

# Structure of saccharides

## Polysaccharides

It is estimated that approximately  $4 \times 10^{11}$  tons of carbohydrates are biosynthesized each year on earth by plants and photosynthesizing bacteria. The majority of these carbohydrates are produced as polysaccharides. Polysaccharides are macromolecules consisting of a large number of monosaccharide residues. They are sometimes also called glycans. (The term 'glycan' is also used for the saccharide component of a glycoprotein even though the chain length may not be large; cf. chapter 6). For polysaccharides which contain a substantial proportion of amino sugar residues the term glycosaminoglycan is a common one. Polysaccharides which consist of only one kind of monosaccharide are called homopolysaccharides (homoglycans); when they are built up of two or more different monomeric units they are named heteropolysaccharides (heteroglycans). In the latter type, the monosaccharide units are usually linked to each other in a definite pattern, rather than randomly. Certain sequences of monomeric building blocks are often found to be regularly repeated as so-called repeating units. Homo- as well as heteropolysaccharides can be linear or branched.

For polysaccharides, as for every polymer, it is not possible to attribute one distinct molecular weight as they are polydisperse molecules, which are characterized by an average molecular weight. The number of monosaccharide units in a polysaccharide is termed degree of polymerization or d.p. The size of a polysaccharide varies between approximately 16,000 and 16,000,000 daltons (Da).

Polysaccharides exist in an enormous structural diversity as they are produced by a great variety of species, including microbes, algae, plants and animals. Among these are fructans, xanthans, fucans, bacterial gel polysaccharides, capsule polysaccharides of bacteria, or agar, which is a mixture of two polysaccharides and is obtained from red-purple seaweeds. The most well-known polysaccharides are starch, glycogen, cellulose and chitin.

## *Starch*

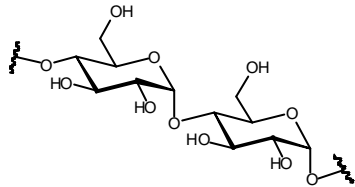
Starch is a mixture of two glucans (polysaccharides built from glucose), which are called  $\alpha$ -amylose and amylopectin. It is synthesized by plants as their principal food reserve and deposited in the plant cell cytoplasm as insoluble granules.

$\alpha$ -Amylose is a linear polymer of several thousand glucose residues,  $\alpha$ -(1,4)-glycosidically linked. This polymer adopts an irregularly aggregating helically coiled conformation containing regular left-handed helix regions. Amylopectin on the other hand, carries  $\alpha$ -(1,6)-connected branches every 24 to 30 glucose residues of the  $\alpha$ -(1,4)-linked chain, resulting in a tree- or brush-like structure. It contains up to a million glucose residues which makes it among the largest molecules occurring in nature.

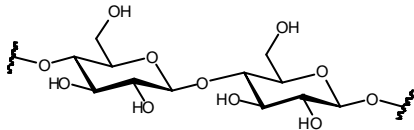
Starch is degraded by enzymes called amylases, which randomly hydrolyze the  $\alpha$ -(1,4)-glycosidic bonds digesting the polysaccharide into oligosaccharide fragments, such as maltose and maltotriose as well as oligosaccharides containing  $\alpha$ -(1,6)-branches, the latter being called dextrans. The oligosaccharides produced by amylase digestion are further hydrolyzed to glucose by specific glucosidases and by debranching enzymes, which remove the  $\alpha$ -(1,6)-branches.

## *Glycogen*

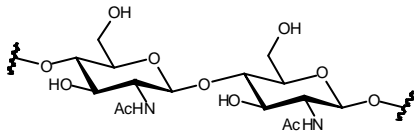
Glycogen is the storage polysaccharide of animals and is present in all cells but most prevalently in skeletal muscle and liver, where it occurs in cytoplasmic granules. The primary structure of glycogen only differs from that of amylopectin in that it is more highly branched with branching points occurring every 8 to 12 glucose residues of the  $\alpha$ -(1,4)-linked glucan chain. The degree of polymerization of glycogen is similar to that of amylopectin. In the cell, glycogen is degraded for metabolic use by glycogen phosphorylase, which cleaves the  $\alpha$ -(1,4)-linkages sequentially inwards from its non-reducing ends to release glucose-1-phosphate which can be fed into the citric acid cycle. The branching points are hydrolyzed by glycogen debranching enzyme. Glycogen contains about 1% covalently linked protein.



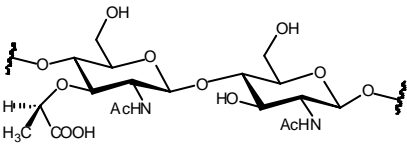
Glucose moieties,  $\alpha$ -(1,4)-linked such as in starch and glycogen; these polymers are further modified by  $\alpha$ -(1,6)-branches, which are not shown.



Glucose moieties,  $\beta$ -(1,4)-linked such as in cellulose. As a consequence of the  $\beta$ -linkages the three-dimensional shape and the properties of cellulose are fundamentally different from those of starch and glycogen.



*N*-Acetylglucosamine residues,  $\beta$ -(1,4)-linked such as in chitin, which is similar to cellulose in shape and properties.



The disaccharidic unit of murein. This structure differs from that of chitin only by an *O*-lactic acid group in the 3-position of every second GlcNAc residue.

## Cellulose

Cellulose is an abundant carbohydrate of commercial and biological importance, found in all plants as the major structural component of the cell walls. Cellulose in wood is mixed with many other polymers such as hemicelluloses and lignin. It has to be split from these components to be used for paper production. The fluffy fiber found in the cotton ball is the purest naturally occurring form of cellulose.

Cellulose is the  $\beta$ -isomer of amylose consisting of  $\beta$ -(1,4)-linked glucose residues. The different stereochemistry of the glycosidic linkage compared to amylose gives cellulose totally different properties. In contrast to amylose, the  $\beta$ -linkages in cellulose allow the polymer to fold in a fully extended conformation to form a sheet-like secondary structure. The tertiary structure of cellulose is characterized by parallel-running intermolecular hydrogen-bonded cellulose chains further associated by hydrogen bonds and van der Waals forces to produce three-dimensional microfibrils. This gives cellulose fibres exceptional strength and makes them water insoluble despite their hydrophilicity. The cellulose microfibrils give an X-ray diffraction pattern that indicates regular, repeating microcrystalline structures interspersed by less-ordered paracrystalline regions.

As a consequence of its three-dimensional structure, cellulose cannot be hydrolyzed by starch-degrading enzymes. The cellulose-degrading enzymes, called cellulases, are produced by microorganisms.

### *Chitin*

Chitin is the principal structural component of the exoskeleton of invertebrates such as crustaceans, insects, and spiders and is also present in the cell walls of most fungi and many algae, thus representing the widespread occurrence of carbohydrates on earth, their abundance being almost equivalent to that of cellulose. The structure of chitin is related to that of cellulose. It is a homopolymer of  $\beta$ -(1,4)-linked *N*-acetyl-D-glucosamine residues. X-ray analysis indicates that chitin and cellulose have similar secondary and tertiary structures. De-*N*-acetylated chitin is called chitosan.

### *Murein*

Murein, also named 'peptidoglycan', is an aminosugar polymer consisting of two  $\beta$ -(1,4)-linked hexoses, *N*-acetylglucosamin (GlcNAc) and *N*-acetyl-D-muramic acid (NAMA), the latter differing from GlcNAc in that the 3-position is substituted with an *O*-lactic acid group. The carbohydrate moieties are linked to short oligopeptides, which cross-link the polysaccharide chains. Murein is the structural component of the cell wall of all bacterial species. There are also other kinds of carbohydrates found in the bacterial cell wall, but it is murein that is the major unifying structure.

### *Pectins*

Pectins are polysaccharides occurring in all plants primarily in their cell walls. They act as intracellular cementing material that gives body to fruits and helps them keep their shape. When fruit becomes overripe, the pectin is broken down into its monosaccharide constituents. As a result, the fruit becomes soft and loses its firmness. One of the most prominent characteristics of pectins is their ability to form gels. All pectins are composed of D-galactopyranosyl uronic acid units, which are  $\alpha$ -(1,4)-linked. They contain methyl esters and acetyl groups to various degrees and show a typical average molecular size of 100,000 Da.

### *Dextrans*

It has long been known that sucrose solutions convert into viscous solutions, gels, or flocculent precipitates. The material that produced such changes in sucrose solutions was isolated and found to be a polysaccharide that was called dextran. The enzymes capable of synthesizing polysaccharides from sucrose are produced by Gram-positive bacteria of the type *Leuconostoc* and *Streptococcus*.

A dextran is defined as a glucan that is enzymatically synthesized from sucrose and has contiguous  $\alpha$ -(1,6)-linked D-glucofuranose units in the main chains. All known dextrans are branched at different branching points and to different extents. Differences in the three-dimensional structures of different dextrans are due to the percentage and manner in which the branches are arranged.

### *Structure analysis of polysaccharides*

The analysis of polysaccharide structures is of general interest but also of industrial relevance as the structure and properties of polysaccharides are closely related. Polysaccharide analysis requires specialized techniques, which differ from those methods used for the characterization of small molecules.

For structure analysis of polysaccharides the following aspects have to be elucidated:

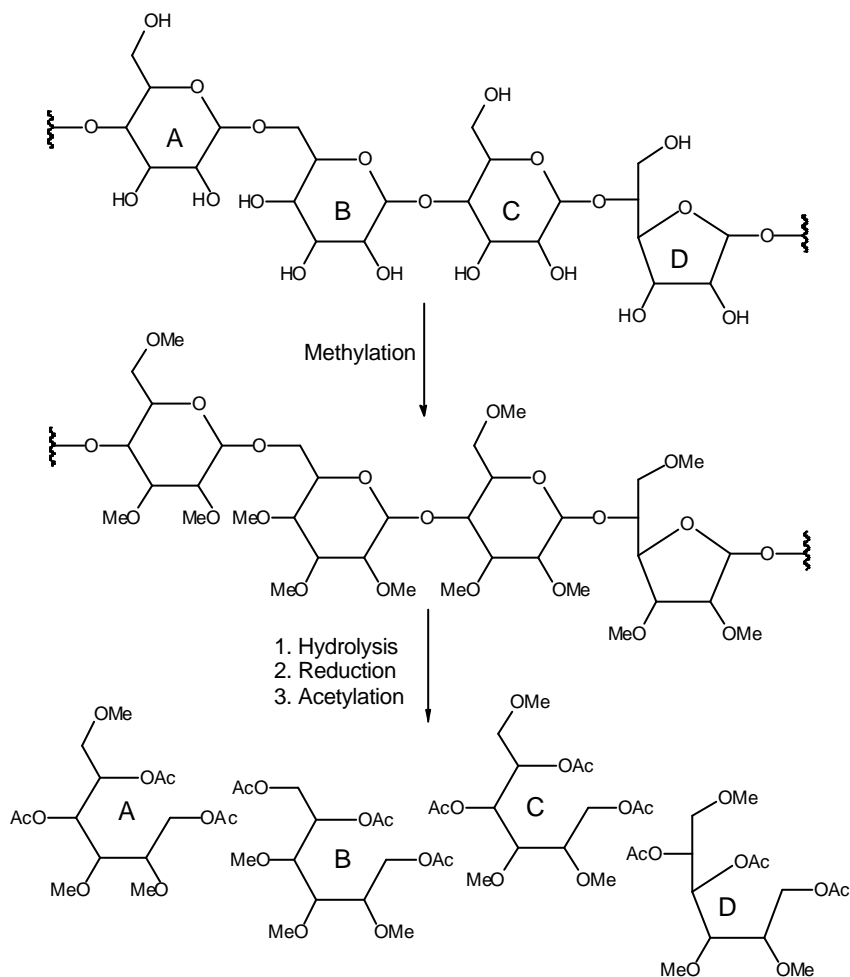
- (i) nature and molar ratios of the contained monosaccharide building blocks;
- (ii) positions of the glycosidic linkages;
- (iii) distinction of furanosidic and pyranosidic forms;
- (iv) anomeric configuration;
- (v) monomer sequences and identification of repeating units; and
- (vi) position and nature of OH-modifications such as *O*-phosphorylation or *O*-sulfation.

The challenges listed above, which do not even include supramolecular considerations, show that the structural analysis of polysaccharides as well as of complex oligosaccharides, can be a complex and demanding task. Mass spectrometry can provide much information regarding sequence analysis and the identification of building blocks. However, for the analysis of the individual monomer types, the polysaccharide is normally subjected to a total hydrolysis under strongly acidic conditions, followed by reduction and peracetylation of the resulting monomeric units. The acetylated alditols that are formed are then subjected to chromatographic analysis and their retention times recorded.

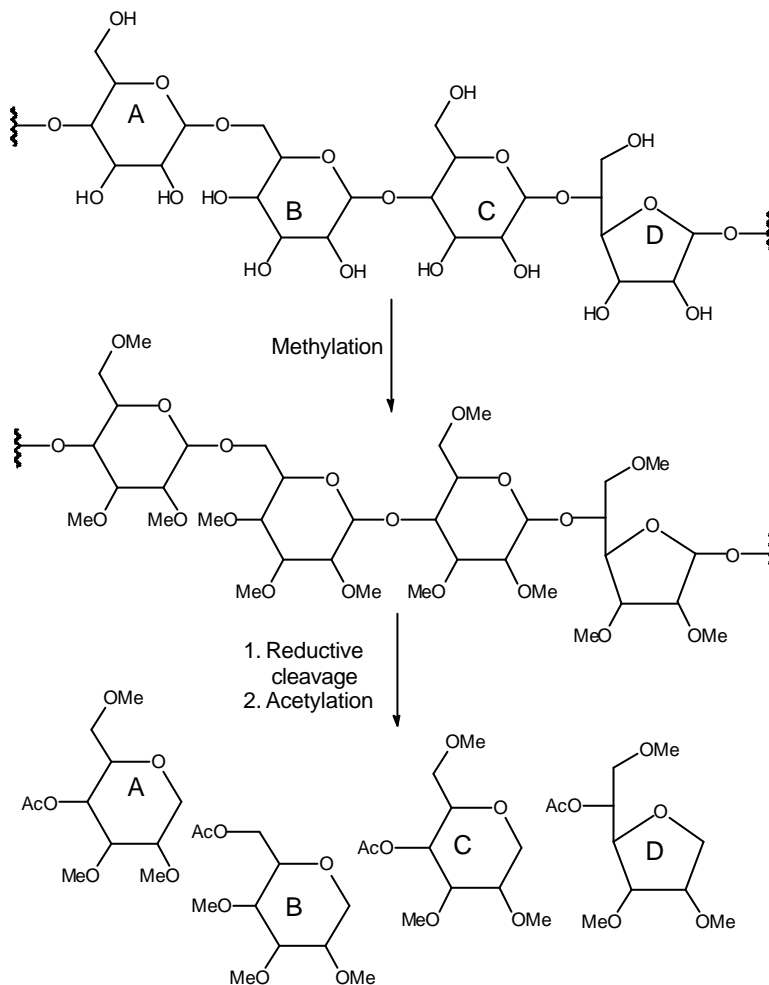
Elucidation of linkage positions is achieved by permethylation of the polysaccharide. Acidic hydrolysis of the resulting poly-methylethers cleaves only the interglycosidic linkages and leaves the methylether bonds intact. Reduction and acetylation then yields partially methylated alditols, which are acetylated at the former linkage positions. The products of this so-called standard methylation analysis are then characterized by gas chromatography and mass spectrometry.

Standard methylation analysis however, has one major drawback and that is that during the reduction step leading to the alditols, structural information is lost, for example by the formation of *meso*-alditols. Furthermore, the same alditol structure is formed in this procedure regardless of whether a 4-*O*-linked aldopyranose or the corresponding 5-*O*-linked aldofuranose was present in the polysaccharide. A solution to this problem was found by using a reductive cleavage protocol, during which the methylated polysaccharide is cleaved using a Lewis acid instead rather than a Brønsted acid; this is then followed by acetylation of the fragments obtained. This methodology was introduced by G. Gray. It yields partially methylated anhydro alditols in which the anomeric position is deoxygenated and only the second linkage position of the sugar ring is acetylated. Thus different reaction products are obtained depending on whether furanosides or pyranosides were cleaved. A combination of trimethylsilylmethanesulfonic acid (TMS-mesylate) and  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  proved to be very successful in this procedure.

### Standard methylation analysis



### Reductive cleavage method



By standard methylation analysis of polysaccharides less structural information is obtained than by employing the reductive cleavage method. While standard analysis does not allow the two residues C and D to be distinguished, when they have the same relative configurations at the stereogenic centers, their different ring forms (pyranose and furanose form, respectively) are conserved in the reductive cleavage method.