Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 338 (2003) 2889–2898
CARBOHYDRATE RESEARCH
www.elsevier.com/locate/carres

Acetan:glucomannan interactions—a molecular modeling study

Rengaswami Chandrasekaran,[a,*] Srinivas Janaswamy,[a] Victor J. Morris[b]

[a] *Whistler Center for Carbohydrate Research, Purdue University, 745 Agriculture Mall Drive, West Lafayette, IN 47907-2009, USA*
[b] *Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA, UK*

Received 14 July 2003; accepted 28 August 2003

Abstract

X-ray fiber diffraction patterns from deacylated acetan and glucomannan (konjac mannan) blends are diagnostic of good orientation and modest polycrystallinity. The meridional reflection on the sixth layer line suggests that the binary complex is a 6-fold helix of pitch 55.4 Å. A molecular modeling study incorporating this information reveals that a double helix in which one strand is acetan and the other glucomannan is stereochemically feasible. While the backbone and side groups are sufficiently flexible to allow the chains to associate with the same or opposite polarity, the parallel model is superior in terms of unit cell packing. The results are compatible with the observed synergy; namely the weak gelation behavior of the complex. The molecular model can be generalized for the binary system when acetan is replaced by xanthan or glucomannan by galactomannan.

Keywords: Acetan; Xanthan; Glucomannan; Galactomannan; Polysaccharide; X-ray diffraction; Molecular modeling; Gelation

1. Introduction

Whilst polysaccharides such as alginate, carrageenan, curdlan, gellan and pectin are well-known gelling agents, aqueous solutions of xanthan, acetan, glucomannan and galactomannan are good thickeners that do not gel under normal conditions. Systematic X-ray investigations to date have demonstrated that single, double or triple helices are the preferred molecular morphologies of these hydrocolloids.[1,2] Mannan-like ribbon structures (180° twist and 5.2 Å axial rise for the monomer) are common to both glucomannans and galactomannans.[3] The occasional occurrence of equatorial hydroxyl at C-2 in the former and frequent galactosyl side groups at O-6 in the latter are mainly responsible for their water solubility and ensuing viscosity. Careful examination of the three-dimensional structures reveals that the molecular basis of the observed physical properties does not merely rest on the polymer shape but is formulated by the intricate interactions taking place among the polysaccharide chains, solvent molecules and certain cations that may be present.[2] For example, the ability of anionic gellan to form stronger gels in the presence of calcium rather than potassium is related to the corresponding decrease in the strength of the carboxylate -X- carboxylate interactions taking place between the helices as X changes from Ca^{2+} in the divalent to the triplet K^+–water–K^+ in the monovalent form.[4] Similar structure–gelation correlation is noticed when the interactions involving the sulfate groups of adjacent helices are examined in the monovalent and divalent salts of ι-carrageenan.[5,6]

Although the bacterial polysaccharide xanthan has a simple cellulosic main chain, the trisaccharide side group in every cellobiose repeat unit is apparently detrimental to a unique and stable helical structure.[2] Xanthan mutants containing shorter mono- and disaccharide side groups also fall under this category.[7] The same is true for acetan, a xanthan-like polymer having a pentasaccharide instead of the trisaccharide side group.[8] Absence of order within and between polymer molecules of these cellulose derivatives leads to poor quality X-ray fiber diffraction patterns. Whereas the structural details are still unknown, it is not in doubt that a 5-fold helix of pitch about 47.4 Å is common to the entire xanthan family of polysaccharides.[9,10]

* Corresponding author. Tel.: +1-765-494-4923; fax: +1-765-494-7953.
E-mail address: chandra@purdue.edu (R. Chandrasekaran).

0008-6215/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2003.08.018

Blends of two unrelated polysaccharides may show synergistic behavior. Some interesting examples are κ-carrageenan:galactomannan,[11] κ-carrageenan:glucomannan,[12] and agarose:glucomamnnan[13] complexes since they exhibit gel properties that are distinct from those of κ-carrageenan or agarose itself. On the other hand, glucomannans such as konjac mannan as well as certain galactomannans interact with xanthan or acetan to yield gelling complexes[9,14] under conditions for which the individual components do not gel. Destabilization of the xanthan or acetan helices through deacetylation enhances the gelation of the mixtures. Using preliminary X-ray diffraction patterns and modeling calculations, a 5-fold double helix of pitch 47.4 Å in which a xanthan chain is intertwined with an antiparallel galactomannan chain has previously been proposed for the hybrid.[15] However, additional X-ray data obtained by us suggest that the xanthan:glucomannan and deacetylated acetan:glucomannan complexes are novel 6-fold helices of longer pitch of about 55.4 Å.[10,16,17] We have now extended the analysis for the deacetylated acetan:glucomannan binary system incorporating the recent experimental observations and conclude that the hybrid duplex has far more conformational freedom than thought hitherto.

2. Materials and methods

2.1. Sample preparation

Acetan, produced by batch synthesis of *Acetobacter xylinum* NRRL B42 strain M1, was prepared and purified as the sodium salt following the MacCormick et al. procedure.[18] Deacetylated acetan was prepared by alkali treatment using the methods described by Ojinnaka et al.[19] Konjac mannan glucomannan, isolated from *Amorphophallus konjac*, was obtained from Senn Chemicals, Switzerland. Concentrated solutions of the two polymers were prepared by heating aqueous dispersions to 90 °C in sealed glass tubes. The konjac mannan samples were centrifuged (4500 × *g* for 1.5 h) to remove insoluble material. Hot (90 °C) 1:1 mixtures were poured onto PTFE sheets, allowed to cool to room temperature and partially dried to thin films. This material was used to produce stretched (100%) fibers. The density of a fiber was measured by the flotation method using a mixture of chlorobenzene and carbon tetrachloride. Henceforth, unless otherwise specified, acetan refers to the deacetylated polymer in this paper.

2.2. X-ray data

Diffraction patterns were recorded on photographic films in a pinhole camera, flushed with helium at a relative humidity of 98%, on a microfocus X-ray generator using nickel-filtered Cu$K\alpha$ radiation of wavelength 1.5418 Å. One of the best patterns is shown in Fig. 1. Background scattering extends to the outer edge, yet few sharp Bragg reflections and some diffuse spots stand out in the interior. The distribution of intensity is

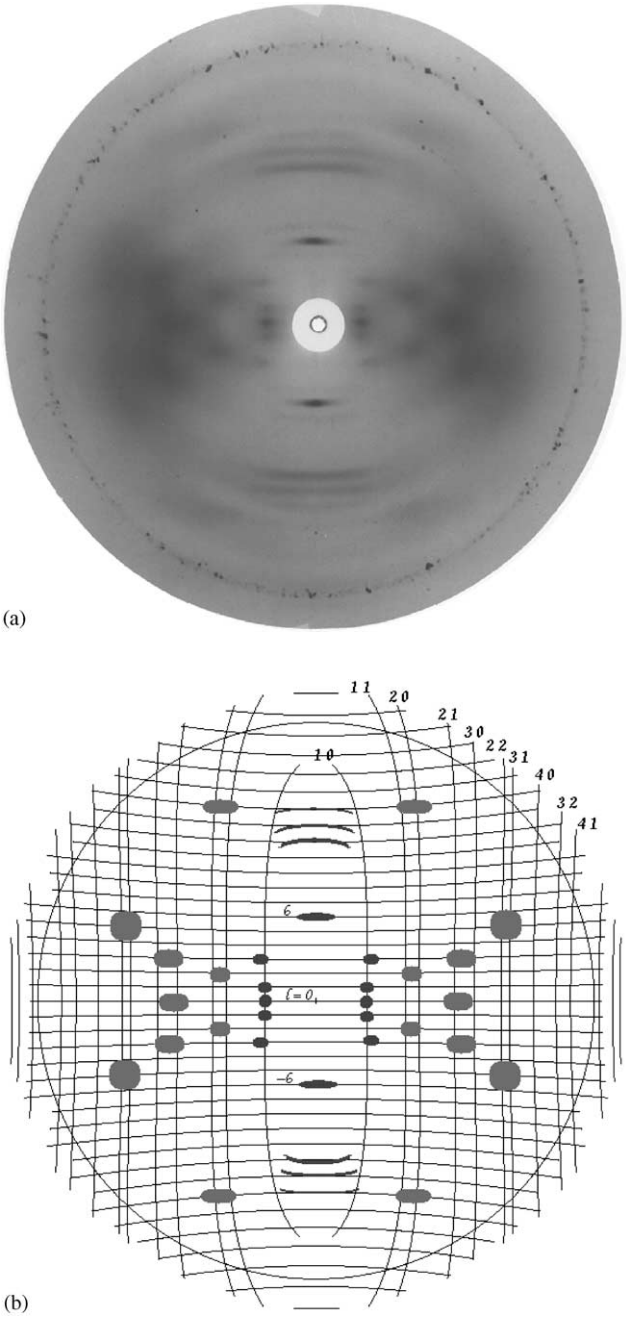

Fig. 1. (a) Diffraction pattern from a fiber of the 1:1 acetan:glucomannan complex characterized by a sharp meridional reflection on the 6th layer line and a few diffuse spots elsewhere. The ring is from calcite (*d*-spacing 3.035 Å) for internal calibration. (b) A schematic of the pattern is shown superposed on the Bernal chart for the hexagonal unit cell $a =$ 17.3 and $c = 55.4$ Å. The (*hk*) indices of the row lines, and layer lines 0, 6 and -6 are marked.

consistent with good axial orientation and short-range lateral organization of the helices in the fiber. The first meridional reflection on the 6th layer line indicates 6-fold helix symmetry. The diffraction data can be reconciled with a hexagonal unit cell of dimensions $a = 17.3$ and c (fiber axis) $= 55.4$ Å.

3. Modeling analysis

3.1. Model building and refinement

Six-fold double helices of pitch 55.4 Å composed of an acetan chain intertwining with a glucomannan chain were constructed and examined using the Linked-Atom Least-Squares (LALS) program.[20] The computations included four types of models that account for right- and left-handed chiralities coupled with parallel and antiparallel dispositions of the chains. The heptasaccharide repeat (I to VII) of acetan and the disaccharide repeat (VIII and IX) of glucomannan (coiling along with I and II) that constitute the basic motif of the complex are shown in Fig. 2. A helix is formed when the I–II:VIII–IX segment of the duplex accounts for $\pm 60^\circ$ in twist and 9.23 Å projected length along the helix axis. Except for Rha in $^1C_4$, all the pyranosyl rings conform to $^4C_1$ chair conformations. Both VIII and IX were hypothetically allowed to have two oxygen atoms from C-2, O-2 and O-2* in equatorial and axial orientations, respectively, so as to represent any arbitrary glucomannan sequence. The bond angle at each bridge oxygen atom was kept at 116.5°. While retaining the bond lengths and bond angles as rigid parameters at their standard values, the conformation angles (ϕ, ψ) around the interglycosidic bonds and orientations (χ) of the hydroxymethyl and carboxylate groups were varied. The function minimized in the least-squares procedure is given by

$$\begin{aligned} \Omega &= \sum_{m=1}^{M} e_m \Delta\theta_m^2 + \sum_{j=1}^{J} k_j \Delta c_j^2 + \sum_{n=1}^{N} \lambda_n G_n \\ &= \boldsymbol{E} + \boldsymbol{C} + \boldsymbol{L}. \end{aligned} \quad (1)$$

The term $\boldsymbol{E}$ ensures that the M conformation angles and related parameters refined are in their standard or expected domains; $\boldsymbol{C}$ relieves short contacts and monitors the J non-bonded interactions including hydrogen bonds; and $\boldsymbol{L}$ vanishes when the N constraints on helix symmetry are fully satisfied. The limits for OH···O bond were set at 2.6–3.0 Å for distance and 90–150° for the angle P-OH···O, P being the precursor atom of the donor group. The parameters e_m and k_j are the weights associated with the observations in the first two summations, respectively. Ω, $\boldsymbol{E}$ and $\boldsymbol{C}$ were used as the statistics for comparing the relative merits of the models.

3.2. Glucan:glucomannan core

The main chain conformation angles that should satisfy the helical parameters are the four pairs of (ϕ_1, ψ_1), (ϕ_2, ψ_2), (ϕ_8, ψ_8) and (ϕ_9, ψ_9). The corresponding hydroxymethyl orientations χ_{II}, χ_I, χ_{IX} and χ_{VIII} are unknowns. While the starting (ϕ, ψ) values were adopted from the structure of cellulose, all the three staggered domains for χ were tested and those promoting intra/inter-chain hydrogen bonds selected for further analysis. Proper juxtaposing of VIII–IX to I–II, in forming the helix core, was achieved by refining the orientation μ (rotation about) and translation w (fraction of c) along the helix axis of the glucomannan chain.

3.3. Acetan:glucomannan

The acetan side group geometry is defined by 15 conformation angles in five sets of (χ_{III}, ψ_3, ϕ_3), (χ_{IV}, ψ_4, ϕ_4), (χ_V, ψ_5, ϕ_5), (χ_{VI}, ψ_6, ϕ_6) and (χ_{VII}, ψ_7, ϕ_7), and

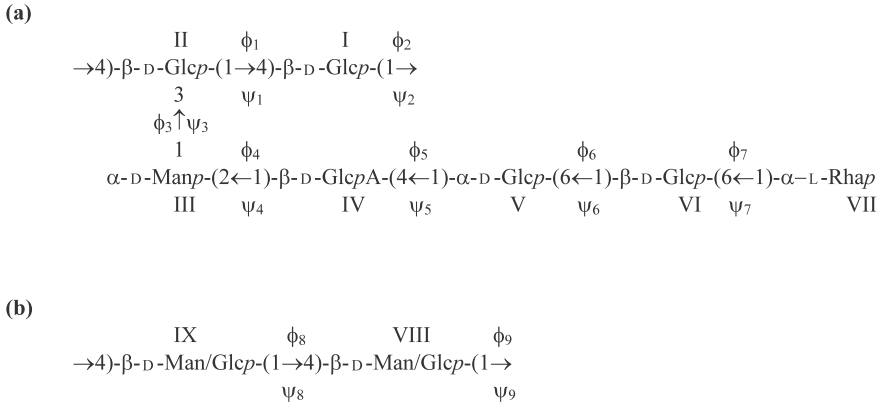

Fig. 2. Chemical structure of the repeating unit of (a) acetan and (b) glucomannan and the $(1 \rightarrow n)$ glycosidic conformation angles, ϕ = O-5–C-1–O-n–C-n and ψ = C-1–O-n–C-n–C-n+1. Note that the terminal atom defining ψ in the $(1 \rightarrow 6)$ linkage is C-n − 1 and not C-n+1. The hydroxymethyl/carboxyl orientation χ refers to C-4–C-5–C-6-O-6/O-61. Roman numerals designate the residues from the reducing to nonreducing end.

the preferred values must emerge from the modeling calculations. Relevant disaccharide contact maps were computed, the (ϕ, ψ) values for attaching III, IV and V adapted from the centers of the allowed domains in their respective maps and refined for optimizing contacts. Holding χ at 60°, 180° and −60°, three sets of hard sphere maps for V–VI and VI–VII were obtained. Using the center of the allowed domain in each map as the starting point while attaching VI and VII in nine distinct (χ_V, χ_{VI}) sets, the conformation angles were refined against the intrahelical interactions as observations for completing the entire side group attachment. Finally, the unit cell contents were investigated by refining all the above conformation angles of the duplex and the pertinent packing parameters so as to obtain favorable intermolecular association.

4. Results

4.1. Glucan and glucomannan geometry

The modeling calculations swiftly demonstrated that the initial net twist of 0° and axial rise of 10.4 Å for the cellobiose (mannobiose) repeat in cellulose (mannan)—wherein the helix axis almost coincides with the molecular axis—could be readily reduced to −60° and 9.23 Å, respectively, by positioning the repeat unit away from the helix axis at about 4.5 Å and changing the corresponding backbone (ϕ, ψ) and χ angles. The resulting left-handed helix was free from steric hindrance and retained one O–3H···O–5 bond (2.65 Å) per disaccharide repeat unit across the (1→4) linkage. Similar calculations failed to produce a satisfactory right-handed helix (+60° twist). The major reason was steric compression that brought the above atoms too close to comfort (<2.45 Å). Could this be remedied during glucan:glucomannan association?

4.2. Glucan:glucomannan complex

The next step was to test if the glucan and glucomannan helices could conveniently interact when both had the same polarity. The melding was performed by mildly relaxing the conformations of these two helices while simultaneously letting the latter to adopt suitable μ and w. The resulting left-handed, parallel, double helix (Fig. 3a) is stereochemically acceptable and exhibits a series of hydrogen bonds. These include intrachain O-3^{I}···O-5^{II} and O-2^{II}···O-6^{I} in glucan and O-3^{VIII}···O-5^{IX} in glucomamnnan, and four interchain O-6^{II}···O-5^{IX}, O-3^{I}···O-2^{VIII}, O-6^{IX}···O-6^{II} and O-3^{VIII}···O-6^{II} bonds, together lending structural stability to the core. A follow-up calculation convincingly proved that a left-handed, antiparallel, duplex (Fig. 3b) is also a valid model. Notable interchain hydrogen bonds in this case are O-2^{I}···O-6^{IX}, O-3^{I}···O-6^{IX}, O-2*^{VIII}···O-6^{II}, O-6^{II}···O-2^{VIII} and O-3^{VIII}···O-6^{II}.

In contrast, when two right-handed glucan and glucomannan helices were mated, a serious difficulty was encountered irrespective of their polarities. The previously spotted short O-3···O-5 distance (2.45 Å) in the glucan chain contracted further to 2.2 Å suggesting that right-handed chirality is not a good choice. This key finding led us to focus on left-handed structures, only.

4.3. Acetan:glucomamnnan complex

Attachment of the side group to II of glucan (Fig. 2) was straightforward. Nevertheless, optimization of the contacts in the left-handed helix had to be conducted separately for each of the nine sets of χ_V and χ_{VI}, as described in Section 3.3. The final statistics listed in Table 1 for both the parallel (↑↑) and antiparallel (↑↓) helices are useful in comparing the relative merits and selecting the preferred models of each type. A foremost observation is that all the three staggered domains are allowed for χ_V. This trend extends to χ_{VI} as well (sets 1 to 3) when the former is *gauche*+. On the other hand, when χ_V becomes *trans*, χ_{VI} is bimodal in the other two domains (sets 4 and 6). When χ_V shifts to *gauche*−, χ_{VI} is confined to *trans* only (set 8). Such a correlated behavior is evident from the relatively larger values of Ω (>160) for sets 5, 7 and 9 than for others (<143). Set 4 is the best ($\Omega = 115$) and set 6 a close contender for the parallel, but Hamilton's test[21] is in favor of set 4 for the antiparallel model. Consequently, set 4 [χ_V *trans* and χ_{VI} *gauche*+] was chosen to represent the acetan:glucomamnnan parallel and antiparallel double helices. Since there are no steric flaws in either case, both models were pursued in the packing analysis.

4.4. Molecular packing

The measured fiber density of 1.49 g/cc accounts for one hybrid helix in the unit cell, positioned at (0, 0), along with nearly four water molecules per saccharide. A refinement as stated in Section 3.2, including μ as the packing variable, was conducted. The results indicate that parallel duplexes nestle nicely forming numerous intermolecular hydrogen bonds. In the antiparallel case, there is not only loss of some hydrogen bonds within the helix, but also steric compression between the helices (such as 2.6 Å for C···O and 2.4 Å for O···O). Thus, at this stage, the final preference for the acetan:glucomamnnan hybrid is the left-handed, parallel double helix whose packing arrangement is shown in Fig. 4.

The conformation angles around the glycosidic bonds along with χ are given in Table 2. The (ϕ, ψ) angles at II–I and I–II′ (where the prime refers to the reducing end) are very close to those in IX–VIII and VIII–IX′, respectively, as they differ at most by 9°. The χ angles

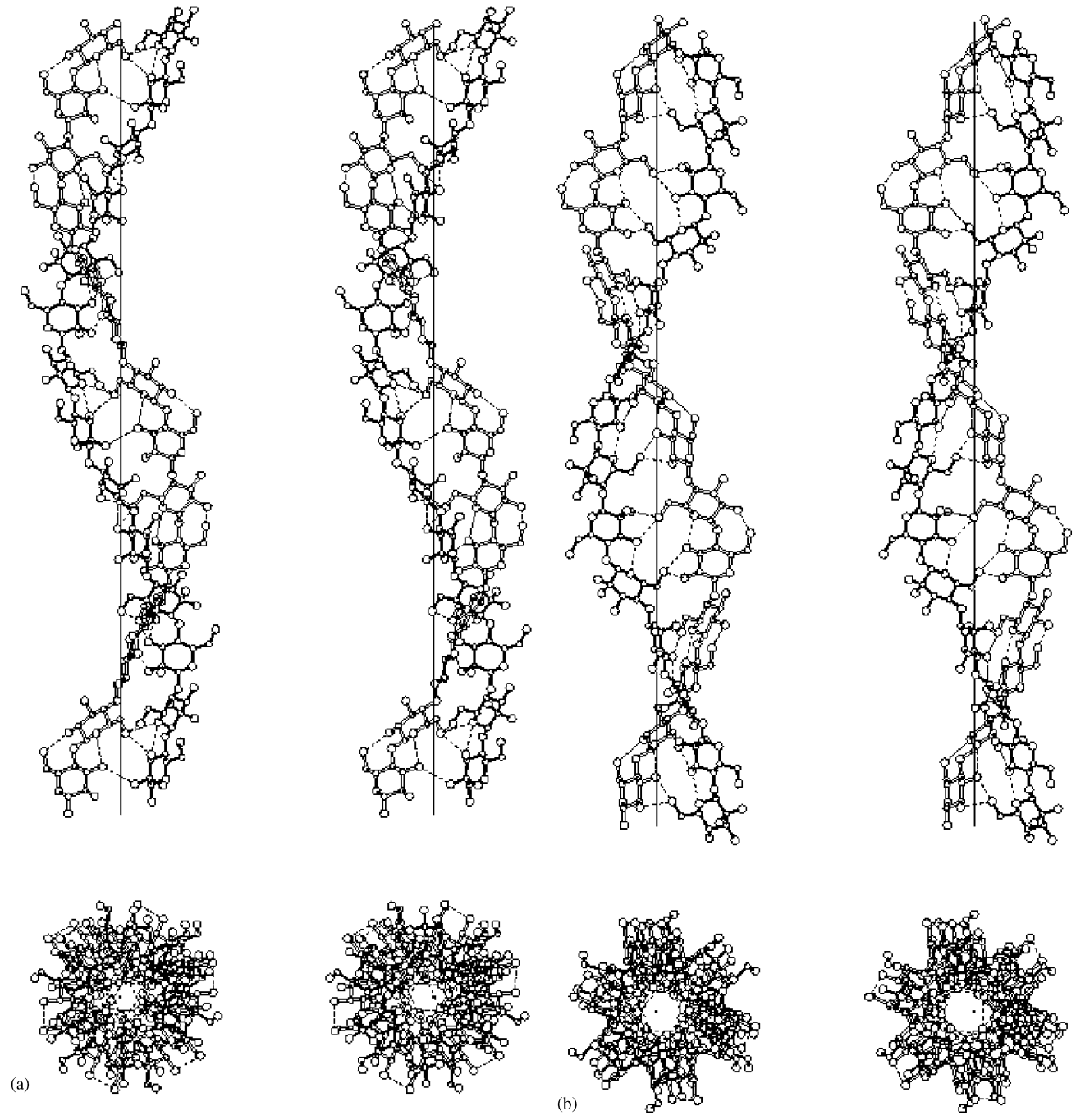

Fig. 3. Stereo projections (top) normal to and (bottom) along the helix axis of two prototype models of one turn of the 6-fold, left-handed, double helix composed of glucan in one chain and glucomannan (filled bonds) running (a) parallel and (b) antiparallel relative to the helix axis (vertical line). Intra- and inter-chain hydrogen bonds (dashed lines) provide structural stability.

follow the same trend with regard to their specific domains. Thus, the main chains are quite similar. However, since one ϕ angle (-147° at I–II′ and -150° at VIII–IX′) is off by nearly -50°, the backbone geometry of neither chain resembles that of cellulose. Instead, as shown in Fig. 3a, the two chains wrap around a cylinder of radius about 4.8 Å, which represents the displacement of virtual bond O-1···O-4 from the helix axis, in a concerted fashion and thus give rise to a stable 6-fold, left-handed, double helix.

It is clear from Fig. 5 that while the glucomannan chain is bare, the acetan chain that is periodically decorated with long side groups is bulky. This increases the helix radius from 8.2 Å for the core to 15.7 Å. The side group, oriented along the nonreducing end, spans nearly 13 Å up to the penultimate unit VI. However,

Table 1
Statistics for the acetan:glucomannan preliminary 6-fold, left-handed, parallel (↑↑) and antiparallel (↑↓) models in nine different conformational sets of χ (°) [a]

Set	χ_V	χ_{VI}	*E*		*J*		*C*		Ω	
			↑↑	↑↓	↑↑	↑↓	↑↑	↑↓	↑↑	↑↓
1	60	60	6	9	96	101	126	129	132	138
2	60	180	6	9	96	101	124	127	130	136
3	60	−60	7	9	96	101	127	130	134	139
4	180	60	6	12	96	99	109	115	115	127
5	180	180	14	19	115	111	214	160	228	179
6	180	−60	7	12	95	109	113	130	120	142
7	−60	60	22	29	115	120	174	178	196	207
8	−60	180	12	19	104	106	126	132	138	151
9	−60	−60	24	52	110	108	140	174	164	226

[a] ***E***, ***C*** and Ω are the terms minimized in Eq. (1). The number of observations *M* contributing to ***E*** is 15 in any set.

because VI is flanked by (1→6) linkages that are just two bonds apart, the terminal unit VII swings around towards the reducing end. Such a reversal in direction of progress has been noticed in the structure of RMDP17 in which the disaccharide side group also contains similar flanking (1→6) linkages.[22]

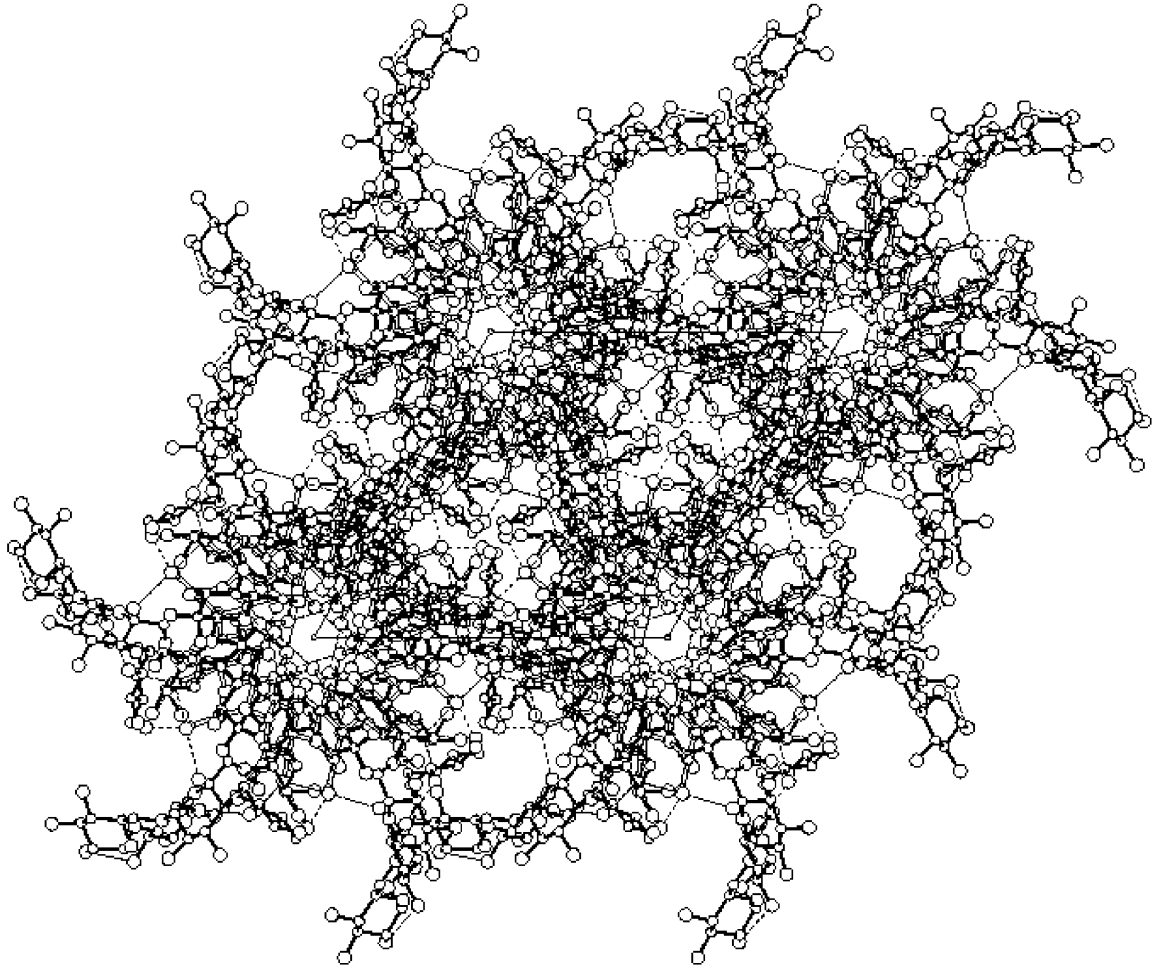

Fig. 4. The *c*-axis projection of the packing arrangement, one parallel double helix of the acetan:glucomannan complex at each corner of the unit cell, shows nestling of neighboring molecules hydrogen bonded via side groups. The peripheral unit seen is monomer VI.

Table 2
Major conformation angles in the final 6-fold, left-handed, parallel, double helical model of the acetan:glucomannan complex [a]

Parameter (°)	Linkage								
	II–I (1→4)	I–II' (1→4)	IX–VIII (1→4)	VIII–IX' (1→4)	III–II (1→3)	IV–III (1→2)	V–IV (1→4)	VI–V (1→6)	VII–VI (1→6)
ϕ	−107	−147	−99	−150	90	−59	107	−126	−100
ψ	−153	−137	−150	−146	141	106	−144	167	−124
χ [a]	178	−60	180	−89	−168	15	179	69	−53

[a] For the first unit in the linkage.

Hydrogen bonds (Table 3) are present within and between side groups (especially III, IV, V and VII) and also from the side group to the main chains within and between helices. In particular, the carboxylate oxygen atoms O-61^{IV} and O-62^{IV} are acceptors from O-6^{I} and O-6^{VIII} in intra- and interhelical interactions, respec-

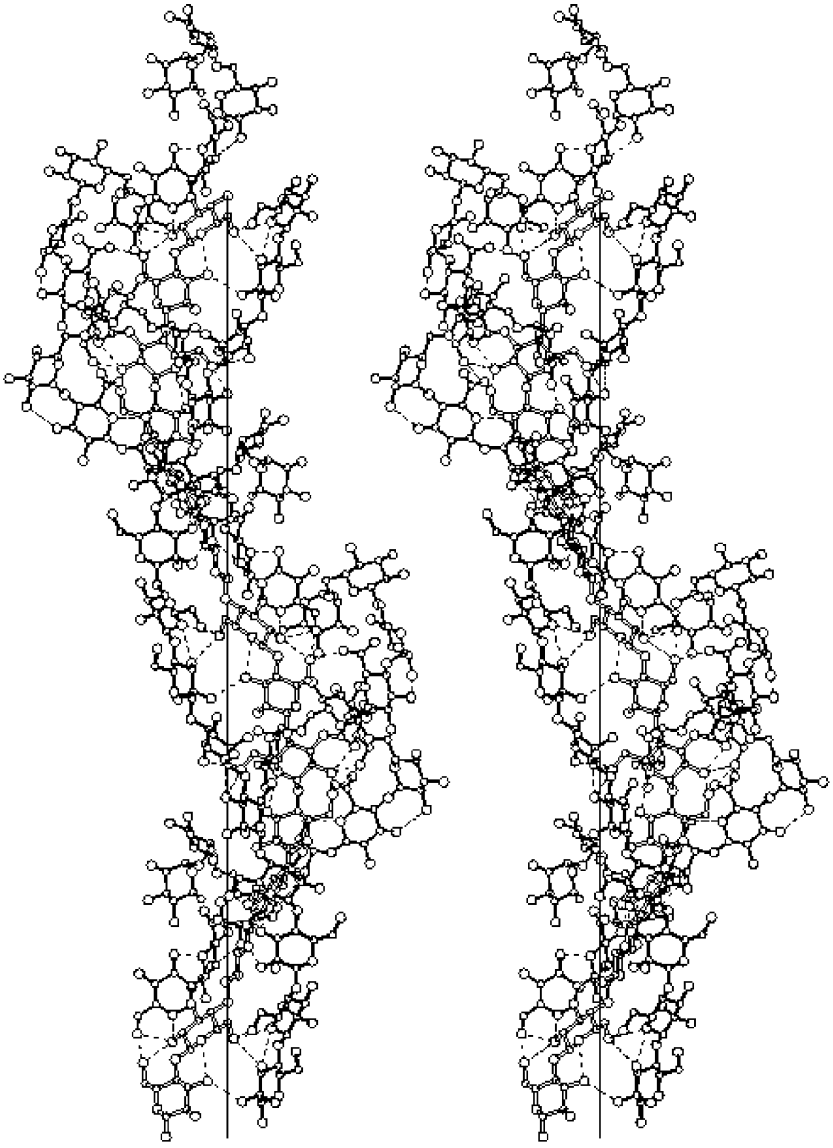

Fig. 5. Stereo side view of the left-handed, parallel double helix of acetan:glucomannan. The glucomannan chain (filled bonds) is turned nearly −96° and moved up 1.1 Å from the acetan chain in this model. The side group (filled, thinner bonds), shielded by the acetan backbone, is away from the glucomannan chain.

Table 3
Intra- and intermolecular hydrogen bonds in the acetan:glucomannan complex [a]

Hydrogen bond	Atom X	Atom Y	$X\cdots Y$ (Å)	Precursor P	$P-X\cdots Y$ (°)
Core					
Intra					
1	O-2^{II}	O-6^{I}	2.71	C-2^{II}	107
2	O-3^{I}	O-5^{II}	2.88	C-3^{I}	99
3	O-3^{VIII}	O-5^{IX}	2.73	C-3^{VIII}	101
Inter					
4	O-6^{II}	O-5^{IX}	2.71	C-6^{II}	90
5	O-3^{I}	O-2^{VIII}	2.80	C-3^{I}	131
6	O-6^{IX}	O-6^{II}	2.62	C-6^{IX}	98
7	O-3^{VIII}	O-6^{II}	2.69	C-3^{VIII}	99
Side group					
Intra					
8	O-3^{III}	O-5^{IV}	2.47	C-3^{III}	106
9	O-2^{V}	O-3^{IV}	2.83	C-2^{V}	110
Inter					
10	O-2^{IV}	O-2^{VII} (1 0)	2.74	C-2^{IV}	103
11	O-2^{VII}	O-3^{IV} (0 1)	2.71	C-2^{VII}	129
Core–side group					
Intra					
12	O-2^{II}	O-5^{III}	2.63	C-2^{II}	105
13	O-2^{II}	O-6^{III}	2.63	C-2^{II}	129
14	O-6^{I}	O-62^{IV}	2.64	C-6^{I}	106
15	O-6^{III}	O-6^{I}	2.82	C-6^{III}	113
Inter					
16	O-2^{IV}	O-2^{VII}(1 0)	2.74	C-2^{IV}	103
17	O-3^{V}	O-6^{IX}(1 0)	2.65	C-3^{V}	109
18	O-4^{V}	O-6^{IX}(1 0)	2.60	C-4^{V}	111
19	O-2^{VII}	O-3^{IV}(0 1)	2.71	C-2^{VII}	129
20	O-3^{VII}	O-2^{IX}(1 1)	2.78	C-3^{VII}	100
21	O-6^{VIII}	O-61^{IV}(0 -1)	2.60	C-6^{VIII}	114

[a] The superscript in the atom name refers to residue number. The digits in parentheses, if any, are a and b translations in the unit cell.

tively. Surprisingly, VI (β-D-glucose) does not have any such role. Nevertheless, the bulky side group not only stabilizes the main chain but also promotes helix–helix association.

5. Discussion

It is a remarkable phenomenon that certain binary blends of polysaccharides are bestowed with a unique physical behavior that is innate to neither. The origin of the synergistic interactions at the molecular level owes to the unprecedented shape of the acetan:glucomannan hybrid emerging from our modeling analysis. The adoption of the orthodox 2-fold structure, common to cellulose and mannan, by glucomannan is readily understood from conventional wisdom. The previously obtained X-ray diffraction patterns are undoubtedly diagnostic of 5-fold helix symmetry for xanthan as well as acetan, but too poor in quality to determine their structures.[9,10] Consequently, whether each will form a single or double helix, right- or left-handed, and parallel or antiparallel duplex, are pertinent questions. Answers are not in sight unless and until polycrystalline and well-oriented specimens become available.

The diffraction pattern shown in Fig. 1 is strong evidence that the acetan:glucomannan mixed system is neither 5-fold nor 2-fold, but a novel 6-fold helix whose axial rise per main chain disaccharide repeat, however, remains the same (9.2 Å) as in acetan.[9] While the latter is not a surprise, the role of the additional twist to the helical structure is a priori perplexing. What is the driving force? The 2-fold structure of cellulose or mannan has 0° twist per disaccharide repeat unit (180° per monomer) and is reinforced by an O-3H$\cdots$O-5 bond across each glycosidic linkage. Any perturbation to this hydrogen bond will destabilize the ribbon, and a coil-like structure is imminent as revealed by related poly-

mers and this analysis. For example, upon replacing its hydroxymethyl group by a hydrogen atom, glucopyranose becomes xylopyranose, and due to less crowding, the resulting (1→4)-β-D-xylan gains greater rotational freedom around C-1–O-4 and O-4–C-4 bonds; this provokes atom O-3 to break away from O-5 and pair instead with a water molecule nearby.[23,24] As the transformation propagates, the chain becomes a 3-fold helix with a negative twist (-120°), and the axial rise per monomer shortens to 5.0 Å from 5.2 Å in cellulose.[23]

Another example is chitosan [poly (1→4)-β-D-glucosamine]. While its type I secondary structure is rigid, similar to cellulose, the type II structure has a relaxed geometry, i.e., a 2-fold helix composed of a tetrasaccharide repeat for 180° twist and 20.3 Å axial rise.[25] Some of the O-3H···O-5 bonds are either lost or weakened as adjacent monomers in the repeat adopt different conformations, and the polymer chain coils in a left-handed fashion.

The gel-forming polysaccharide gellan[4] and the branched polymers such as welan[26] and RMDP17[22] in the gellan family constitute the third example. Common to them is the main chain tetrasaccharide repeat [→3)-β-D-Glc-(1→4)-β-D-GlcA-(1→4)-β-D-Glc-(1→4)-α-L-Rha-(1→], nearly 75% cellulose-like. The (1→3)-linkage and Rha unit, however, disrupt the regular cellulose sequence thereby preventing the formation of a rigid ribbon structure. Instead, the polymer chain coils in a left-handed fashion (-120° twist and 18.9 Å axial rise). Nevertheless, one of the two possible O-3H···O-5 bonds in each chemical repeat remains intact. Two such helices associate in a half-staggered, parallel fashion so as to produce a coaxial double helix stabilized by interchain hydrogen bonds (O-6H···O-62) periodically linking hydroxymethyl to carboxylate groups.[4] In this context, the glucan:glucomannan duplex shown in Fig. 3a can be adapted and perceived as the "cellulose analog" of gellan, because two successive disaccharide repeats together in either glucan chain will have -120° twist and 18.5 Å axial rise, just 0.4 Å shorter than in gellan. Although not half-staggered, as mentioned earlier, this duplex will be reinforced by a series of interchain hydrogen bonds (O-3H···O-6) between hydroxyl and hydroxymethyl groups.

Substitution at atom O-3 could also affect the ribbon structure for cellulosic polymers. Nature apparently has adopted this approach conveniently in producing branched polysaccharides such as xanthan, acetan, welan and RMDP17. The overpowering of side groups on main chain seems to have tremendous ill effect on the lateral organization of xanthan and acetan helices that their molecular structures yet remain elusive. In contrast, the side groups being at most half the main chain size, the details of the preferred molecular shapes of welan and RMDP17 and the interactions between their helices in regulating the functional properties are known from X-ray studies. Thus, the examples cited above support the fact that a cellulosic polysaccharide tends to form a helix if allowed to abandon one or more of the O-3H···O-5 hydrogen bonds, similar to that of the acetan:glucomannan complex.

Recapitulate that some of the main chain hydroxymethyl groups take part in interchain hydrogen bonds (Table 3) and impart stability to the double helix. If acetylated, O-6 being a preferred site, these interchain hydrogen bonds are lost. Consequently, both the double helix and the assembly of helices are considerably weakened. Native acetan is partially acetylated at O-6 on the branched backbone glucose residue and there may only be small segments of the backbone that can interact with the glucomannan. This would explain why the synergistic interaction between native acetan and konjac mannan is significantly enhanced upon deacetylation.[14]

The involvement of the carboxylate group in interhelical interactions (Table 3) suggests that cations in the vicinity also have a key role in the association of the acetan:glucomannan molecules. Although the details are unknown, by extrapolation of the structural features of gellan, carboxylate–carboxylate interactions mediated by cations and water molecules must persist in the assembly of these hybrid helices. However, the long side groups might limit the ordering to a short range only. The resulting junction zones are appropriate for the onset of weak gelation as observed.[14] Likewise, without loss of generality, it is also compatible with the xanthan:glucomannan hybrids and consistent with the corresponding X-ray diffraction patterns.[10,16]

Our modeling calculations have confirmed that there are no restrictions within the acetan:glucomannan double helix for replacing the glucomannan with a galactomannan chain that has a (1→6)-linked α-D-galactosyl unit on every second residue in the backbone. This can be further verified from Fig. 5 which shows that such side groups sticking out of the helix surface do not clash with the other atoms. Therefore, the 6-fold, left-handed, parallel, double helix presented for acetan:glucomannan is a possible model for the acetan:galactomannan complex, which also exhibits weak gelation. However, the X-ray diffraction patterns obtained for the analogous xanthan:galactomannan mixed gels suggest a junction zone based around a modified galactomannan diffraction pattern.[27,28]

Acknowledgements

This research was supported by the Industrial Consortium of the Whistler Center for Carbohydrate Research and the BBSRC core strategic grant to the Institute of Food Research. P. Cairns is thanked for experimental assistance.

References

1. Chandrasekaran, R. *Adv. Carbohydr. Chem. Biochem.* **1997**, *52*, 311–439.
2. Chandrasekaran, R. *Adv. Food Nutr. Res.* **1998**, *42*, 131–210.
3. Chandrasekaran, R.; Bian, W.; Okuyama, K. *Carbohydr. Res.* **1998**, *312*, 219–224.
4. Chandrasekaran, R.; Puigjaner, L. C.; Joyce, K. L.; Arnott, S. *Carbohydr. Res.* **1988**, *181*, 23–40.
5. Janaswamy, S.; Chandrasekaran, R. *Carbohydr. Res.* **2001**, *335*, 181–194.
6. Janaswamy, S.; Chandrasekaran, R. *Carbohydr. Res.* **2002**, *337*, 523–535.
7. Millane, R. P.; Narasaiah, T. V.; Arnott, S. In *Biochemical and Biotechnological Advances in Industrial Polysaccharides*; Crescenzi, V.; Dea, I. C. M.; Paoletti, S.; Stivala, S. S.; Sutherland, I. W., Eds.; Gordon and Breach: New York, 1989; pp 469–478.
8. Jansson, P.-E.; Lindberg, J.; Wimalasiri, K. M. S.; Dankert, M. *Carbohydr. Res.* **1993**, *245*, 303–310.
9. Morris, V. J.; Brownsey, G. J.; Cairns, P.; Chilvers, G. R.; Miles, M. J. *Int. J. Macromol.* **1989**, *11*, 326–328.
10. Brownsey, G. J.; Cairns, P.; Miles, M. J.; Morris, V. J. *Carbohydr. Res.* **1988**, *176*, 329–334.
11. Miles, M. J.; Morris, V. J.; Carroll, V. *Macromolecules* **1984**, *17*, 2443–2445.
12. Cairns, P.; Miles, M. J.; Morris, V. J. *Carbohydr. Polym.* **1988**, *8*, 99–104.
13. Goycoolea, F. M.; Richardson, R. K.; Morris, E. R.; Gidley, M. J. *Biopolymers* **1995**, *36*, 643–658.
14. Ridout, M. J.; Brownsey, G. J.; Morris, V. J. *Macromolecules* **1998**, *31*, 2539–2544.
15. Chandrasekaran, R.; Radha, A. *Carbohydr. Polym.* **1997**, *32*, 201–208.
16. Morris, V. J. In *Biopolymer Mixtures*; Harding, S. E.; Hill, S. E.; Mitchell, J. R., Eds.; Nottingham University Press: Nottingham, 1996; pp 289–314.
17. Ridout, M. J.; Cairns, P.; Brownsey, G. J.; Morris, V. J. *Carbohydr. Res.* **1998**, *309*, 375–379.
18. MacCormick, C. A.; Harris, J. E.; Gunning, A. P.; Morris, V. J. *J. Appl. Bacteriol.* **1993**, *74*, 196–199.
19. Ojinnaka, C.; Brownsey, G. J.; Morris, E. R.; Morris, V. J. *Carbohydr. Res.* **1998**, *305*, 101–108.
20. Smith, P. J. C.; Arnott, S. *Acta Crystallogr., Sect. A* **1978**, *34*, 3–11.
21. Hamilton, W. C. *Acta Crystallogr.* **1965**, *18*, 502–510.
22. Bian, W.; Chandrasekaran, R.; Rinaudo, M. *Carbohydr. Res.* **2002**, *337*, 45–56.
23. Nieduzynski, I.; Marchessault, R. *Biopolymers* **1972**, *11*, 1335–1344.
24. Almond, A.; Sheehan, J. K. *Glycobiology* **2003**, *13*, 255–264.
25. Okuyama, K.; Noguchi, K.; Kananeri, M.; Egawa, T.; Osawa, K.; Ogawa, K. *Carbohydr. Polym.* **2000**, *41*, 237–247.
26. Chandrasekaran, R.; Radha, A.; Lee, E. J. *Carbohydr. Res.* **1994**, *252*, 183–207.
27. Cairns, P.; Miles, M. J.; Morris, V. J. *Nature* **1986**, *332*, 89–90.
28. Cairns, P.; Miles, M. J.; Morris, V. J.; Brownsey, G. J. *Carbohydr. Res.* **1987**, *160*, 411–423.