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Molecular modeling of substituted polysaccharides

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X-ray diffraction patterns of gellan, high acyl native gellan and branched polymer welan correspond to polycrystalline and well oriented specimens. Their molecular structures are strikingly similar, but their packing arrangements are not. The benzyl ester of gellan diffracts like gellan implying that a gellan-like double helix can sustain this substitution. Likewise, the benzyl ester of hyaluronan can adopt any of the reported single- and double-helical forms of hyaluronan itself. Carboxymethylhydroxypropyl guaran (CMHPG) diffracts similarly to guaran. The hydroxypropyl and carboxymethyl groups can cause favorable association of a pair of CMHPG chains which can further be stabilized by divalent cations, one for two carboxymethyl groups belonging to different chains. X-ray data combined with modeling calculations have revealed the details of the molecular architectures in each case to varying extents.

INTRODUCTION

Polysaccharides are widely used in food as well as non-food industries because of their cost-effective availability from plants, bacteria, animals and seaweed. While the structural polysaccharides cellulose, mannan and chitin are water-insoluble, the industrial gums xanthan, guaran and gellan are water-soluble and hence termed hydrocolloids. The dramatic differences in functional properties between cellulose and xanthan, both of which have the same main chain, owe their origin to the regular branching observed in xanthan and can be understood from their respective molecular structures and their interactions with surrounding molecules. The ribbon-like cellulose chains are water-insoluble since they interact more favorably among themselves than with water. In contrast, the charged side chains in xanthan, apart from increasing the molecular dimensions relative to cellulose, lead to excellent hydration, which in turn, imparts high viscosity in aqueous solution that persists over a wide range of temperatures and pH. This observation highlights the importance of substitution in altering physical properties. Consequently, chemical modification of specific sites of native polysaccharides is a powerful method for the production of designer polymers for particular applications.

As part of an ongoing research program on X-ray diffraction and molecular modeling of polysaccharides towards understanding their structure–function relationships, we have described some important results on three distinct polymer systems. The geometries of three structurally related polysaccharides in the gellan family, determined by X-ray fiber diffraction analysis, illustrate the effect of substitution on molecular morphology (Chandrasekaran *et al.*, 1988, 1992, 1994). Regulation of the effective charge density of ionic polymers by the desired degree of substitution decreases solvation, but results in the formation of hydrogels. Their ability to swell to several times their initial volume and hold large amounts of water or other material has already found uses in biomedical applications such as controlled drug release as a function of time, temperature or chemical change (Crescenzi *et al.*, 1992*a*, *b*). Calculations based on preliminary X-ray diffraction patterns indicate that the native helical forms of gellan and hyaluronan are stereochemically amenable for their respective benzyl ester derivatives. Molecular modeling of carboxymethylhydroxypropyl guaran (CMHPG) in relation to that of guaran has provided the first visualization of ion-mediated crosslinking of the substituted chains. In all the above modified systems, the molecular structures are minor variants of the corresponding parent polymers. However, intermolecular interactions before and after substitution are not the same.

MATERIALS AND METHODS

The major experimental tool for this structural study was X-ray fiber diffraction. For each polymer investigated, attempts were made to obtain polycrystalline and well oriented fibers so that both the molecular structure

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and packing arrangement could be determined with precision via the linked-atom least-squares (LALS) refinement procedure (Smith & Arnott, 1977). This method was relatively successful for gellan (Chandrasekaran *et al.*, 1988), native gellan (Chandrasekaran *et al.*, 1992), and welan (Chandrasekaran *et al.*, 1994). However, only oriented specimens could be prepared for the benzyl esters of gellan and hyaluronate and also for CMHPG. A stereochemically viable model for the benzyl ester of gellan was generated by appropriately attaching a benzyl moiety to the carboxyl oxygen atom in each repeating unit of a rigid gellan double helix. The conformation of the substituent, specified by the three successive angles χ_1, χ_2 and χ_3 in the fragment -C–O–CH_2–C_6H_5, was maintained to be the same throughout the helix. These angles were assigned initial values from an inspection of the gellan molecule and subsequently refined by the LALS method until the pendant atoms were in conformationally sound domains. A number of helical structures (Arnott & Mitra, 1983) had to be considered for hyaluronan. For each structure, one or more models incorporating the benzyl ester groups were developed by following the same procedure as for gellan.

The polymer software from Biosym Technologies, San Diego, California on a Silicon Graphics Indigo workstation was primarily used to examine substituted and unsubstituted galactomannan structures. All models were idealized as 2-fold helices of pitch 10.3 Å. For convenience, an α-D-galactosyl (G) unit was 1 → 6 linked to every second mannosyl (M) unit of the rigid backbone so that the galactomannan structure approximated to that of guaran which has a G/M ratio of 0.6. Thus, an M–MG trisaccharide constituted the repeating unit of the galactomannan molecule prior to substitution. The three conformation angles (ϕ_G, ψ_G, ω_G) at the G(1 → 6)M linkage, defining the orientation of G relative to M in the MG disaccharide, were the major variables, g^+, g^- and t, being the three preferred domains for ω_G. Each domain was considered separately in analyzing the influence of substitution on conformational freedom. At the time of writing, results were known for only one model in which ω_G was fixed at −60°. In principle, one or more free hydroxyls in the repeating unit may be primary appendage sites for either carboxymethyl (CM) or 2-hydroxypropyl (HP) groups. But following McNeil *et al.* (1984) and Kesavan & Prud'homme (1992), this was pinpointed to the three most frequently occupied sites. Single as well as multiple sites were examined using the Biosym energy minimization software. The galactomannan chain was kept rigid and only the conformation angles within and around each substituent were varied in this analysis. The resulting low energy structure was used to further visualize the lateral association of two CMHPG chains.

RESULTS

Gellan, native gellan and welan

The three-dimensional structures of these three microbial polysaccharides in the gellan family are now known, but details of the other members are sparse. The complexity of chemical structure increases from gellan to welan, gellan being the commercial gel-forming polysaccharide obtained after alkali treatment of native gellan. Gellan has the tetrasaccharide repeating unit → 3)-β-D-Glc-(1 → 4)-β-D-GlcA-(1 → 4)-β-D-Glc-(1 → 4)-α-L-Rha-(1 →, denoted as A-B-C-D. The other two also have the same sequence in the main chain, but are substituted; native gellan has an L-glyceryl group at *O*2 and acetyl group (50%) at *O*6 of residue A; and welan has an acetyl group (80%) at *O*2 of residue A and a side chain E, α-L-Rha or α-L-Man (in the ratio 2:1), linked to *O*3 of residue C. The hard and brittle gels of gellan, and the soft and rubbery gels of native gellan, are useful to the food industry. In contrast, welan is an attractive viscosifier for oil well drills and also an additive in concrete mix.

X-ray diffraction patterns from polycrystalline and well oriented fibers of the monovalent salts of gellan and native gellan, and the calcium salt of welan are remarkably similar in overall intensity distribution (Chandrasekaran *et al.*, 1988, 1992, 1994). All three polymers form half-staggered, parallel, double-helical structures (Fig. 1) stabilized by interchain hydrogen bonds between carboxylate (residue B) and hydroxymethyl (residue C) groups. Detailed analyses confirm that their molecular structures are similar and suggest that the double helix is robust and the backbone conformation is unaffected by substituents. On the other hand, substituents cause appreciable differences in packing arrangements as reflected by variations in unit cell dimensions and contents (Table 1). The change in *c* is small, but the intermolecular separation is much higher for welan relative to gellan than for native gellan. This is easily understood in terms of relative repeat size. However, three welan molecules per unit cell, instead of two for gellan, or native gellan, is rather unexpected, but is certainly a consequence of side chain induced lateral re-organization. Substituents make specific interactions with neighboring carboxylate groups. For example, the glyceryl group in native gellan hydrogen bonds with an adjacent carboxylate group within the chain, reduces potassium occupancy to 0.5, and also dislodges the ion by approximately 5.5 Å relative to that in gellan (Chandrasekaran *et al.*, 1992). Therefore, ion-mediated interactions among native gellan double helices are not as strong as those observed in gellan. Side chain (E) in welan is on the helix surface, shields a carboxylate group by forming two hydrogen bonds and bestows additional stability on the helix. Two calcium ions are shared by three carboxylate groups. Two

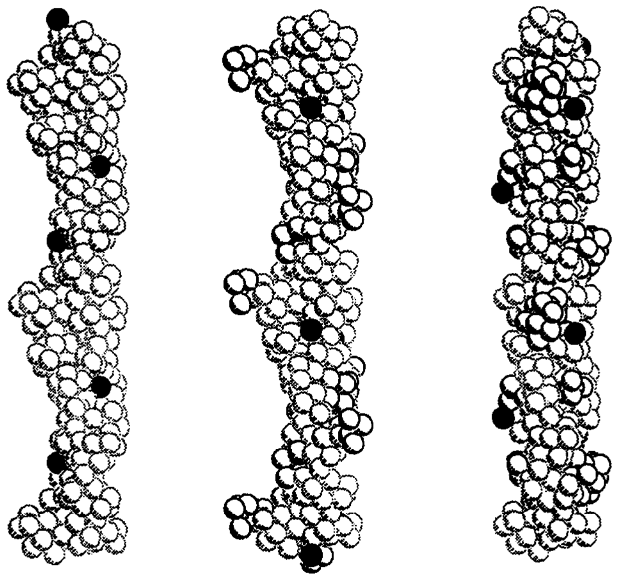

Fig. 1. Computer drawn space filling models of approximately one pitch-length of the double helix of (a) gellan; (b) native gellan; and (c) welan. The filled circles are potassium ions in (a) and (b), and calcium ions in (c). Substituents and side chains are highlighted. The indentations in gellan are slightly filled in by glyceryl groups in native gellan in which the acetyl groups appear as little protrusions. The side chains in welan are on the surface; the width of the molecule appears to be nearly the same throughout its length.

Table 1. Comparison of unit cell (trigonal, $a = b$, $\alpha = \beta = 90°$ and $\gamma = 120°$) dimensions, number of molecules (Z) passing through unit cell and intermolecular separation (s) in gellan, native gellan and welan

Polymer	a (Å)	c (Å)	Z	s (Å)
K^+ Gellan	15.75	28.15	2	9.1
K^+ Native gellan	16.47	28.42	2	9.5
Ca^{2+} Welan	20.83	28.69	3	12.0

double helices (I and III) in the unit cell are pointing 'up' and a third (II) is pointing 'down' and hence they do not have equivalent surroundings. Firstly, direct carboxylate–calcium–carboxylate crosslinks between II and III, such that one or more hydroxyls in the side chains serve as ligands to the ion, represent the strongest possible interactions between welan double helices (Chandrasekaran *et al.*, 1994). Secondly, interactions between I and II involve side chains alone and are considered to be relatively weak. Finally, the crosslinks between I and III through carboxylate–calcium–water–carboxylate interactions are of intermediate strength. While the medium and weak interactions might be related to welan's usefulness as a viscosifier at low and high temperatures, respectively, the strong interactions might dominate when welan is exploited as an additive in concrete mix.

Benzyl ester of gellan

Two derivatized samples of gellan, benzyl substitutions 18 and 100%, were made available to us by Professor Vittorio Crescenzi, University of Rome, Italy. The former was converted to its potassium salt by dialysis before stretching fibers using water as solvent. Fibers of the latter sample were prepared from a polymer solution in DMSO/water (70/30 mixture). In either case, the diffraction pattern appeared as in Fig. 2a. There was some orientation in the fiber along the molecular axis and lateral organization was limited to a short range. Overall intensity distribution was diagnostic of a gellan-like double-helical structure. It is instructive to note that the monovalent salt forms of welan and two other branched polysaccharides, S-657 and rhamsan in the gellan family, also produced diffraction patterns which resembled Fig. 2a. Subsequent molecular modeling calculations, similar to those conducted for native gellan (Chandrasekaran & Thailambal, 1990), swiftly predicted that their side chains would not interfere with double helix formation (Lee & Chandrasekaran, 1991). Later, independent X-ray structure determination validated these theoretical results for welan (Chandrasekaran *et al.*, 1994). This emphasizes that prediction methods are sound and reliable in the study of structurally related polymers.

Current calculations show that the gellan double helix can also accommodate a benzyl ester group in each repeating unit. One of the oxygen atoms of the carboxylate group is an acceptor for the interchain hydrogen bond that stabilizes the double helix. The other oxygen atom is the natural site for substitution. While the conformation of the methylene carbon atom in the substituent is sterically confined to the g^+ domain, there is more freedom of movement for the remaining atoms. One of the sterically allowed conformations (Fig. 3) corresponds to $\chi_1 = 100°$, $\chi_2 = -142°$ and $\chi_3 = -76°$. Positioned towards the reducing end, the peripheral benzene ring has partial overlap with glucose A and is oriented almost parallel to the chain direction. The radius of the helix is stretched out marginally to approximately 8.1 Å from 7.9 Å in the unsubstituted form. The predicted conformation for the isolated molecule represents one of the possible solution structures. In the solid state, however, intermolecular forces might discipline the flexibility of side chains.

The quality of X-ray diffraction pattern (Fig. 2a) is inadequate to permit a complete structural analysis that would confirm the fine details of the proposed model and also reveal how the double helices might associate in the presence of aromatic rings. Intermolecular separation must be slightly greater than that in gellan (9.1 Å). In fact, the measured *d*-spacings in the diffraction pattern of 18% benzyl ester of gellan do suggest modest expansion, of approximately 0.2 Å relative to gellan, in the *a* and *b* values of its unit cell. Until

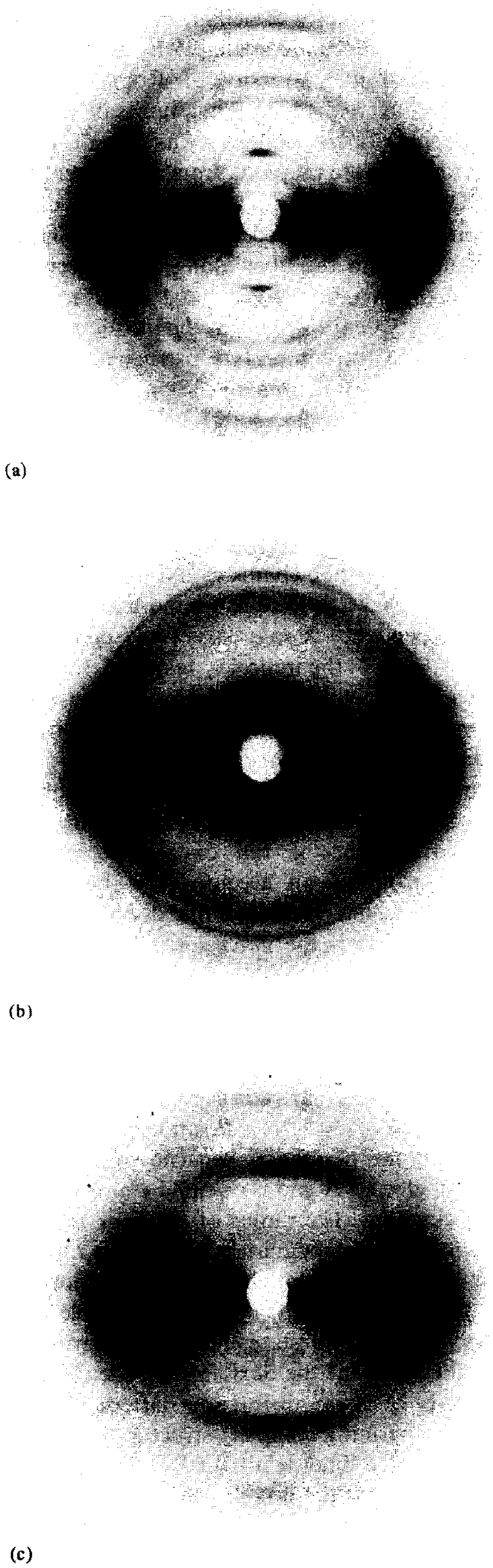

Fig. 2. X-ray diffraction pattern (using Cu*K*α radiation) from an oriented and non-crystalline stretched fiber of (a) the benzyl ester of gellan (18% substitution); (b) the benzyl ester of hyaluronate (75% substitution); and (c) CMHPG.

superior X-ray patterns are obtained, experimental details on the influence of these aromatic rings on the aggregation properties of the benzyl ester derivative will remain elusive.

Benzyl ester of hyaluronan

Hyaluronic acid, an ubiquitous polysaccharide in the glycosaminoglycan family, is found largely in soft connective tissues including umbilical cord, synovial fluid and vitreous humour, and also in bacteria. Its repeating unit is the disaccharide → 4)-β-D-glucuronic acid-(1 → 3)-β-D-*N*-acetylglucosamine-(1 →. It is perhaps one of the most investigated fibrous polymers using a variety of experimental techniques to understand the molecular basis of its interesting and intriguing physical, chemical and biological properties. Pioneering X-ray studies in the 1970s and 1980s on glycosaminoglycans, summarized by Arnott & Mitra (1983), generated a wealth of structural information on a variety of molecular helices of hyaluronic acid and their packing arrangements which were controlled by a combination of the type of cations (Na, K and Ca), humidity and pH (4–10). Table 2 lists some important morphological parameters of seven allomorphs (I–VII) fully characterized at the atomic level. The variability in helical parameters (turn angle *t* and axial rise *h*) is a reflection of the stretching and contracting ability of the polysaccharide chain. The polymer has been trapped in both single and double-helical states in polycrystalline and well oriented specimens. Six of these were reported to be left-handed single-helices. They can be grouped under three distinct forms corresponding to helix symmetry 4_3 and 3_2 with a disaccharide, and 2_1 with a tetrasaccharide repeating unit. The 2_1 helix (IV) is a minor variant of the 4_3 helix (I) in that the two halves of the tetrasaccharide repeating unit, are marginally different in conformation from that of a 4_3 helix. While *t* per disaccharide varies from −77 to −120°, *h* fluctuates from 8.5 to 9.5 Å in an uncorrelated fashion. In addition, potassium hyaluronate has been induced to adopt an antiparallel, left-handed, double-helical structure (VII).

Thus, the four canonical forms of interest are the 4-, 3- and 2-fold single helices and the 4-fold double helix. Whether the partially or fully derivatized benzyl ester of hyaluronate has the potential to retain one or more of these secondary structures has now been examined. Samples of this material were provided to us in two different forms by Fidia Advanced Biopolymers, Italy. One sample was in the form of oriented and noncrystalline fibers extruded from DMSO in a commercial process, and a second was freeze-dried material. We prepared fibers from the second material. In either case, a typical diffraction pattern (Fig. 2b) resembled that from a similar unsubstituted hyaluronate specimen, and the observed intensity distribution was

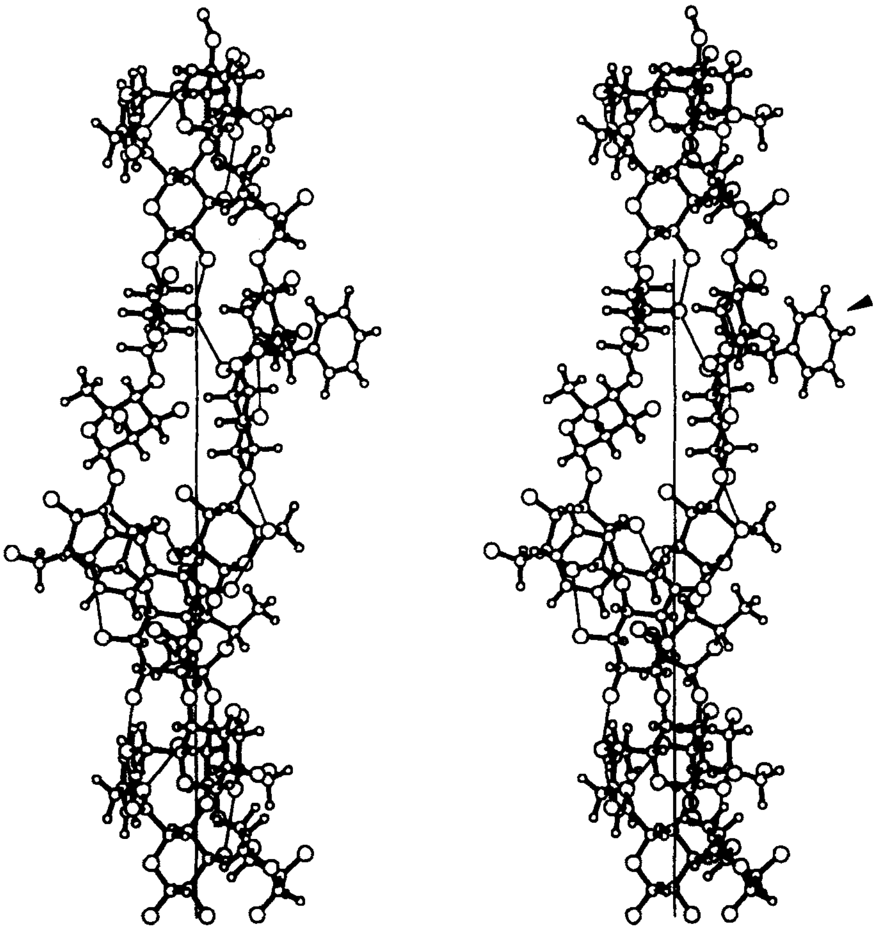

Fig. 3. A stereo view of a double-helical model of the benzyl ester of gellan. The vertical line in the middle corresponds to its *c*-repeat. Hydrogen bonds are denoted as thin lines. The arrow shows one of the peripheral benzyl groups.

Table 2. Comparison of unit cell ($\alpha = \beta = 90°$) dimensions, helix symmetry, turn angle (t), and axial rise (h) for seven different allomorphs of hyaluronate helices

Structure	Cation	a (Å)	b (Å)	c (Å)	$\gamma°$	Helix	t (°)	h (Å)	Ref.
I	Na	9.9	9.9	33.9	90	4_3	−90	8.5	Guss *et al.* (1975)
II	K	10.0	10.0	37.9	90	4_3	−90	9.5	Mitra *et al.* (1983*a*)
III	Na	11.7	11.7	28.5	120	3_2	−120	9.5	Winter *et al.* (1975)
IV	Na	11.5	9.9	33.9	90	2_1	−77	8.5	
							−103	8.5	Guss *et al.* (1975)
V	K	11.7	9.3	35.4	90	2_1	−82	8.8	
							−98	8.9	Mitra *et al.* (1983*b*)
VI	Ca	20.9	20.9	28.3	120	3_2	−120	9.4	Winter & Arnott (1977)
VII	K	17.1	17.1	32.8	90	4_3	−90	8.2	Arnott *et al.* (1983)

Structures I–VI are single helices and VII is a double helix.

nearly the same for several fibers examined. The degree of substitution apparently had no influence on backbone structure. On a fingerprint basis, the pattern suggested that, as for the benzyl ester of gellan, this hyaluronate derivative also had a regular structure identifiable with that of hyaluronate itself.

All four helical forms were individually examined and verified for their ability to include a benzyl group at every carboxyl oxygen atom without affecting the original polymer geometry. Figure 4 illustrates the resulting structures and Table 3 gives the three angles χ_1, χ_2 and χ_3 which define the conformation of the substituent in each case. The fact that the orientations of the benzene rings are not the same in all models is a consequence of the basic differences in the morphologies of the starting structures. Likewise, the two independent substituents in the 2-fold model have different surroundings and hence altered conformations.

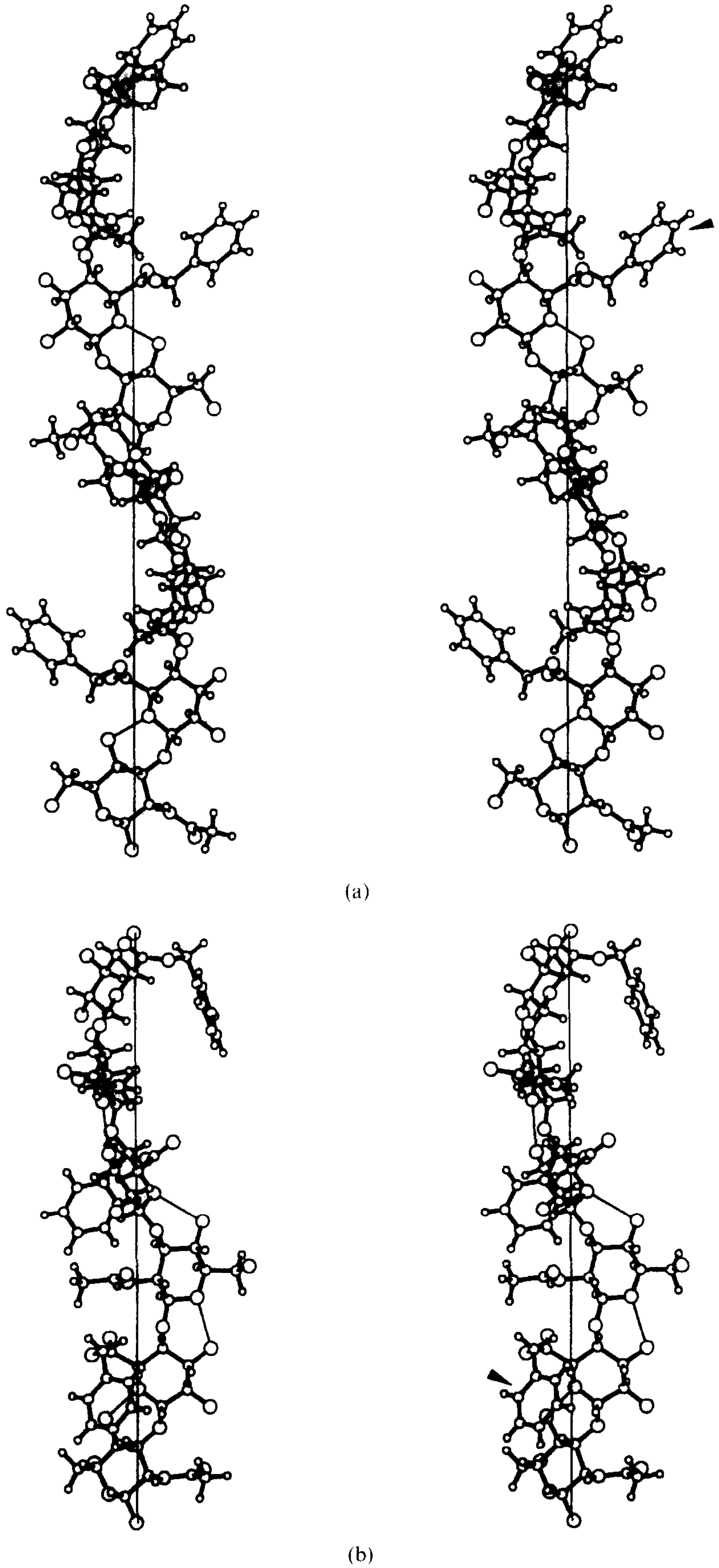

Fig. 4. Stereo views of the benzyl esters of hyaluronate helices: (a) 4-fold; (b) 3-fold; (c) 2-fold single helix; and (d) 4-fold double helix. Symbols and lines are the same as in Fig. 3.

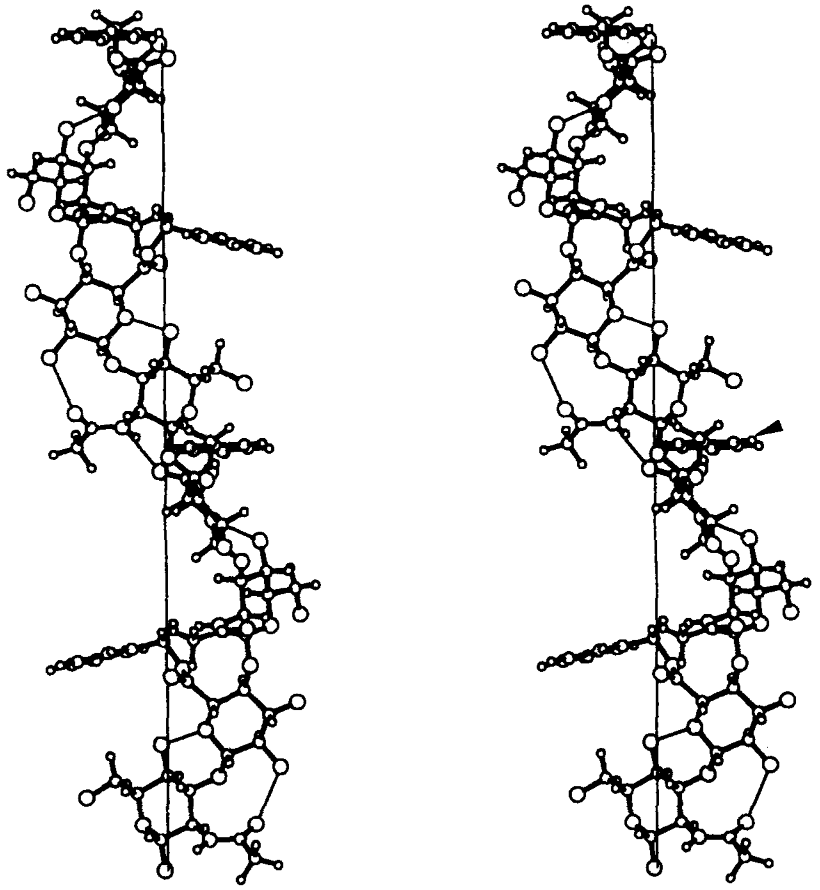

Fig. 4. (c)

Computations further suggest that the substituent is fairly flexible within the isolated helix and alternatives are within easy reach. Packing forces are expected to have some influence on the flexibility (rigidity) of the substituent. Further details would be necessary to understand and rationalize the molecular basis of hydrogel formation.

Carboxymethylhydroxypropyl guaran (CMHPG)

X-ray diffraction patterns from galactomannans are known to correspond to long range lateral organization of 2-fold helical structures (Chien & Winter, 1985). The galactosyl side chains are responsible for solubility in water. Borate mediated crosslinking can lead to gelation and may involve salt crosslinks between pairs of *cis*-hydroxyls in the free mannosyl units on different polymer chains (Maier *et al.*, 1993). Structural details are, however, unavailable.

X-ray diffraction patterns, typical of that shown in Fig. 2c from CMHPG fibers with low levels of substitution, suggest a galactomannan-like structure and only short range lateral organization. Each of the three substitution sites in the M–MG trisaccharide repeating unit (Fig. 5) were initially appended with an HP group, one at a time, in generating a preliminary molecular model for hydroxypropyl guaran. All three sites were found to be stereochemically satisfactory. Energy minimization did not reveal a preference for any particular site. When all three sites were occupied by hydroxypropyl groups, the resulting model demonstrated that the hydroxypropyl groups on the mannosyl units stretched out on one side as much as the galactosyl side chains on the other side, in a direction perpendicular to the length of the main chain.

We then generated a low energy conformation of a CMHPG chain in which it was found that no more than one CM group was admissible per M–MG repeat. Results of energy minimization showed that HP(1), CM(2) and HP(3) was one of the preferred patterns of substitution, where the numbers in parentheses refer to the sites marked in Fig. 5. When this set-up is used, the relative enhancement of molecular dimensions from mannan to galactomannan and CMHPG can be seen in Fig. 6. The width of the CMHPG chain is 15.7 Å, more than double that of mannan.

A pair of CMHPG chains may associate in a number of ways. One possible mode in which the two chains are parallel, and separated laterally by approximately 7.8 Å which is consistent with the *d*-spacings measured from Fig. 2c, is shown in Fig. 7a. Packing parameters (rotation and translation) about the helix axes were the

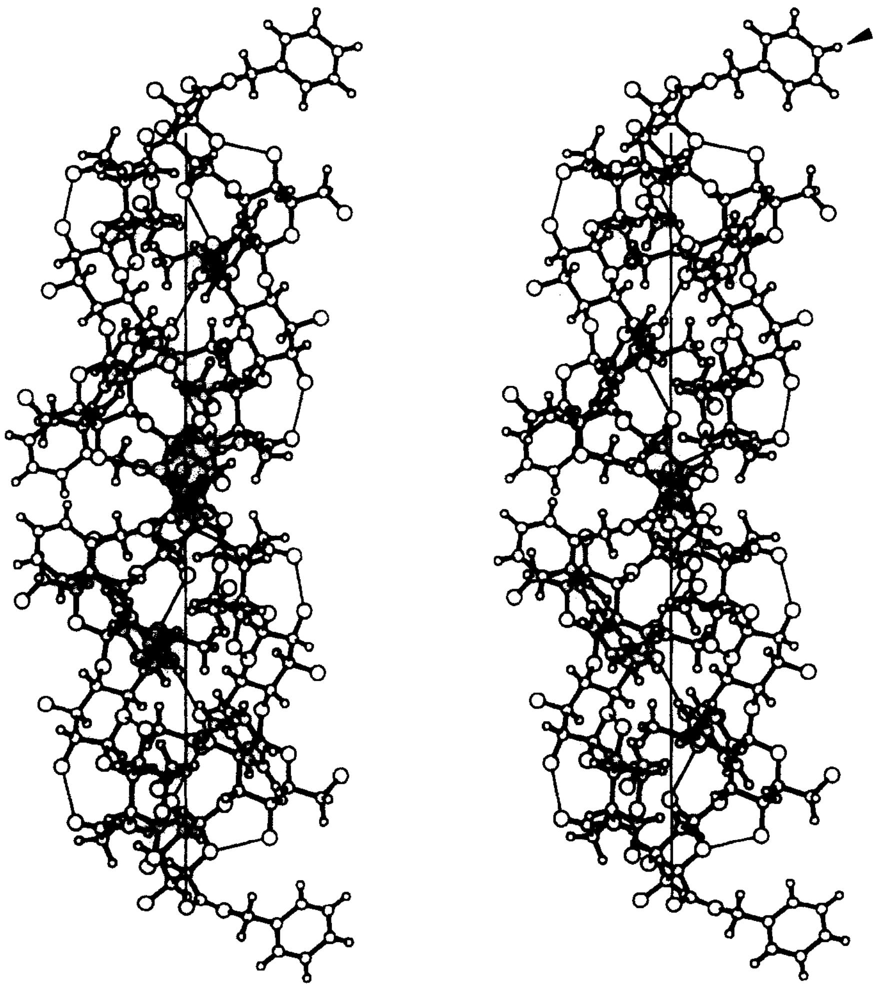

Fig. 4. (d)

Table 3. Conformation angles χ_1, χ_2 and χ_3 of the substituent in four different helical models of the benzyl ester of hyaluronan

Model	Structure	χ_1°	χ_2°	χ_3°
1	II	−118	−102	−83
2	III	39	−129	45
3	V	−145	−117	−89
		−165	−101	−79
4	VII	136	−170	112

only variables in energy minimization. This complex was stabilized by interactions between a carboxyl group in one chain and a bridge oxygen, as well as atom *O*2, both belonging to a galactosyl unit in the other chain. An axial view of the complex (Fig. 7b) further shows that carboxylate groups belonging to the two chains have the proper orientation and separation to include a divalent ion in the centre. This constellation provides a total of up to six ligands at distances of approximately 3 Å. They include the carboxylate oxygen atoms and atom *O*3 of a mannosyl unit, 6-linked to a galactose, from each chain. This provides the first overall picture

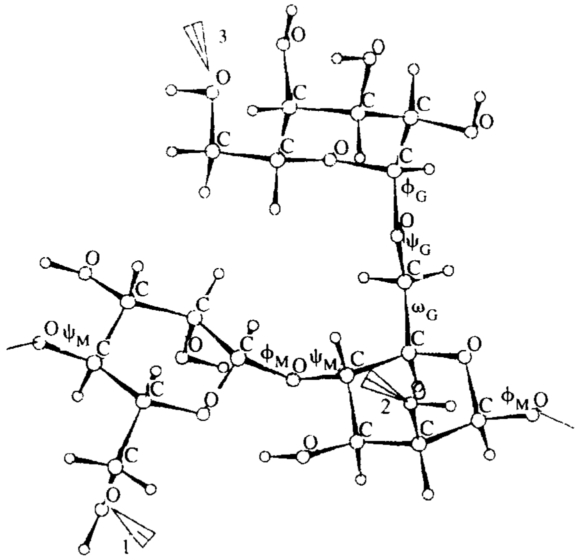

Fig. 5. A schematic representation of the M–MG trisaccharide repeating unit of the idealized guaran chain. The three most frequently substituted sites (1–3) at atoms *O*6M, *O*2M and *O*6G, respectively, are indicated by arrows and labeled. The major conformation angles are marked.

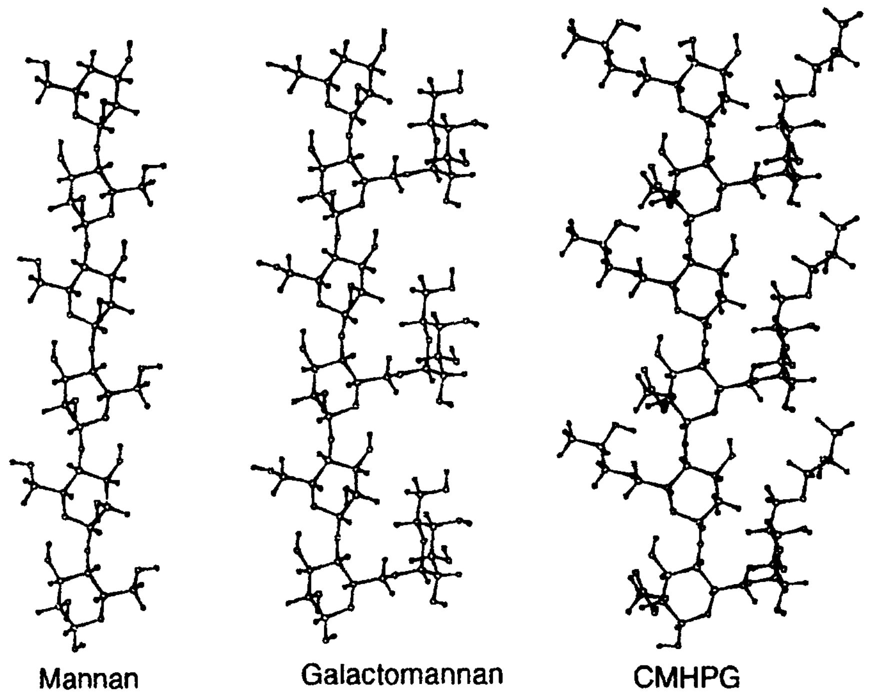

Fig. 6. Side views of three pitch-lengths of mannan, galactomannan and CMHPG. The side chains in galactomannan are all on the right-hand side. Substituents in CMHPG are almost equally distributed on either side of the mannan backbone.

of a probable crosslinking of CMHPG chains by divalent ions. At this stage, this gives an indication of contacts which might arise from fortuitous coincidence of short alternating sequences on contiguous chains. The details must await further refinement which is now in progress.

CONCLUSIONS

X-ray diffraction analysis and modeling calculations were used to probe the influence of chemical substitutions on molecular morphology in three polysaccharide families. The results show that the gellan double helix is unperturbed either by minor substituents such as acetyl and glyceryl groups as in native gellan, or by rhamnosyl (or mannosyl) side chains as in welan. However, the substituents induce considerable variations in intermolecular interactions. These can explain the observed differences in rheological properties. Benzyl esters of gellan and hyaluronan can also adopt the respective parent structures; these consist of a double helix for gellan, and three single- and one double-helical forms for hyaluronan. Benzyl esterification reduces negative charge on the polysaccharide, but the substituent can be accommodated without disrupting the original chain geometry. Bulky aromatic rings, however, will modify the associative properties in each case. Hydroxypropyl and carboxymethyl substituents have the potential to create energetically favorable associations between a pair of CMHPG chains via hydrogen bonds and also via divalent cations, one for every two carboxylate groups belonging to different chains.

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REFERENCES

Arnott, S. & Mitra, A.K. (1983). X-ray diffraction analyses of glycosaminoglycans. In *Molecular Biophysics of the Extracellular Matrix*, eds S. Arnott, D.A. Rees & E.R. Morris. Humana Press, New Jersey, USA, pp. 41–67.

Arnott, S., Mitra, A.K. & Raghunathan, S. (1983). Hyaluronic acid double helix. *J. Mol. Biol.*, **169**, 861–872.

Chandrasekaran, R., Puigjaner, L.C., Joyce, K.L. & Arnott, S. (1988). Cation interactions in gellan: an X-ray study of the potassium salt. *Carbohydr. Res.*, **181**, 23–40.

Chandrasekaran, R., Radha, A. & Lee, E.J. (1994). Structural roles of calcium ions and side chains in welan: an X-ray study. *Carbohydr. Res.*, **252**, 183–207.

Chandrasekaran, R., Radha, A. & Thailambal, V.G. (1992). Roles of potassium ions, acetyl and L-glyceryl groups in native gellan double-helix: an X-ray study. *Carbohydr. Res.*, **224**, 1–17.

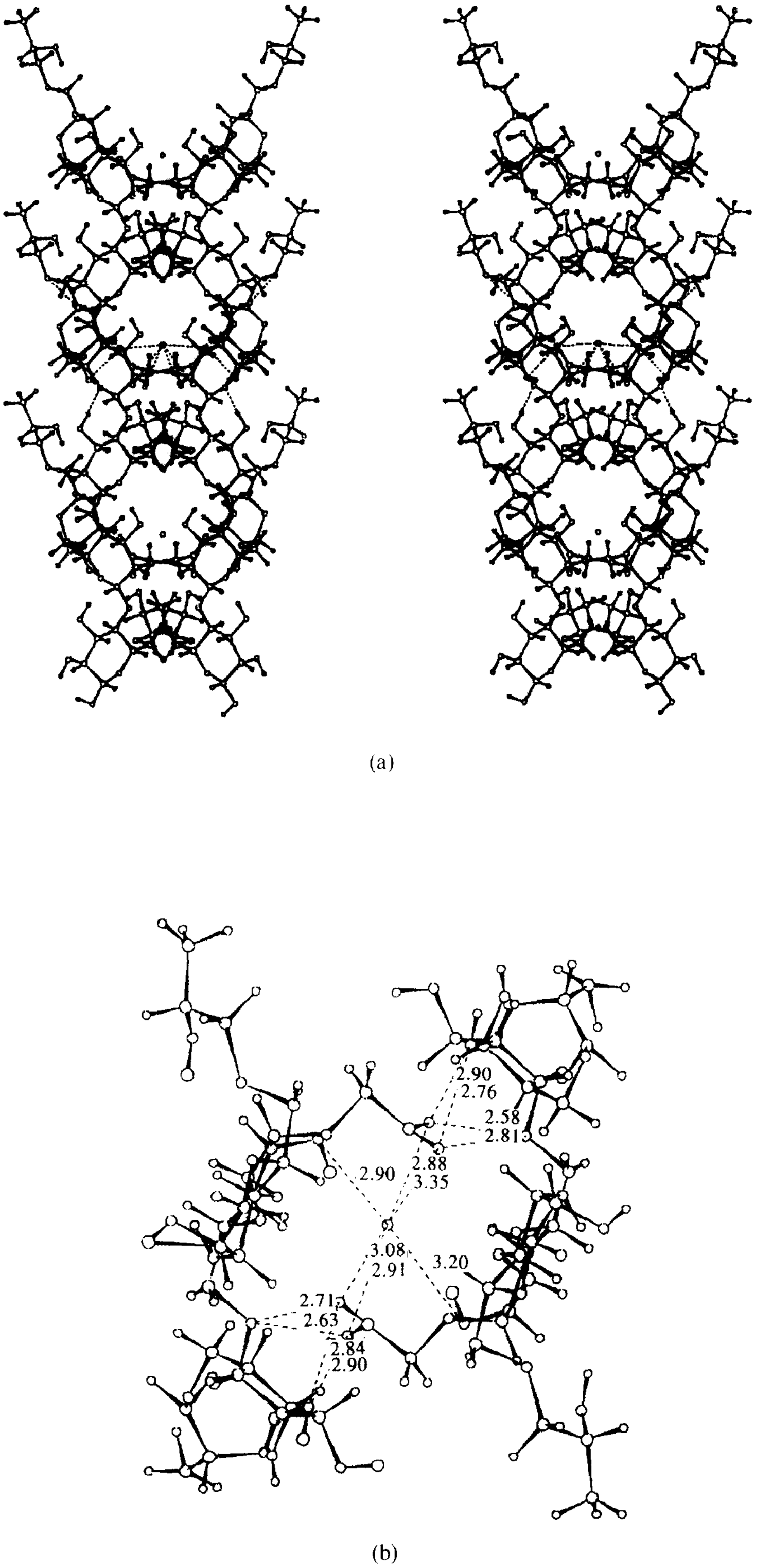

Fig. 7. A plausible model depicting the association of a pair of CMHPG chains stabilized by interchain interactions involving the carboxylate groups and a divalent cation: (a) a stereo side view; and (b) an axial view which highlights (dashed lines) up to six ligands for the divalent ion at the center. Distances < 3.4 Å are marked.

Chandrasekaran, R. & Thailambal, V.G. (1990). The influence of calcium ions, acetate and L-glycerate groups on the gellan double-helix. *Carbohydr. Polym.* **12,** 431–442.

Chien, Y.Y. & Winter, W.T. (1985). Accurate lattice constants for tara gum. *Macromolecules,* **18,** 1357–1359.

Crescenzi, V., Dentini, M. & Callegaro, L. (1992*a*). Potential biomedical applications of partial esters of the polysaccharide gellan. In *Harnessing Biotechnology for the 21st Century*, eds M.R. Ladisch & A. Bose. American Chemical Society, Washington DC, USA, pp. 88–91.

Crescenzi, V., Dentini, M., Segatori, M., Tiblandi, C., Callegaro, L. & Benedetti, L. (1992*b*). Synthesis and preliminary characterisation of new esters of the bacterial polysaccharide gellan. *Carbohydr. Res.,* **231,** 73–81.

Guss, J.M., Hukins, D.W.L., Smith, P.J.C., Winter, W.T., Arnott, S., Moorhouse, R. & Rees, D.A. (1975). Hyaluronic acid: molecular conformations and interactions in two sodium forms. *J. Mol. Biol.,* **95,** 359–384.

Kesavan, S. & Prud'homme, R.K. (1992). Rheology of guar and HPG cross-linked by borate. *Macromolecules,* **25,** 2026–2032.

Lee, E.J. & Chandrasekaran, R. (1991). X-ray and computer modeling studies on gellan related polymers: molecular structures of welan, S-657, and rhamsan. *Carbohydr. Res.,* **214,** 11–24.

Maier, H., Anderson, M., Karl, C., Magnuson, K. & Whistler, R.L. (1993). Guar, locust bean, tara, and fenugreek gums. In *Industrial Gums*, eds R.L. Whistler & J.N. BeMiller. Academic Press, California, USA, pp. 181–226.

McNeil, M., Szalecki, W. & Albersheim, P. (1984). Quantitative methods for determining the points of *O*-(carboxymethyl) substitution in *O*-(carboxymethyl)-guar. *Carbohydr. Res.,* **131,** 139–148.

Mitra, A.K., Arnott, S. & Sheehan, J.K. (1983*a*). Hyaluronic acid: molecular conformation and interactions in the tetragonal form of the potassium salt containing extended chains. *J. Mol. Biol.,* **169,** 813–827.

Mitra, A.K., Raghunathan, S., Sheehan, J.K. & Arnott, S. (1983*b*). Hyaluronic acid: molecular conformations and interactions in the orthorhombic and tetragonal forms containing sinuous chains. *J. Mol. Biol.,* **169,** 829–859.

Smith, P.J.C. & Arnott, S. (1977). LALS: a linked-atom least-squares reciprocal-space refinement system incorporating stereochemical restraints to supplement sparse diffraction data. *Acta Crystallogr.,* **A34,** 3–11.

Winter, W.T. & Arnott, S. (1977). Hyaluronic acid: the role of divalent cations in conformation and packing. *J. Mol. Biol.,* **117,** 761–784.

Winter, W.T., Smith, P.J.C. & Arnott, S. (1975). Hyaluronic acid: structure of a fully extended 3-fold helical sodium salt and comparison with less extended 4-fold helical forms. *J. Mol. Biol.,* **99,** 219–235.