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Molecular structure of the rhamsan-like exocellular polysaccharide RMDP17 from *Sphingomonas paucimobilis*

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Abstract

X-Ray diffraction analysis of the sodium salt of the polysaccharide RMDP17, a 2-deoxy rhamsan analog, reveals that it adopts a gellan-like, half-staggered, threefold, left handed, double helix of pitch 57.4 Å. The side chain of the branched polymer is hydrogen bonded to the main chain. Sodium ions, linked to the carboxylate groups, promote the association of helices via water molecules. Two helices of opposite polarity occupy a trigonal unit cell of dimensions $a = 17.6$ and $c = 28.7$ Å. The packing arrangement displays a series of hydrogen bonds involving main chain and side chain atoms, as well as some water bridges, between the helices.

Keywords: RMDP17; X-ray diffraction; Three-dimensional structure; Rhamsan analog

1. Introduction

There is always a quest for novel biopolymers that would exhibit excellent rheological properties, be cost effective and above all more efficient than those already available on the market for industrial applications. Ever since the discovery of the gelling agent gellan in 1977, the number of polysaccharides, excreted by bacteria from diverse species, yet having primary structures related to gellan has been steadily growing[1] and they are shown in Fig. 1. Classified under the gellan family, these polysaccharides have the same or similar main chain as gellan has, i.e., composed of an anionic tetrasaccharide repeating-sequence. Some of them also have a side chain in every repeat. For example, the side chain is a monosaccharide in welan but a disaccharide in S-657 and rhamsan. The sequence, linkage type and position of side chain in S-657 are different from those in rhamsan. Such variations in chemical structure manifest in significant changes in the rheological behavior.

In order to understand the molecular basis of the observed physical properties, we started in the middle 1980s, a systematic investigation of the polysaccharides in the gellan family by X-ray fiber diffraction analysis. The parent gellan morphology was first determined to be a threefold, left-handed, half-staggered, parallel double helix.[2] Monovalent or divalent cations were found to be responsible for the association of helices through carboxylate–X–carboxylate interactions, where X is an ion–water–ion bridge in the presence of monovalent ions such as sodium and potassium,[3] or a divalent ion itself as in the case of calcium.[4] The structural results created a vivid picture of the strong junction zones that would be formed in gellan gels.

Based on these details, together with modest diffraction patterns from non-crystalline fibers of the monovalent salt forms of the branched polymers, molecular modeling computations swiftly pointed out that the gellan helix would prevail in welan, S-657 and rhamsan also.[5] The results further suggested that the side chains would fold back on the helix so as to shield the carboxylate groups from the surroundings, more so in S-657 than in welan. This effect is fully consistent with the observed higher thermal stability of viscosity of S-657 than welan in solution.[6] On the other hand, due

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to greater flexibility, the side chains in rhamsan would project out of the helix leading to entanglement of the polymer chains in solution. No shielding of the carboxylate groups was perceived in this case.

Our subsequent X-ray study not only confirmed the predictions relevant for welan but also helped to identify the interactions responsible for holding the helices together at their carboxylate groups via calcium ions and water molecules.[7] Experimental verification has remained elusive for S-657 or rhamsan since good quality fibers from them could not be stretched. However, the rhamsan analog RMDP17 (also named I-886),[8] whose sequence is shown in Fig. 1, has recently become tractable. For the first time, we have obtained reasonable X-ray diffraction patterns from its sodium salt and used them to establish the molecular structure of the deoxy-polysaccharide as well as its preferred packing arrangement. The structural results are presented in this paper.

2. Experimental

Sample preparation.—Native RMDP17 broth was obtained from ARD (Pomacle, France). It was first deacetylated by a brief alkali treatment and then centrifuged in order to remove any bacterial material. The polysaccharide was precipitated by alcohol in the pres-

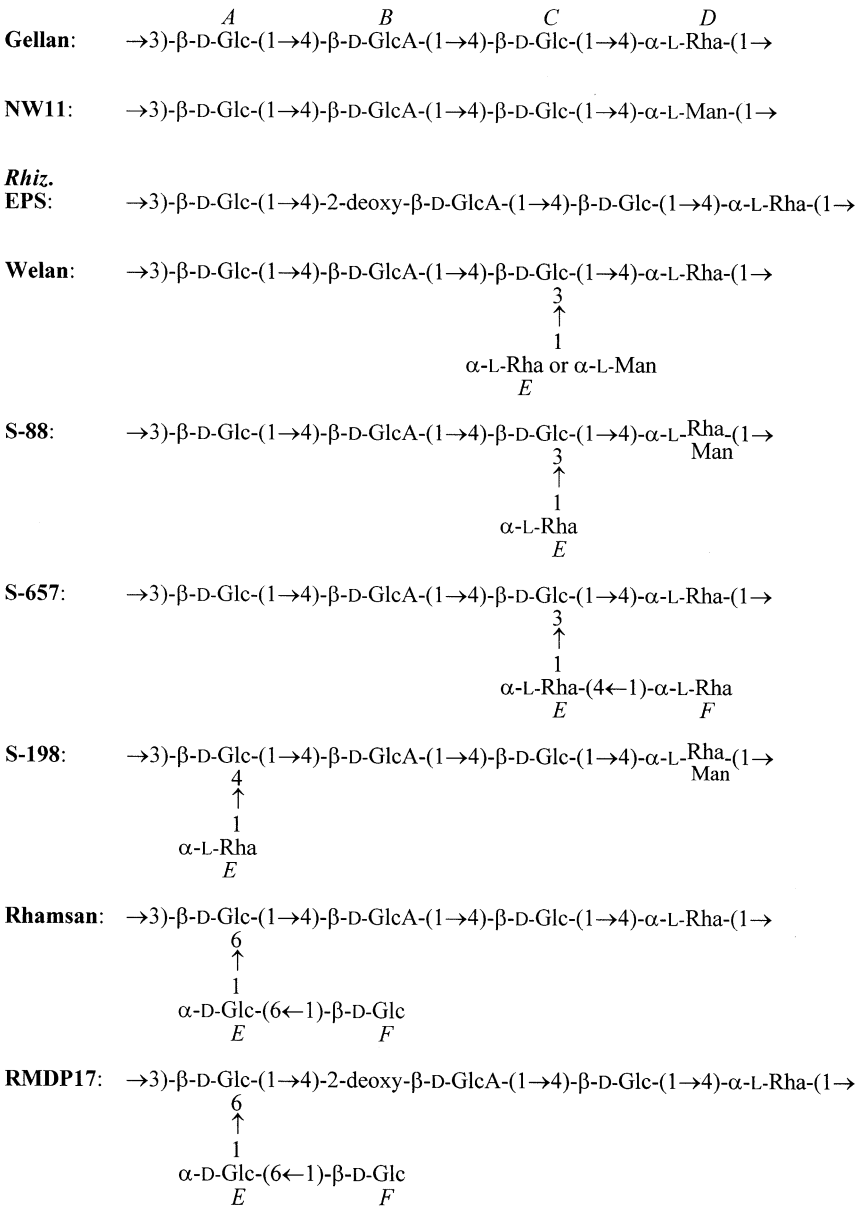

Fig. 1. Comparison of the chemical repeats of nine polymers in the gellan family of polysaccharides showing similarities in the backbone and differences in the side chains.

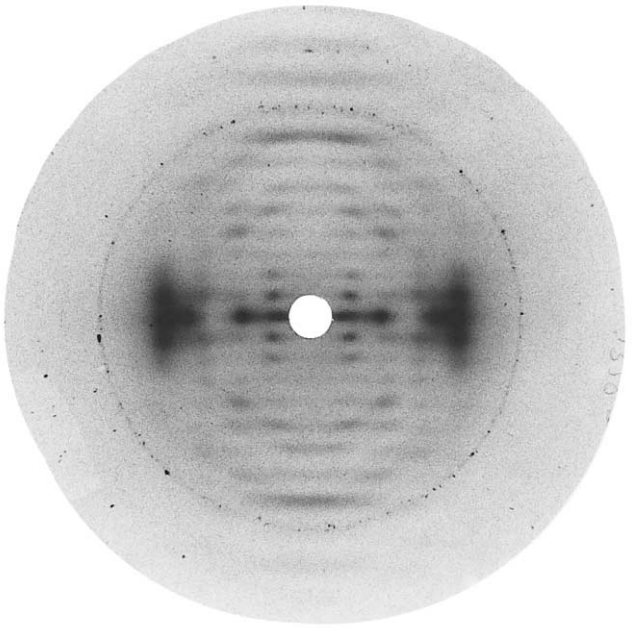

Fig. 2. Diffraction pattern from a well oriented fiber of the sodium salt of RMDP17 kept at 75% relative humidity shows moderate polycrystallinity. The fiber is slightly tilted towards the incident X-ray beam (Cu K_α radiation, wavelength 1.5418 Å). The calcite ring corresponds to *d*-spacing 3.035 Å.

ence of 0.5 M NaCl, washed with EtOH–water mixtures stepwise from 80 to 100% (v/v) and then dried under vacuum.[9] Drops of a concentrated solution of this sample in water were used to prepare oriented and polycrystalline fibers.

X-ray data.—Fiber diffraction patterns were recorded on photographic films in a pinhole camera on a microfocus X-ray generator. The pattern shown in Fig. 2 was chosen for structure analysis. All the Bragg reflections could be indexed on a trigonal unit cell of dimensions $a = 17.6(1)$ and c (fiber axis) $= 28.7(2)$ Å. The meridional reflections on layer lines 3 and 6 imply that RMDP17 has threefold helix symmetry. In terms of the measured fiber density (1.53 g/cc), the unit cell has room for six hexasaccharide repeats as well as a sodium ion and 11 water molecules for each repeat.

Intensities of the reflections were measured with a Joyce Loebl Mark III microdensitometer. They were converted into observed structure amplitudes after applying Lorentz and polarization corrections. Ten out of a total of 41 reflections within the field of view, up to 2.8 Å resolution, were too weak to be seen. The lowest measured intensity was assigned as the threshold value for each of them. A weak reflection was included in the structure refinement if its F_c was greater than F_o, but omitted otherwise.

Additional details on fiber preparation and data collection may be obtained from a previous publication.[10]

3. Structure analysis

Model building and refinement.—The overall intensity distribution (Fig. 2) is strikingly similar to that reported for gellan in the presence of monovalent ions.[2,3,5] Both polymers have trigonal unit cells. With 0.5 Å elongation along *c*, and 1.8 Å increase along *a* and *b*, relative to gellan, the RMDP17 cell is 26% larger so as to accommodate its side chains. The two molecular structures, and also the packing arrangements, are therefore isomorphous. However, unlike for gellan, the lateral organization of RMDP17 is severely restricted to short-range only as inferred from the diffuse nature of the reflections (Fig. 2).

The linked-atom least-squares (LALS) program[11] was used to generate threefold, left-handed, half-staggered, parallel double helix of pitch ($2c$) 57.4 Å. The preferred chair conformations, $^1C_4$ in D, and $^4C_1$ in the remaining pyranosyl rings were incorporated in the model. Out of the 17 major conformation angles relevant for describing the geometry of the hexasaccharide repeat, eight of them—(ϕ_1, ψ_1), (ϕ_2, ψ_2), (ϕ_3, ψ_3), and (ϕ_4, ψ_4)—controlled the helical geometry, χ_B and χ_C the orientations of the carboxylate and hydroxymethyl groups in the main chain, and the remaining seven—(χ_A, ψ_5, ϕ_5), (χ_E, ψ_6, ϕ_6) and χ_F—defined the side chain disposition. The first eight angles could be adapted from gellan, but there was no prior information on the other angles leading to the correct morphology of RMDP17. The bond angles at the six glycosidic bridge oxygen atoms were initially set at the expected value of 116.5°.

Following the $P3_1$ scheme for gellan,[2,3] the packing parameters were (μ_1, 2/3, 1/3, 0) and (μ_2, 1/3, 2/3, w_2) for helices I and II which were aligned antiparallel in the unit cell, μ being the orientation of the helix relative to the a^*-axis, and w the fractional translation of the helix along the c-axis from the ab-plane. The major task was to determine the preferred conformation of the side chain along with the best values of μ_1, μ_2 and w_2.

Once a reasonable model of the crystal structure was obtained, both the molecular and packing parameters were allowed to vary in a joint X-ray and contact refinement by the LALS method.[11] Briefly, the function minimized was of the form

$$\Omega = \sum w_m \Delta F_m^2 + \sum k_i \Delta c_i^2 + \sum e_j \Delta \theta_j^2 + \sum \lambda_h G_h \qquad (1)$$

$$= \quad X \quad + \quad C \quad + \quad E \quad + \quad L \qquad (2)$$

The terms X and C optimized the model against the X-ray data and non-bonded as well as hydrogen bonding interactions, respectively; E retained the varied conformation angles and related parameters near standard or expected domains; and L ensured that constraints on helix symmetry and ring closure were fully satisfied.

Table 1
Cartesian and cylindrical polar atomic coordinates of a repeating unit of the RMDP17 helix, and those of cations and water molecules in an asymmetric unit

Group	Atom	x (Å)	y (Å)	z (Å)	r (Å)	ϕ (°)
Glucose (*A*)	C-1	−1.639	−2.599	15.371	3.073	−122.24
	C-2	−1.131	−2.230	16.758	2.501	−116.88
	C-3	−1.872	−3.020	17.826	3.554	−121.80
	C-4	−3.377	−2.853	17.656	4.421	−139.80
	C-5	−3.784	−3.176	16.223	4.940	−139.99
	C-6	−5.253	−2.919	15.961	6.009	−150.94
	O-1	−1.031	−1.762	14.444	2.042	−120.32
	O-2	0.269	−2.478	16.827	2.493	−83.81
	O-3	−1.479	−2.562	19.121	2.959	−120.00
	O-4	−4.076	−3.731	18.537	5.526	−137.53
	O-5	−3.046	−2.357	15.302	3.852	−142.26
	O-6	−5.990	−4.134	15.835	7.278	−145.39
	H-1	−1.440	−3.664	15.177	3.936	−111.46
	H-2	−1.278	−1.154	16.929	1.722	−137.93
	H-3	−1.606	−4.082	17.715	4.387	−111.48
	H-4	−3.660	−1.817	17.895	4.086	−153.59
	H-5	−3.582	−4.237	16.014	5.548	−130.21
	H-61	−5.672	−2.319	16.781	6.128	−157.77
	H-62	−5.363	−2.326	15.040	5.845	−156.55
Glucuronate (*B*)	C-1	0.196	−2.026	10.486	2.035	−84.47
	C-2	−0.994	−2.897	10.866	3.063	−108.94
	C-3	−1.592	−2.435	12.186	2.910	−123.18
	C-4	−0.514	−2.364	13.259	2.419	−102.27
	C-5	0.665	−1.532	12.765	1.670	−66.54
	C-6	1.820	−1.518	13.744	2.370	−39.82
	O-1	0.788	−2.564	9.350	2.682	−72.92
	O-3	−2.617	−3.345	12.592	4.247	−128.03
	O-4	−1.031	−1.762	14.444	2.042	−120.32
	O-5	1.168	−2.070	11.532	2.376	−60.56
	O-61	1.868	−2.446	14.579	3.078	−52.63
	O-62	2.639	−0.579	13.641	2.702	−12.36
	H-1	−0.139	−0.989	10.340	0.998	−98.02
	H-21	−1.758	−2.839	10.077	3.339	−121.77
	H-22	−0.668	−3.944	10.957	4.000	−99.62
	H-3	−2.047	−1.442	12.055	2.504	−144.84
	H-4	−0.173	−3.385	13.485	3.390	−92.93
	H-5	0.340	−0.493	12.607	0.599	−55.40
Glucose (*C*)	C-1	1.535	−1.343	5.452	2.039	−41.17
	C-2	1.520	−0.236	6.498	1.538	−8.81
	C-3	1.637	−0.822	7.897	1.831	−26.66
	C-4	0.571	−1.888	8.113	1.972	−73.18
	C-5	0.614	−2.912	6.985	2.976	−78.09
	C-6	−0.497	−3.936	7.081	3.967	−97.19
	O-1	1.294	−0.774	4.208	1.508	−30.87
	O-2	2.587	0.670	6.239	2.672	14.52
	O-3	1.483	0.217	8.866	1.499	8.32
	O-4	0.788	−2.564	9.350	2.682	−72.92
	O-5	0.469	−2.257	5.715	2.305	−78.27
	O-6	−1.689	−3.486	6.439	3.874	−115.85
	H-1	2.496	−1.877	5.496	3.123	−36.95
	H-2	0.583	0.335	6.416	0.673	29.84
	H-3	2.634	−1.268	8.026	2.923	−25.71
	H-4	−0.416	−1.401	8.129	1.462	−106.53
	H-5	1.574	−3.448	7.013	3.791	−65.46
	H-61	−0.167	−4.880	6.623	4.883	−91.96
	H-62	−0.708	−4.149	8.140	4.209	−99.68

Table 1 (*Continued*)

Group	Atom	x (Å)	y (Å)	z (Å)	r (Å)	ϕ (°)
Rhamnose (*D*)	C-1	3.832	0.311	1.054	3.845	4.64
	C-2	3.215	1.426	1.889	3.517	23.92
	C-3	2.002	0.912	2.651	2.200	24.50
	C-4	2.424	−0.284	3.492	2.441	−6.68
	C-5	2.997	−1.365	2.564	3.293	−24.49
	C-6	3.499	−2.590	3.305	4.353	−36.51
	O-1	2.959	0.000	0.000	2.959	0.00
	O-2	4.215	1.899	2.782	4.623	24.25
	O-3	1.507	1.990	3.422	2.497	52.86
	O-4	1.294	−0.774	4.208	1.508	−30.87
	O-5	4.124	−0.825	1.853	4.206	−11.31
	H-1	4.757	0.675	0.582	4.804	8.08
	H-2	2.911	2.253	1.230	3.681	37.74
	H-3	1.225	0.603	1.937	1.365	26.22
	H-4	3.199	0.036	4.204	3.199	0.65
	H-5	2.225	−1.678	1.845	2.787	−37.02
	H-61	2.644	−3.144	3.720	4.108	−49.94
	H-62	4.052	−3.238	2.609	5.187	−38.63
	H-63	4.165	−2.277	4.123	4.747	−28.67
Glucose (*E*)	C-1	−7.339	−3.980	15.477	8.348	−151.53
	C-2	−8.194	−4.711	16.503	9.452	−150.10
	C-3	−7.829	−6.187	16.548	9.979	−141.68
	C-4	−7.894	−6.790	15.150	10.412	−139.30
	C-5	−7.066	−5.955	14.179	9.241	−139.88
	C-6	−7.182	−6.437	12.749	9.644	−138.13
	O-1	−5.990	−4.134	15.835	7.278	−145.39
	O-2	−7.999	−4.123	17.784	8.999	−152.73
	O-3	−8.732	−6.879	17.413	11.116	−141.77
	O-4	−7.380	−8.120	15.158	10.973	−132.27
	O-5	−7.511	−4.589	14.196	8.802	−148.57
	O-6	−5.929	−6.382	12.068	8.711	−132.89
	H-1	−7.580	−2.909	15.410	8.119	−159.00
	H-2	−9.258	−4.555	16.272	10.318	−153.80
	H-3	−6.812	−6.302	16.950	9.280	−137.23
	H-4	−8.940	−6.818	14.811	11.243	−142.67
	H-5	−6.006	−5.999	14.469	8.488	−135.03
	H-61	−7.560	−7.470	12.739	10.628	−135.34
	H-62	−7.593	−7.457	12.738	10.643	−135.52
Glucose (*F*)	C-1	−5.888	−5.700	10.841	8.195	−135.93
	C-2	−5.622	−4.227	11.118	7.034	−143.07
	C-3	−5.425	−3.466	9.816	6.438	−147.43
	C-4	−4.351	−4.136	8.970	6.003	−136.45
	C-5	−4.663	−5.620	8.803	7.302	−129.68
	C-6	−3.572	−6.367	8.066	7.300	−119.29
	O-1	−5.929	−6.382	12.068	8.711	−132.89
	O-2	−6.709	−3.685	11.861	7.654	−151.22
	O-3	−5.043	−2.119	10.101	5.470	−157.21
	O-4	−4.290	−3.536	7.678	5.560	−140.50
	O-5	−4.801	−6.244	10.089	7.876	−127.56
	O-6	−2.425	−6.578	8.889	7.010	−110.24
	H-1	−6.830	−5.867	10.297	9.003	−139.34
	H-2	−4.690	−4.124	11.692	6.245	−138.67
	H-3	−6.372	−3.443	9.257	7.243	−151.61
	H-4	−3.373	−4.020	9.459	5.247	−130.00
	H-5	−5.598	−5.737	8.236	8.016	−134.30
	H-61	−3.959	−7.337	7.722	8.337	−118.35
	H-62	−3.280	−5.800	7.170	6.664	−119.49

Table 1 (*Continued*)

Group	Atom	*x* (Å)	*y* (Å)	*z* (Å)	*r* (Å)	ϕ (°)
Sodium	Na-1	−1.227	−2.959	−6.702	3.203	−112.53
	Na-2	−5.066	12.687	−5.551	13.661	111.77
Water	*W*-1	−0.988	−3.642	−9.211	3.773	−105.18
	W-2	−5.114	10.988	−3.605	12.119	114.96
	W-3	−8.393	13.085	−5.959	15.546	122.68
	W-4	−9.077	12.886	−3.290	15.762	125.16
	W-5	5.068	2.098	−1.671	5.485	22.49
	W-6	5.188	−4.601	−7.753	6.934	−41.57
	W-7	5.127	−5.183	−5.112	7.290	−45.31
	W-8	3.592	−4.430	−3.016	5.704	−50.96
	W-9	−4.354	5.925	−1.554	7.353	126.31

The cylindrical coordinates of the next two hexasaccharides in one chain of the double helix are (r, $\phi-120°$, $z+2c/3$) and (r, $\phi+120°$, $z+4c/3$). The second chain is generated from the first by adding c to their z coordinates. For a down-pointing double helix, the coordinates are (r, $-\phi$, $-z$). The packing parameters are:

Molecule	Sense	μ (°)	*u*	*v*	*w*
Helix I	up	96.5	2/3	1/3	0.0
Helix II	down	-3.2	1/3	2/3	-0.0628
Na and Water		0.0	2/3	1/3	0.0

Table 2
Observed and calculated structure amplitudes for sodium RMDP17

h k	*l* = 0	1	2	3	4	5	6	7	8
0 0	M [*2022*]	N [*0*]	N [*0*]	M [*57*]	N [*0*]	N [*0*]	M [*27*]	N [*0*]	N [*0*]
1 0	235 *92*	165 *102*	145 *95*	94 *155*	119 *79*	187 *159*	172 *127*	156 *104*	275 *158*
1 1 2 0	491 *400*	156 *261*	140 *254*	154 *212*	276 *185*	224 *196*	193 *250*	285 *210*	287 *186*
2 1	(96) (*122*)	449 *357*	286 *366*	280 *334*	191 *296*	206 *255*	307 *328*	209 *229*	151 *219*
3 0	550 *532*								
2 2	948 *841*	543 *591*	470 *496*	331 *384*	(142) (*143*)	[149] [*117*]	(157) (184)	(166) (*176*)	[176] [*142*]
3 1									
4 0	(151) (*165*)	[153] [*84*]	[155] [*112*]	[159] [*55*]					

In each reflection box, the observed amplitude is given in the first line and the calculated (italic) in the second. The curved and rectangular brackets refer to below threshold reflections included in, and rejected from, the least-squares refinement, respectively. M and N denote respectively meridional and systematically absent reflections, respectively. The calculated structure amplitudes include a temperature factor with $B = 6.0$ Å^2.

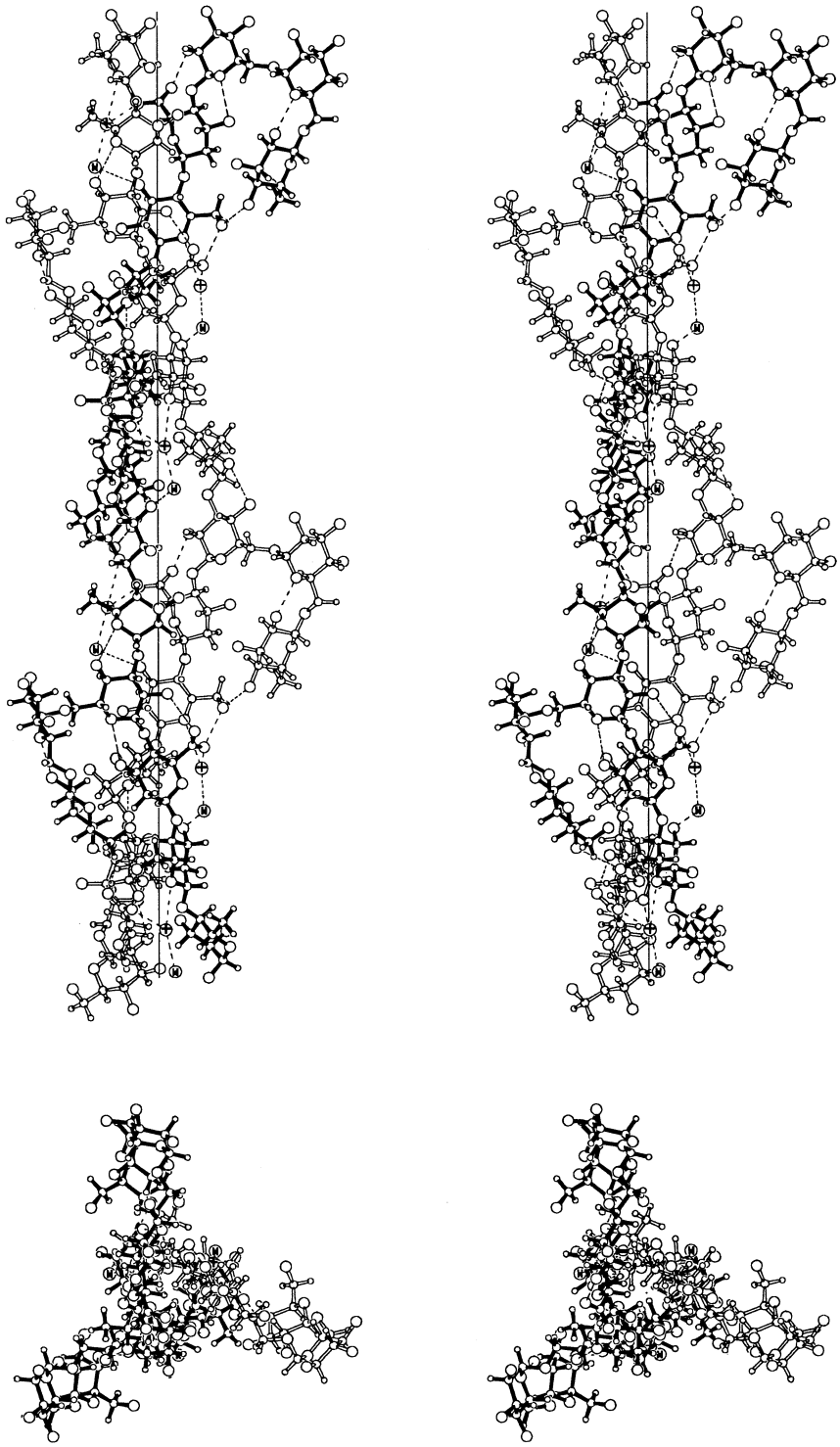

Fig. 3. Stereo drawing of two mutually perpendicular views of the RMDP17 double helix stabilized by interchain O-6H*C*···O-62*B* and five intrachain hydrogen bonds (dashed line) including O-4H*F*···O-6*C* that is formed as the side chain folds towards the main chain. The sodium ion (crossed circle) and water molecule (*W*) enhance structural stability by connecting the carboxylate group to the hydroxyl group in the neighboring unit *C*. All the hydrogen atoms are included only in this drawing. For distinction, the bonds in one chain are filled. The vertical line is the helix axis.

Three-dimensional difference electron density maps were subsequently computed, using normal atomic scattering factors,[12] for a third of the unit cell, namely, the crystallographic asymmetric unit ($z = 0$ to $c/3$), and examined carefully for validating and amending the model, and for locating cations and water molecules surrounding the helices. Addition of guest molecules in augmenting the crystal structure was always subject to

lowering of the crystallographic R-value ($=\Sigma||F_o|-|F_c||/\Sigma|F_o|$). In order to compensate for the intractable water molecules, water smeared atomic scattering factors[13] were used in computing the R-values.

4. Results

Molecular structure.—Out of the nine possible combinations arising from the three staggered domains for χ_A and χ_E each, two were identified for the preferred models for rhamsan[5] based on the gellan structure. We first adapted model 1, which was more compact than the other, as the starting helix for RMDP17. The main chain conformation angles had to change by a few degrees to cope with the slight axial extension mentioned above. There were no short non-bonded contacts within the helix.

Unit cell contents.—In order to pack the helices I and II properly in the unit cell, the approximate values for μ_1, μ_2, and w_2 were first evaluated as follows. Keeping the helix as a rigid body, both intermolecular contacts and R-values in the three-dimensional space were examined at small intervals for μ and w. In the region near (100°, 0°, −0.1), R was low (0.36) and contacts were satisfactory. When the polymer geometry and these packing parameters were allowed to vary simultaneously in a refinement, the conformation angles for the terminal unit in the side chain changed up to 45° so as to re-optimize the helix–helix contacts and the R-value came down to 0.30. During a similar calculation, model 2[5] was rejected because its larger molecular diameter introduced severe steric compression everywhere. The same was true for the remaining seven possibilities also. Therefore, the packing mode of model 1 only was scrutinized further.

A difference Fourier map confirmed that the molecular geometry selected was correct. In particular, the side chain atoms needed no major movement. Two positive peaks, located near the carboxylate groups of the two helices, were named sodium ions, and five others in the interstices were selected to be water molecules. Upon including them in the crystal structure one at a time, and refining their positions, the R-value decreased to 0.26. A difference map for the augmented structure helped to identify four more water molecules. A final round of refinement of the crystal structure, in which the bond angles at the bridge oxygen atoms were also varied, improved the X-ray fit further ($R=0.23$). This accounts for 31 observed and five out of 10 unobserved reflections. With very small changes in the varied parameters, the refinement was deemed complete and concluded. All the non-bonded contacts in the structure were found to be above the acceptable limits. The final atomic coordinates are given in Table 1. The observed and calculated structure amplitudes are listed in Table 2.

Morphological features.—The RMDP17 double helix is shown in Fig. 3 both normal to and along its helix axis. It can be seen in the top panel that the polysaccharide chain coils around this axis with a left-handed twist. The side chain draped on the backbone folds down towards the reducing end. It stretches out considerably in the axial projection as seen in the bottom panel so that the triangular shape is much larger than in gellan. The major conformation angles are given in Table 3 along with those previously predicted for rhamsan.[5] Comparison shows that the experimental ϕ and ψ values are within 20° from theory implying that RMDP17 is able to retain the gellan geometry remarkably well. Further, the hydroxymethyl group orientations in A and C have changed by −27 and 29°, respectively, and that of the carboxylate group by 11° with respect to gellan. However, the side-chain conformation angles differ up to 62° from the predicted values in the rhamsan model. Such large differences are essentially due to crystal packing forces.

Although the helix diameter for the backbone is 14.6 Å up to atom O-6A, E attached to A sticks out farther and increases this diameter to almost 22.2 Å. Terminal F folds down to interact with unit C such that some of its atoms approach the carboxylate group in the second chain. This has repercussions for the ion binding ability of RMDP17 (explained later). The main chain flexibility is tempered by three hydrogen bonds per repeat,

Table 3
Major conformation angles (and e.s.d.) in degrees in (a) RMDP17 (this study) and (b) rhamsan model[5]

Parameter	a	b	Location
ϕ_1(O-5–C-1–O-3–C-3)	−104(3)	−124	D-(1→3)-A
ψ_1(C-1–O-3–C-3–C-4)	82(3)	88	D-(1→3)-A
χ_A(C-4–C-5–C-6–O-6)	−108(9)	−81	hydroxymethyl
ϕ_2(O-5–C-1–O-4–C-4)	−107(3)	−101	A-(1→4)-B
ψ_2(C-1–O-4–C-4–C-5)	−151(3)	−136	A-(1→4)-B
χ_B(C-4–C-5–C-6–O-61)	21(8)	10	carboxylate
ϕ_3(O-5–C-1–O-4–C-4)	−137(4)	−154	B-(1→4)-C
ψ_3(C-1–O-4–C-4–C-5)	−150(3)	−144	B-(1→4)-C
χ_C(C-4–C-5–C-6–O-6)	87(7)	58	hydroxymethyl
ϕ_4(O-5–C-1–O-4–C-4)	−155(4)	−150	C-(1→4)-D
ψ_4(C-1–O-4−C-4–C-5)	92(3)	86	C-(1→4)-D
χ_D(C-4–C-5–C-6–H-61)	70(10)	77	methyl
ϕ_5(O-5–C-1–O-6–C-6)	116(9)	102	E-(1→6)-A
ψ_5(C-1–O-6–C-6–C-5)	−175(3)	−173	E-(1→6)-A
χ_E(C-4–C-5–C-6–O-6)	−140(8)	179	hydroxymethyl
ϕ_6(O-5–C-1–O-6–C-6)	−155(6)	−100	F-(1→6)-E
ψ_6(C-1–O-6–C-6–C-5)	−127(5)	171	F-(1→6)-E
χ_F(C-4–C-5–C-6–O-6)	75(7)	60	hydroxymethyl

Table 4
Attractive interactions among RMDP17 helices, sodium ions and water molecules

Type	No.	Atom *X*	Atom *Y*	Precursor *P*	*X*···*Y* (Å)	*P*–*X*···*Y* (°)
Intrachain	***1***	O-3*B*	O-5*A*	C-3*B*	2.92	99
	2	O-2*A*	O-61*B*	C-2*A*	2.76	122
	3	O-4*A*	O-5*D*	C-4*A*	2.82	101
	4	O-2*F*	O-5*E*	C-2*F*	2.63	124
	5	O-4*F*	O-6*C*	C-4*F*	2.88	116
Interchain	***6***	O-6*C*	O-62*B*	C-6*C*	2.79	126
Sodium coordination	***7***	O-62*B*(I)	Na-1	C-6*B*	2.54	136
		O-5*C*(I)	Na-1	C-5*C*	3.04	135
		W-1	Na-1		2.61	
	8	O-62*B*(II)	Na-2	C-6*B*	2.51	158
		O-5*C*(II)	Na-2	C-5*C*	2.64	114
		W-2	Na-2		2.58	
Interhelix	***9***	O-2*D*(I)	O-61*B*(II)	C-2*D*	2.54	139
	10	O-2*F*(II)	O-61*B*(I)	C-2*F*	2.40	145
	11	O-2*C*(II)	O-5*F*(I)	C-2*C*	2.85	129
	12	O-3*E*(I)	O-4*A*(II)	C-3*E*	2.75	75
		O-3*E*(I)	O-2*E*(II)	C-3*E*	2.82	160
	13	O-4*E*(I)	O-4*A*(II)	C-4*E*	2.96	96
		O-4*E*(I)	O-5*D*(II)	C-4*E*	2.54	110
	14	O-6*F*(I)	O-2*C*(II)	C-6*F*	2.58	118
		O-6*F*(I)	O-3*C*(II)	C-6F	2.45	97
Water bridges	***15***	O-62*B*(II)	*W*-2	C-6B	2.83	109
		O-3*C*(II)	*W*-2	C-3*C*	2.65	145
	16	O-2*A*(II)	*W*-9	C-2*A*	2.70	131
		O-3*F*(II)	*W*-9	C-3*F*	2.74	131
	17	O-4*A*(I)	*W*-6	C-4*A*	2.78	157
		O-5*D*(I)	*W*-6	C-5*D*	2.81	121
		W-6	*W*-7		2.70	
		W-7	*W*-8		2.70	
		O-2*E*(II,0-1)	*W*-8	C-2*E*	2.69	136
		O-3*E*(I,0-1)	*W*-8	C-3*E*	2.83	115
		O-6*F*(II,10)	*W*-8	C-6*F*	2.71	155
	18	O-3*C*(I)	*W*-1	C-3*C*	2.79	139
		W-1	*W*-5		2.72	
		W-5	*W*-4		2.79	
		W-4	*W*-3		2.76	
		O-62*B*(II)	*W*-3	C-6B	2.73	122
		O-6*C*(II)	*W*-3	C-6*C*	3.01	142

labeled ***1***–***3*** in Table 4, where the dominant attractive interactions (less than 3.1 Å) between the functional groups are tabulated. The first two among them are across *A*-(1→4)-*B* as in gellan[3] and the third is across *D*-(1→3)-*A*. Glucosyl unit *C* is involved in interchain hydrogen bond with the carboxylate group (***6***) and strengthens the double helix. In addition, the side chain promotes two intrachain hydrogen bonds; one of them (***4***) is within the disaccharide itself and the other (***5***) is with the hydroxymethyl group of *C*. Together, these six hydrogen bonds are vital for the structural stability of the RMDP17 molecule.

Sodium ions and water molecules.—One sodium ion and 4.5 out of 11 water molecules have been identified per hexasaccharide repeat. The rest of them are presumably amorphous. For charge balance, each carboxylate group is linked to a sodium ion that is already connected to a water molecule. As seen in Fig. 3, this ion is located below the carboxylate group (that is, the reducing end) as in potassium native gellan.[14] The association of Na-1 and *W*-1 with helix I (***7***) and, likewise, Na-2 and *W*-2 with II (***8***), suggests that the sodium–water pair is an integral part of the helix; atom O-5*C* is the third ligand for the ion in each case. With

only three ligands, the coordination of sodium is reminiscent of that reported for native gellan where the potassium ion also has only three ligands.[14]

Helix–helix interactions.—The two, one up and one down, RMDP17 helices of identical geometry are separated by 10.1 Å in the trigonal unit cell as shown in Fig. 4. There is considerable nestling between the helices, and the side chains in particular encroach on neighboring unit cells and initiate several significant tertiary interactions; some are directly between the functional groups of the helices and others are mediated by water molecules that contribute to the stability of the crystal structure. There are six hydrogen bonds (***9***–***14***) per repeat between the helices as illustrated in Fig. 5. Two of them, ***9*** and ***10***, involve the carboxylate oxygen atoms; ***11*** incorporates a ring oxygen atom; and the rest (***12***, ***13*** and ***14***) appear to be bifurcated. Except for the first, side chains are responsible for these hydrogen bonds.

Among water molecules, *W*-2 (***15***) and *W*-9 (***16***) are able to communicate with both chains of helix II. The former makes hydrogen bonds with atoms in the main chains only and the latter chooses both main chain and side chain atoms as partners. In contrast to these two short interchain, intrahelix water bridges, two others are gluing the helices together in this structure. In the first instance (***17***), three contiguously hydrogen bonded water molecules, *W*-6–*W*-7–*W*-8, form a long spacer between two oxygen atoms in the main chain of helix I and three oxygen atoms in the side chain, one from each of three neighboring helices. In the second (***18***), the spacer consists of four similarly strung water molecules, *W*-1–*W*-5–*W*-4–*W*-3. Atom O-3*C* of I is near one end of the spacer and a carboxylate, as well as a hydroxymethyl oxygen atom of II is near the other end. Since *W*-1 is common to both ***7*** and ***18***, this spacer can further be extended to include Na-1 so that the carboxylate oxygen atoms of I and II are linked by the longest five atom spacer, namely, Na-1–*W*-1–*W*-5–*W*-4–*W*-3. All of these bridges, readily seen in Fig. 5, are greatly responsible for holding the RMDP17 helices together in an organized state.

5. Discussion

Based on computer modeling, it was predicted a decade ago that rhamsan would form a gellan-like double helix and that its flexible side chain could take up any of several extended or folded conformations.[5] This is yet to be verified because its three-dimensional structure has not yet been determined. However, we have now demonstrated from detailed X-ray structure analysis that the prediction is indeed valid for the rhamsan analog RMDP17 and that the preferred state corresponds to a folded side chain conformation that facilitates the hydrogen bond O-4*F*···O-6*C* (***5***) between side chain and main chain that enhances helix stability. We have also shown that RMDP17 adopts a gellan-like packing arrangement.

Our long-term X-ray investigations on industrially useful polysaccharides has led to the important obser-

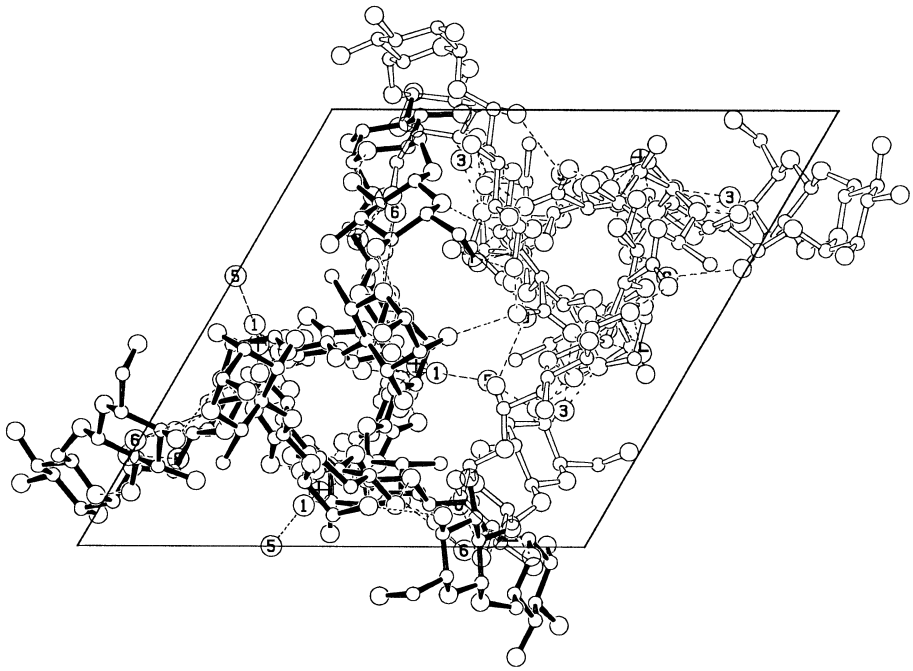

Fig. 4. A projection of the trigonal unit cell down the *c*-axis. Helix I (filled bonds) and II, are respectively pointing up and down and connected by direct hydrogen bonds as well as some water molecules (numbered circles).

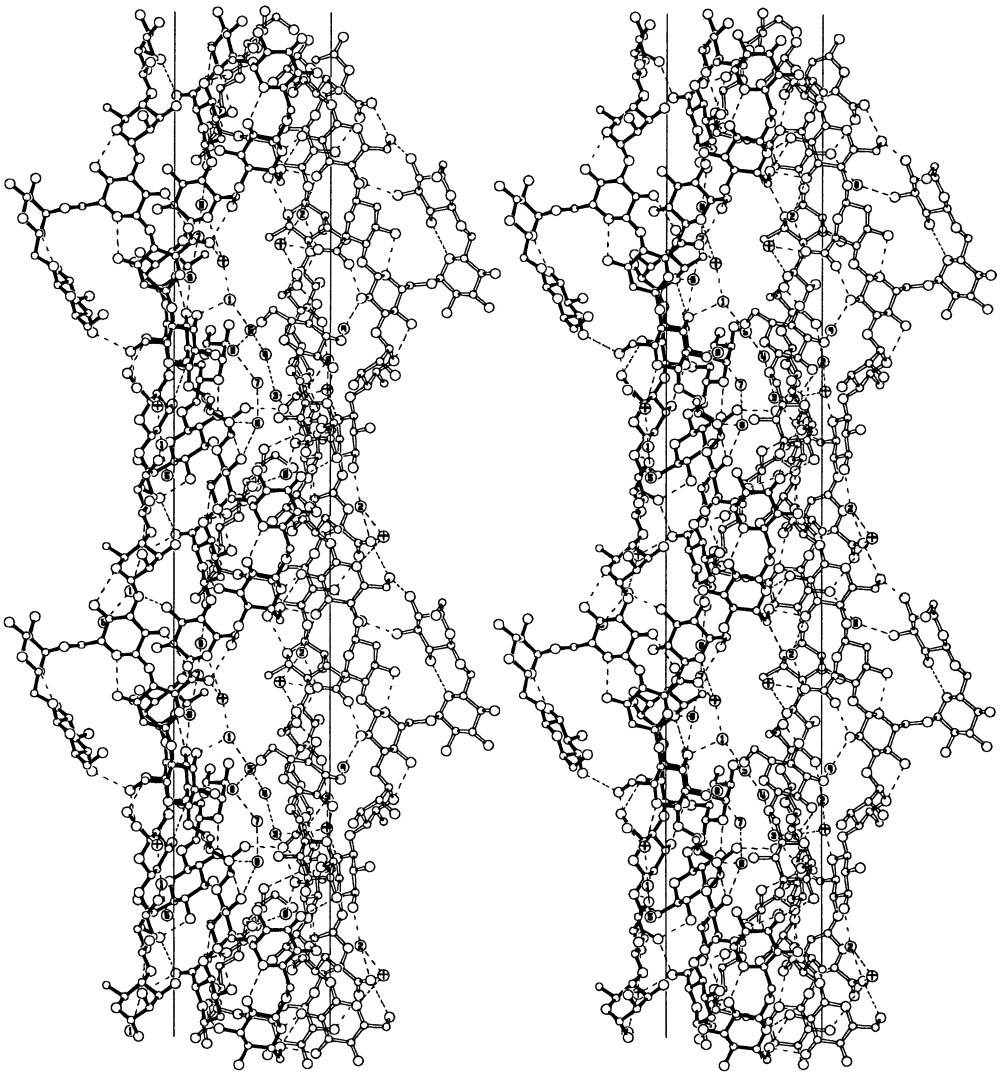

Fig. 5. Stereo drawing showing the interactions between RMDP17 helices separated by 10.1 Å in the unit cell viewed normal to the helix axis. Hydrogen bonds and coordination bonds for sodium ions are in dashed lines. Water molecules (numbered circles) form long bridges between the helices.

vation that, with or without side chains, the morphology of gellan is conserved in five different structures so far in the family. Two are of the parent polymer: the lithium salt of gellan,[2] and the potassium salt of gellan.[3] One has substitutents: the potassium salt of high acyl and glyceryl gellan (also known as native gellan).[14] And the last two have side chains: the calcium salt of welan[7] and the sodium salt of RMDP17 (this study). Analysis of these structures reveals certain common features. First of all, the conformation angles in the main chain around the glycosidic bonds are all very similar and this ensures that the backbone of the helix remains robust. Rotations around C-5–C-6 bonds defining hydroxymethyl and carboxylate orientations are confined to specific domains only. Second, without and with acetyl groups (gellan and native gellan, respectively) or with side chains (welan and RMDP17), atom O-6*A* prefers only the *gauche minus* region. Third, as expected for carboxylate groups, the rotation angle for atom O-61*B* is close to 0° (values ranging from -8 to 37°). Finally, atom O-6*C* goes into the *gauche plus* domain that facilitates a crucial interchain hydrogen bond with atom O-62*B*, ensuring structural rigidity for the double helix.

In contrast to this conservation of molecular conformation, affinity for ion is fairly sensitive to both substituents and side chains. For instance, when a monovalent ion (potassium) binds gellan, it is surrounded by six atoms in an octahedral coordination, namely, O-2*A*, O-61*B* and O-62*B* in one chain, O-2*B* and O-6*C* in the second chain and a water molecule.[3] In native gellan, since this preferred site for the ion, which is above the carboxylate group, is preempted by the terminal oxygen atom in the glyceryl group, the ion drifts to a site below the carboxylate group.[14] A similar feature is now seen in RMDP17 also where the side

chain unit *F* appears to be responsible for the drift (Fig. 3). In both cases, with only three ligands for the ion, the polymer-ion interactions must be weaker than in gellan.

In anionic polysaccharides, carboxylate–carboxylate interactions mediated by ions and water molecules are a major factor for the association of helices and their details may be used to correlate with the observed rheological properties. When two RMDP17 double helices are in register as in Fig. 5, the adjacent duplexes are antiparallel and 10.1 Å apart, 1 Å larger than in gellan. In each *c*-repeat, a sodium ion in one helix is about 4.6 Å from another in the second helix. With a putative water molecule (*W*) in the middle, the carboxylate–Na–*W*–Na–carboxylate interactions would stabilize the packing arrangement in a manner analogous to that reported for potassium gellan.[3] However, in view of the weaker binding between the carboxylate group and the sodium ion, as well as the larger inter helical separation, the aggregation of RMDP17 helices might be restricted to short range only. This translates to poorer gelation behavior compared to gellan on the one hand, and smaller crystallite size resulting in larger spots in the X-ray diffraction pattern as observed (Fig. 2) on the other.

The only difference between rhamsan and RMDP17, and similarly gellan and the newly discovered *Rhizobium* EPS[15] (Fig. 1), is in the chemistry of uronic acid: glucuronic acid in the former and '2-deoxyglucuronic' acid in the latter, in each pair. In this regard, our computations suggest that the RMDP17 morphology, after some small conformational changes, can accommodate the 2-hydroxyl group comfortably so that the resulting rhamsan helix will have the gellan-like intrachain O-2*B*···O-6*C* hydrogen bond restored. While this will bestow extra stability to the helix, it is not clear whether rhamsan could still sustain the interchain O-6*C*···O-62*B* link that stabilizes the RMDP17 double helix, and the side chain to main chain O-4*F*···O-6*C* hydrogen bond that leads to its compact packing arrangement. If that were the case, rhamsan molecule might be more robust. However, both optical rotation and differential scanning calorimetry measurements ascribe higher thermal stability to RMDP17.[9] A knowledge of the three-dimensional structure of rhamsan would therefore be helpful for understanding the reported physicochemical behavior of these polymers at the molecular level.

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References

1. Chandrasekaran, R.; Radha, A. *Trends Food Sci. Technol.* **1995**, *6*, 143–148.
2. Chandrasekaran, R.; Millane, R. P.; Arnott, S.; Atkins, E. D. T. *Carbohydr. Res.* **1988**, *175*, 1–15.
3. Chandrasekaran, R.; Puigjaner, L. C.; Joyce, K.; Arnott, S. *Carbohydr. Res.* **1988**, *181*, 23–40.
4. Chandrasekaran, R.; Thailambal, V. G. *Carbohydr. Polym.* **1990**, *12*, 431–442.
5. Lee, E. J.; Chandrasekaran, R. *Carbohydr. Res.* **1991**, *214*, 11–24.
6. Moorhouse, R. In *Industrial Polysaccharides*: *Genetic Engineering*, *Structure/Property Relations and Applications*; Yalpani, M., Ed.; Elsevier: Amsterdam, 1987; pp. 187–1205.
7. Chandrasekaran, R.; Radha, A.; Lee, E. J. *Carbohydr. Res.* **1994**, *252*, 183–207.
8. Falk, C.; Jansson, P.-E.; Rinaudo, M.; Heyraud, A.; Widmalm, G.; Hebbar, P. *Carbohydr. Res.* **1996**, *285*, 69–79.
9. Villain-Simonnet, A.; Milas, M.; Rinaudo, M. *Int. J. Biol. Macromol.* **1999**, *26*, 55–62.
10. Janaswamy, S.; Chandrasekaran, R. *Carbohydr. Res.* **2001**, *335*, 181–194.
11. Smith, P. J. C.; Arnott, S. *Acta Crystallogr., Sect. A* **1978**, *34*, 3–11.
12. International Tables for X-ray Crystallography; Kluwer Academic: Dordrecht, 1974; Vol. IV, pp. 99–101.
13. Chandrasekaran, R.; Radha, A. *J. Biomol. Str. Dynam.* **1992**, *10*, 153–168.
14. Chandrasekaran, R.; Radha, A.; Thailambal, V. G. *Carbohydr. Res.* **1992**, *224*, 1–17.
15. Guentas, L.; Pheulpin, P.; Michaud, P.; Heyraud, A.; Gey, C.; Courtois, B.; Courtois, J. *Carbohydr. Res.* **2001**, *332*, 167–173.