

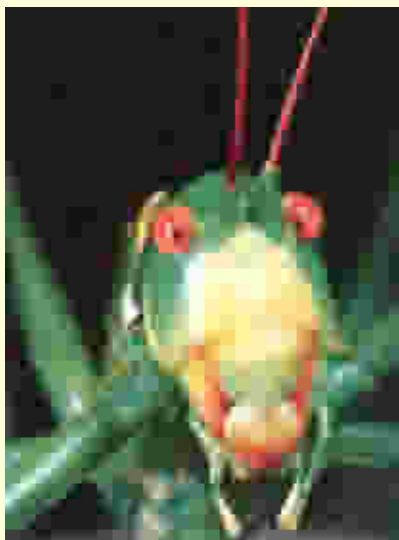


# Source

Fundamentals of Biochemistry  
(J L Jain)

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The glistening outer skeleton of this grasshopper is primarily made up of **chitin** one of the most abundant biopolymer on the Earth.

## CHAPTER

## 8

# Carbohydrates-IV

## Polysaccharides

**P**olysaccharides (variously called as *glycanes* or *polyholosides* or *polyosides*) are high molecular weight carbohydrates which, on hydrolysis, yield mainly monosaccharides or products related to monosaccharides. They may also be regarded as **polymeric anhydrides of simple sugars**. D-glucose is the commonest component of polysaccharides. However, D- and L-galactose, D-mannose, D-xylose, L-arabinose as well as D-glucuronic, D-galacturonic, D-mannuronic acids, D-glucosamine, D-galactosamine and amino uronic acids also occur as constituents of polysaccharides. The various polysaccharides differ from one another not only in the composition of the constituent monosaccharide but also in the molecular weight, in the nature of the chain (whether linear or branched), in the type of glycosidic bond (whether  $\alpha$  or  $\beta$ ) and in the type of linkage (whether 1 $\rightarrow$ 2, 1 $\rightarrow$ 3, 1 $\rightarrow$ 4, or 1 $\rightarrow$ 6) involved in the respective monosaccharide

The small arrow shows the direction of the glycosidic bond which is always from the hemiacetal hydroxyl to some alcohol or hemiacetal group of the following sugar.

units. A great majority of carbohydrates of nature occur as polysaccharides. Chemically, the polysaccharides may be distinguished into **homopolysaccharides** (or **homoglycanes**), which yield, on hydrolysis, a single monosaccharide and **heteropolysaccharides** (or

**heteroglycanes**), which produce a mixture of monosaccharides on hydrolysis.

Based on their functional aspect, the polysaccharides may be grouped under two heads :

- Nutrient** (or **digestible**) **polysaccharides**. These act as metabolic reserve of monosaccharides in plants and animals, *e.g.*, starch, glycogen and inulin.
- Structural** (or **indigestible**) **polysaccharides**. These serve as rigid mechanical structures in plants and animals, *e.g.*, cellulose, pectin and chitin and also hyaluronic acid and chondroitin.

## HOMOPOLYSACCHARIDES

These yield, on hydrolysis, a single monosaccharide. They serve as both storage (starch, glycogen, inulin) and structural (cellulose, pectin, chitin) polysaccharides.

### Starch

**Occurrence.** It is the most important reserve food material of the higher plants and is found in cereals, legumes, potatoes and other vegetables. *More than half the carbohydrate ingested by humans is starch.* Sago starch is obtained from sago palm, *Meteroxylyon rumphii* ; the arrowroot from *Maranta arundinacea* and the tapioca, a starchy food, from *Manihot utillissima*. It is usually present inside the plant cells as compact insoluble granules which may be spherical, lens-shaped or ovoid, and which have a distinctly layered structure.

**Chemistry.** Natural starches consist of two components : *amylose* (15–20%), a long unbranched straight-chain component and *amylopectin* (80–85%), a branched chain polysaccharide. Starch from waxy corn is notable as it consists practically of amylopectin component, there being no amylose.

**$\alpha$ -amylose** or simply **amylose** (Fig. 8–1) has a molecular weight range of 10,000 to 50,000. It may be formed in plant cells by elimination of a molecule of water from glycosidic OH group of one  $\alpha$ -D-glucose molecule and alcoholic OH group on carbon 4 of the adjacent  $\alpha$ -D-glucose molecule. The linkage in amylose is, thus, an  $\alpha$ -1, 4-glucoside, like that in maltose. Enzymic hydrolysis of amylose with *amylase*, henceforth, yields maltose units mainly. Amylose may be considered as an anhydride of  $\alpha$ -D-glucose units.

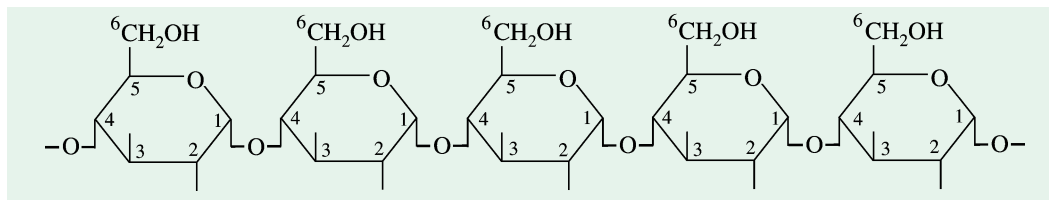


Fig. 8–1. Structure of amylose (= A-fraction)

Various types of starches differ in the amount of the amylose component present in them (Table 8–1).

**$\beta$ -amylose** or **isoamylose** or **amylopectin** (Fig. 8–2) has a high molecular weight range of 50,000 to 1,000,000, thus indicating the presence of 300–5,500 glucose units per molecule. This possesses the same basic chain of  $\alpha$ -1, 4-glucoside linkage like that of amylose but has, in addition, many side chains attached to the basic chain by  $\alpha$ -1, 6-glucoside linkages, similar to those in isomaltose. It may, thus, be seen that the glucose unit, present at each point of branching, has substituents not only on carbon atoms 1 and 4 but also on carbon atom 6. In other words, these glucose units have 3 points of attachment to serve as branching points. The average chain length is about 24 glucose units. Amylopectin, upon incomplete hydrolysis, yields the disaccharide isomaltose.

Table 8-1. Amylose contents of some starches

Starch	Amylose component (in %)
Sugary mutant corn	70
Steadfast pea	67
Alderman pea	65
Buckwheat	28
Barley	27
<i>Sorghum</i>	27
Commercial corn	26
Wheat	25
White potato	23
Arrowroot	21
Sweet potato	20
Tapioca	18
Waxy barley	3
Waxy corn	0-6

(Adapted from Whistler RD and Smart CL, 1953.)

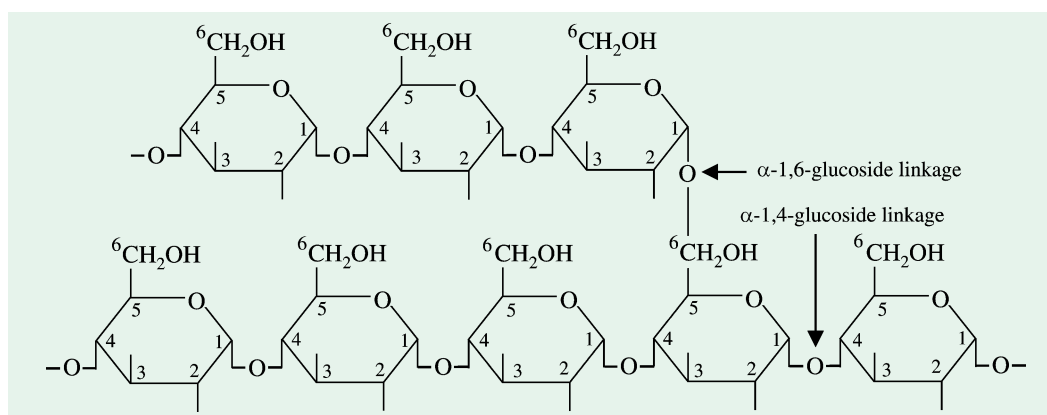
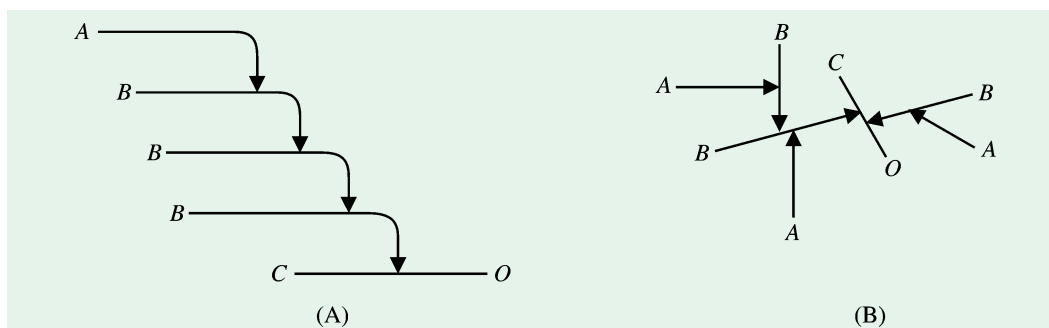


Fig. 8-2. Structure of amylopectin (= B-fraction)

The detailed structure of amylopectin (Fig. 8-3) is still speculative. The general consensus appears to be that amylopectin is composed of 3 types of chain, A, B and C ; each chain consisting of 24 glucose units. *A-chains* are linked to *B-chains* in 1, 6, or 1, 3 manner. These *B-chains* are further linked to other *B-chains*, the terminal one of which is linked to a single *C-chain*, which characteristically possesses a free reducing group. There are 2 different types of combinations of these chains which explain reasonably well the properties of amylopectin : the laminated structure as proposed by Haworth in 1937 and the randomly highly-branched structure as given by Meyer in 1940.

**Properties.** Starch is a white soft amorphous powder and lacks sweetness. It is insoluble in water, alcohol and ether at ordinary temperature. The specific rotation of starch,  $[\alpha]_D^{20}$  is  $+196^\circ$ . The microscopic form of the starch grains is characteristic of the source of starch. Starch, on partial hydrolysis by boiling with water under pressure at about  $250^\circ\text{C}$ , breaks down into large fragments called **dextrins**. The resulting dextrins then confer stiffness to clothes that have been starched and ironed. Starch molecule is highly hydrated since it contains many exposed hydroxyl groups. With the



**Fig. 8-3. Detailed structure of amylopectin**

(A) Laminated structure (B) Randomly branched structure

result, starch, when extracted from granules with hot water, forms turbid colloidal solutions. In common with other polysaccharides, starch is a nonreducing carbohydrate since carbonyl groups of all units (except one of the two terminal ones) participate in the glycosidic linkages.

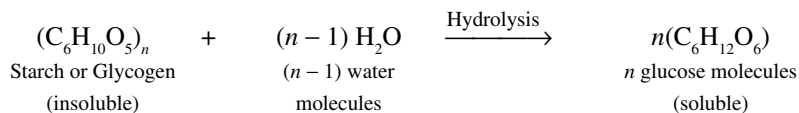
The two components of starch differ in following points :

- I. Amylose has a simpler structure and hence more soluble in water than amylopectin. Because of this difference in solubility, the two components may be separated partially by keeping starch in water for a prolonged period. When potatoes are boiled, amylose is extracted by the hot water turning it milky and opalescent. The amylopectin left behind accounts for most of the starch in boiled potatoes.
- II. Amylose is soluble without swelling in hot water whereas amylopectin swells in water.
- III. Amylose is readily dispersed in water but does not form the characteristic gel or starch paste. However, when dispersed in sufficient concentrations, the amylopectin forms the typical starch gel.
- IV. Amylose produces a typical blue colour with iodine whereas amylopectin gives a purplish colour with iodine. In fact, amylose is an open, helical molecule. The inside diameter of the helix is just large enough to accommodate an iodine molecule and it is the consequent change in the light-absorbing properties of the halogen that is responsible for the blue colour given in the starch-iodine test. By contrast, most other polysaccharides, including amylopectin, give only dull, reddish-brown colours.

**Hydrolysis.** One enzyme,  $\alpha$ -*amylase*, found in the digestive tract of animals (in saliva and the pancreatic juice) hydrolyzes the linear **amylose** chain by attacking  $\alpha$  (1  $\rightarrow$  4) linkages at random throughout the chain to produce a mixture of maltose and glucose.  $\beta$ -*amylase*, an enzyme found in plants, attacks the nonreducing end of amylose to yield successive units of maltose (The prefixes  $\alpha$  and  $\beta$  used with the amylases do not refer to glycosidic linkage, but simply designate these two enzymes). **Amylopectin** can also be attacked by  $\alpha$ - and  $\beta$ -amylases, but the  $\alpha$  (1  $\rightarrow$  4) glycosidic bonds near the branching point in amylopectin and the  $\alpha$  (1  $\rightarrow$  6) bond itself are not hydrolyzed by these enzymes. A separate “debranching” enzyme, an  $\alpha$  (1  $\rightarrow$  6) *glucosidase* can hydrolyze the bond at the branch point. Therefore, the combined action of  $\alpha$ -amylase and the  $\alpha$  (1  $\rightarrow$  6) glucosidase will hydrolyze amylopectin ultimately to a mixture of glucose and maltose. Starch is readily hydrolyzed by mineral acid with the final production of glucose. The course of hydrolysis may be followed by the gradual change in colour when treated with iodine (blue  $\longrightarrow$  purple  $\longrightarrow$  red  $\longrightarrow$  none) and also by the increase in concentration of reducing sugar. A compound which yields only glucose molecules on hydrolysis is called a **glucosan**. Hence, starch is a glucosan.

The suffix **-an** is used for designating polymers; for example, starch is a *glucosan* as it yields only glucose molecules on hydrolysis; similarly, inulin is a *fructosan* as it yields only fructose molecules on hydrolysis.

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If hydrolysis of starch is interrupted early, dextrins are formed. The dextrins are white powders, and are used for making adhesives and confectionery, for sizing paper, etc. These materials possess adhesive properties and are used as adhesives on paper products.

**Glycogen**

**Occurrence.** It is the major reserve food in animals and is often called '**animal starch**'. Glycogen is stored in the liver and muscles of animals. It is especially abundant in the liver where it may attain up to 7% of the wet weight. It occurs in animal cells as particles much smaller than the starch grains.

Although of animal origin, a glycogen-like polysaccharide has also been described in *maize*.

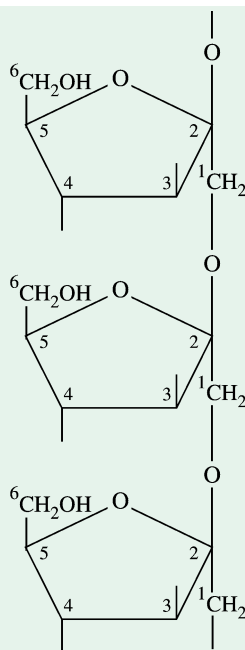
**Chemistry.** Glycogen is a branched-chain polysaccharide and resembles amylopectin very much in structure, rather than amylose (for which there is no 'animal' equivalent), but has somewhat more glucose residues per molecule and about one-and-a-half times as many branching points. Also the chains are shorter (10–20 glucose units), and hence the molecule is even more highly branched and more compact. These differences, however, do not alter the functional behaviour of the molecule to any significant extent. Its molecular weight is  $1 - 2 \times 10^7$ .

**Properties.** It is a white powder and is more soluble in water than amylopectin. Hence, it readily forms suspension even in cold water. Glycogen is precipitated from aqueous solutions by addition of ethyl alcohol and is fairly stable in hot alkali. It is a nonreducing sugar and gives red colour with iodine. The red colour, however, disappears on boiling and reappears on cooling.

**Hydrolysis.** On incomplete hydrolysis with  $\alpha$ -1,4-glucanmaltohydrolase, glycogen yields maltose. However, the acids completely hydrolyze it into glucose.

**Inulin**

**Occurrence.** It is the storage form of carbohydrate in the members of the family—Compositae, such as dahlias (*Dahlia sp.*), artichokes (*Helianthus tuberosus*) and dandelions (*Taraxacum officinale*). Inulin is stored in the tubers of the dahlia and artichoke and in the roots of dandelion. It is also found in onion and garlic.



**Fig. 8-4. Structure of inulin**

[The two glucose units have not been shown for the sake of simplification.]

**Chemistry.** Inulin (Fig. 8-4) has a molecular weight of about 5,000 and consists of about 30–35 fructose units per molecule. It is formed in the plants by eliminating a molecule of water from the glycosidic OH group on carbon atom 2 of one  $\beta$ -D-fructose unit and the alcoholic OH group on carbon atom 1 of the adjacent  $\beta$ -D-fructose unit. In inulin, the fructose residues (in furanose form) are, thus, joined together in a straight chain by  $\beta$ -2, 1-fructosidic linkages. Inulin is, therefore, 2  $\rightarrow$  1- $\beta$ -polyfructose.

On hydrolysis, however, inulin also yields a small amount of glucose besides fructose molecules. It is now thought that there are 2 glucose units in the inulin molecule, one somewhere in the centre and the other at the reducing end of the chain.

**Properties.** It is a white tasteless powder and is insoluble in cold water. In warm water, it easily dissolves forming a colloidal solution which does not form a gel on cooling. Inulin solution does not give any colour with iodine. It is a nonreducing sugar.

**Hydrolysis.** Inulin, on complete hydrolysis by dilute acids, yields D-fructose and hence it is a **fructosan** or **levan**.

### Cellulose

**Occurrence.** Cellulose is not only the most abundant extracellular structural polysaccharide of the plant world but is also *undoubtedly the most abundant of all biomolecules in the biosphere*. It is present in all land plants, but is completely lacking in meat, egg, fish and milk. It is, however, not metabolized by the human system. Some  $10^{15}$  kg of cellulose is synthesized and degraded on earth each year! It shares as much as 50% of all the carbon in vegetation. It is the most widely distributed carbohydrate of the plants, although certain tunicates also possess it. Cellulose occurs in the cell walls of plants where it contributes in a major way to the structure of the organism. Lacking a skeleton of bone onto which organs and tissues may be organized, the higher plant relies on its cell walls to bear its own weight, whether it is the smallest *Wolffia* flower or the tallest *Sequoia* tree. Cellulose is often found associated with other structural substances such as lignin. Plant residues in soil consist of 40–70% cellulose. This high cellulose content emphasizes the importance of cellulolytic organisms in the mineralization process and in the carbon cycle. It occurs in nearly pure form in cotton (94%), flax (90%) and in the wood of many plants (60%). It is not usually found in bacterial cell walls, but it is the substance which keeps the cells of *Sarcina ventriculi* together in large packets or bunches.

**Chemistry.** The molecular weight of cellulose (Fig. 8-5) ranges between 200,000 and 2,000,000, thus corresponding to 1,250–12,500 glucose residues per molecule. It may be formed by taking out a molecule of water from the glycosidic OH group on carbon atom 1 of one  $\beta$ -D-glucose molecule and the alcoholic OH group on carbon atom 4 of the adjacent  $\beta$ -D-glucose molecule. It, thus, resembles in structure with amylose except that the glucose units are linked together by  $\beta$ -1, 4-glucoside linkages. Cellulose may, henceforth, be regarded as an anhydride of  $\beta$ -D-glucose units.

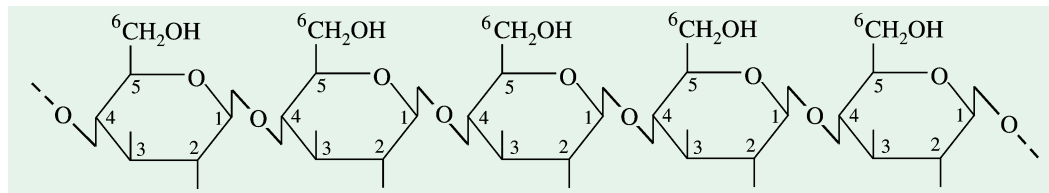
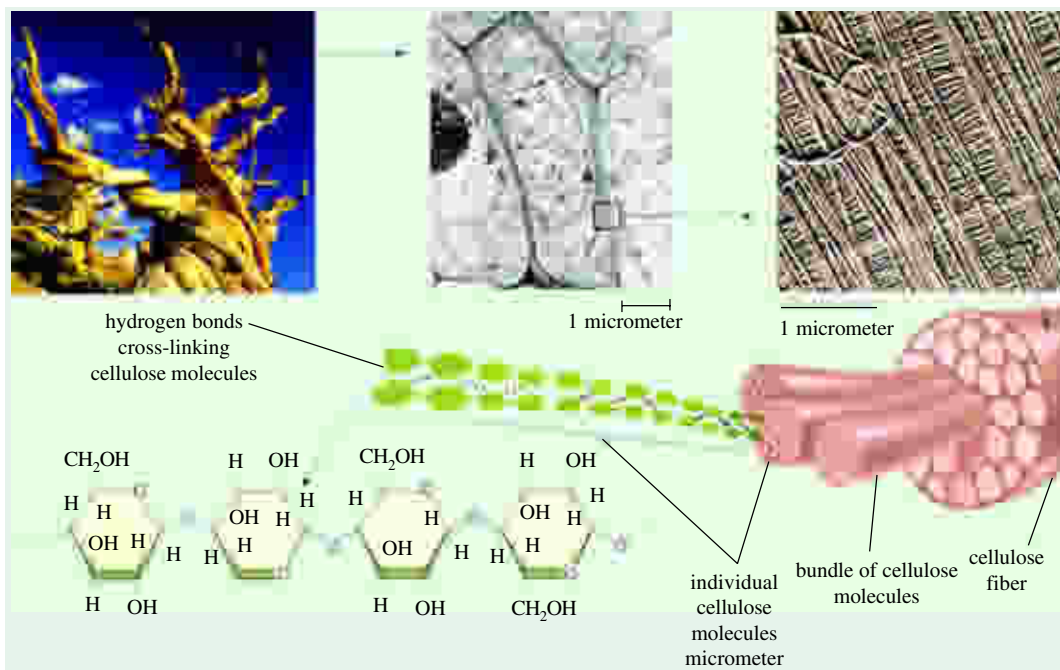


Fig. 8-5. Structure of cellulose

The correlation between the structure of cellulose molecule and the function it perform is illustrated in Fig. 8-6.

**Properties.** It is a fibrous, tough, white solid, insoluble in water but soluble in ammoniacal cupric hydroxide solution (Schweitzer's reagent). It gives no colour with iodine and lacks sweetness. Although insoluble in water, cellulose absorbs water and adds to the bulk of the fecal matter and acilitates its removal.





**Fig. 8-6. Interrelation between cellulose structure and function**

Cellulose, like starch, is composed of glucose monomers but the orientation of the bond between monomers in cellulose is different such that every other glucose molecule is “upside down.” Unlike starch, cellulose has great structural strength, due partly to the difference in bonding and partly to the arrangement of parallel molecules of cellulose into long, cross-linked fibres. Plant cells often deposit cellulose fibres in layers that run at angles to each other, resulting in resistance to tearing in both directions. The final product can be incredibly tough, as this 3000-year-old bristlecone pine testifies.

Because of the lack of chemical reactivity, the cellulose is of no nutritive value unlike starch. However, the same characteristics have made it so useful as fibres for paper and cloth. In man, the cellulose is not digested since it does not possess an enzyme capable of catalyzing the cleavage of  $\beta$ -glucoside bonds. Hence, it serves as an important source of “bulk” in the diet. However, the ruminants (cattles, sheep, goats, camels, giraffes) and certain wood-eating insects are able to digest it because the microorganisms, present in their digestive tract, do possess such digesting enzymes. Also certain wood-eating insects such as termites readily digest cellulose because their intestinal tract harbours a parasitic protozoan, *Trichonympha*. The latter secretes *cellulase*, a cellulose-hydrolyzing enzyme which enables termites to digest wood. Wood-rot fungi and bacteria also produce cellulase.

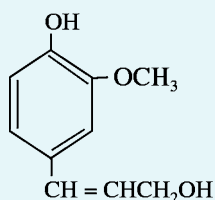
**Hydrolysis.** Cellulose is a relatively inert material and is completely degraded only under the most drastic conditions. For example, it may be hydrolyzed to glucose when treated with conc.  $H_2SO_4$  or HCl or with conc. NaOH.

**Biological importance.** Huge quantities of cellulose are made annually by the plants of the globe. It is estimated that every day some 50 kg of cellulose is synthesized by the plant world for each human being on the earth. Cellulose is also a very useful substance. Wood, cotton, paper and cardboard are all largely cellulose. Dried wood contains about 65% cellulose, 30% lignin and 2% pentosans. In the manufacture of **paper**, wood is cooked with water, lime and  $SO_2$  to remove lignin leaving behind the cellulose. These fibres are then spread into mats and rolled into sheets which may be coated to provide a smooth writing surface. When heavy paper is treated with  $H_2SO_4$ , the translucent parchment paper is obtained.

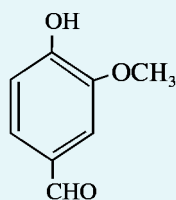


The amount of **lignin** in wood varies in different species and with age in the same species. It makes up about 23-33% of softwoods, but only about 16-25% of hardwoods.

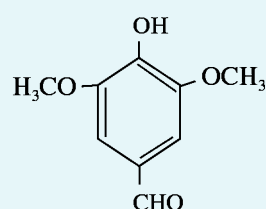
When wood passes from the green pliable stage, it is hardened by the interpenetration of a highly complex non-polysaccharide polymeric substances called *lignin*. This substance is formed by the cross-linking of a number of *non-sugar aromatic compounds*, such as coniferyl alcohol, vanillin, syringaldehyde etc. The process of lignification has some chemical resemblance to that which strengthens the proteins of the insect cuticle.



Coniferyl alcohol

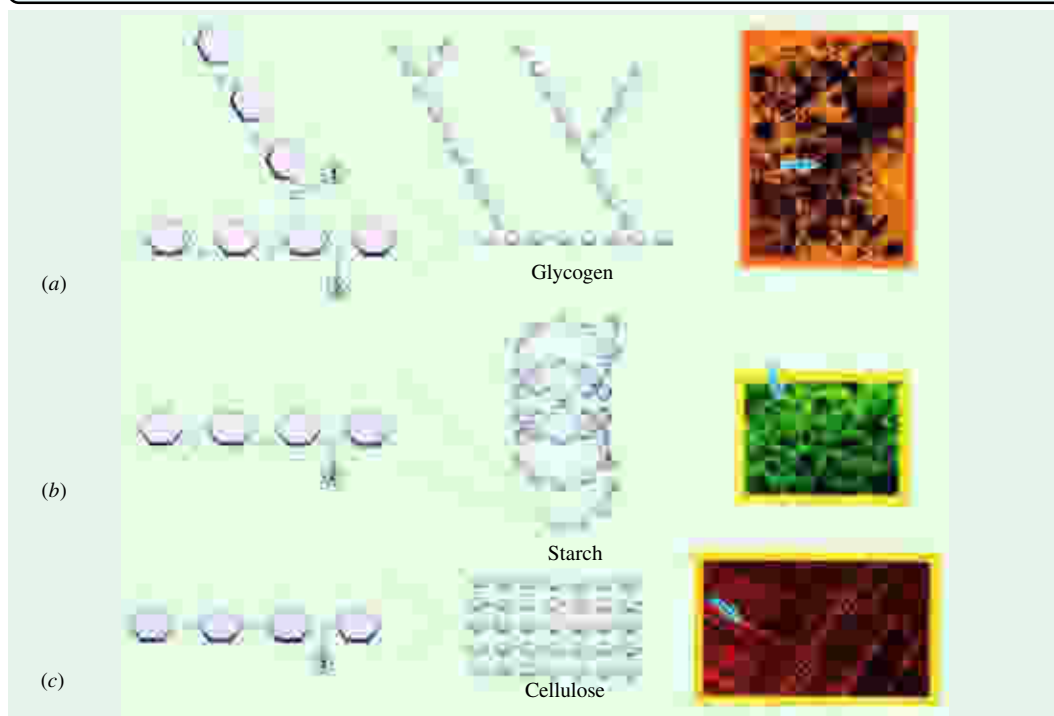


Vanillin



Syringaldehyde

Fruits with seeds and lignified cells of pear are important sources of lignin. Woody nondigestible parts of vegetables too are rich in lignin. Lignin is water-insoluble and non-digestible. It adsorbs organic substances like bile acid from the intestinal lumen and thus facilitates its fecal excretion. Lignin is an intractable material that is difficult to remove from the wood.



**Fig. 8.7. Three polysaccharides with identical sugar monomers but dramatically different properties** Glycogen (a), starch (b), and cellulose (c) are each composed entirely of glucose subunits, yet their physical and chemical properties are very different due to the distinct ways that the monomers are linked together (three different types of linkages are indicated by the circled numbers). *Glycogen* molecules are the most highly branched, *starch* molecules assume a helical arrangement, and *cellulose* molecules are unbranched and highly extended. Whereas glycogen and starch are energy stores, cellulose molecules are bundled together into tough fibres that are suited for their structural role. Coloured electron micrographs show glycogen granules in liver cell, starch grains (amyloplasts) in a plant seed, and cellulose fibres in a plant cell wall; each is indicated by an arrow.

[Photo insets : (Top) Don Fawcett; (Centre) Jeremy Burgess; (Bottom) Cabisco]

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Cotton treated with alkali becomes somewhat translucent, acquires a silky lustre, and is called **mercerized cotton**. Rayon is made from cellulose by dissolving the material in NaOH and CS<sub>2</sub>. The viscous material is forced through small jets into an acid solution of sodium bisulfite, regenerating the cellulose in small threads.

**Cellulose nitrates** are used in explosives, lacquers, celluloid and collodion. **Cellulose acetate** is widely used in photographic film and packing materials.

It may, thus, be easily visualized that the way the constituent monomeric units of various polysaccharides (glycogen, starch and cellulose, for example) are linked together has a profound bearing on the physical and chemical properties of these molecules, although the polysaccharides are made up of identical glucose units (Fig. 8-7).

### Pectin

**Occurrence.** Pectins are found as intercellular substances in the tissues of young plants and are especially abundant in ripe fruits such as guava, apples and pears. They are components of the middle lamella found between the cell wall and adjacent cells.

**Chemistry.** Pectin is a polysaccharide of  $\alpha$ -D-galacturonic acid where some of the free carboxyl groups are, either partly or completely, esterified with methyl alcohol and others are combined with calcium or magnesium ions. Chemically, they are called polygalacturonides (Fig. 8-8) and have high molecular weights.

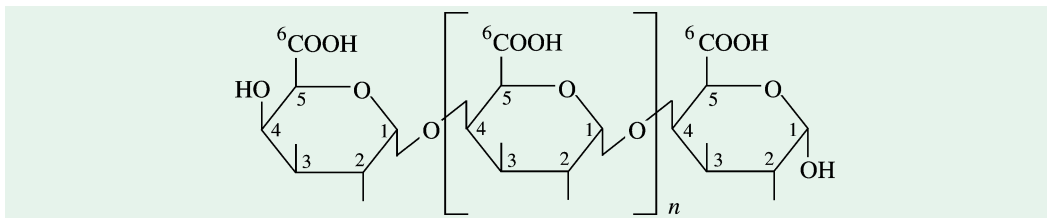


Fig. 8-8. Structure of  $\alpha$ -polygalacturonic acid

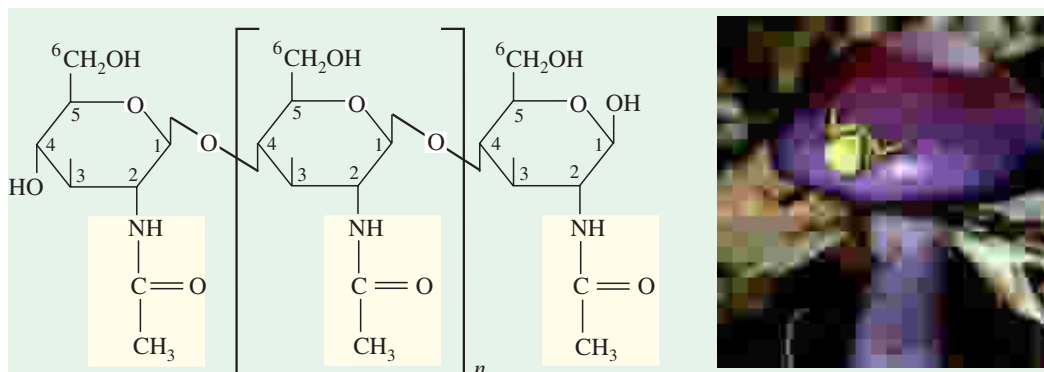
**Biological properties.** The importance of pectin rests not so much on their quantitative role but rather on the functional part they play in plant stability and solidity. The characteristic property of pectins is the ability of their solutions to gelate, *i.e.*, form jellies. Pectins, along with sugar, furnish the gelling characteristics of jellies and preserves made from fruit.

### Chitin

**Occurrence.** *Chitin is probably the most abundant polysaccharide of nature after cellulose.* It is also one of the most abundant biopolymer on the earth. It is found in fungi but principally among the arthropods (crabs and insects). The armour of crabs and the exoskeleton (= cuticula) of insects consist mostly of chitin and some protein. The excellent mechanical properties of insect skeleton are due to chitin. The chitin framework of lobster and crabs shells is impregnated and hardened with calcium carbonate.

**Chemistry.** Chitin (Fig. 8-9) is closely related to cellulose. Here the alcoholic OH group on carbon atom 2 of  $\beta$ -D-glucose units is replaced by an N-acetylamino group. It is, thus, a linear polymer of N-acetyl-D-glucosamine units joined together by  $\beta$ -1,4-glucosidic linkages. Chitin's pronounced stability is based on the hydrogen bonding of the N-acetyl side chains. Like cellulose, chitin is indigestible by vertebrate animals.

**Hydrolysis.** On hydrolysis with mineral acids, chitin yields 2 final end products, namely, glucosamine and acetic acid. Glucosamine is an important component of some glycoproteins (=mucoproteins) such as mucin of saliva. *Chitinases* (from the gastric juice of snails or from bacteria), however, decompose the chitin to N-acetylglucosamine.



**Fig. 8-9. Structure of chitin, a unique polysaccharide**

Chitin has the same “alternating upside down” bonding of glucose molecules as cellulose has, but in chitin the glucose subunits are modified by the replacement of one of the hydroxyl groups with a nitrogen-containing functional group (yellow). Tough, slightly flexible chitin supports the otherwise soft bodies of arthropods (insects, spiders, and their relatives) and fungi.

### Hemicelluloses

Xylan and other related substances are collectively referred to as hemicelluloses. They are not structurally related to cellulose, nor do they contain the same building blocks but they are, at least partially, soluble in water or alkali. The hemicelluloses consist of either pentoses (xylose, arabinose) or hexoses (glucose, mannose, galactose) as well as uronic acids. Unrefined cereals, some fruits and vegetables and whole wheat are rich sources of hemicelluloses. They adsorb water and are partially digestible. They function as storage and supporting substances in plants. The designation ‘hemicelluloses’ has been discontinued since a large number of similar polysaccharides were discovered in fungi and bacteria.

Xylan is representative of the group and is, therefore, being discussed below.

### Xylan

**Occurrence.** Xylan is the next most abundant and widely distributed carbohydrate after cellulose. Straw and bark consist of upto 30% xylan; residues of sugarcane also contain about 30% xylan. It also occurs in conifer wood (7–12%) and deciduous wood (20–25%).

**Chemistry.** Xylan is a linear homopolymer of D-xylose (an aldopentose) in  $\beta$ -1 $\rightarrow$ 4 linkage, bearing side chains of 4-*O*-methylglucuronic acid and/or arabinose. Xylan can be derived from a cellulose chain by substituting hydrogen atoms for the  $-\text{CH}_2\text{OH}$  groups, but its polymer size (number of units per polymer) is considerably lower (30–100).

**Properties.** Xylan is more rapidly degraded by a large number of microorganisms than cellulose. Many cellulose-degrading organisms (such as *Sporocytophaga myxococcoides*) also produce *xylanase*. The ability to utilize xylans is very common among fungi. Xylan is even an excellent substrate for the cultivation of mushrooms. In bacteria, xylanase is formed constitutively in some organisms (*e.g.*, Clostridia) and in others it is inducible by xylan.

## HETEROPOLYSACCHARIDES

These yield, on hydrolysis, a mixture of monosaccharides. They are numerous in both plants and animals.

The ‘specific soluble sugar’ of pneumococcus type III (Fig. 8–10) is one of the simplest heteropolysaccharides. It contains repeating units of a mixed disaccharide consisting of glucose and glucuronic

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acid. Apparently, on hydrolysis, it yields equimolar amounts of glucose and glucuronic acid.

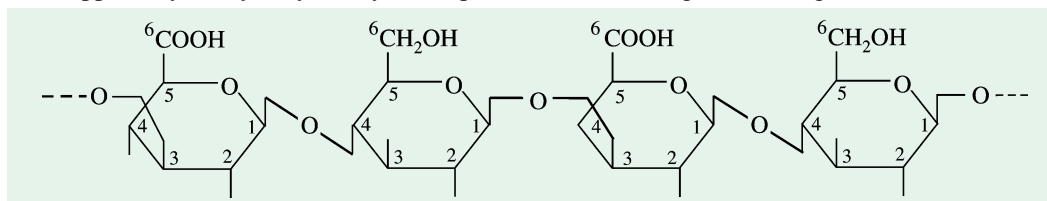


Fig. 8-10. Structure of 'specific soluble sugar' of pneumococcus type III

### Mucopolysaccharides

Polysaccharides that are composed not only of a mixture of simple sugars but also of derivatives of sugars such as amino sugars and uronic sugars are called mucopolysaccharides. They are gelatinous substances of high molecular weights (up to  $5 \times 10^6$ ). Most of these act as structural support material for connective tissue or mucous substances of the body. They serve both as a lubricant and a cementing substance.

Structurally, they have a common feature. They consist of disaccharide units in which a uronic acid is bound by a glycosidic bond to the C<sub>3</sub> of an acetylated amino acid (1 → 3 linkage). These disaccharide residues are polymerized by 1 → 4 linkages to give a linear macromolecule. The uronic and sulfuric acid residues impart a strong acidic character to these substances. Table 8-1 lists the structural features of some common mucopolysaccharides.

Table 8-2. Some common mucopolysaccharides

Mucopolysaccharide	Two components of the disaccharide units	Linkages*	
		β-1 → 3	β-1 → 4
Hyaluronic acid	D-glucuronic acid + N-acetyl-D-glucosamine	β-1 → 3	β-1 → 4
Chondroitin sulfate A	D-glucuronic acid + N-acetyl-D-galactosamine-4-sulfate	β-1 → 3	β-1 → 4
Chondroitin sulfate C	D-glucuronic acid + N-acetyl-D-galactosamine-6-sulfate	β-1 → 3	β-1 → 4
Dermatan sulfate (Chondroitin sulfate B)	L-iduronic acid + N-acetyl-D-galactosamine-4-sulfate	α-1 → 3	β-1 → 4
Keratosulfate	D-galactose + N-acetyl-D-glucosamine-6-sulfate	β-1 → 4	β-1 → 3

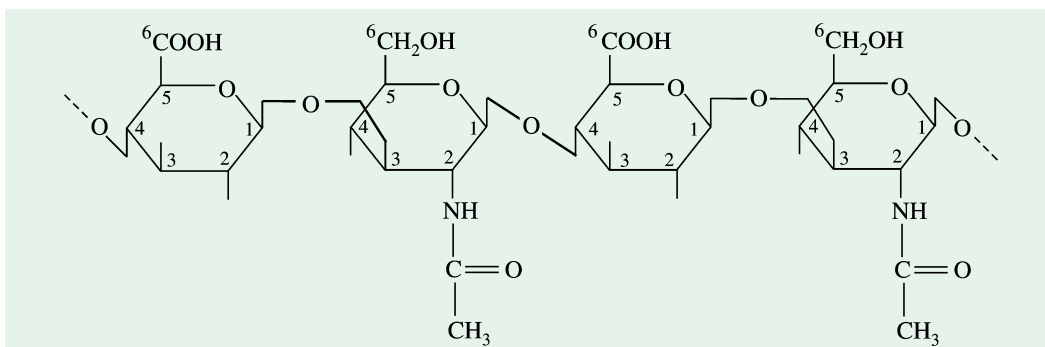
\* Linkage of the first column represents the linkage involved between the two monosaccharides of the disaccharide unit whereas the linkage of the second column is the one involved between the repeating disaccharide units.

### Hyaluronic acid

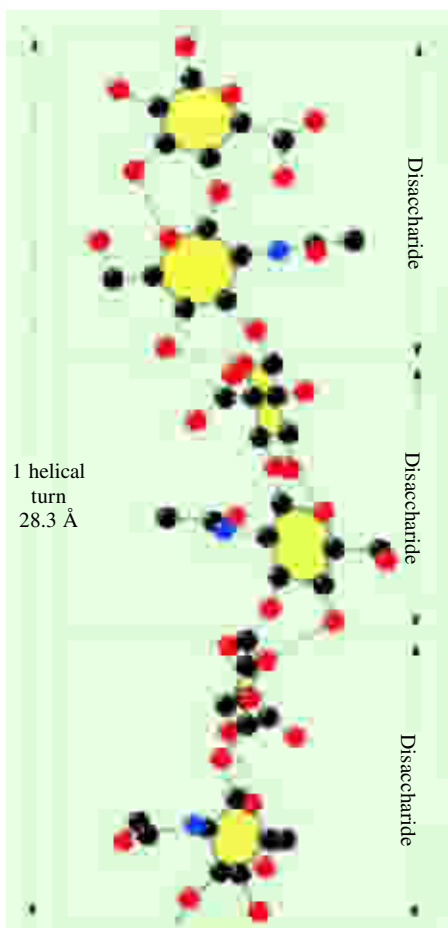
**Occurrence.** It is the most abundant member of mucopolysaccharides and is found in higher animals as a component of various tissues such as the vitreous body of the eye, the umbilical cord and the synovial fluid of joints. The high viscosity of the synovial fluid and its role as biological lubricant is largely due to the presence of its hyaluronic acid content (about 0.03%). Frequently, it is prepared from umbilical cord.

**Chemistry.** Hyaluronic acid (Fig. 8-11) has the least complicated structure among mucopolysaccharides. It is a straight-chain polymer of D-glucuronic acid and N-acetyl-D-glucosamine (NAG) alternating in the chain. Its molecular weight approaches approximately, 5,000,000. Here, apparently two linkages are involved, β-1 → 3 and β-1 → 4. Hyaluronic acid is an acidic substance,

because the carboxyl groups are largely ionized at cellular pH.



**Fig. 8-11.** Structure of hyaluronic acid



**Fig. 8-12.** The X-ray fibre structure of  $\text{Ca}^{2+}$  hyaluronate. The hyaluronate polyanion forms an extended left-handed single-stranded helix with three disaccharide units per turn that is stabilized by intramolecular hydrogen bonds (dashed lines). H atoms and  $\text{Ca}^{2+}$  ions are omitted for clarity.

(After Winter WT and Arnott S. 1977)

The  $\text{Ca}^{2+}$  polyanionic form of hyaluronic acid is depicted in Fig. 8-12. It is an extended left-handed helix, which is stabilized by hydrogen bonds.

**Hydrolysis.** Hyaluronic acid, upon hydrolysis, yields an equimolar mixture of D-glucuronic acid, D-glucosamine and acetic acid.

It is noteworthy that hyaluronic acid is split swiftly by the enzyme *hyaluronidase*. The enzyme brings about depolymerization of hyaluronic acid (leading to a drop in its viscosity) and cleavage to smaller fragments. Hyaluronidase is a 'spreading factor' of skin and connective tissue. The depolymerization effect allows any foreign bodies (such as pigments, pathogenic bacteria) to penetrate the tissue, since the cementing substance is being dissolved. The enzyme also has a physiologic role in fertilization. The sperm is rich in hyaluronidase and hence can advance better in the cervical canal and finally fertilize the ovum.

### Chondroitin

**Occurrence.** Chondroitin is of limited distribution. It is found in cartilage and is also a component of cell coats. It is a parent substance for two more widely distributed mucopolysaccharides, chondroitin sulfate A and chondroitin sulfate B.

**Chemistry.** Chondroitin is similar in structure to hyaluronic acid except that it contains galactosamine rather than glucosamine. It is, thus, a polymer of  $\beta$ -D-glucuronido-1, 3-N-acetyl-D-galactosamine joined by  $\beta$ -1  $\rightarrow$  4 linkages.

**Hydrolysis.** On hydrolysis, chondroitin produces equimolar mixture of D-glucuronic acid, D-galactosamine and acetic acid.

### Chondroitin Sulfates

**Occurrence.** The two chondroitin sulfate A and C are widely distributed and form major structural components of cartilage, tendons and bones. They are very often associated with collagen and probably with other proteins too.

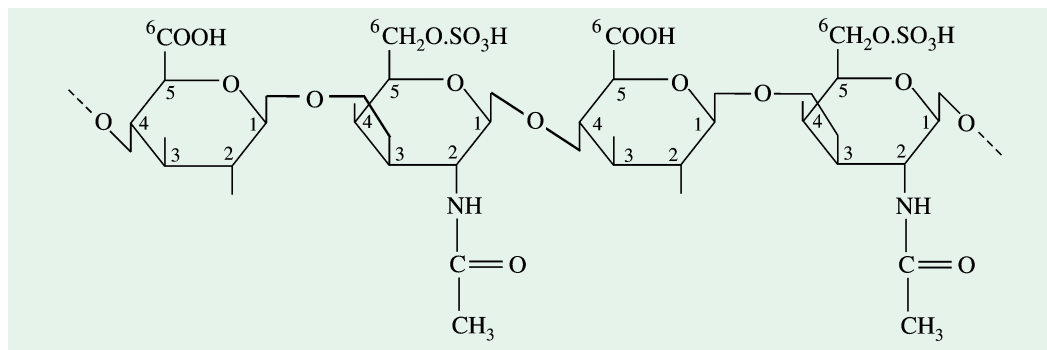


Fig. 8–13. Structure of chondroitin sulfate C

**Chemistry.** Chondroitin sulfates may be regarded as derivatives of chondroitin where, in the galactosamine moiety, a sulfate group is esterified either at carbon 4 as in chondroitin sulfate A or at carbon 6 as in chondroitin sulfate C (Fig. 8–13). The two linkages involved in both types of chondroitin sulfate would, obviously, be the same. These are  $\beta$ -1  $\rightarrow$  3 and  $\beta$ -1  $\rightarrow$  4.

**Hydrolysis.** On hydrolysis, chondroitin sulfates A and C yield approximately equivalent amounts of D-glucuronic acid, D-galactosamine, acetic acid and sulfuric acid.

### Dermatan Sulfate

Dermatan sulfate is a mucopolysaccharide structurally similar to chondroitin sulfate A except that the D-glucuronic acid is replaced by L-iduronic acid (the two uronic acids differ in configuration only at C<sub>5</sub>). The two linkages involved are  $\alpha$ -1  $\rightarrow$  3 and  $\beta$ -1  $\rightarrow$  4. Dermatan sulfate is also known by its conventional name, **chondroitin sulfate B**. This is, however, a misnomer since dermatan sulfate differs from both chondroitin sulfate A and C in the composition of their repeating disaccharide unit.

### Keratosulfate

Keratosulfate differs from other mucopolysaccharides in that the uronic acid component is replaced by D-galactose. Here, the second acetylated amino sugar component (which is N-acetyl-D-glucosamine in this case) is esterified by a sulfate group at carbon 6. Although, the two alternating linkages involved are  $\beta$ -1  $\rightarrow$  4 and  $\beta$ -1  $\rightarrow$  3, in this case the linkage between the repeating disaccharide units is  $\beta$ -1  $\rightarrow$  3 rather than  $\beta$ -1  $\rightarrow$  4.

### Heparin

**Occurrence.** Related to the sulfated mucopolysaccharides is heparin. It is present in liver, lung, arterial walls and, indeed, wherever mast cells are found, possibly for the purpose of neutralizing biogenic amines (*e.g.*, histamine).

**Chemistry.** Heparin (Fig. 8–14) is a heteropolysaccharide composed of D-glucuronic acid units, most of which (about 7 out of every 8) are esterified at C<sub>2</sub> and D-glucosamine-N-sulfate (= sulfonylaminoglucose) units with an additional O-sulfate group at C<sub>6</sub>. Both the linkages of the polymer are alternating  $\alpha$ -1  $\rightarrow$  4. Thus, the sulfate content is very high and corresponds to about 5–6 molecules per tetrasaccharide repeating unit. The relative positions of the sulfate residues may also vary. Its molecular weight ranges between 17,000 and 20,000.

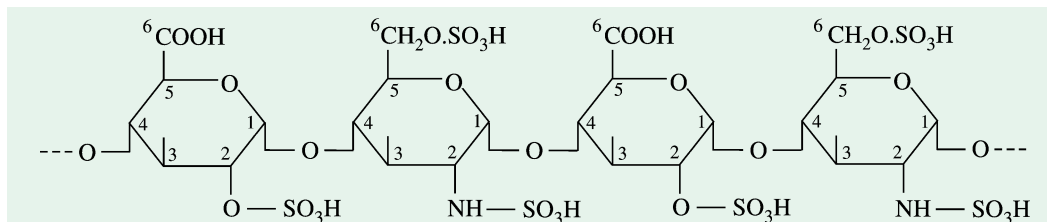


Fig. 8-14. Structure of heparin

Heparin acts as an anticoagulant. It prevents coagulation of blood by inhibiting the prothrombin-thrombin conversion. This eliminates the effect of thrombin on fibrinogen.

The heteropolysaccharides described above contain two different sugars in their repeating units or monomers. However, there are many heteropolysaccharides which contain more than 2 carbohydrates in their repeating units. Vegetable 'gums' and agar-agar are two notable examples.

**Vegetable 'gums'.** These contain as many as 4 different monosaccharide units. Most common of these are D-glucuronic acid, D-mannose, D-xylose (the second most abundant sugar in the biosphere) and L-rhamnose.

**Agar-agar.** It is a gelatinous polysaccharide produced by certain marine red algae (= rhodophycean members) such as species of *Gelidium*, *Gracilaria*, *Gigartina*, *Eucheuma*, *Campylaephora*, *Ahnfeltia*, *Hypnea*, etc. Japan is the major producer of agar-agar. It consists of D- and L-galactose, predominantly with 1 → 3 bonds and always contains some amount of sulfuric acid. On hydrolysis, it yields D- and L-galactose in a ratio of 9 : 1. It forms highly viscous gels and has melting point between 90 and 100°F. At lower temperature, it solidifies. It is insoluble in cold but soluble in hot water.

Agar-agar is of great value in the preparation of foodstuffs, particularly as articles of diet for invalids. It is used extensively in biological laboratories as the base material for the preparation of culture media, especially for bacteria and fungi. It is also used in the preparation of some medicines, and in cosmetics and leather industry. It is largely used as a solidifying agent in desserts, as a laxative and as a sizing material for textiles. It also finds application as an emulsifier in dairy products. Agar-agar is not utilized by man and hence adds to the bulk of the feces and helps in its propulsion.

A wide variety of heteropolysaccharides are found in animals and microbes. These, on hydrolysis, yield various types of monosaccharides. These more complex polysaccharides are found bound to proteins (**glycoproteins**) and lipids (**glycolipids**). Carbohydrates occur in them as an oligosaccharide moiety.

### Analysis of Carbohydrates

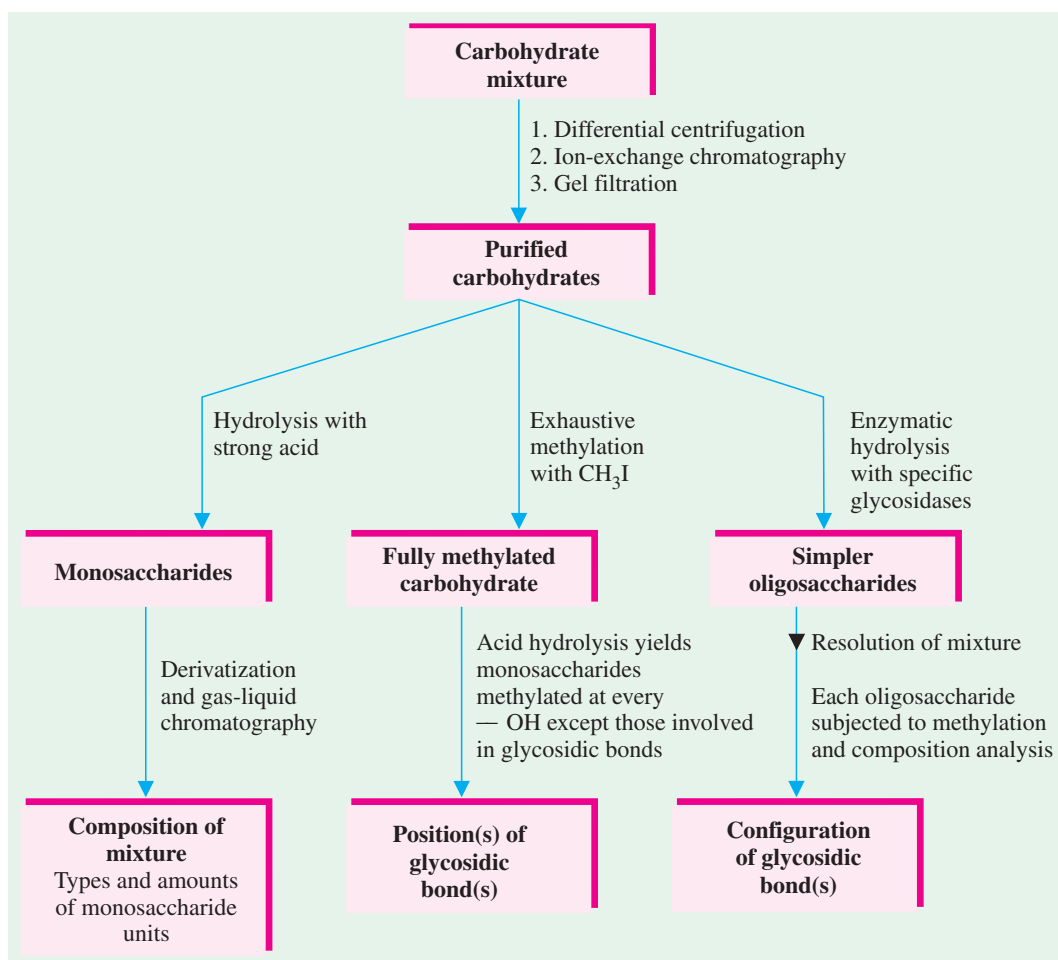
Mixtures of carbohydrates can be resolved into their individual components (Fig. 8-15) by many techniques (such as differential centrifugation, ion exchange chromatography and gel filtration) which are also used in protein and amino acid separation. Each carbohydrate separated in the first stage of analysis is subjected to the following 3 analytic routes for complete characterization. The 3 routes are as follows :

1. Hydrolysis in strong acid yields a mixture of monosaccharides, which after conversion to suitable volatile derivatives may be separated, identified and quantified by gas-liquid chromatography to yield the overall composition of the polymer.
2. For simple linear polymers such as amylose, the position of the glycosidic bond between monosaccharides is determined by treating the intact polysaccharide with methyl iodide to convert all free hydroxyls to acid-stable methyl esters. When the methylated polysaccharide is hydrolyzed in, the only free hydroxyls present in the monosaccharides produced are those that were involved in glycosidic bonds.



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3. To determine the stereochemistry at the anomeric carbon, the intact polymer is tested for sensitivity to purified glycosidases known to hydrolyze only  $\alpha$ - or only  $\beta$ -glycosides. Total structure determination for complex heteropolysaccharides is much more difficult. Stepwise degradation with highly specific glycosidases, followed by isolation and identification of the products, is often helpful. Mass spectral analysis and high-resolution nuclear magnetic resonance (NMR) spectroscopy are highly powerful analytic tools for carbohydrates.



**Fig. 8-15. Some common methods for the analysis of complex carbohydrates**

Each carbohydrate separated in the first stage of the analysis requires all 3 analytic routes for complete characterization.

A summary chart of the important homopolysaccharides is given on the next page.

**Table 8-3. Summary of occurrence, structure and properties of some important homopolysaccharides**

Name	Occurrence	Constituent monosaccharides*	Oxide linkage (s) involved	Groups involved in linkage	Properties	Hydrolysis
<b>Sucrose</b>	Major reserve food of higher plants; found in cereals, legumes, potatoes and other vegetables	$\alpha$ -D-glucose	(In amylose) $\alpha$ -1, 4-glucoside (In amylopectin) $\alpha$ -1, 4-glucoside and $\alpha$ -1, 6-glucoside $\alpha$ -1, 4-glucoside and $\alpha$ -1, 6-glucoside and $\alpha$ -1, 6-glucoside Chains are shorter and much more branched than amylopectin	glycosidic OH on C <sub>1</sub> alcoholic OH on C <sub>4</sub> glycosidic OH on C <sub>1</sub> alcoholic OH on C <sub>4</sub> alcoholic OH on C <sub>6</sub>	amylose gives blue colour with iodine; amylopectin gives purple colour with iodine; dextrorotatory	Yields glucose units only on hydrolysis, hence it is a glucosan
<b>Glycogen</b>	Major reserve food of animals; stored in liver and muscles of animals	$\alpha$ -D-glucose	$\alpha$ -1, 6-glucoside and $\alpha$ -1, 4-glucoside Chains are shorter and much more branched than amylopectin	glycosidic OH on C <sub>1</sub> alcoholic OH on C <sub>4</sub> alcoholic OH on C <sub>6</sub>	a nonreducing sugar; gives red colour with iodine	Yields glucose units only on complete hydrolysis, hence a glucosan
<b>Inulin</b>	Found in tubers and roots of certain composite plants such as dahlias, artichokes and dandelions	$\beta$ -D-fructose	$\beta$ -2, 1-fructoside	glycosidic OH on C <sub>2</sub> alcoholic OH on C <sub>1</sub>	a nonreducing sugar; gives no colour with iodine	Yields fructose units only on complete hydrolysis, hence a fructosan
<b>Cellulose</b>	Most abundant organic compound found in world; widely distributed in plants such as cotton and flax	$\beta$ -D-glucose	$\beta$ -1, 4-glucoside	glycosidic OH on C <sub>1</sub> alcoholic OH on C <sub>4</sub>	gives no colour with iodine	Yields glucose units only on complete hydrolysis, hence a glucosan
<b>Pectin</b>	Fruits of many plants	$\alpha$ -D-galacturonic acid	$\alpha$ -1, 4-galactoside	glycosidic OH on C <sub>1</sub> alcoholic OH on C <sub>4</sub>	—	—
<b>Chitin</b>	Most abundant in nature after cellulose; found in fungi and arthropods	N - a c e t y l - D - glucosamine	$\beta$ -1, 4-glucoside	glycosidic OH on C <sub>1</sub> alcoholic OH on C <sub>4</sub>	a nonreducing sugar	Yields N-acetyl-glucosamine on hydrolysis with chitinases

\* All the constituent monosaccharides are present in pyranose ring form except  $\beta$ -D-fructose which has a furanose ring structure.

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### PROBLEMS

1.  $\alpha$ -amylose is an unbranched glucose polymer. Why would this polymer not be as effective a storage form for glucose as glycogen ?
2. The stems of bamboo, a tropical grass, can grow at the phenomenal rate of 0.3 m/d under optimal conditions. Given that the stems are composed almost entirely of cellulose fibres oriented in the direction of growth, calculate the number of sugar residues per second that must be added enzymatically to growing cellulose chains to account for the growth rate. Each D-glucose unit in the cellulose molecule is about 0.45 nm long.
3. Both cellulose and  $\alpha$ -amylose consist of (1 $\rightarrow$ 4)-linked D-glucose units and can be extensively hydrated. Despite this similarity, a person on a diet consisting predominantly of  $\alpha$ -amylose (starch) will gain weight, whereas a person on a diet of cellulose (wood) will starve. Why ?
4. The practically pure cellulose obtained from the seed threads of the plant genus *Gossypium* (cotton) is tough, fibrous, and completely insoluble in water. In contrast, glycogen obtained from muscle or liver disperses readily in hot water to make a turbid solution. Although they have markedly different physical properties, both substances are composed of (1 $\rightarrow$ 4)-linked D-glucose polymers of comparable molecular weight. What features of their structures cause these two polysaccharides to differ in their physical properties ? Explain the biological advantages of their respective properties.
5. A polysaccharide of unknown structure was isolated, subjected to exhaustive methylation, and hydrolyzed. Analysis of the products revealed three methylated sugars : 2, 3, 4-tri-*O*-methyl-D-glucose, 2, 4-di-*O*-methyl-D-glucose, and 2, 3, 4, 6-tetra-*O*-methyl-D-glucose, in the ratio 20 : 1 : 1. What is the structure of the polysaccharide ?
6. One of the critical functions of chondroitin sulfate is to act as a lubricant in skeletal joints by creating a gel-like medium that is resilient to friction and shock. This function appears to be related to a distinctive property of chondroitin sulfate : the volume occupied by the molecule is much greater in solution than in the dehydrated solid. Why is the volume occupied by the molecule so much larger in solution ?
7. Why is it not possible to write on an oily paper ?
8. Why does an apple turn orangish when kept in the air ?
9. What is gun cotton ?
10. In what forms carbohydrates are stored in plants and animals ?
11. If one has diabetes, should he avoid potatoes because they raise one's blood pressure ?
12. Why starch forms turbid colloidal solution, when extracted from granules with hot water ?
13. Why do we need to fold a paper to tear it along a straight line ?
14. Why do the smooth pages of paper, after being dipped in water and dried, get wrinkled ?