X-ray and Molecular Modeling Studies on the Structure-function Correlations of Polysaccharides

Rengaswami Chandrasekaran

Whistler Center for Carbohydrate Research, 1160 Food Science Building, Purdue University, West Lafayette, IN 47907, USA

SUMMARY: X-Ray fiber diffraction analysis and computer molecular modeling have revealed the three-dimensional structures of several important industrially useful polysaccharides such as algin, carrageenan, galactomannan, gellan, pectin, welan, xanthan and xanthan:guaran complex. Consistent with their chemical structures, their molecular morphologies, which include single and double helices, are quite distinct. The helix-helix associations are promoted by cations and/or water molecules and the patterns are not always the same. The polymer-dependent structural details can be correlated with, and used to understand the molecular basis of, the observed physical properties.

Introduction

Water soluble polysaccharides, known as hydrocolloids or gums, produce viscous aqueous solutions at low concentrations. Some of them, however, form gels at higher concentration. Such polysaccharides are widely used as additives in order to eliminate or reduce "defects" pertaining to appearance, texture and stability in foods. Both in solution and in the solid state, these polysaccharides tend to adopt helical conformations. This paper gives an overview of the molecular morphologies of certain polysaccharide helices as determined by the X-ray fiber diffraction technique during the past 25 years in conjunction with molecular modeling[1]. The results suggest that it is not just the shape of the helix, but the intimate interactions of these helices with each other, and with the surrounding ordered water molecules and cations that are responsible for the observed physical properties of a polymer. This type of structural knowledge helps to understand the molecular basis of the solution behavior of gums. It also paves a cost effective way for chemical modification of an existing gum for improved industrial applications.

 CCC 1022-1360/99/$ 17.50+.50/0

Carrageenan, algin, pectin and gellan are some of the most common food polysaccharides to date and their three-dimensional structures have enabled to visualize the "junction zone", which is necessary for the onset of gelation in each case. Welan, xanthan and galactomannan are non-gelling polysaccharides which exhibit high viscosity when dissolved in water; according to current data, the side chains in them influence their molecular structures and physical properties. On the other hand, as molecular modeling demonstrates, intermolecular association between the two polymers is responsible for the gelation behavior of the xanthan:galactomannan complex.

In order to be self-contained, this article first gives a short account of the basic principles of X-ray diffraction as applied to non-crystalline polymer, methodology of structure determination and refinement protocol. This is followed by a description of the molecular morphologies of polysaccharide helices. In each case, the preferred interactions between helices, solvent molecules and cations, and how they correlate with the observed physical properties are presented.

X-Ray Diffraction

Diffraction of X-rays by single crystals is the most powerful experimental tool for determining the three-dimensional structure of, or the spatial disposition of atoms in, molecules. Although single crystals are out of the question, polycrystalline and well-oriented fibers are within the reach of helix-forming polymers. In these fibers, there is short range lateral organization as well as near parallelism along the molecular axes of the helices. Diffraction of X-rays, usually of wavelength 1.5418 Å, by a polycrystalline and oriented specimen is equivalent to that from a rotating single crystal. Recorded on a photographic film, with specimen-to-film distance of about 4 cm in the camera, and the fiber kept normal to the incident beam, the diffraction pattern displays a series of Bragg reflections on layer lines which are parallel to the equator that passes through the center of the film. Since the meridian in the pattern is reckoned parallel to the fiber axis, the equator and the other layer lines are perpendicular to this axis. Starting with zero for the equator, the layer line numbers are sequentially numbered 1, 2, 3 and so on. The first meridional reflection usually occurs on the layer line whose number specifies the number of repeating units per turn of the helix. It is sufficient to state here that the positions of the reflections are geometrically related to the dimensions [a, b, c, α, β and γ] of the unit cell which is the building block of the crystal. Note that a, b and c are in Ångstroms; α, β and γ are in degrees. Generally, the helix pitch is c, but sometimes as explained later, it is an integral multiple of c. In principle, by measuring and making use of the intensities of all the reflections in the pattern, the structural details can be synthesized.

Structure Analysis

The X-ray data from a fiber alone are inadequate to solve the structure as they are far fewer than the number of atomic positions in the helix repeat to be determined. This difficulty is overcome by supplementing bond lengths and bond angles as known stereochemical information so that helical models could be constructed and refined by treating the major conformation angles only as the main variables. The density of the fiber, space group symmetry and unit cell volume further help to define the number of helices and guest molecules such as solvent and cations in the unit cell. The Linked-Atom Least-Squares procedure is designed to meet the requirements for solving helical structures efficiently[2]. The variables in the refinement jointly against the X-ray data and steric compression within and between the unit cell contents include, but are not restricted to, the conformation angles about the glycosidic bonds in the main chain, and the packing parameters that position the helices appropriately in the unit cell.

Consistent with the observed helical parameters, alternatives to be examined are: single, double and multistranded helices; right and left-handed chirality; parallel and antiparallel polarity when either two or more chains intertwine about a common helix axis or more than one helix occupy the unit cell. Comparison of competing models is based on the X-ray fit between experimental data and those calculated for the model, and freedom from steric anomalies. Once the choice for the best model is made, difference electron density maps are computed and closely examined in order to verify its correctness and to locate the guest molecules in the unit cell which are further refined as part of the augmented crystal structure in subsequent stages.

Carrageenan

Carrageenans are gel-forming sulfated polysaccharides found in the marine red algae *Rhodophyceae*. Two of them, ι-carrageenan and κ-carrageenan, are well known thermoreversible gelling agents. Their respective disaccharide repeats, denoted as (A-B) are given by

Iota: →3)-β-D-Gal4SO_3^--(1→4)-3,6-anhydro-α-D-Gal2SO_3^--(1→

Kappa: →3)-β-D-Gal4SO_3^--(1→4)-3,6-anhydro-α-D-Gal-(1→

Clear and elastic gels are formed by *i*-carrageenan that neither synerese nor undergo hysteresis effects. On the other hand, κ-carrageenan gels are hazy and brittle, and prone to both syneresis and hysteresis effects.

According to Arnott et al., who first determined its three-dimensional structure, *i*-carrageenan forms a 3-fold, right-handed, parallel, half-staggered double helix[3]. That is, the two chains

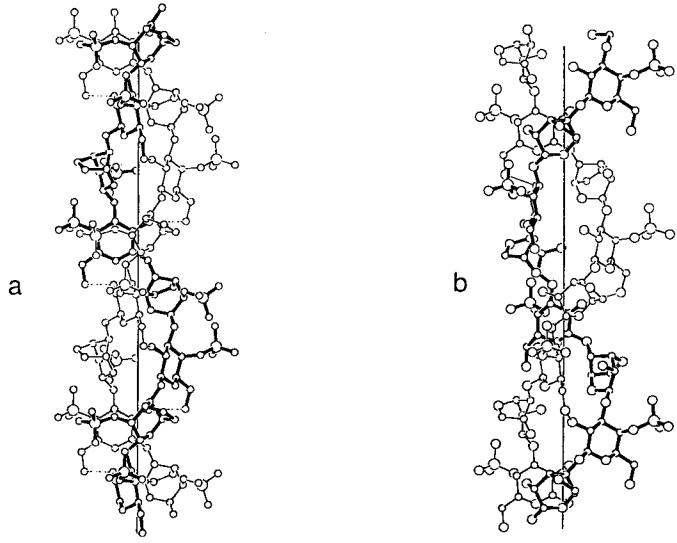


Fig. 1. Three-fold double helix of (a) iota-carrageenan and (b) kappa-carrageenan.

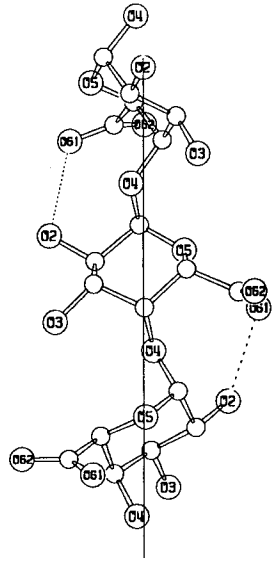


Fig. 2. Three-fold helix of pectic acid.

intertwine such that when one chain is shifted by *c* along the helix axis, it superposes on the other. Hence, the pitch is $2c = 26.6$ Å. The A and B monomers in the helix, which is very sinuous as shown in Fig. 1a, are in the preferred $^4C_1$ and $^1C_4$ chair conformations, respectively. The vertical line in this and in subsequent figures represents the helix axis, and the dashed lines denote hydrogen bonds. The inner and outer diameter of the helix are 3.4 and 14.2 Å, respectively. The two chains in the helix are connected by O6H···O2 hydrogen bonds at each galactosyl unit. The sulfate groups are in the periphery exposed to the environment. The packing arrangement, one helix per trigonal unit cell, suggests that the strong sulfate···Ca^{2+}···sulfate interactions between the anhydrogalactosyl units of adjacent helices are responsible for polymer aggregation during gelation.

The molecular structure of *k*-carrageenan is also a double helix as above except that one polysaccharide chain in it is distinctly off-set from the half-staggered position by about 1.1 Å along, and 28° about, the common helix axis. Hence, as shown in Fig. 1b, only half the number of galactosyl units are able to form the O6H···O2 hydrogen bonds between the chains. Also, the pitch of the helix is shorter (25 Å), and the diameter is 1 Å larger, than in ι-carrageenan. The sulfate groups on the galactosyl units describe the periphery of the helix. Fewer sulfate groups and fewer hydrogen bonds should render lower stability so that *k*-carrageenan helices associate weaker than in the case of ι-carrageenan. These structural details mirror the observed differences in the gel properties of the two polymers.

Pectin

Pectin is a heterogeneous structural plant polysaccharide found, for example, in apple pomace and citrus peel. Its backbone is made up of (1→4)-linked α-D-galacturonic acid repeating units in which some of the carboxyl groups are methyl esterified. The regular main chain is often interrupted by (1→2)-linked α-L-rhamnosyl units. The high viscosity and gelling properties of pectin are dependent on the type and amount of cations, as well as the degree of methylation. X-ray studies show that the molecular structures of sodium pectate and pectic acid are very similar; they form 3-fold, right handed, single helices of pitch 13.3 Å as shown in Fig. 2. Stabilized by O2H···O61 hydrogen bond across each bridge oxygen atom, the helix has inner and outer diameters as 0.4 and 7.0 Å, respectively. While the helices associate via sodium ions in the salt form, they are directly connected by hydrogen bonds involving the carboxyl groups in the acid form[5]. In the case of a divalent salt form, say calcium pectate, or pectinic acid, the helices are believed to be of the same type. The details are however sketchy due to poorer diffraction patterns[6].

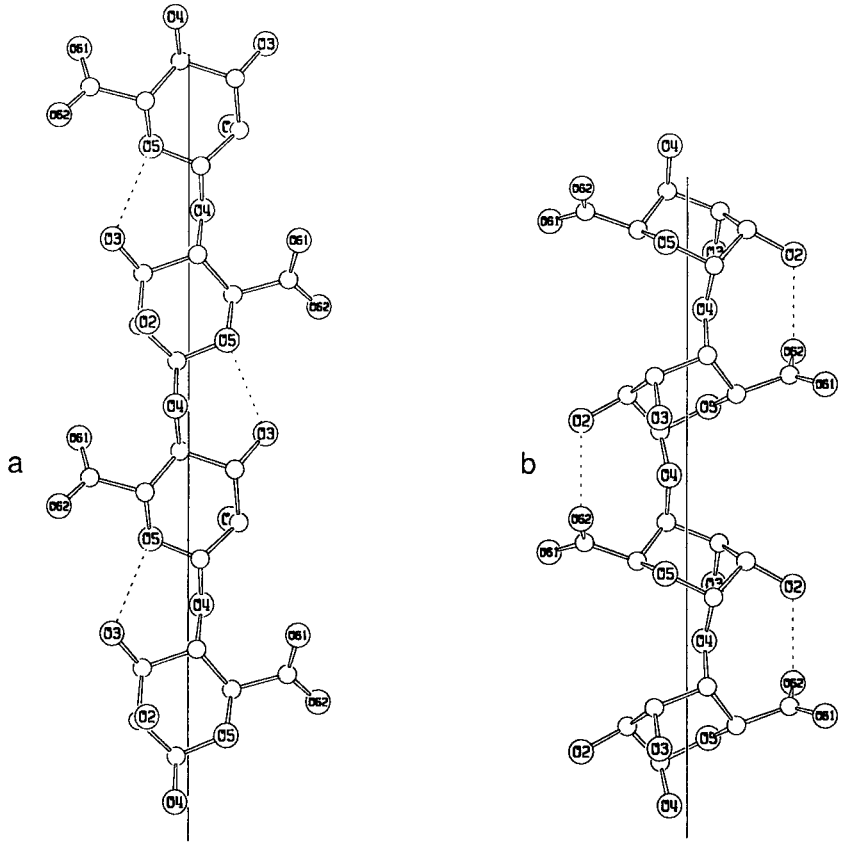


Fig. 3. Two-fold helix of (a) polymannuronic acid and (b) polyguluronic acid.

Algin

Algin constitutes one of the structural polysaccharides of marine brown algae and some bacteria. Initially biosynthesized as a linear polymer of (1→4)-linked β-D-mannuronic acid (M), mannuronan C5 epimerase later converts β-D-mannuronic acid to α-L-guluronic acid (G) in some blocks. As a result, the final polymer consists of M blocks alternating with G blocks of varying size. The composition and block length are species dependent. Alginic acid is widely used in the food and pharmaceutical industries because of its cation dependent gelling properties. The hexopyranose rings of M and G prefer $^4C_1$ and $^1C_4$ chair conformations, respectively. The linkages in the respective blocks are diequatorial and diaxial. Therefore, the two polymers have radically distinct secondary structures.

Atkins et al. reported a preliminary molecular model for polymannuronic acid[7]. This has recently been revised[8] after correcting some fundamental errors in the original work. The new results confirm that the helix, as shown in Fig. 3a, is 2-fold, fully extended, 7.2 Å wide and has a pitch of 10.4 Å similar to that observed for mannan. The tight packing in an orthorhombic unit cell leaves no room for guest molecules to come between the helices. This implies that polymannuronic acid is intended more as a structural polysaccharide than as a gelling agent.

In the case of polyguluronic acid, Atkins et al. reported a preliminary model[9] which also suffered from similar drawbacks. The correct structure has now been obtained[10]. According to this recent work, the polymer forms a 2-fold helix of pitch 8.7 Å and width 7.2 Å as shown in Fig. 3b. The buckled shape is in sharp contrast to that of the polymannuronic acid helix. The packing of helices in an orthorhombic unit cell is mediated by calcium ions and water molecules. The structural details demonstrate that a stable junction zone in calcium alginate gels would be composed of not less than four polyguluronate helices surrounding a central column of calcium ions and/or water molecules such that four ligands, one per helix, are linked to each calcium ion or water molecule. This is the first picture to give the structural details at molecular level of a schematic "egg-box" model that was proposed 25 years ago[11] for the junction zone of calcium alginate gels.

Gellan Related Polymers

Gellan and welan are just two of the eight anionic polysaccharides secreted by unrelated bacteria, all having the same tetrasaccharide (A-B-C-D) repeat in their main chains as illustrated below. Native gellan denotes the polysaccharide as secreted by *Pseudomonas elodea* and contains both

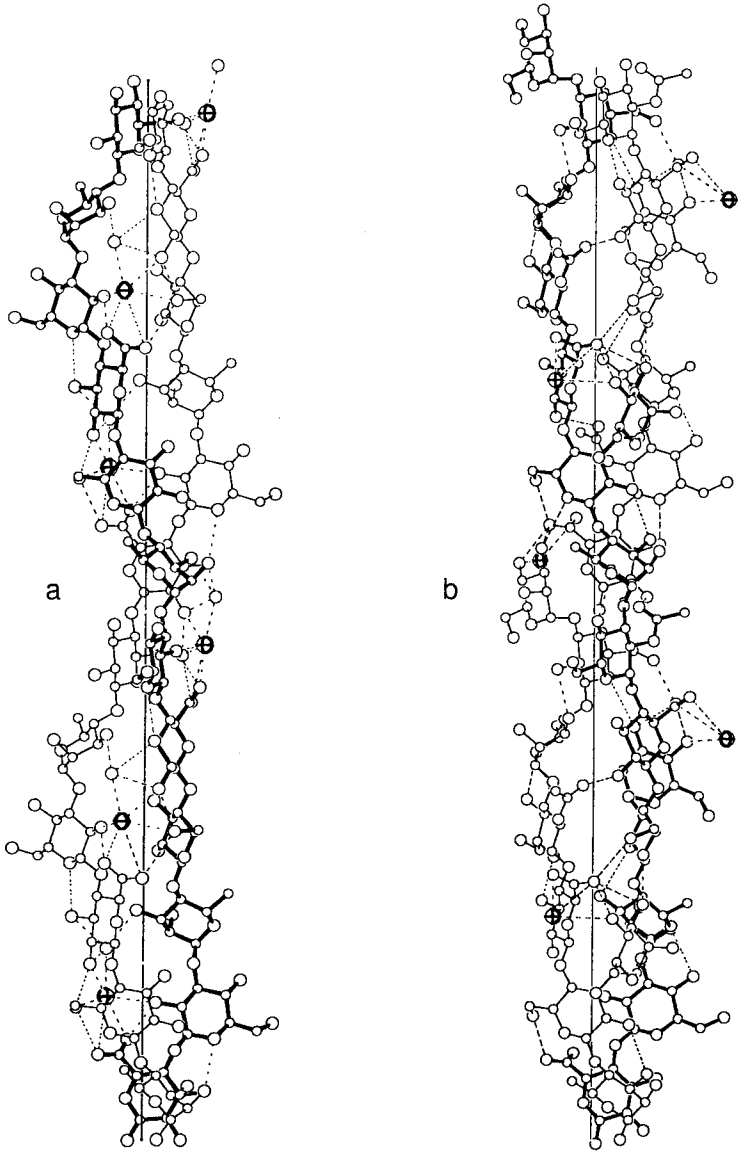


Fig. 4. Three-fold double helix of (a) gellan and (b) welan.

glyceryl and acetyl substituents. Gellan refers to the commercially de-esterified polymer. In contrast to the soft and spongy gels of native gellan, hard and brittle gels are obtained with gellan. The branched polymer welan has side chain E and is an excellent viscosifier. The roles of substituents and side chains are now understood from their respective three-dimensional structures[12].

O-L-glycerate
↑
2
Native gellan: →3)-β-D-Glc-(1→4)-β-D-GlcA-(1→4)-β-D-Glc-(1→4)-α-L-Rha-(1→
6
↑
O-acetate (0.5 occupancy)

Gellan: →3)-β-D-Glc-(1→4)-β-D-GlcA-(1→4)-β-D-Glc-(1→4)-α-L-Rha-(1→
A B C D

Welan: →3)-β-D-Glc-(1→4)-β-D-GlcA-(1→4)-β-D-Glc-(1→4)-α-L-Rha-(1→
2 3
↑ ↑
O-acetate (0.85 occupancy) 1
α-L-Rha or α-L-Man (2:1)
E

The X-ray analysis of the potassium salt of gellan[13] has revealed that gellan forms a 3-fold, left-handed, half-staggered, double helix of pitch 56.3 Å as shown in Fig. 4a. Except for α-L-rhamnose in the $^1C_4$ chair conformation, the remaining monosaccharides are in $^4C_1$ chair conformations. The inner and outer diameters of the helix are 1.1 and 15.8 Å, respectively. Notable is that the carboxyl group in every repeat, linked to a potassium ion, is fully exposed to the surroundings and is hydrogen bonded to the hydroxymethyl group (in C) in the other chain within the helix. Two such helices are packed in an antiparallel fashion within a trigonal unit cell in such a way that the carboxylate groups of adjacent helices are juxtaposed and connected via a K^+···water···K^+ bridge. By extrapolation, computer modeling[14] has predicted that a similar but direct connection could be made by a divalent ion such as Ca^{2+}. This type of strong aggregation represents the junction zone in, and correlates with the observed nature of, gellan gels.

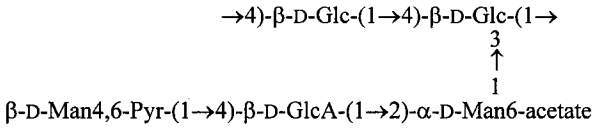
On the basis of the gellan structure, Chandrasekaran and Thailambal[14] proposed that the glyceryl groups in native gellan would actively interfere with its ability to retain the exact gellan geometry near the carboxylate groups. This prediction was subsequently proved when the fiber structure of native gellan was determined[15]. The X-ray results indicate that native gellan helices, as expected, are unable to establish cation mediated interactions between their carboxylate

groups as efficiently as in the case of gellan. This is the structural explanation for the soft and spongy nature of native gellan gels.

The parent gellan X-ray structure was instrumental for an initial proposal[16] that welan would also adopt a gellan-like double helix. This was swiftly confirmed when the fiber structure of the calcium salt of welan was determined[17]. The pitch of the welan double-helix, shown in Fig. 4b, is 57.4 Å, 1 Å higher than that of gellan. The interchain hydrogen bond between every carboxyl group and a nearby hydroxymethyl group (in C) is retained. The peripheral side chain E folds towards the non-reducing end and shields, as well as hydrogen bonds with, a vicinal carboxylate group; this enhances the stability of the welan helix. Despite the side chain, its inner and outer diameter being 1.2 and 13.0 Å, respectively, this helix is 2.8 Å slimmer than that of gellan. Three welan helices, two pointing-up and one pointing-down, are packed in a trigonal unit cell. This gives rise to three distinct modes of interhelical interactions: carboxylate···Ca^{2+}···carboxylate, carboxylate···water···Ca^{2+}···carboxylate and side chain···side chain hydrogen bonds, in decreasing order of strength. In known practical applications, the strong interactions might be relevant for cement mixes, while the medium and weak interactions are appropriate for viscous solutions at low and high temperatures, respectively.

Xanthan

The bacterial polysaccharide xanthan from *Xanthomonas campestris* has a pentasaccharide repeat composed of a disaccharide in the main chain and trisaccharide in the side chain as shown below:



It is used in the food industry because its viscosity in aqueous solution is not only very high, but also independent of pH in the range 2 to 12 and temperature from 18 to 80° C. Since the X-ray diffraction patterns from xanthan fibers are dominated by diffuse continuous intensity on all the layer lines, complete structure analysis has not yet been successful. The X-ray data suggest that xanthan has 5-fold helix symmetry and a pitch of 47 Å. Using this information, several speculative models, which include single and double helices, parallel and antiparallel chains, left and right-handed chiralities, and up to four chains in an orthorhombic unit cell have been proposed to date[18,19]. None of them provide a satisfactory solution with a good X-ray fit.

Although the molecular details are still elusive, it is clear that the side chains are disruptive to the lateral organization of xanthan molecules.

Galactomannan

Galactomannans constitute a family of plant polysaccharides containing a mannan [(1→4)-linked polymer of β-D-mannosyl units] backbone and (1→6)-linked α-D-galactosyl units as random side chains. Depending on its source, the galactose/mannose ratio varies from 0.3 in carob, 0.6 in guaran to 0.9 in fenugreek galactomannan. As they are water soluble and produce high viscosity, galactomannans are utilized as thickening agents in foods. Recent X-ray analysis has revealed the three-dimensional structure of guaran [20]. The polymer has 2-fold helix symmetry and pitch of 10.3 Å. The backbone is essentially similar to that of mannan and the galactosyl side chain folds towards the reducing end as shown in Fig. 5a. This conformation is stabilized by systematic hydrogen bonds between the side chain and the main chain. The four galactomannan helices in an orthorhombic unit cell form two hydrogen bonded sheets. The side chains are actively involved in the association within and between the sheets via ordered water molecules. Thus, the ability of the galactosyl units to promote favorable interactions with the surrounding solvent molecules is the structural reason for the solubility of galactomannan in water.

Xanthan:Galactomannan Complex

It is an interesting observation that a complex between xanthan and galactomannan forms gels, but the individual polymers do not. It has been believed that in these mixed gels, the backbone of xanthan helix associates with the naked regions of galactomannan chain as sketched in schematic drawings[21,22]. However, a modeling study guided by limited X-ray data from fibers of the xanthan:guaran complex has revealed some promising molecular details[23]. One possibility is that both xanthan and guaran adopt similar 2-fold helices as in cellulose and mannan so that the interactions involve either the backbone or the side chains or both. A second possibility is that the complex forms a double helix of pitch 47.4 Å in which the xanthan and galactomannan chains intertwine. Whether the chains are parallel or antiparallel, and whether the helix is right or left-handed, are yet uncertain. One putative model[23] of this kind is shown in Fig. 5b. This type of association would orient the carboxyl groups in the xanthan side chain on the outside of the helix so that a junction zone involving a pair of helices and intervening ions, similar to that observed for gellan[13], is not unthinkable for explaining the gelation behavior of this complex.

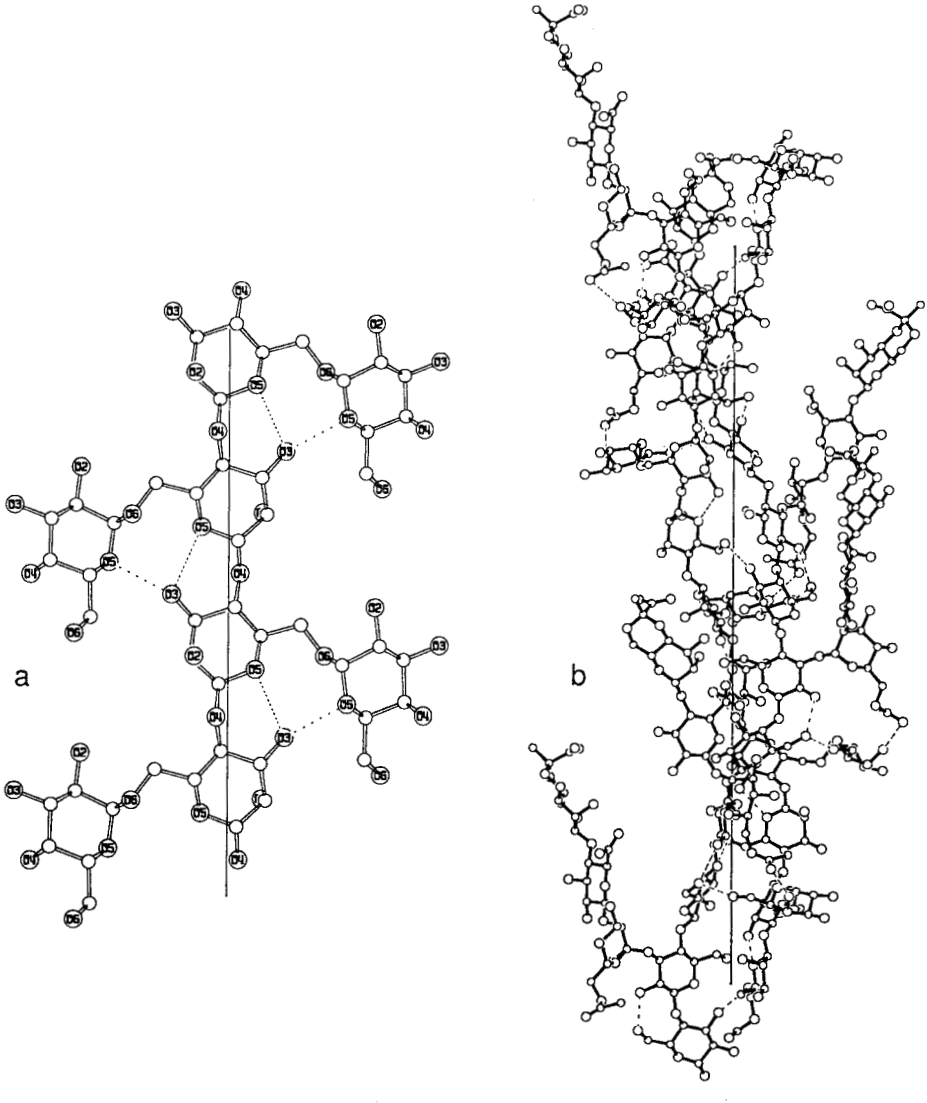


Fig. 5. (a) Two-fold helix of galactomannan and (b) 5-fold double helix of xanthan:guaran.

References

1) R. Chandrasekaran, *Adv. Food Nutr. Res.* **42**, 131 (1998).

2) P.J.C. Smith, S. Arnott, *Acta Crystallogr.* **A34**, 3 (1978).

3) S. Arnott, W.E. Scott, D.A. Rees, C.G.A. McNab, *J. Mol. Biol.* **90**, 253 (1974).

4) R.P. Millane, R. Chandrasekaran, S. Arnott, I.C.M. Dea, *Carbohydr. Res.* **182**, 1 (1988).

5) M.D. Walkinshaw, S. Arnott, *J. Mol. Biol.* **153**, 1055 (1981).

6) M.D. Walkinshaw, S. Arnott, *J. Mol. Biol.* **153**, 1075 (1981).

7) E.D.T. Atkins, I.A. Nieduszinski, H.H. Will, W. Mackie, K.D. Parker, E.E. Smolko, *Biopolymers* **12**, 1865 (1973).

8) B.A. Ryan, *M.S. Thesis, Purdue University* (1997).

9) E.D.T. Atkins, I.A. Nieduszinski, W. Mackie, K.D. Parker, E.E. Smolko, *Biopolymers* **12**, 1879 (1973).

10) W. Bian, *Unpublished Results.*

11) G.T. Grant, E.R. Morris, D.A. Rees, P.J.C. Smith, D. Thom, *FEBS Letters* **32**, 195 (1973).

12) R. Chandrasekaran, A. Radha, *Trends Food Sci. Technol.* **6**, 143 (1995).

13) R. Chandrasekaran, L.C. Puigjaner, K.L. Joyce, S. Arnott, *Carbohydr. Res.* **181**, 23 (1988).

14) R. Chandrasekaran, V.G. Thailambal, *Carbohydr. Polym.* **12**, 431 (1990).

15) R. Chandrasekaran, A. Radha, V.G. Thailambal, *Carbohydr. Res.* **224**, 1 (1992).

16) E.J. Lee, R. Chandrasekaran, *Carbohydr. Res.* **214**, 11 (1991).

17) R. Chandrasekaran, A. Radha, E.J. Lee, *Carbohydr. Res.* **252**, 183 (1994).

18) R. Moorhouse, M.D. Walkinshaw, S. Arnott, *Am. Chem. Soc. Symp. Ser.* **45**, 90 (1977).

19) K. Okuyama, S. Arnott, R. Moorhouse, M.D. Wakinshaw, E.D.T. Atkins, Ch. Wolf-Ullish, *Am. Chem. Soc. Symp. Ser.* **145**, 411 (1980).

20) R. Chandrasekaran, W. Bian, K. Okuyama, *Carbohydr. Res.* in press (1998).

21) P. Cairns, M.J. Miles, V.J. Morris, *Nature* **322**, 89 (1986).

22) N.W.H. Cheetham, E.N.M. Mashimba, *Carbohydr. Polym.* **14**, 17 (1991).

23) R. Chandrasekaran, A. Radha, *Carbohydr. Polym.* **32**, 201 (1997).