yeast (*Debaryomyces subglobosus* and *Endomycopsis chodati*) through fermentation of D-glucose, D-mannose, and sucrose [133].

12.3 Sugar Alcohols in Teichoic Acids

The teichoic acids are bacterial polymers of sugar alcohols (glycerol or ribitol) and phosphoric acid joined end to end by phosphodiester linkages to the primary alcohol groups. They are found in conjunction with the peptidoglycan of Gram-positive bacterial cell walls [134]. The C2 hydroxy group of glycerol is frequently acylated by D-alanine or glycosylated by *N*-acetyl-D-glucosamine or D-glucopyranose [135]. In some *Bacillus* species phosphodiester linkages join glycerol units between the C1 hydroxy of one unit to the C2 hydroxy of the next unit. Ribitol residues are joined together between the C1 hydroxyl of one unit to the C5 hydroxy of the adjoining unit. The C3 or C4 hydroxy groups can be acylated by D-alanine and the C2 hydroxyl group can be glycosylated by a number of different carbohydrate residues, for example, *N*-acetyl-D-glucosamine, D-glucopyranose, and di- or tri-saccharides of D-glucopyranose. More complex teichoic acids occur that have a repeating sequence of glycerol joined (1→4) to *N*-acetyl-D-glucosamine by a phosphodiester linkage and *N*-acetyl-D-glucosamine joined (1→3) by a phosphodiester linkage to the next glycerol unit [136].

13 Properties and Occurrence of Deoxy Sugars

The most abundant and probably best known deoxy sugar is 2-deoxy-D-ribofuranose, which is found as the carbohydrate component in the genetic polymer, deoxyribonucleic acid, the carrier of genes in the chromosomes of living organisms. Other deoxy sugars include 6-deoxy-L-mannose (L-rhamnose), which is found in glycosides and in *Salmonella* sp. *O*-antigen polysaccharides (see *Sect. 10.2*). The third deoxy sugar is 6-deoxy-L-galactose (L-fucose), found in glycocoproteins, such as the blood group substances (see *Sect. 14*). The fourth deoxy sugar, 6-deoxy-D-glucose (D-quinovose) is found in acarbose, the naturally occurring pseudotetrasaccharide, produced by *Actinoplanes* sp. fermentation. Acarbose is an inhibitor of α-glucosidase [137]. It also occurs in some of its analogues, such as

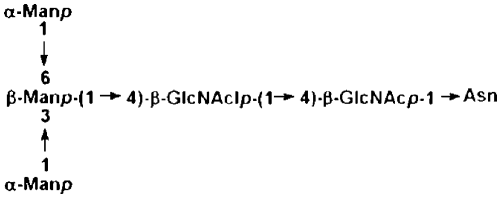
α-acarviosine-(1→6)-cellobiose and α-acarviosine-(1→6)-lactose, which act as inhibitors of β-glucosidases and β-galactosidase [138]; and α-maltohexaose-(1→4)-acarbose and α-maltododecaose-(1→4)-acarbose, which are potent inhibitors of α-amylases in the nM range [139]. Four naturally occurring 3,6-dideoxy sugars appear in the different *Salmonella* sp. *O*-antigen capsular polysaccharides, see *Sect. 10.2*, for their names and structures. Four 2,6-dideoxy sugars (2,6-dideoxy-D-ribo-hexaose [D-digitoxose]; 2,6-dideoxy-3-*O*-methyl-D-ribo-hexose [D-cymarose]; 2,6-dideoxy-D-xylo-hexose [D-boivinose]; and 2,6-dideoxy-3-*O*-methyl-D-xylo-hexose [D-sarmentose]) are found in a number of plants, as the carbohydrate component of the so-called cardiac glycosides [140].

14 Properties and Occurrence of Carbohydrates in Glycoproteins

Glycoproteins make up a large class of important biological compounds. It is estimated that over 75% of the known (~3,000) proteins are glycosylated. The carbohydrates are believed to mediate a number of biological functions: (1) the correct folding of a protein tertiary structure after biosynthesis, (2) establishment and stabilization of protein conformation, (3) secretion of proteins through membranes, (4) control of protein turnover, (5) protection of proteins from proteinase hydrolysis, (6) increase in protein water-solubility, (7) biological recognition involved in growth, cell differentiation, organ formation, fertilization, processes of bacterial and viral infections, formation of tumors, tumor metastasis, allergies, and autoimmune diseases.

Carbohydrates are primarily attached to proteins in two ways: (1) by linkage of C1 to the amide nitrogen of L-asparagine, giving *N*-linked carbohydrate proteins and (2) by formation of acetal linkages with the hydroxyl group of L-serine or L-threonine, giving *O*-linked carbohydrate proteins. The carbohydrate can be a single monosaccharide residue or it can be an oligosaccharide, containing several monosaccharide residues. There are six major carbohydrates involved in glycoproteins; they are *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, D-mannopyranose, D-galactopyranose, L-fucose, and *N*-acetyl-D-neuraminic acid [141].

The attachment of carbohydrate to nitrogen is invariably by *N*-acetyl-D-glucosamine and the attachment to oxygen is invariably by *N*-acetyl-D-galactosamine. *N*-linked carbohydrates are invariably composed of a "core" pentasaccharide [142] of the following structure:



Scheme 5
Core oligosaccharide for *N*-linked glycoproteins

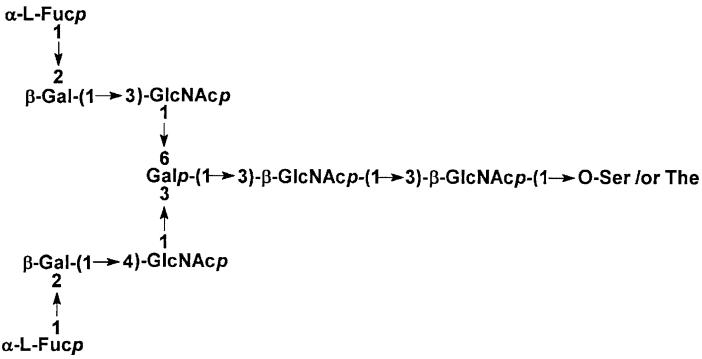
The *N*-linked glycosides can be classified into three families that result from the further attachment of monosaccharides to the two branched D-mannopyranose residues at the nonreducing-end of the core pentasaccharide. These additional residues make up the variable regions of the oligosaccharides. The first family is the "high mannose family" that has additional

α-D-mannopyranose residues attached to the two terminal D-mannopyranose residues of the core pentasaccharide. These residues are linked α-(1→3) and α-(1→6). Substitution is terminated by α-(1→2) linkages of the D-mannopyranose residues. An example of this type of *N*-glycoside is found attached to ovalbumin.

The second family of *N*-linked oligosaccharides are called the "lactosamine family" in which the D-mannose residues of the core pentasaccharide are substituted 1→2 by lactosamine, which is a lactose analogue with *N*-acetyl-D-glucosamine substituted for D-glucopyranose at the reducing-end of lactose. The lactosamine is frequently substituted by *N*-acetyl-D-neuraminic acid (for the structure of *N*-acetyl-D-neuraminic acid, see the monomer residue in colominic acid, *Sect. 8.2* and *Fig. 7*) linked 2→3 or 2→6 [142,143]. The third family has a mixed structure of the high mannose and lactosamine families [144,145,146,147].

The *O*-linked saccharides are not as common as the *N*-linked saccharides. A relatively simple saccharide is that attached to L-threonine of the highly glyosylated (1 out of every 3 amino acid residues), antifreeze protein found in the blood sera of fish living in the Arctic and Antarctic waters. This glycoside is a disaccharide, β-D-Galp-(1→3)-α-D-GalNAc *p* [147].

Lactosamine and isolactosamine (lactosamine analogue with D-galactopyranose linked β-(1→6) to *N*-acetyl-D-glucosamine) are a well characterized set of *O*-linked oligosaccharides that make up the ABO human blood group substances [148]. They are on the surface of erythrocytes and divide human blood into four distinct types. The following core structure makes up the *O*-blood type and is found in all four blood types:



Scheme 6
Type O human blood group oligosaccharide

The core is composed of nine monosaccharide residues with two chains terminating in β-D-galactopyranose residues linked (1→4) and (1→3). The other three human blood groups have two additional monosaccharide residues added to the two β-D-galactopyranose residues of the two chains. A-type human blood group has two α-D-galactosamine residues, one each linked (1→3) to the ends of the two chains; B-type has two α-D-galactopyranose residues, one each linked (1→3) to the ends of the two chains; and AB-type has a mixture of α-D-galactosamine and α-D-galactopyranose residues, one each linked (1→3) to the two chains. *O*-Type blood is the universal blood donor and can give blood to all four types, but can accept blood only from *O*-type donors; AB-type blood is the universal blood acceptor and can accept blood from all four types; A-type and B-type can accept blood from *O*-type donors or from donors

with their own blood type, as they make antibodies against either the A- or B-types, and AB-type donors, precipitating the blood [148,149,150].
There are additional blood group variations. A common variation is an isomerization in which α-L-fucopyranose is moved from β-D-Galp to β-D-GlcNAcp and linked (1→4) to give the Lewis-a blood type. A second and related variation is the addition of another α-L-fucopyranose residue to β-D-GlcNAcp linked (1→4) to give two α-L-Fucp residues on the first chain, giving Lewis-b blood type. These kinds of variations can occur for each of the ABO blood types, giving *O*-type-Lewis-a, *O*-type-Lewis-b, A-type-Lewis-a, and so forth: *O*-Le^a, *O*-Le^b, A-Le^a, A-Le^b, B-Le^a, B-Le^b, AB-Le^a, AB-Le^b [150].

15 Separation and Purification of Carbohydrates

The source and the specific physical and chemical properties of carbohydrates determine the methods that are used for their separation and purification. Mono-, di-, tri- and sometimes higher-saccharides, for example maltodextrins, isomaltodextrins, and raffinose-sucrose dextrin series, are usually quite soluble in water. Carbohydrates, thus, are often obtained by the extraction of natural materials with hot water. As many impurities as possible are removed in an extraction mixture, such as salts, proteins, and lipids. Salts can be removed by precipitation and/or the use of ion exchangers. Lipids are removed with organic solvents, such as a 2:1 mixture of chloroform and methanol, and proteins are precipitated with acids and heat. High amounts of alkali and acid, however, should be avoided. Frequently, some of the last impurities in the aqueous extract, especially colored yellow to brown materials, can be removed by adding activated charcoal and filtering it out to give a clear solution before the extract is concentrated. The concentrated carbohydrate extract is obtained at an elevated temperature (50–60 °C) and an organic solvent such as methanol or ethanol is slowly added to the point where the clear solution just becomes cloudy. The solution is then cooled to ≈20 °C to give crystallization of the carbohydrate and then 4 °C to obtain additional crystals. Monosaccharides and disaccharides will often crystallize, while higher oligosaccharides are frequently obtained as amorphous precipitates that can be removed by centrifugation or filtration and dehydrated.
Many different chromatographic methods of separation (on charcoal, BioGel, silica gel, hydroxyapatite, paper) can be used on a preparative scale to give pure materials that can be studied and used even though they are not crystalline. Two typical examples are given for the isolation, purification, and crystallization of a monosaccharide, α-D-xylopyranose, and a disaccharide, lactose, from natural sources.

15.1 Isolation and Purification of α-D-Xylopyranose from Corn Cobs

Coarsely ground corn cobs or crude xylan can be used as starting materials. The xylan in either source is hydrolyzed with 7% (v/v) sulfuric acid by refluxing for 2.5 h. The mixture is filtered through cloth on a Büchner funnel with as much liquid as possible obtained by suction. The residue is washed with an equal volume of water by suspension as thin slurry and then filtered. A few drops of 1-octanol are added to the combined filtrates that are neutralized with barium carbonate. The solids (primarily barium sulfate) in the mixture are filtered and the residue washed by suspension in water and filtered. If corn cobs are used as the starting material,

approximately one-sixth of a cake of baker's yeast is finely suspended in 10–15 mL of water and added to the clear filtrate that is covered with a cotton plug. Fermentation is allowed to go ≈15 h at 37 °C to remove D-glucose (if crude xylan is used, this step can be omitted). After removal of the yeast, activated charcoal is added with an equal weight of Celite 535 and the mixture is filtered by suction. The filtrate is concentrated to a syrup under reduced pressure at 50–60 °C, and three volumes of methanol are added with stirring. The solution is filtered and concentrated to syrup under reduced pressure. The syrup is dissolved in water and passed through a column (5.5 × 50 cm) of equal amounts of activated charcoal and Celite 535. The column is then washed with 6 L of water and the washings and the original filtrate are combined and concentrated under pressure to a syrup (~30 mL) that is filtered through a coarse sintered-glass filter. The filtrate is allowed to stand at 20 °C until crystallization of α-D-xylopyranose is complete. The crystals are removed by filtration and washed with cold (4 °C) 85% (v/v) aqueous methanol. A second crop of crystals are usually obtained by placing the supernatant at 4 °C [151].

15.2 Isolation and Purification of Lactose from Milk

Commercial skimmed (defatted) milk contains ~3% casein, 0.7% albumin, 4–5% lactose, and 1% minerals, along with small amounts of lactosamine and lactosamine oligosaccharides, with the remainder being water. The casein is first precipitated by warning to 40 °C and the addition of 1:10 (v/v) glacial acetic acid and water to 200 mL of milk, with continuous stirring. The dilute acetic acid is added until casein no longer separates. The precipitated casein is removed by centrifugation. Then 5 g of calcium carbonate is immediately added and stirred for ~5 min and then the solution is heated to boiling for ~10 min. This produces almost complete precipitation of the albumin, which is removed by vacuum filtration. The filtrate is concentrated by roto-vacuum evaporation to ~30 mL. Then 166 mL of hot ethanol is added, along with 5 g of activated charcoal; after it has been mixed well, the warm solution is filtered through a bed of Celite. The clear filtrate is allowed to stand 15–25 h at 20 °C or longer for crystallization. When crystallization is complete, the crystals are removed by filtration and a second crop of crystals are obtained by placing the clear solution at 4 °C.

15.3 Analysis, Isolation, and Purification of Monosaccharides and Oligosaccharides

Individual monosaccharides and their reduced sugar alcohols can be separated and analyzed by multiple ascent silica-gel, thin-layer chromatography [152], as well as the more complex mixtures of a series of homologous oligosaccharides, such as maltodextrins, isomaltodextrans, cellodextrins, chitosan- and chito-dextrins, cyclomaltodextrins, and the raffinose-sucrose dextrins can be quantitatively analyzed by multiple ascent silica-gel, thin-layer chromatography (TLC), followed by scanning densitometry [152,153,154]. See ❯ *Fig. 8* for a TLC separation of maltodextrins and cyclomaltodextrins. Pure individual oligosaccharides can be obtained in 50–200 mg amounts by preparative descending paper chromatography, using 70:30 (v/v) propanol-1/water solvent on 23 × 54 cm Whatman 3MM paper for 24–36 h on which the saccharides are separated, and detected by $AgNO_3$/NaOH/$Na_2S_2O_3$ development of a 1-cm strip

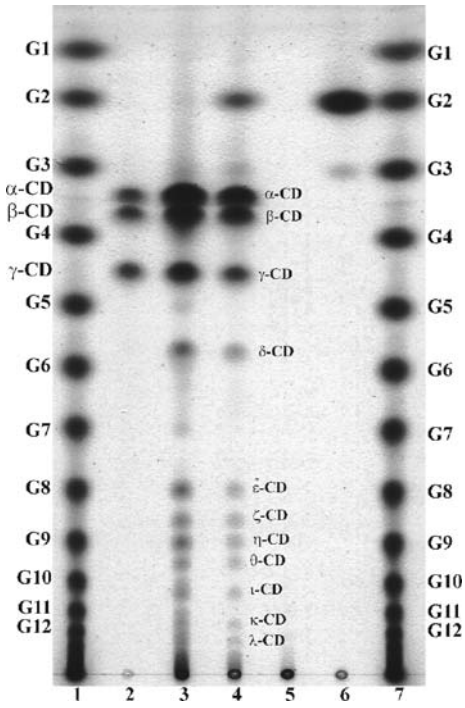


Figure 8

Thin-layer silica-gel chromatographic (TLC) separation of maltodextrins and cyclomaltodextrins and using Whatman K5 silica gel plate, irrigated 18 cm three-times with 85:25:55:50 volume proportions of acetonitrile, ethyl acetate, propanol-1, water solvent. The carbohydrates were visualized on the plate by dipping it into a methanol solution, containing 0.3% (w/v) *N*-(1-naphthyl)ethylene diamine and 5% (v/v) sulfuric acid, dried, and heated at 120 °C for 10 min. The saccharides can be quantitated by scanning densitometry [152,153]. Lanes 1 and 7 are D-glucose (G1) and maltodextrins (G2 to G12); lane 2, cyclomaltohexaose (α-CD), cyclomaltoheptaose (β-CD), and cyclomaltooctaose (γ-CD); lane 3, a mixture of maltodextrins and cyclomaltodextrins; lane 4, cyclomaltodextrins (α-CD) to (λ-CD), with 6 to 16 D-glucopyranose residues; and lane 6 is maltose and maltotriose. From [166], reproduced by permission of the publisher, Elsevier Press

on each side of the paper. The paper is sectioned, and then the individual saccharides are eluted from the sectioned pieces of paper in pure form [155]. They can also be obtained in pure form in larger quantities by charcoal-Celite column chromatography: for example, cellodextrins [156], isomaltodextrins [157], maltodextrins [158], and xylodextrins [159] have been prepared in this way. Sialyl oligosaccharides from human milk have been separated by ion-exchange chromatography [160] and maltodextrins have been separated by high performance liquid chromatography (HPLC) [161,162].

Capillary electrophoresis has been used to separate and analyze synthetically modified carbohydrates in the nanogram to milligram range [163]. Fluorophore-assisted capillary electrophoresis (FACE) has successfully been used to separate nanogram amounts of maltodextrins, containing 4–76 D-glucose residues [164,165,166], see *Fig. 9*. Many carbohydrates can be analytically separated by matrix-assisted, laser desorption, ionization-time of flight,

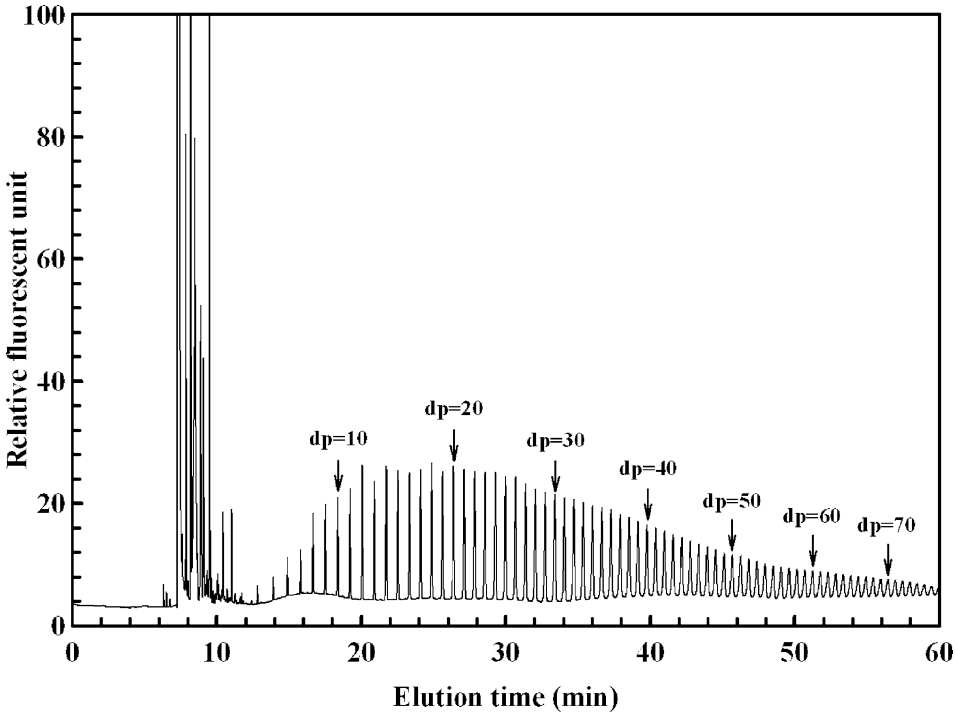


Figure 9
Fluorophore-assisted capillary electrophoresis (FACE) analysis of maltodextrins from G2 to G77. From [166], reproduced by permission of the publisher, Elsevier Press

mass spectrometry (MALDI-TOF MS). For example, cyclomaltodextrins, containing 6–25 D-glucose residues are readily analyzed by this technique [166] (see *Fig. 10*).

15.4 Separation and Purification of Water-Soluble Polysaccharides

Water soluble polysaccharides found in bacterial fermentations or produced enzymatically, such as the dextrans or xanthan, and so forth can be obtained and purified from the aqueous solutions by the addition of two volumes of ethanol, centrifugation, and the resulting pellet redissolved by slowly adding it to boiling water or by suspending it in water and autoclaving at 121 °C for 30 min and then reprecipitating it with two volumes of ethanol. The resulting precipitate can be obtained as a dry powder by treating it several times (5–10) with anhydrous acetone and then once with anhydrous ethanol, and dried in a vacuum oven at 40–50 °C for 12 h. The acetone removes the bulk of the water and the ethanol removes the last traces of water as the 95% azeotrope.

Dextrans with different structures synthesized by distinct dextransucrases that were elaborated by the same strains of *Leuconostoc mesenteroides* have been separated by differential ethanol precipitation, using different concentrations of ethanol, for example strains B-742,

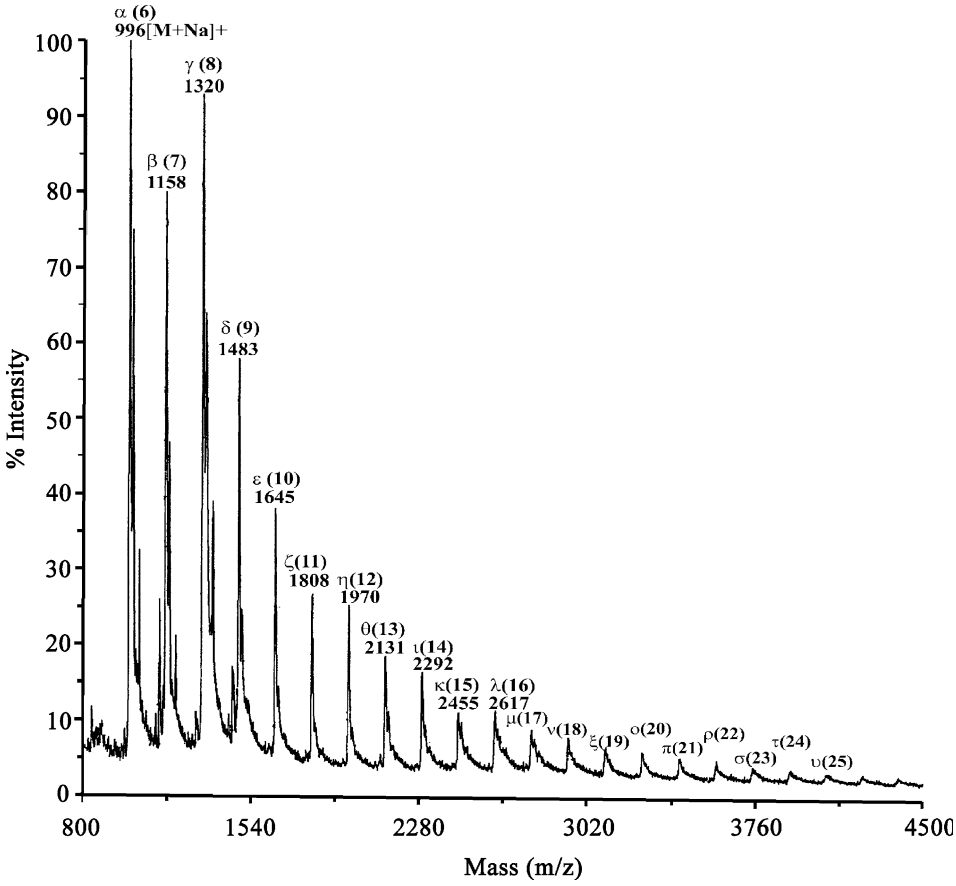


Figure 10
Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis of cyclomaltodextrins with 6 to 27 D-glucopyranose residues. The numbers in parentheses above the peaks are the number of D-glucopyranose residues in the cyclomaltodextrins. From [166], reproduced by permission of the publisher, Elsevier Press

B-1254, and B-1299 each gave two dextrans with different structures that were separated from each other; dextran and alternan, produced by strain B-1355, were also separated in this way [167].

15.5 Separation and Purification of Water-Insoluble Polysaccharides, Starch and Cellulose

The separation and purification of starch granules from plant extracts (see ❯ Sect. 2 in ❯ Chap. 6.2) and the fractionation of amylose and amylopectin is given in ❯ Sect. 5 in ❯ Chap. 6.2. The separation of cellulose and hemicelluloses from plant materials and from each other is given in ❯ Sect. 3.2 in ❯ Chap. 6.3.

15.6 Separation and Purification of Cyclomaltodextrins

The cyclomaltodextrins (α-CD, β-CD, and γ-CD) can be selectively obtained from a fermentation culture or an enzyme digest of cyclomaltodextrin glucanotransferase reaction with solubilized starch. The majority of the cyclomaltohexaose (α-CD) can be separated from cyclomaltoheptaose (β-CD) and γ-CD by their selective precipitation with p-cymene from the culture supernatant or from an enzyme digest [168]. The α-CD can then be precipitated from the supernatant with cyclohexene, which is extracted with acetone to remove the cyclohexene and the α-CD can be crystallized from water or a propanol-1/water solution [169]. The p-cymene precipitates of β-CD and γ-CD are put into a water solution and β-CD selectively precipitated from γ-CD with fluorobenzene. The γ-CD is then precipitated with anthracene saturated in diethyl ether. After the removal of the fluorobenzene from β-CD with acetone or ethanol extraction, β-CD can be crystallized from water, and after the removal of anthracene with acetone or ethanol extraction from γ-CD, it can also be crystallized from water [170,171]. The selective precipitations of the cyclomaltodextrins with various organic molecules is based on the selective formation of complexes of the organic molecules with the specific sizes of the cyclomaltodextrins and the relatively hydrophobic interior cavities of the cyclomaltodextrins [166,167,168].

15.7 Release of Oligosaccharides from Glycoproteins

The *O*-linked oligosaccharides are relatively easily released from the protein by a β-elimination reaction, using mild alkali (0.05–0.5-M NaOH) and temperatures of 0–45 °C for 15–216 h [172,173,174]. A standard procedure is 0.1-M NaOH at 37 °C for 48 h. Conditions, however, must be determined for each glycoprotein. The asparagine *N*-linked glycosides can be cleaved by hydrazinolysis [175]. The glycoprotein is heated at 100 °C with anhydrous hydrazine for 8–12 h in a sealed tube. Various endoglycosidases, such as endo-β-*N*-acetylglucosaminidase have also been used to release the oligosaccharides from glycoproteins [176,177]. A chemical method that releases both *O*- and *N*-linked oligosaccharides from glycoproteins involves trifluoromethane sulfonic acid (TFMS) [178]. TFMS reactions are performed at 0 °C for 0.5–2 h under nitrogen. After reaction, the mixture is cooled below −20 °C in a dry ice-ethanol bath and slowly neutralized by the addition of 60% (v/v) aqueous pyridine that is previously cooled to −20 °C. More information on the structure and analysis of glycoproteins may be found in Chap. 8.

References

1. Bonner WA (1991) Origins Life Evolut Biosph 21:72
2. Bailey J, Chrysostomou A, Hough JH, Gledhill TM, MeCall A, Clark S, Ménard F, Tamura M (1998) Science 281:672
3. Lobry de Bruyn CA, Alberda van Ekenstein W (1895) Rec Trav Chim 14:156; 203; (1896) 15:92; (1897) 16:241, 262, 274, 282; (1899) 18:147
4. French D (1954) Adv Carbohydr Chem 9:149
5. Avigad G, Feingold DS, Hestrin S (1956) Biochim Biophys Acta 20:129
6. Feingold DS, Avigad G, Hestrin S (1957) J Biol Chem 224:295

7. Avigad G (1957) J Biol Chem 229:121
8. Stodola FH, Sharpe ES, Koepsell HJ (1956) J Am Chem Soc 78:2514
9. Sharpe ES, Stodola FH, Koepsell HJ (1960) J Org Chem 25:1062
10. Khan R (1976) Adv Carbohydr Chem Biochem 33:236
11. Ballard JM, Hough L, Richardson AC, Fairclough PH (1973) J Chem Soc Perkin Trans I 1524
12. Hough L, Phadnis SP, Tarelli E (1975) Carbohydr Res 44:37
13. Parolis H (1976) Carbohydr Res 48:132
14. Hough L, Phadnis SP (1976) Nature 263:800
15. Hough L, Khan R (1978) Trends Biol Sci 3:61
16. Kuhn R, Gauhe A (1962) Chem Ber 95:518
17. Got R, Font J, Bourrillon R, Cornillot P (1963) Biochim Biophys Acta 74:247
18. Kuhn R, Ekong D (1963) Chem Ber 96: 683
19. Kalf GF, Rieder SV, J (1958) Biol Chem 230:691
20. Clegg JS, Filosa MF (1961) Nature 192:1077
21. Stewart LC, Richtmeyer NK, Hudson CS (1950) J Am Chem Soc 72:2059
22. Ingram J, Bartels D (1996) Ann Rev Plant Physiol Plant Molec Biol 47:377
23. Beattie GM, Leibowitz G, Lopez DA, Levine F, Hayek A (2000) Cell Transplant 9:91
24. Han B, Bischof N (2004) Cell Preserv Technol 2:91
25. Jane J-l, Kasemsuwan T, Leas S, Zobel H, Robyt JF (1994) Starch/Stärke 46:121
26. Hizukuri S, Takeda Y, Yasuda M, Szuki A (1981) Carbohydr Res 94:205
27. Hizukuri S (1991) Carbohydr Res 217:251
28. Leach HW, Schoch TJ (1962) Cereal Chem 39:318
29. Banks W, Greenwood CT (1975) Starch and its Components, Edinburgh University Press, Edinburgh, pp 15, 30
30. Whistler RJ, Daniel JR (1984) In: Whistler RJ, BeMiller JN, Paschall EF (eds) Starch: Chemistry and Technology. Academic Press, New York, pp 153–178
31. BeMiller JN, Whistler RL (1996) Starch Derivatives. In: Fennema OR (ed) Food Chemistry. Marcel Dekker, New York, pp 201–203
32. Fox JD, Robyt JF (1992) Carbohydr Res 227:163
33. Robyt JF, Choe J-Y, Hahn RS, Fuchs EB (1996) Carbohydr Res 281:203
34. Robyt JF, Choe J-Y, Fox JD, Hahn RS, Fuchs EB (1996) Carbohydr Res 283:141
35. Ruttenberg MW, Solarek D (1984) Starch Derivatives: Production and Uses. In: Whistler RJ, BeMiller JN, Paschall EF (eds) Starch: Chemistry and Technology, 2nd edn. Academic Press, San Diego, pp 311–388
36. Geddes R, Harvey JD, Wills PR (1977) Biochem J 30:257
37. Wanson J-C, Drochmans P (1968) J Cell Biol 38:130; (1972) 54:206
38. Geddes R (1985) Glycogen. In: Aspinall GO (ed) The Polysaccharides, vol. 3. Academic Press, San Diego, p 316
39. Preiss J, Walsh DA (1981) The Comparative Biochemistry of Glycogen and Starch. In: Ginsburg V, Robbins P (eds) Biology of Carbohydrates, vol. 1. Wiley, New York, p 203
40. Okada G, Hehre E (1973) Carbohydr Res 26:240; Tao BY, Reilly PJ, Robyt JF (1988) Carbohydr Res 181:163
41. Robyt JF (1995) Adv Carbohydr Chem Biochem 51:133
42. Jeanes A, Haynes WC, Wilham CA, Rankin JC, Melvin EH, Austin MJ, Cluskey JE, Fisher BE, Tsuchiya HM, Rist EE (1954) J Am Chem Soc 76:5041
43. Tsiyisaka Y, Mitsuhashi M (1993) Pullulan. In: Whistler RL, BeMiller JN (eds) Industrial Gums: Polysaccharides and Their Derivatives, 3rd edn. Academic Press, San Diego, pp 447–460
44. Bender H, Lehmann J, Wallenfels K (1959) Biochim Biophys Acta 36:309
45. Bouveng HO, Kiessling B, Lindberg B, McKay J (1962) Acta Chem Scand 16:615; (1963) 17:792
46. Wallenfels K, Keilich G, Bechtler G, Freudenberger D (1965) Biochem Z 341: 433
47. Catley BJ, Whelan WJ (1971) Arch Biochem Biophys 143:138
48. French D, Rundle RE (1942) J Am Chem Soc 64:1651; Freudenberg K, Cramer F (1948) Z Naturforsch 36:464; French D, Knapp DV, Pazur JH (1950) J Am Chem Soc 72:5150
49. Penninga D, Strokopytov B, Bozeboom HJ, Lawson CL, Dijkstra BW, de Vries GE, Bergsma J, Dijkhuizen L (1995) Biochemistry 34:3368; Mori S, Hirose, S, Oya T, Kitahata N (1994) Biosci Biotech Biochem 58:1968
50. Pulley AO, French D (1961) Biochem Biophys Res Commun 5:11; Endo T, Ueda H, Kobayashi S, Nagai T (1995) Carbohydr Res 269:369; Endo T, Nagase H, Ueda H, Kobayashi S, Nagai T (1997) Chem Pharm Bull 45:532

51. Szejtli J (1976) Stärke 29: 26; (1978) 30: 427; (1981) 33:387
52. Oguma T, Horiuchi T, Kobayashi M (1993) Biosci Biotech Biochem 57:1225
53. Côté G, Biely P (1994) Eur J Biochem 226:641
54. Aga H, Nishimoto T, Kuniyoshi M, Maruta K, Yamashita H, Higashiyama T, Nakada T, Kubota M, Fukuda S, Krimoto M, Tsujisaka Y (2003) J Biosci Bioeng 95:215
55. Kim Y-K, Kitaoka M, Hayashi K, Kim C-H, Côté GL (2003) Carbohydr Res 338:2213
56. Takaha T, Yanase M, Takata H, Okada S, Smith SM (1996) J Biol Chem 271:2902
57. Takata H, Takaha T, Okada S, Takagi M, Imanka T (1996) J Bacteriol 178:1600; Gorin PAF, Spencer JFT, Westlake DWS (1961) Can J Chem 39:1067; Zevenhuizen LPTM, Scholten-Koerselman HJ (1979) Antonie Leewenhoek 45:165; York WS, McNeil M, Darvill AG, Albersheim P (1980) J Bacteriol 142:243; Da Castro JM, Bruneteau M, Mutaftshiev S, Truchet G, Michel G (1983) FEMS Microbiol Lett 18:269
58. McIntire FC, Peterson WH, Riker AJ (1942) J Biol Chem 143:491
59. Dylan T, Helinski DR, Ditta GS (1990) J Bacteriol 172:1400; Cangelosi GA, Hung L, Pvanesarajah V, Stacey G, Ozga DA, Leigh JA, Nester EW (1987) J Bacteriol 169:2086
60. Miller KJ, Gore RS, Johnson R, Benesi AJ, Reinhold VN (1990) J Bacteriol 172:136; Rolin DB, Pfeffer PE, Osman SF, Szergold BS, Kappler F, Benesi AJ (1992) Biochim Biophys Acta 1116:215
61. Pfeffer PE, Osman SF, Hotchkiss A, Bhagwat AA, Keister DL, Valentine KM (1996) Carbohydr Res 296:23
62. O'Sullivan AC (1997) Cellulose 4:173
63. Marchessault RH, Sundararajan PR (1983) In: Aspinall GO (ed) The Polysaccharides, vol. 2. Academic Press, San Diego, pp 25–65
64. Hess K, Mahl G, Gütter E (1957) Kolloid Z 155:1
65. Ross P, Mayer R, Benziman M (1991) Microbiol Rev 55:35
66. Hus DS, Reeves RE (1967) Carbohydr Res 5:202
67. Aspinall GO, Krishnamurthy TN, Rosell K-G (1977) Carbohydr Res 55:11
68. Aspinall GO (1959) Adv Carbohydr Chem 14:429
69. Timell TE (1964) Adv Carbohydr Chem 19:247; (1965) 20:409
70. Aspinall GO, Molloy A, Craig JWT (1969) Can J Biochem 47:1063; Gould SEB, Rees DA, Wright NJ (1971) Biochem J 124:47
71. Bauer WD, Talmadge KW, Keestra K, Albersheim P (1973) Plant Physiol 51:174
72. Timell TE (1964) Adv Carbohydr Chem 19:247; (1965) 20:409
73. Stephen AM (1983) Other Plant Polysaccharides. In: Aspinall GO (ed) The Polysaccharides, vol. 2. Academic Press, San Diego, pp 166–169
74. Saka S (1990) Xyloglucans and Xylans. In: Hon DN-S, Shiraishi N (eds) Wood Cellulosic Chemistry. Marcel Dekker, New York, pp 59–88
75. Rolin C (1993) Pectin. In: Whistler RL, BeMiller JN (eds) Industrial Gums: Polysaccharides and Their Derivatives, 3rd edn. Academic Press, San Diego, pp 257–282
76. Karrer P, Francois G (1929) Helv Chem Acta 12:986
77. Ward K Jr, Seib PA (1970) Chitin. In: Pigman W, Horton D (eds) The Carbohydrates, vol. IIA. Academic Press, New York, pp 435–437
78. Rudall KM (1963) Adv Insect Physiol 1:257
79. Horton D, Lineback DR (1965) Methods Carbohydr Chem 5:403
80. Ueno H, Mori T, Fujinaga T (2001) Adv Drug Deliv Rev 52:105
81. Sanford PA (1989) Applications of Chitosan. In: Skjåk-Bræk G, Anthonsen T, Sanford PA (eds) Chitin and Chitosan. Elsevier Applied Science, London, pp 51–69
82. Vorlop KD, Klein J (1981) Biotech Lett 3:9
83. Jeon Y-J, Shahid F, Kim S-K (2000) Food Revs Int 16:159
84. Vandenberg GW, De La Noue J (2001) J Microencap 18:433
85. Jeanloz RW, Sharon N, Flowers HM (1963) Biochem Biophys Res Commun 13:20
86. Tipper DJ, Ghuysen J-M, Strominger JL (1965) Biochemistry 4:468
87. Sharon N, Osawa T, Flowers HM, Jeanloz RW (1966) J Biol Chem 242:223
88. Tipper DJ, Strominger JL (1966) Biochem Biophys Res Commun 22:48
89. Krulwich TA, Ensign JC, Tipper DJ, Strominger JL (1967) J Bacteriol 94:734
90. Ghuysen J-M (1968) Bacteriol Rev 32:425
91. Weidel W, Pelzer H (1964) Adv Enzymol 26:193
92. Fransson L-Å (1985) Mammalian Polysaccharides. In: Aspinall GO (ed) The Polysaccharides, vol. 3. Academic Press, San Diego, pp 338–386
93. Casu B (1985) Adv Carbohydr Chem Biochem 43:51

94. Painter TJ (1983) Algal Polysaccharides. In: Aspinall GO (ed) The Polysaccharides, vol. 2. Academic Press, San Diego, pp 263–264
95. Clare K (1993) Algin. In: Whistler RL, BeMiller JN (eds) Industrial Gums, Polysaccharides, and Their Derivatives, 3rd edn. Academic Press, San Diego, pp 108–118
96. Haug A, Larsen B (1962) Acta Chem Scand 16:1908
97. McNeely WH, Pettit DJ (1973) Algin. In: Whistler RJ, BeMiller JN (eds) Industrial Gums 2nd edn. Academic Press, San Diego, pp 74–75
98. Skjåk-Bræk G, Smidsrød O, Larsen B (1986) Int J Biol Macromol 8:330
99. Scott CD (1987) Enzyme Microb Technol 9:66
100. Linker A, Jones RS (1966) J Biol Chem 241:3845; Evans LR, Linker A (1973) J Bacteriol 116:915; Gorin PAJ, Spencer JFT (1966) Can J Chem 44:993; Pindar DF, Bucke CC (1975) Biochem J 152:617
101. Anderson DMW, Gill MCL, Jeffrey AM, McDougal FJ (1985) Phytochemistry 24:71
102. Aspinall GO, Hirst EL, Wickstrom A (1955) J Chem Soc 1160; Aspinall GO, Auret BJ, Hirst EL (1958) J Chem Soc 4408
103. Aspinall GO, Baille J (1963) J Chem Soc 1702
104. Sanford PA (1979) Adv Carbohydr Chem Biochem 36:266; Robyt JF (1998) Essentials of Carbohydrate Chemistry. Springer, Berlin, Heidelberg, New York, pp 193–218
105. Sloneker JH, Jeanes A (1962) Can J Chem 40:2066
106. Sloneker JH, Orentas DG (1962) Can J Chem 40:2188
107. Sloneker JH, Orentas DG, Jeanes A (1964) Can J Chem 42:1261
108. Lindberg B, Lorngren J, Thompson JF (1973) Carbohydr Res 28:351
109. Melton LD, Mindt L, Rees DA, Serson GR (1976) Carbohydr Res 46:245
110. Kang KS, Veeder GT, Mirrasoul PJ, Kaneko T, Cottrell IW (1982) Appl Environ Microbiol 43:1086
111. Jansson PE, Lindberg B, Widmalm G, Sanford PA (1984) Carbohydr Res 139:217; Kuo M-S, Mort AJ, Dell A (1986) Carbohydr Res 156:173; Jansson P-E, Lindberg B, Lindberg J, Maekawa E, Sanford PA (1986) Carbohydr Res 156:157
112. Lüderitz O, Staub AM, Westphal O (1966) Bacteriol Rev 30:193; Robbins PW, Uchida T (1962) Biochemistry 1:323
113. Bagdian G, Lüderitz O, Staub AM (1966) Ann NY Acad Sci 133:849
114. How MJ, Brimacombe JS, Stacey M (1964) Adv Carbohydr Chem 19:303; Larm O, Lindberg B (1976) Adv Carbohydr Chem Biochem 33:295; Lindberg B (1990) Adv Carbohydr Chem Biochem 48:279
115. McGuire EJ, Binkley SB (1964) Biochemistry 3:247
116. Bhattacharjee AK, Jennings HJ, Kenny CP, Martin A, Smith ICP (1975) J Biol Chem 250:1926
117. Egan W, Lui T-Y, Dorow D, Cohen JS, Robbins JD, Gotschlich EC, Robbins JB (1977) Biochemistry 16:3687
118. French AD, Waterhouse AL (1993) Structural Chemistry of Inulin. In: Suzuki M, Chatterton NJ (eds) Science Technology of Fructans. CRC Press, Boca Raton, FL, pp 41–82
119. Hendry GAF, Wallace RK (1993) Occurrence of Inulins and Levans. In: Suzuki M, Chatterton NJ (eds) Science Technology of Fructans. CRC Press, Boca Raton, FL, pp 119–140
120. Baird JK, Longyear VMC, Ellwood DC (1973) Microbios 8:143; Rossel K-G, Birkhead D (1974) Acta Chem Scand Ser B 28:589; Ebisu S, Kato K, Kotani S, Misaki A (1975) J Biochem (Tokyo) 78:879; Corrigan AJ, Robyt JF (1979) Infect Immun 26:387
121. Avigad G (1968) Bacterial Levans. In: Mark HF, Gaylard NG, Bikales NM (eds) Encyclopedia of Polymer Science Engineering, vol. 8. Wiley Interscience, New York, pp 71–78
122. Garzozynski SM, Edwards JR (1973) Arch Oral Biol 18:239; Eshrlich J, Stivala SS, Bahary WS, Garg SK, Long LW, Newbrun E (1975) J Dent Res 54:290; Marshall K, Weigel H (1976) Carbohydr Res 49:351
123. Boussingault J (1972) Compt Rend 74:939
124. Asahina Y, Shimoda H (1930) J Pharm Soc Japan 50:1; Haas P, Hill TG (1932) Biochem J 26:987
125. Strain HH (1937) J Am Chem Soc 56:2264
126. Proust M (1806) Ann Chim Phys 57:144
127. Jrier E (1893) Compt Rend 117:498
128. Bidwell RGS (1958) Can J Botany 36:337
129. Archibald AR, Baddiley J (1966) Adv Carbohydr Chem 21:354
130. Frèryacque M (1939) Compt Rend 208:1123
131. Lindberg B, Misiorny A, Wachtmeister CA (1953) Acta Chem Scand 7:591; Aghoramarthy K, Sarma KG, Seshadri TR (1961) Tetrahedron 12:173
132. Richtmyer NK (1970) Carbohydr Res 12:135
133. Anderson FB, Harris G (1963) J Gen Microbiol 33:137; Hajny GJ (1964) Appl Microbiol 12:87

134. Armstrong JJ, Baddiley J, Buchanan JG, Carss B, Greenberg GR (1958) J Chem Soc 4344
135. Archibald AR, Baddiley J (1966) Adv Carbohydr Chem 21:323
136. Archibald AR, Baddiley J, Burton D (1968) Biochem J 110:543; Archibald AR, Baddiley J, Heckels JE, Heptinstall S (1971) Biochem J 125:353
137. Truscheit E, Frommer W, Junge B, Muller L, Schmidt DD, Wingender W (1981) Angew Chem Int Ed Engl 20:744
138. Lee S-B, Park KH, Robyt JF (2001) Carbohydr Res 33:13
139. Yoon S-H, Robyt JF (2003) Carbohydr Res 338:1969
140. Courtois JÉ, Percheron F (1970) Phenanthrene Glycosides. In: Pigman W, Horton D (eds) The Carbohydrates, vol. IIA. Academic Press, New York, pp 216, 221–222
141. Montreuil J (1980) Adv Carbohydr Chem Biochem 37:158
142. Montreuil J (1975) Pure Appl Chem 42:431
143. Fournet B, Strecker G, Montreuil J, Dorl L, Haverkamp J, Vliegenthart JFG, Schmid K, Binette JP (1978) Biochemistry 17:5206
144. Nilsson B, Nordén NE, Svensson S (1979) J Biol Chem 254:4545
145. Tai T, Yamashita K, Setsuko I, Kobata A (1977) J Biol Chem 252:6687
146. Yamashita K, Tachibana Y, Kobata A (1978) J Biol Chem 253:3862
147. Feeney RE, Yeh Y (1978) Adv Prot Chem 32:191
148. Watkins WM (1966) Science 152:172
149. Lloyd KO, Kabat EA (1968) Proc Natl Acad Sci US 61:1470
150. Ginsburg V (1972) Adv Enzymol 36:131
151. Whistler RL, BeMiller JN (1962) Methods Carbohydr Chem 1:88
152. Robyt JF (2000) Thin-layer Chromatography of Carbohydrates. In: Wilson ID, Cooke M, Poole CF (eds) Encyclopedia of Separation Science, vol. 5. Academic Press, San Francisco, pp 2235–2244
153. Robyt JF, Mukerjea R (1994) Carbohydr Res 251:187
154. Han NS, Robyt JF (1998) Carbohydr Res 313:135
155. Robyt JF, White BJ (1987) Biochemical Techniques: Theory and Practice. Waveland Press, Prospect Heights, IL, pp 82–86
156. Miller GL, Dean J, Blum R (1960) Arch Biochem Biophys 91:21
157. Whelan WJ (1962) Methods Carbohydr Chem 1:321
158. French D, Robyt JF, Weintraub M, Knock P (1966) J Chromatog 24:68
159. Havlicek J, Samuelson O (1972) Carbohydr Res 22:307
160. Smith FD, Zopf DA, Ginsburg V (1978) Anal Biochem 85:602
161. Kainuma K, Nakakuki T, Ogawa T, (1981) J Chromatog 212:126
162. Ammeraal RN, Delgado GA, Tenbarge FL, Friedman RB (1991) Carbohydr Res 215:179
163. Kerns RJ, Vlahov IR, Lindhardt RJ (1995) Carbohydr Res 267:143
164. O'Shea MG, Sammuel MS, Konik CM, Morrell MK (1998) Carbohydr Res 307:1
165. Mukerjea Ru, Robyt JF (2003) Carbohydr Res 338:1811
166. Yoon S-H, Robyt JF (2002) Carbohydr Res 337:2245
167. Wilham CA, Alexander BH, Jeanes A (1955) Arch Biochem Biophys 59:61
168. Cramer F (1958) Chem Ber 91:308
169. French D (1957) Adv Carbohydr Chem 12:189
170. Thoma J, Stewart L (1965) Cycloamyloses. In Whistler RJ, Paschall EF, BeMiller JN, Roberts HJ (eds) Starch: Chemistry and Technology, vol. I. Academic Press, New York, pp 209–249
171. Anderson B, Seno N, Sampson P, Reilly JG, Hoffman P, Meyer K (1964) J Biol Chem 239:2716
172. Spiro RG, Bhoyroo VD (1971) Fed Proc Am Soc Exp Biol 30:1223
173. Spiro RG (1972) Methods Enzymol 28:35
174. Takasaki S, Mizuochi T, Kobata A (1982) Methods Enzymol 83:263
175. Muramatsu T (1978) Methods Enzymol 50:555
176. Kobata A (1978) Methods Enzymol 50:560
177. Tarentino AL, Trimble RB, Maley F (1978) Methods Enzymol 50:574
178. Sojar HT, Bahl OP (1987) Methods Enzymol 138:341