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Crystal structures of mannan and glucomannans

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Mannan and glucomannans are related polysaccharides that are widely distributed as reserve materials of certain plants, as well as in the cell walls of some softwoods and a variety of vegetative tissues. They are often crystalline *in vivo* and occur as a number of crystalline allomorphs. The glucomannan from the konjac plant is also exploited commercially as a thickening and gelling agent. X-ray diffraction can be used to visualize hydrated polymer systems at atomic resolution, and the molecular and crystal structures so derived can be used to study structure–function relationships. The different crystalline allomorphs of mannan are described and compared with those of cellulose. The crystal structures of konjac mannan are described and implications for the molecular basis of its gel-forming behaviour discussed.

INTRODUCTION

Mannan is a homopolymer of $1 \rightarrow 4$ linked β-D-mannose that is widely distributed in nature. It is present in the endosperm of certain plants, notably ivory nut, but also palm, date and coffee beans, as well as in the cell walls of softwoods and algae belonging to the families *Codiaceae* and *Dasycladaceae* (Meier & Reid, 1982; Frei & Preston, 1968). Glucomannans are related linear copolymers in which some of the mannose units are replaced, in an apparently random fashion, by $1 \rightarrow 4$ linked β-D-glucose residues. They are found in hemicelluloses of several softwoods, and in the bulbs and endosperm of various plants (Meier & Reid, 1982; Timell, 1965). Pure mannan is similar to cellulose, the homopolymer $\rightarrow 4)$-β-D-Glc-$(1\rightarrow$, both stereochemically, in that it is identical except for the axial configuration of O2 in mannose as opposed to equatorial in glucose, and ultrastructurally, in that they both exist as crystalline microfibrils as structural components of cell walls. The crystal and molecular structures of mannan and related polysaccharides are of interest for a number of reasons. The structural similarity to cellulose offers the opportunity to investigate the effects of configurational alteration at O2 on these structures. Secondly, there are appreciable similarities between the crystal lattice constants of mannan and of the more complex galactomannans such as guar, tara and locust bean gums, suggesting conservation of some packing features in these polysaccharides.

As well as their importance in plant systems, some of these polysaccharides are also used for rheological control of aqueous solutions in the food and other industries, as thickening, stabilizing and gelling agents (Glicksman, 1983). The glucomannan from the konjac plant has useful rheological properties that are exploited commercially. Solution thickening is a result of physical entanglement of conformationally mobile random coils and/or the increased hydrodynamic volume of molecules containing ordered polymer segments. Polysaccharide gels are formed by co-operative associations of structurally regular polymer segments into interchain junction zones that are the tie-points of an infinite network. The chains in the junction zones pack together in an ordered and geometrically regular manner, analogous to chain packing in the solid state (Morris, 1986). The network is solubilized by interconnecting chain segments that are disordered and conformationally mobile, as in solution. Hence, knowledge of the molecular structures and intermolecular interactions of polysaccharides is crucial to understanding the properties of these systems. For thickening polysaccharides, preferred conformations can provide a starting point for estimating chain flexibility and thence hydrodynamic parameters. For gelling polysaccharides, details of ordered structures and the interactions of the polymers with themselves and other components provide models for the types of interactions that may occur in junction zones.

In this paper, the molecular and crystal structures of mannan and konjac glucomannan are described and discussed. As well as providing information on structure–function relationships of the individual polysaccharides, studies on classes of related polysaccharides such as these can provide information on broader principles of structure–property relation-

ships. The structures described have been determined by X-ray fiber diffraction analysis.

Following a brief description of fiber diffraction analysis, the structures of the various allomorphs of mannans and glucomannans are described. These different allomorphs are compared, and are also compared with the different allomorphs of cellulose. The implications of the structures of konjac glucomannan for the molecular basis of its gel-forming behavior is discussed.

X-RAY FIBER DIFFRACTION STUDIES

X-ray diffraction analysis is the most powerful means by which one can accurately visualize a highly hydrated polymer system at atomic resolution (Arnott, 1980; Millane, 1988). Since these molecules do not form regular crystals, they are not suitable for conventional crystallographic analysis. Polysaccharides sometimes occur naturally as more-or-less oriented polysaccharide microfibrils. In other cases, specimens can often be prepared from concentrated solutions as fibers in which the molecular axes are approximately parallel, and sometimes groups of molecules incorporate into small crystallites. In these types of specimens, the orientations of the molecules or crystallites about their long axes are random. It is likely that the ordered secondary structures responsible for many of the physical properties of polysaccharides are essentially the same as those trapped in fibers and stabilized by opportunistic lateral interactions. Hence, although this organization is often artificial, its details can help illuminate the ordered states of polysaccharides that occur in solutions and gels.

The number of diffraction data measured from fibrous specimens is usually small and they are therefore insufficient, on their own, for direct structure determination using traditional crystallographic techniques. However, these data can be systematically augmented with reliable stereochemical information, including the primary polymer structure, helix pitch and symmetry, probable sugar ring geometries, characteristic hydrogen bond and polar interaction geometries, and the requirement that the distances between non-bonded atoms are longer than minimum acceptable values. The melding together of these different kinds of data can lead to a very detailed structure in which most of the atomic positions are defined to within a few tenths of an angstrom, which is a precision adequate for identifying the critical interactions within and between molecules.

Polymer specimens suitable for fiber diffraction analysis are prepared by stretching hydrated films or fibers to induce molecular orientation and crystallinity (Millane, 1990). X-ray diffraction patterns from such specimens contain diffracted intensities that are related to the molecular and crystal structures of the constituent molecules. The pitch and symmetry of the molecular helix and the unit cell dimensions can be derived directly from the diffraction pattern. This information, together with the monomer geometries and other stereochemical constraints, is used to construct all possible *types* of molecular and packing models. The different types of model may correspond to, for example, different chiralities of the molecular helix, different numbers of chains, and different modes of packing. All candidate models are refined (by varying the glycosidic conformation angles, angles defining substituent conformations, and parameters describing the crystal packing) using standard techniques (Arnott, 1980) until the fit with measured X-ray amplitudes and steric factors allows one model to be declared significantly superior to the others by appropriate statistical tests.

MANNAN

Microfibrils of mannan were observed in the cell walls of ivory nut endosperm and date seeds by Meier (1958), who observed two forms, termed A and B, that differed in molecular weight and their granular and microfibrillar appearance, by electron microscopy. These two forms were also observed for algal mannans if extracted under mild conditions, although the granular form was dominant (Mackie & Preston, 1968). A thorough investigation of mannan cell walls in siphoneous green algae was reported by Frei and Preston (1968). The material was granular and was characterized by X-ray fiber diffraction patterns from well-oriented specimens. When the cell wall material was treated with alkali, a different X-ray diffraction pattern was obtained. The materials were moisture-sensitive. The native and alkali-treated allomorphs were named mannan I and mannan II, respectively, by analogy with cellulose I and cellulose II. Subsequently, it was demonstrated by electron diffraction that the alkali-resistant microfibrillar components correspond to mannan II, and the alkali-soluble granular components to mannan I (Chanzy *et al.*, 1984). This is in contrast to cellulose, for which cellulose I is the only native allomorph.

The crystal structure of mannan I has been determined using both X-ray (Atkins *et al.*, 1988) and electron (Chanzy *et al.*, 1987) diffraction. The polysaccharide crystallizes in an orthorhombic unit cell with dimensions $a = 8{\cdot}92$ Å, $b = 7{\cdot}21$ Å and $c = 10{\cdot}27$ Å (Chanzy *et al.*, 1987), and space group $P2_12_12_1$. The molecular axes are parallel to the c-axis and two molecules pass through the unit cell, one at the corner and one at the center of the a–b face (Fig. 1). The polymer chains have two-fold screw symmetry, the molecular axes coinciding with space group two-fold screw axes parallel to the c-axis. The two molecules are antiparallel, being related by space group screw axes perpendicular to the c-axis. The molecular structure is

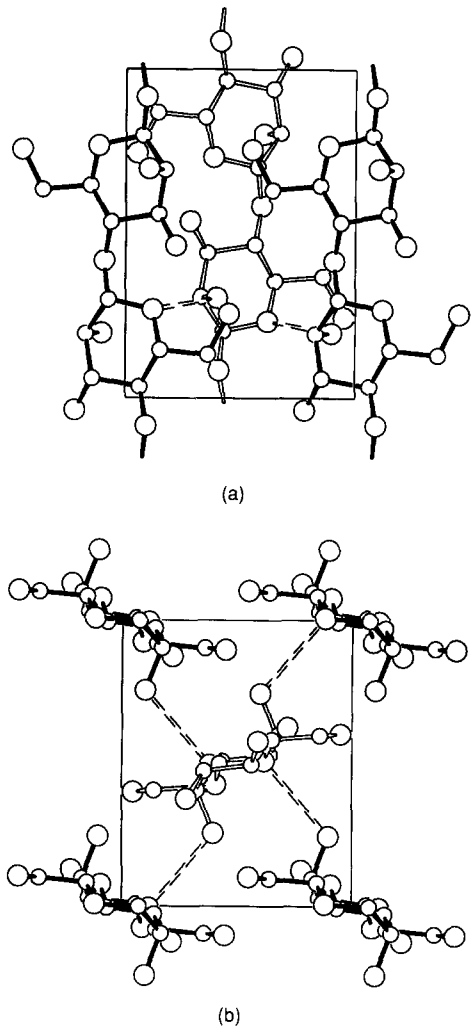

Fig. 1. Projections of the mannan I crystal structure (Chanzy *et al.*, 1987) along (a) the *a*-axis and (b) the *c*-axis. The up and down chains are shown by full and open bonds, respectively, and the hydrogen bonds by broken lines.

very similar to that of cellulose, incorporating the same O3–O5 intramolecular hydrogen bond across the glycosidic linkage, and the hydroxymethyl groups in the *gt* conformation. Oxygens O6 and O3 are also at hydrogen bonding distance across the glycosidic linkage. The structure forms a herringbone pattern when viewed down the *c*-axis and is stabilized by a network of O2–O5 intermolecular hydrogen bonds in which each residue acts as both a donor and an acceptor (Fig. 1). Referring to Fig. 1, the structure can be viewed as sheets of molecules parallel to the *a*-axis, the *b*-axis or the *a*–*b*-diagonal, although the figure suggests that crystal growth occurs on *a*–*b*-diagonal planes. It is possible that the packing does not conform exactly to $P2_12_12_1$ symmetry (Atkins *et al.*, 1988), but the conformational differences are small and probably below the precision of the data.

We have recently determined the crystal structure of algal mannan II from the inner wall of *Cympolia barbata* using X-ray diffraction analysis, and the details of this structure will be published shortly (Millane *et al.*, 1995). The inner wall material was dried under tension and X-ray diffraction data obtained at 98% relative humidity (Frei & Preston, 1968; Millane *et al.*, 1995). The polysaccharide crystallizes in a rectangular unit cell with dimensions $a = 9{\cdot}00$ Å, $b = 16{\cdot}65$ Å and $c = 10{\cdot}35$ Å, with space group I222. Four molecules pass through the unit cell with their molecular axes parallel to the *c*-axis and adjacent molecules (in both the *a* and *b* directions) are antiparallel. The molecular axes coincide with space group two-fold screw axes parallel to the *c*-axis. The four molecules are conformationally identical, being related by crystallographic two-fold rotation axes perpendicular to the *c*-axis. The molecular conformation is essentially identical to that of mannan I including the O3–O5 intramolecular hydrogen bond, but the hydroxymethyl group is in the *tg* conformation. A packing model is shown in Fig. 2, although this is to be subjected to further refinement. The packing is quite different to that for mannan I. The chains pack antiparallel in sheets with their edges approximately parallel to the *b*-axis, and molecules in adjacent sheets are also antiparallel. The X-ray data indicate the presence of a periodic water molecule on a two-fold rotation axis between the sheets (Fig. 2).

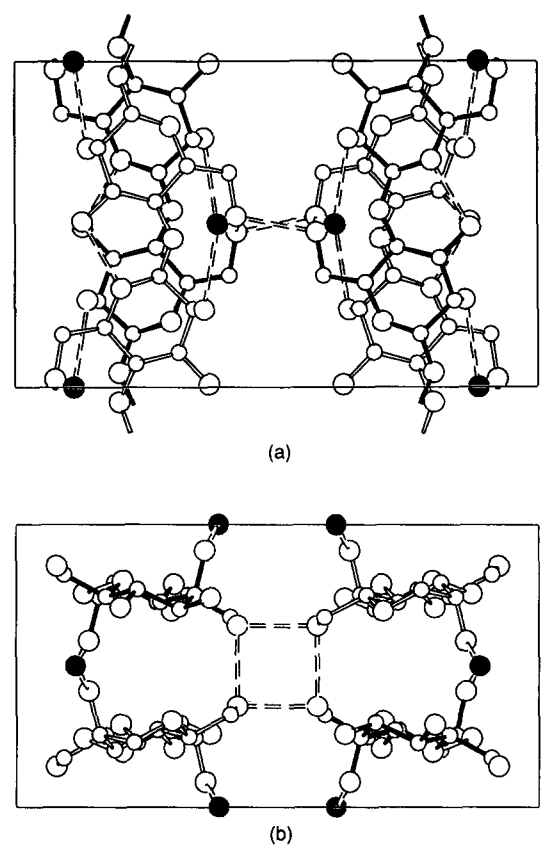

Fig. 2. Projections of the mannan II crystal structure along (a) the *a*-axis and (b) the *c*-axis. The up and down chains are shown by full and open bonds, respectively, and the hydrogen bonds by broken lines. The water molecules are shown by filled circles.

GLUCOMANNANS

Glucomannans are mannose-rich hemicelluloses found in softwoods and some roots, tubers, bulbs and seeds. Glucomannans often contain a small number of D-galactose terminal sidechains, typically about 2–6% per mainchain residue as well as O-acetyl groups to varying degrees (Timell, 1965; Gidley *et al.*, 1991). Glucomannans from softwoods typically have a DP less than 100 and mannose-to-glucose ratios (M/G) between 2 and 4 (Timell, 1965). Pine glucomannan contains about 50% O-acetyl groups per sugar. The position(s) of acetylation is generally unknown, although for pine glucomannan the acetate groups have been shown to be attached to positions 2 and 3 of the mannose residues (Meier, 1961; Lindberg *et al.*, 1973), and are randomly distributed (Kenne *et al.*, 1975). Some of the glucomannan molecules are believed to be associated with cellulose in the cell wall. They can be crystallized with lattice constants very similar to those of either mannan I or mannan II, depending on the conditions. The mannan II form is favored by low temperature and high humidity compared to that for the mannan I form (Chanzy *et al.*, 1982). Softwood glucomannans can also be grown on cellulose microfibrils (Chanzy *et al.*, 1982). Glucomannans also occur in a variety of vegetative tissues and generally have higher molecular weights than those from softwoods (usually DP > 100 and often much larger) (Meier & Reid, 1982). They have M/G ratios between 1·5 and 4, and acetyl group content between 5 and 20% per sugar. In most cases the location of the acetate groups is not known. The crystallization behavior of glucomannans from tubers of the orchids *Tubera salep* and *Amorphophallus konjac* has been studied by electron diffraction (Chanzy *et al.*, 1982). *T. salep* glucomannan has a DP of 600, M/G = 3·2 and an acetyl content of 20%, and could be crystallized in a mannan I or mannan II lattice, the latter at lower temperatures and higher humidity, as for the softwood glucomannans (Chanzy *et al.*, 1982). The glucomannan from *A. konjac* (often referred to as konjac mannan) has a very high molecular weight (average DP = 6000), M/G = 1·6 and an acetyl content of about 15% (Gidley *et al.*, 1991), and could only be crystallized in the mannan II lattice, independent of temperature (Chanzy *et al.*, 1982). Hence the mannan II lattice appears to be favored also by a high molecular weight. The lattice constants appear to be independent of the glucose and acetyl contents. Electron microscopy also showed that a lower molecular weight and a higher mannose content favor larger, more regular crystals (Chanzy *et al.*, 1982). This indicates that long mannan sequences form good crystals, and insertion of glucose residues in the sequence is detrimental to crystal perfection.

The primary structure of konjac mannan has been investigated by enzymatic hydrolysis by a number of investigators using various β-mannanases (Shimahara *et al.*, 1975; Takahashi *et al.*, 1984). The hydrolysis products consist of a variety of oligosaccharides containing both mannose and glucose. The results of these studies preclude a simple repeating structure and do not provide strong evidence of anything but a random sequence, although it is possible that M_1, M_2, M_5, G_1 and G_2 sequences may predominate.

The konjac plant has been cultivated in the East for over 1000 years, and in traditional Japanese cooking it is used to make noodles and gels that are heat-stable in boiling water. The polysaccharide is used as a crude preparation called konjac flour that is dried and milled from the tuber. Konjac mannan is water-soluble giving highly viscous and pseudoplastic solutions. It forms strong elastic gels when heated with mild alkali, that are heat-stable and acid-stable. The gel is unusual in that it increases in strength as it is heated. Konjac mannan interacts synergistically with other polysaccharides such as kappa-carrageenan (increasing gel strength and elasticity) and xanthan gum (promoting gelation), and with corn starch (increasing viscosity). Konjac flour also has film-forming and coating properties, and can be used to make an edible film that is semi-permeable to moisture and oxygen.

The crystal structures of two konjac mannan specimens have been studied by X-ray fiber diffraction. In one of these, the konjac mannan was completely acetylated and prepared as stretched films that were then completely deacetylated (Yui *et al.*, 1992). In the other study, fibers were prepared directly from the native (partially acetylated) polysaccharide (Brownsey *et al.*, 1988; Hendrixson *et al.*, 1995). In both cases the polysaccharide crystallized in a rectangular unit cell with dimensions almost identical to those of mannan II (Table 1). In addition, an overall similarity in the intensity distribution between the glucomannan and mannan II diffraction patterns indicates similar structures, implying isomorphous replacement of some of the mannose residues by glucose.

The structure determined for deacetylated konjac mannan is quite similar to that for mannan II (Yui *et al.*, 1992). The random copolymer was simulated by including an equatorial oxygen atom at the 2-position of the mannose residues, and the occupancy of the axial and equatorial oxygen atoms adjusted to represent the relative proportions of the two sugars in the glucomannan. The packing symmetry is identical, and the

Table 1. Unit cell dimensions (Å) for mannan and konjac mannan crystal structures

	a	*b*	*c*
Mannan I	8·92	7·21	10·27
Mannan II	9·00	16·65	10·35
Konjac mannan (deacetylated)	9·01	16·73	10·40
Konjac mannan (native)	9·18	16·60	10·30

positions of the chains in the unit cell are very close, to those of mannan II (Fig. 3). Acceptable packing and X-ray agreement does indeed indicate isomorphous replacement of some of the mannose residues by glucose in the crystal structure. The *gt* and *gg* conformations of the primary hydroxyl group were found to be acceptable, although there was a preference for the *gt* conformation, based on a more stable hydrogen bonding scheme. This model contains two independent water molecules in the unit cell, located on symmetry axes, giving an average of one water molecule per sugar residue. The water molecules are positioned between the sheets of molecules as in mannan II, but their positions and interactions are somewhat different. One water molecule is hydrogen bonded to O3 and O6, and the other to O2 of mannose and O6 of the sugar residues, and the water molecules form bridges between molecules both within and between sheets.

The diffraction data from native konjac mannan are not as well resolved as for the deacetylated polysaccharide, and the effect of the acetate substituents was investigated based on the mannan II crystal structure (Hendrixson *et al.*, 1995). As with the deacetylated polysaccharide, an equatorial oxygen atom at C2 could be included in the mannan II crystal structure without any steric difficulties. The water molecule was removed from the structure and an acetate group attached at each available hydroxyl group (O2 (axial), O2 (equatorial), O3 and O6) in turn, since the actual positions of the acetate substituents are not known for konjac mannan. The structures were optimized by varying the conformation of the acetate group while keeping the glucomannan backbone fixed. These refinements showed that acceptable crystal structures could not be generated with acetate substituents in three of the four positions. For the remaining position (O2 of mannose), although the structure was not totally unacceptable it was sterically compressed with some non-bonded interatomic distances shorter than 0·4 Å less than the sum of van der Waals radii. Since the degree of substitution of the acetate groups is low, and since the most sterically accessible position for acetate substitution produces a structure which is still sterically compressed, the most plausible explanation is that the diffraction pattern is due to crystalline regions in the specimen that incorporate only unsubstituted segments of the glucomannan backbone. If the acetate substituents are randomly distributed on one in every 15 sugars, then the average length of the unsubstituted stretches is 14 sugars. If the packing of the chain is disrupted by the presence of an acetate group, then the average length of the crystalline regions would be about 80 Å. Estimates of the crystallite dimensions from the breadths of the reflections on the diffraction pattern give a length of ~50 Å, which is consistent with this proposal.

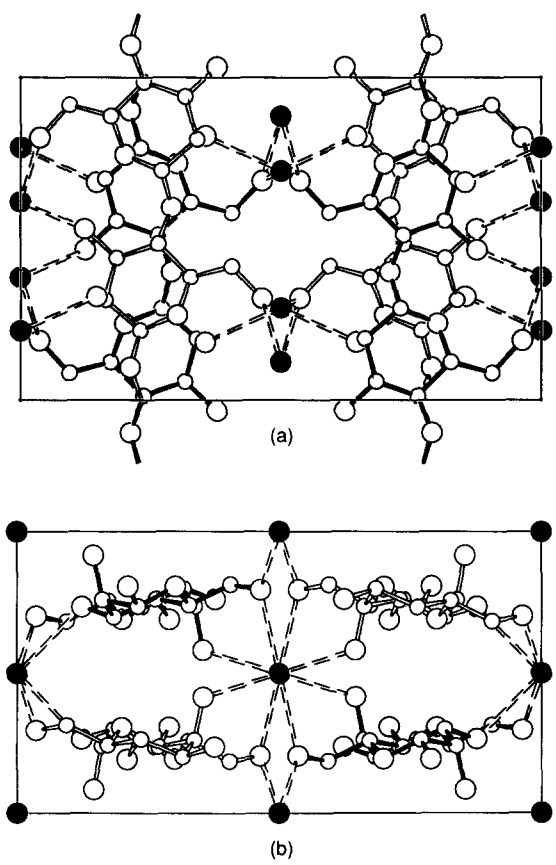

Fig. 3. Projections of the deacetylated konjac mannan crystal structure (Yui *et al.*, 1992) along (a) the *a*-axis and (b) the *c*-axis. The up and down chains are shown by full and open bonds, respectively, and the hydrogen bonds by broken lines. The water molecules are shown by filled circles.

DISCUSSION

Despite the chemical similarity, there are substantial differences between the crystal structures of mannan I and II, and cellulose I and II. The molecules in mannan I form an interleaved pattern, whereas the molecules are disposed edge-to-edge forming narrow sheets in mannan II and cellulose I (Sarko & Muggli, 1974; Gardner & Blackwell, 1974) and, to an extent, in cellulose II (Kolpak & Blackwell, 1976; Stipanovic & Sarko, 1976) (Fig. 4). This leads to a tightly hydrogen bonded network of the chains in mannan I, in which each mannose unit occupies 165 $Å^3$. The molecules form sheets in mannan II with the molecules in neighboring sheets being adjacent. Adjacent molecules, both within and between sheets, are antiparallel. The structure has a lower density, each mannose unit (and associated water molecules) occupying 194 $Å^3$. The molecules in cellulose I form sheets as do those in mannan II, but the packing is different in many respects. The molecules in cellulose I are all parallel (both within and between sheets) and the sheets are staggered along the *a*-axis so that the molecules in one sheet lie adjacent to the gaps between

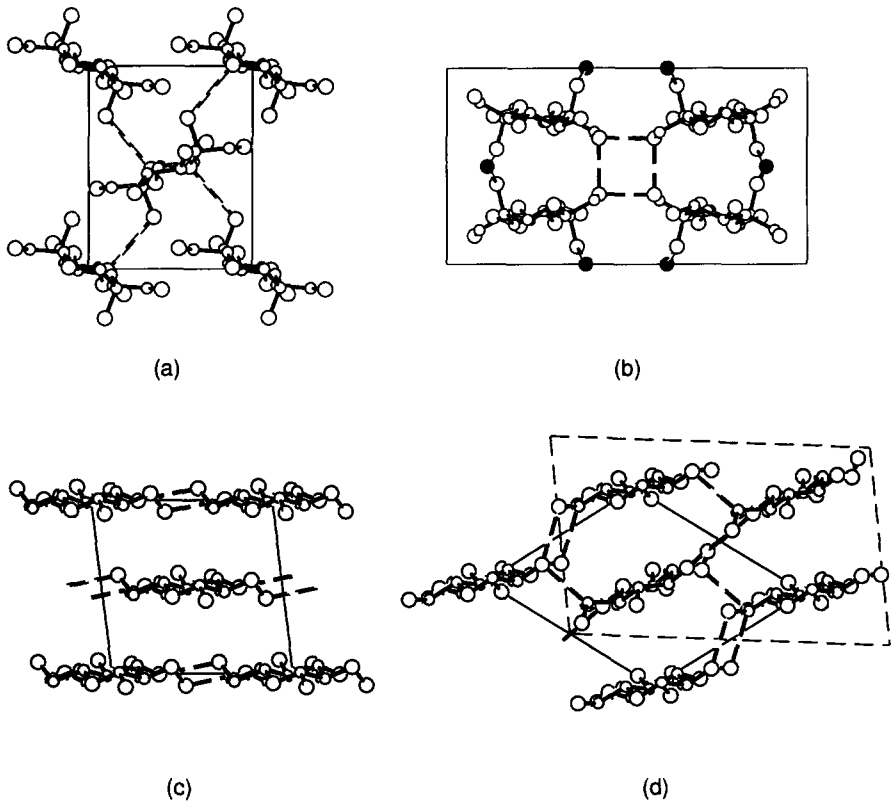

Fig. 4. Crystal packing, viewed along the *c*-axis, of (a) mannan I, (b) mannan II, (c) cellulose I, and (d) cellulose II. In (d) the dashed lines denote a unit cell with twice the volume of the fundamental unit cell.

molecules in the neighboring sheet (Fig. 4c) (Sarko & Muggli, 1974; Gardner & Blackwell, 1974). The density of cellulose I (one sugar residue in 163 Å^3) is similar to that of mannan I. Cellulose II is obtained from cellulose I by swelling with alkali. Although the unit cell and crystal symmetry of cellulose II (Kolpak & Blackwell, 1976; Stipanovic & Sarko, 1976) are quite different to those of mannan II, the packing of the chains is, in fact, quite similar (Fig. 4d). The cellulose II crystal structure may be described in terms of a unit cell (dashed lines in Fig. 4d) that has twice the volume of the fundamental unit cell, with dimensions $a = 8{\cdot}38$ Å, $b = 13{\cdot}76$ Å, $c = 10{\cdot}36$ Å and $\gamma = 92°$. The packing of the molecules in this unit cell is similar to the packing in the mannan II unit cell. The molecules can be viewed as forming sheets along the new *b*-axis, or along the original *a*–*b*-diagonal. Adjacent molecules within these sheets are antiparallel, and molecules in neighboring sheets are adjacent and antiparallel, as in mannan II. Differences between the two structures are related to different rotational relationships between the molecules, a different hydrogen bonding network and the density of cellulose II (one sugar in 165 Å^3) being higher than that of mannan II. In fact the density of cellulose II is similar to that of cellulose I and mannan I, which may be a result of all of these allomorphs being anhydrous. Recent results have shown that some algal native celluloses exist as a mixture of the cellulose I crystal form described here (Iβ) as well as a triclinic crystal form (Iα) (Sugiyama *et al.*, 1991). The triclinic form has not been characterized in detail, but the unit cell dimensions indicate that the packing is similar to that of cellulose I.

The cellulose I to cellulose II conversion involves swelling of the structure and proceeds through a number of crystalline alkali–cellulose complexes. It is believed to involve aggregation of individual molecules from randomly 'up' and 'down' pointing microfibrils (each containing parallel molecules) to form crystallites containing antiparallel chains (Okano & Sarko, 1985). The conversion from mannan I to mannan II is not as well characterized, however. Frei and Preston (1968) observed that mannan I is converted to mannan II on swelling with alkali, however other data (Chanzy *et al.*, 1984) suggest that the alkali treatment may selectively remove the mannan I component, making a pre-existing mannan II component more apparent. In any case, if any conversion from mannan I to mannan II does occur, since both structures involve antiparallel chains the conversion is, at least conceptually, easier to visualize than that for cellulose. In fact, referring to Figs 1 and 2, such a conversion can be achieved by rotation and translation of some of the chains in the mannan I structure without any longer range rearrangements.

The crystal structure of deacetylated konjac mannan is almost identical to that of mannan II although there are differences in the precise packing, primary hydroxyl

conformations and solvent interactions. Native (acetylated) konjac mannan packs in the solid state essentially isomorphously with mannan II, showing that replacement of mannose by glucose does not disrupt the molecular associations adopted by hydrated mannan. The packing of the glucomannan chains does not appear to accommodate the acetate substituents at any position, however. As described above, this suggests that crystalline regions in the specimen incorporate only unsubstituted stretches of the glucomannan backbone.

The structural results, therefore, suggest the following mechanism for the behavior of native konjac mannan. In the solid state (moist fibers), the lower chain entropy associated with a sterically restricted environment allows acetate-free stretches of the native polysaccharide to aggregate in a manner isomorphous to that of mannan II, forming crystallites of limited extent. In solution, however, association of unsubstituted stretches of the native polysaccharide is unfavorable because the associated enthalpy decrease is not large enough to overcome the decrease in conformational and configurational entropy of the chain segments. On alkali treatment, deacetylation increases the lengths of the unsubstituted segments. Cooperative interactions are then more favorable because there is a larger decrease in enthalpy per segment, that is sufficient to overcome the decrease in entropy. Junction zones involving many different chains then develop, leading to a network and gel formation.

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