Among the biopolymers of nature, the high-molecular-weight glucans are probably the most widely distributed. These homopolysaccharides with α- and β-1,4-, 1,3-, and 1,6-linkages account for huge quantities of such natural substances as starch, cellulose, pseudorogerian, curdlan and paramylon, dextran, and pustulan. All of these polymers are either naturally crystalline or can be easily induced to crystallize, which suggests a high degree of stereoregularity as well as chain linearity. Consequently, all have been studied by diffraction methods, and we now know a great deal about their structure and how the latter is related to biological function.

Of particular interest to us have been the α-1,4- and β-1,3-glucans amylose and paramylon, because these food-storage polysaccharides exhibit multiple-helical structures, crystalline granular morphologies, and a high degree of hydration. During their study by X-ray diffraction methods, specialized structure analysis techniques had to be developed.

Structure determination

Both amylose and paramylon are naturally highly crystalline, but they occur in small, difficult-to-handle granules—amylose in starch, and paramylon in even smaller granules found in the alga *Euglena gracilis*. Detailed structure analysis on the basis of powder X-ray patterns obtainable from the granules is not possible. For this reason, both substances had to be recrystallized as highly oriented fibres from which fibre X-ray patterns could be obtained. For amylose, this was accomplished by first converting it to its triacetate derivative, casting the latter into film solution, heat-stretching the film into a fibre, and desacetelytating the fibre in the solid state under tension. With this procedure, fibres of both A- and B-forms of crystalline amylose were obtained, corresponding to the A-starch of cereals and B-starch of tubers (Wu & Sarko, 1978a,b).

Fibres of the β-1,3-glucan were obtained by using a simpler procedure. Curdlan, a bacterial β-1,3-glucan with the same chemical structure as paramylon, was solubilized in dimethyl sulphoxide and extruded as a fibre into a coagulating bath. Heat-annealing the fibre in the presence of water resulted in a highly crystalline fibre whose well-resolved fibre X-ray pattern corresponded exactly to the powder pattern of paramylon (Chuah et al., 1983).

The patterns were indexed and the intensities of the individual reflections determined. The intensities constituted the primary data set for the determination of the crystal structure. The analysis process was carried out with a specially developed computer program for the refinement of polymer crystal structure (Fugemaier & Sarko, 1976, 1980). In this process, the structure was refined to the best possible agreement with the primary intensity data, as well as proper molecular stereochemistry and minimum potential energy crystalline packing. The resulting structure solutions were detailed and of a high degree of reliability, as indicated by the usual X-ray analysis criteria.

Crystal structures

Amyloses. The two crystalline forms of amylose (the A-form and the B-form) possess identical, double-stranded helical conformations (shown in Fig. 1), in which both chains of the duplex have a parallel orientation. The two forms differ, however, in their crystal packing (as shown in Fig. 1). The B-form possesses an antiparallel hexagonal packing in which six duplexes (three pointing in the ‘up’ direction and three pointing in the ‘down’ direction) surround a cavity which is filled with water (approx. 30% w/w). The water molecules in the cavity are mobile and not in crystalllographic positions. In contrast, the A-form has no such cavity, although the packing is still roughly hexagonal. To accommodate water, the packing in the A-form is somewhat expanded in comparison with the B-form, and the water molecules surround each duplex. Despite these apparent differences in packing, the A- and B-structures are similar; the A-form can be thought of as a B-form in which the central cavity has been filled with another duplex.

In both forms, the strongest hydrogen bonds are found within the duplex, i.e., linking together the two strands of the duplex. This accounts for the solubility behaviour of crystalline amylose in water: it is insoluble in cold water, but soluble at temperatures exceeding 100°C.

Curdlan and paramylon. All β-1,3-glucans (curdlan and paramylon, the slightly branched lentivan and pachyan, as well as the substituted schizophrenian and scleroglucan) crystallize in the form of a triple-stranded helix (cf. Fig. 2). In the triplex, the three polysaccharide chains have a parallel orientation, are wound together in phase, and as a result, are strongly hydrogen-bonded together.

In crystalline packing, the structure of curdlan and paramylon resembles that of amylose in that the packing is hexagonal (cf. Fig. 2), but without the cavity of the B-amylose. The water present in the structure (approx. 20% w/w) is also mobile and non-crystalline, surrounding and separating the triplices. As a result of the continually changing positions of water molecules, the hydroxyls of the polysaccharide chains, located on the periphery of the triplices, are also rotationally disordered. Because of these circumstances, there probably are no fixed hydrogen bonds between the triplices.

There is, however, an important difference in the crystalline packing of amylloses and the β-1,3-glucans. In the former, the duplexes pack in an antiparallel manner, whereas in the latter the triplices are all parallel. As shown below, this has important morphological consequences.

Comparisons of structure and morphology

As indicated above, there is a good deal of similarity in the structures of α-1,4- and β-1,3-glucans. Both possess tight, multiple-stranded helical conformations with parallel orientation of strands and strong, inter-strand hydrogen bonds; relatively loose packing of multiple helices with considerable space available for water molecules; and considerable amounts of mobile, unsaturated water of hydration. However, the differences of packing in the two structures (antiparallel duplexes in amylose, parallel triplices in curdlan and paramylon) are...
Fig. 1. Structure of amylose
(a) Double helix of A- and B-amyloses. Hydrogen bonds are shown by dashed lines; hydrogen atoms are omitted. (b) Comparison of crystalline packing of A-amylose (left) and B-amylose (right). Black dots represent water molecules. (c) Schematic illustration of probable layer structure in starch granules. (The bold lines in the upper part of the diagram depict double helices).
Fig. 2. Structure of curdlan and paramylon

(a) Stereo view of the triple helix of curdlan and paramylon. Hydrogen bonds are shown by broken lines; hydrogen atoms are omitted. Black dots represent water molecules. (b) Crystalline packing of curdlan and paramylon. (Numbers indicate the O-6 hydroxyls of different residues.) Black dots represent water molecules; the hydrogen bond scheme and the water positions that are indicated are only one of many probable schemes. (c) Probable arrangement of triple helices in the paramylon granule.
more important than the similarities, as they determined the granular morphologies of starch and paramylon.

In the starch granule (cf. Fig. 1), the helix axes are oriented radially and the granule grows by the addition of thin layers perpendicular to the helix axes (Borch, 1970). Because the molecular weights of both amylase and the branched amylopectin components of starch are quite high, and the granule becomes crystalline while it grows, it is probable that chain folding takes place during the growth of the granule. The resultant antiparallel packing of helices is reflected in the crystal structure.

In contrast, the helix orientation in the granule of paramylon is tangential, as revealed by electron diffraction (Deslandes, 1979). Because of the all-parallel helix packing and a high degree of crystallinity, it is likely that the chains are added to the paramylon granule during its growth in a spiral fashion, as shown schematically in Fig. 2. The triple helices probably form immediately upon synthesis, because the triple helix is the stable form of the β 1,3-glucan in water solution.

Questions of further interest

It is clear that multiple-helical structures represent a convenient way of obtaining compact, high-density, insoluble, yet highly hydrated forms of reserve polysaccharides. Diffraction studies have helped to understand their structures and morphologies. The complexity of the structures has, in turn, raised some interesting questions. For example, what is the nature of the biosynthetic mechanism that leads to multiple helices? Are two (or three) chains synthesized simultaneously in close proximity, which leads naturally to their intertwining? Is the branching of amylopectin and the substitution of the β 1,3-glucan backbone in schizophyllan and scleroglucan accomplished before or after winding into a multiple helix? How are these storage polysaccharides broken down by enzymes? Does an enzyme molecule require a multiple-stranded substrate or is the latter first unwound in some fashion? Are there lessons to be learned here that would allow up to understand the equally complex interactions between oligomeric heterosaccharides and proteins?

Diffraction studies and the accompanying attempts at structure prediction can again be looked to for answers to these questions. Of particular importance in this connection will be crystallographic analyses of the complexes of enzymes with the carbohydrates and polysaccharides that they synthesize or degrade.

This study was supported by National Science Foundation grants CHE-7501560 and CHE-8107534.


Oligosaccharide binding to glycogen phosphorylase b

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Introduction

Glycogen phosphorylase catalyses the reaction:

$$(\text{Glycogen})_{n-a} + P_i = (\text{Glycogen})_{n} + \text{Glucose-1-phosphate}$$

where subscripts indicate the glucosyl chains $n$ and $(n+1)$ residues long and $P_i$ is inorganic phosphate. Early biochemical studies indicated the existence of an extra binding site for glycogen in addition to the catalytic site. Preincubation of the enzyme with glycogen was shown to enhance activity (Wang et al., 1965) and to promote dissociation of inactive tetramers to active dimers (Metzger et al., 1967). The concept of a second glycogen site was supported by the demonstration that glycogen particles, isolated from muscle by differential centrifugation, contain about 17% of the total phosphorylase present in muscle (Meyer et al., 1970) together with substantial amounts of other enzymes of glycogen metabolism. Confirmation for a distinct glycogen binding site has come from X-ray analysis of crystals of glycogen phosphorylase $a$ and phosphorylase $b$. These studies have shown that there is a separate binding site for $a$-($1-4$)-linked oligosaccharides located some 3.0 nm from the catalytic site and some 3.9 nm from the allosteric effector site (Kasvinsky et al., 1978; Weber et al., 1978). The proposed role of the glycogen storage site in muscle phosphorylase is to provide a point of attachment of the enzyme to its substrate so as to enhance the local concentration of end groups and hence to provide some entropic enhancement of catalysis. Kinetic analysis for a maltoheptaose substrate suggests dissociation constants of 1 mM and 22 mM for the oligosaccharide at the storage site and the catalytic site respectively (Kasvinsky et al., 1978).

This paper describes binding studies of a number of linear oligosaccharides to glycogen phosphorylase $b$ which have led to a detailed interpretation of the contacts made between protein and oligosaccharide at the high-affinity glycogen storage site. No binding of glycogen analogues has been observed at the catalytic site and hence proposals for interactions between these two sites remain speculative.

Experimental

The crystal structure of glycogen phosphorylase $b$ has been solved by conventional heavy atom isomorphous replacement techniques at 0.3 nm resolution (Weber et al., 1978) and the resolution extended to 0.2 nm using data recorded with synchrotron radiation. This structure is being refined by the method of restrained least squares refinement. The crystallographic $R$ value for data between 0.6 and 0.26 nm is 40% with an overall temperature factor of 0.18 nm$^2$ and r.m.s. deviation from ideal bond lengths of 0.01 nm. The refinement of the structure, which is still in progress, will be published elsewhere. Examination of the Fourier synthesis based on combined isomorphous and calculated phases shows that approx. 75% of the 841 amino acid residues have well defined electron density for both main chain and side chain atoms.

Binding studies at 0.3 nm resolution for the compounds listed in Table 1 were carried out by soaking crystals in appropriate solutions of oligosaccharide, and the results analysed by difference Fourier methods using the combined set of phases. The difference syntheses were first examined on a small scale to locate all binding sites and then fitted with the aid of an Evans and Sutherland Picture System 2 on-line to a PDP11/70 computer using the program FRISCO (Jones, 1978). The bond lengths, bond angles and torsion angles obtained from the