Crystallography of highly substituted galactomannans: Fenugreek and lucerne gums

ARTICLE *in* MACROMOLECULES · NOVEMBER 1989

Impact Factor: 5.8 · DOI: 10.1021/ma00196a017

grateful to one reviewer for sharing with us his (her) thorough knowledge of the ionomer literature, for constructive criticism, and for helping us prepare a better paper.

Registry No. DMF, 68-12-2; THF, 109-99-9; Nafion 117, 66796-30-3; Cu^{2+}, 15158-11-9; CH_3OH, 67-56-1; H_2O, 7732-18-5.

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Crystallography of Highly Substituted Galactomannans: Fenugreek and Lucerne Gums

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ABSTRACT: Wide-angle X-ray fiber diffraction methods have been used to study the crystal structure of two highly substituted galactomannans, fenugreek and lucerne gums. Both hydrated polysaccharides crystallize on orthorhombic lattices with a = 9.12 Å, b = 33.35 Å, and c = 10.35 Å (for fenugreek gum) and a = 8.98 Å, b = 33.32 Å, and c = 10.34 Å (for lucerne gum). In each case, the density is consistent with four chains passing through the unit cell and the probable space group symmetry is $P2_12_12$. The results for the a and c parameters are essentially the same as those derived from less highly substituted galactomannans such as guar, tara, or carob, while the b dimension is slightly larger. Under vacuum, the b dimension contracts with little concomitant change in either a or c. This suggests that the same fundamental crystal structure applies to these gums as to the less substituted but commercially important guar and carob species.

Introduction

Galactomannans are neutral, polysaccharide gums which find widespread application as materials of commerce in applications ranging from enhanced oil recovery,[1-4] paper sizing, textile,[5] and food[6] industries and are derived from the seed endosperm of various *Leguminosae*. In the seed, the polysaccharides serve the dual function of protecting the embryo from desiccation[7] and providing a source of nutrient.[8] These biogums have linear core poly((1→4)-β-D-mannan) main chains with varying degrees (DS) of α-D-galactosyl substituents attached at the C6 primary hydroxyl groups as shown in Figure 1. Polysaccharides obtained from different species differ largely in the abundance and, possibly, the distribution of D-galactose substituents.[9] The gums obtained from fenugreek (*Tri-*

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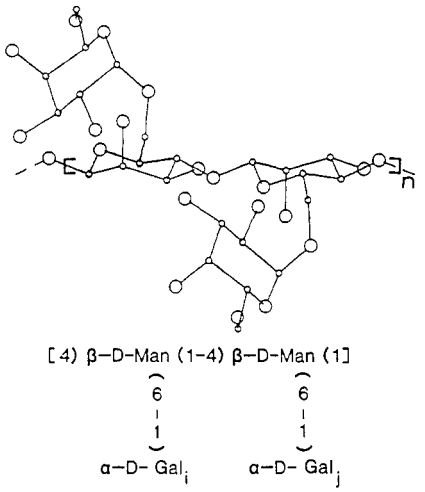

Figure 1. Primary structure of the average crystallographic repeating unit in fenugreek and lucerne gums. Galactose side chains are present approximately 93% of the time. The designations Gal_i and Gal_j are intended to denote that successive galactosyl units are crystallographically independent and may have different linkage conformations.

gonella foenum-graecum) and lucerne or alfalfa (*Medicago sativa*) are amon the chemically more-regular members of this carbohydrate class since their DS are typically 0.92–0.95. With this nearly complete substitution pattern, these polymers provide an important benchmark for a theory previously proposed by us that all galactomannans have essentially the same three-dimensional structure, an anti-parallel sheet stabilized by mannan–mannan hydrogen bonds.[10-13]

Materials and Methods

Samples of fenugreek and lucerne (*Medicago sativa*) gums were obtained from B. V. McCleary, NSW Agricultural Institute, Rydalmere, NSW, Australia, and from P. B. Bulpin, Unilever Research, Bedford, UK. Characterization of these fractions has been previously described.[14,15] Both samples were received in the form of white, fluffy, lyophilized products.

The samples were further purified by precipitation from aqueous solution (0.003 g·cm^{-3}) with excess 95% ethanol, redissolved in glass distilled water (0.001 g·cm^{-3}), and dialyzed exhaustively against distilled water at 4 °C before lyophilization and storage for future use. Cylindrical films were formed as coatings on internally heated polyethylene rods by slow evaporation of 0.4% (w/w) solutions over 20 h.[11] The resulting films show appreciable circumferential orientation as evidenced by their extinction when viewed between crossed polarizers in the optical microscope. Orientation was further enhanced by storing the samples for 100–120 h at 95% relative humidity (RH) and under a load of 10–20 g. Initial dimensions of the strips were 0.2 mm × 1.5 mm with a circumference of 12.3 mm. Crystallinity was subsequently enhanced by annealing in a sealed high-pressure bomb at 100 °C over aqueous Na_2CO_3(sat) for 4 h. Final dimensions of the films were 0.15 mm × 1.0 mm × 25 mm (circumference).

X-ray diffraction data were collected with a flat-film camera using Ni-filtered Cu K_α radiation from a Philips sealed tube generator operated at 35 kV and 14 mA. Accurate film-to-specimen distances were determined from external calibration with crystalline calcite (d = 3.0381 Å). Samples were maintained at constant RH values during the X-ray experiments by flushing the cameras with helium previously bubbled through an appropriate saturated salt solution. Diffraction patterns were collected on Agfa Gevaert Osray M3 film.

Results

Diffraction Patterns and Crystallography. Figure 2 shows typical diffraction patterns obtained from oriented crystalline fenugreek and lucerne gums at 71% and 50% RH, respectively, and from dry fenugreek in vacuo.

Table I
Observed and Calculated Interplanar Spacings (Å^{-1} × 10^4) for Fenugreek and Lucerne Gums

	fenugreek				lucerne			
	RH = 71%		RH = 0%		RH = 50%		RH = 0%	
hkl	obsd	calcd	obsd	calcd	obsd	calcd	obsd	calcd
020	590	601	645	678			656	634
110							1185	1157
040	1212	1202			1185	1201	1234	1267
120			1312	1309			1297	1281
130	1396	1420			1445	1432	1484	1463
060	1796	1804			1801	1802		
150			1965	2031				
200	2194	2194			2230	2227		
220	2271	2275	2355	2340	2306	2306	2318	2314
230							2375	2420
240	2511	2502			2510	2530		
170			2651	2624				
260	2815	2840						
280	3237	3255						
001					974	970	979	970
011	1055	1012	1009	1031				
021	1226	1138						
121	1577	1581	1609	1631	1599	1594	1608	1606
141	1930	1893			1939	1903		
051			1965	1954				
061			2291	2272				
211	2424	2416						
002	1864	1932					1910	1940
012					1959	1962		
112	2245	2242	2291	2272	2268	2256	2261	2258
132	2411	2398					2440	2430
152	2695	2682			2691	2694	2758	2740
062			2801	2815				
212					2961	2968		
003	2850	2898					2890	2909
303							3069	3061
113							3144	3131
123	3157	3156						

Table II
Galactomannan Lattice Constants

source	RH, %	G/M	a, Å	b, Å	c, Å
fenugreek	71	0.93	9.12 (4)[a]	33.27 (22)	10.35 (5)
fenugreek	0	0.93	8.94 (10)	29.50 (29)	10.27 (15)
lucerne	50	0.92	8.98 (3)	33.32 (21)	10.33 (5)
lucerne	0	0.92	8.99 (6)	31.09 (42)	10.31 (4)
guar[11,16]	81	0.64	9.13	32.83	10.35
tara[10]	88	0.39	8.91	30.62	10.40
locust bean[11,6]	88	0.32	9.04	30.61	10.24
mannan I[12,21]	0	0.00	8.92	7.21	10.27

[a] Parenthetical values denote the experimental errors in the last figure of the lattice constant.

Measured interplanar spacings, *hkl* indices, and calculated spacings are reported in Table I. Lattice constants for the calculated orthorhombic unit cells derived from these patterns and those of previously reported galactomannans are summarized in Table II. In all cases, the agreement between observed and calculated spacings is better than 99%.

Meridional reflections are absent on the first layer line but do appear on both the second and third layers. Tilting of the samples by 15° from the normal to the X-ray beam fails to fully split the third layer reflection, and we conclude that it has intensity contributions from both the 003 and 013 planes. Thus, the formal 2-fold screw dyad symmetry of mannan I[12] is not exactly preserved in the galactomannans. Crystallographically, this corresponds to a change in symmetry from $P2_12_12_1$ of mannan I[12] to $P2_12_12$ of the galactomannans. The crystallographic repeating unit then becomes the mannobiose together with any attached galactose. This relieves the requirement, implicit

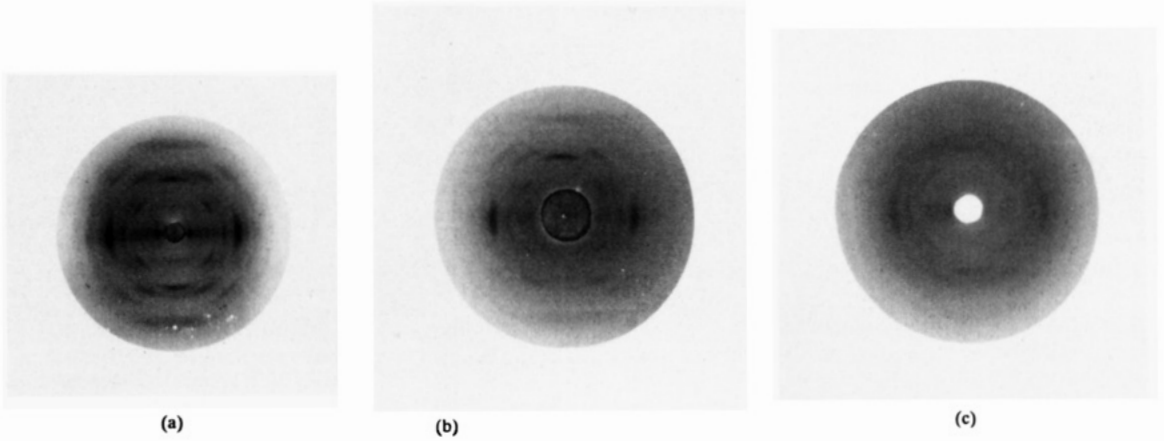

Figure 2. X-ray diffraction patterns of (a) fenugreek gum at 71% RH, (b) lucerne gum at 50% RH, and (c) lucerene gum in vacuo.

in the higher symmetry two-chain model, of statistically equal probabilities for the presence of substituent groups at adjacent sites along the mannan chain. For this reason, we have designated the pendant galactosyl units as Gal_i and Gal_j. The designation implies that the units are not related by either molecular or crystallographic symmetry.

Implications for Other Galactomannans. These results place severe restraints on the allowable packing models for describing galactomannan structures. First, the similarity with published data for guar,[13,15] which has some 30% less galactose, argues strongly against previously proposed models[16] for guar, where smooth, unsubstituted regions were thought to pack in between the highly substituted chains. Instead, it seems clear that a single-packing model must describe the organization of all members of the galactomannan family, and the presence or absence of galactose substitution at a particular site in the crystal is essentially statistical. Given the size of a glactose molecule, it is highly unlikely that such a void can remain empty. Instead, one can expect that such a hole should be filled with water molecules under the crystallization conditions employed in this study and previous studies. The loss of crystallinity upon drying (compare parts a and c of Figure 2) is consistent with this picture since loss of water should result in collapse of the structure. Second, it is immediately apparent that two of the unit cell dimensions, *a* and *c*, are essentially constant over the entire range of galactose substitution from 0% in mannan I to 93% in fenugreek. As is also shown in Table II, changes in hydration primarily affect the long (~30-Å) dimension of the unit cell. Conservation of the 10.35-Å periodicity along *c* is readily explainable in terms of an energetically preferred main-chain conformation. This same repeat is also observed in cellulose[17,18] and chitin[19] polymorphs as well as poly((1→4)-β-D-mannuronic acid)[20] and seems to be ubiquitous for diequatorial (1→4)-linked poly(hexapyranoses) unless C3 serves as a branch point, as in xanthan gum. This periodicity seems to be a consequence of an intramolecular O3···O5 hydrogen bond.

The constancy of the *a* parameter implies the existence of a regular lateral association, which is independent of the degree of galactose substitution in the galactomannans. The structure of the parent mannan I compound has been reexamined recently using electron diffraction intensity data derived from single crystals of ivory nut mannan.[12] It seems clear from this and previous X-ray studies[21] that the chains in mannan I pass through the unit cell in opposite directions and are positioned so as to maximize the opportunity for O2···O5 intermolecular hydrogen bonds. In mannan I, each mannobiose repeating unit participates in four such interactions, two as an O2 donor and two as an O5 acceptor. This results in a three-dimensional hydrogen-bonded network of chains on a crystalline lattice.

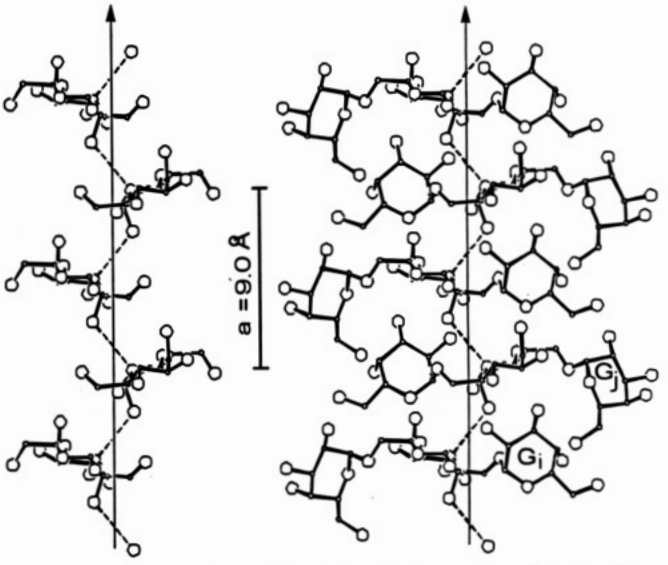

Figure 3. Comparison of (a, left) a hydrogen-bonded sheet in mannan I and (b, right) the same packing arrangement fully populated with (1→6)-linked galactose side chains. Potential hydrogen bonds are designated by dashed lines. Note the differing conformations assumed by galactosyl units on either side of a given mannan main chain.

Figure 3 shows a molecular model of a galactomannan sheet as it exists in mannan I and the same sheet fully substituted with one galactosyl unit at each mannosyl O6. The thickness of this sheet in the *b* dimension is approximately 15 Å, and two such sheets pass through each unit cell. In this model, the more highly substituted galactomannans such as fenugreek and lucerne are the physically more regular species, and in this context, it was interesting to note that these species showed markedly less loss of crystallinity upon drying than was observed for locust bean, tara, or guar. The decrease in *b* of about 3 Å suggests that a substantial amount of water is bound in the interlamellar space. Likewise, any gaps created by the absence of galactose substitution at a given site are also expected to be filled with water at high humidities. Removal of this water upon drying would therefore result in disordering and loss of crystallinity that would be most severe at intermediate galactomannan/mannan (G/M) ratios.

Finally, the relative constancy of the *b* dimension at high humidity over a fairly large range of G/M values is not surprising. If one considers a pair of rigid sheets (the mannan sheet) and begins to place spacers (pairs of ga-

lactose units) between the sheets, then the first few such insertions (three if the sheets are truly rigid) define the intersheet separation. Subsequent additions merely fill the available sites with no significant effect on the separation. Factors that will be sensitive to the number of filled sites in our case will be the ease of hydration, the tendency to form stiff gels by galactose–galactose complexation through suitable intermediates such as borate or heavy metals, and solubility. Recent ^{13}C and ^{11}B NMR also point to such interactions and suggest the particular involvement of galactose O3 and O4 from each of two different chains as the most likely complexation mode.[22,23] This same interaction is also implicit in the detailed modeling that is now in progress in our laboratory, and the structures of several galactomannans are being determined and refined by analysis of the X-ray intensity data.

Acknowledgment. We gratefully acknowledge partial support of this work by the donors of the Petroleum Research Fund, administered by the American Chemical Society, and Centre National de la Recherche Scientifique.

Registry No. Fenugreek gum, 73613-05-5; lucerne gum, 119618-90-5.

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Study on the Interconversion of Unit Structures in Polyaniline by X-ray Photoelectron Spectroscopy

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Received June 28, 1988; Revised Manuscript Received November 28, 1988

ABSTRACT: XPS N_{1S} and valence spectra of various forms of polyaniline were studied and the compositions of four unit structures in some films were determined. A proposed scheme of interconversion of the unit structures by reduction/oxidation and acid/base treatment is useful to understand the interconversion mechanism of polyaniline. Reduced-base-treated polyaniline (1A, white) is taken as a standard material because it consists solely of imino-1,4-phenylene (IP) units (—NHC_6H_4—). Electrochemical oxidation of 1A in nonaqueous medium converts part (46%) of IP into its radical cation ($IP^{\bullet+}$) (doped 1A). Acid treatment of 1A changes some IP (28%) to its cation (IP^+) (1S). Base treatment of doped 1A converts two $IP^{\bullet+}$–IP parts into two IP parts and one nitrilo-2,5-cyclohexadiene-1,4-diylidenenitrilo-1,4-phenylene (NP) (—N=C_6H_4=N—C_6H_4—) part to give a sample which is practically the same as base-treated polyaniline (2A). Washing of as-polymerized polyaniline changes some IP^+ to IP and some $IP^{\bullet+}$ to NP ($2S(H_2O)$). An earlier conclusion that the radical cation of imino-1,4-phenylene plays an important role in electrical conduction in polyaniline has been supported, and it is pointed out that a highly conducting form of polyaniline exhibits a finite density of state at the Fermi energy. A large change in the valence structure is also observed at 8.7 eV in polyaniline containing $IP^{\bullet+}$. The advantage of an electrochemical redox process in nonaqueous media over that in aqueous media for practical purposes is explained.

Introduction

Polyaniline is an excellent material for electronic devices such as field-effect transistors,[1] electrochromic display,[2] solar battery,[3] etc. Actually it has been successfully applied to practical use as a rechargeable polymer lithium battery.[4,5] In order to improve such applied devices, it is important to elucidate the relation between polyaniline's structure and electrical property.

Polyaniline is known to take various forms via acid/base treatment and oxidation/reduction. In a preceding paper,[6] vibrational spectra of polyaniline were studied in detail and four unit structures in its various forms were identified. However, the composition in each form except the reduced-base-treated form (1A) has remained uncertain.

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0024-9297/89/2222-2644$01.50/0