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Three-dimensional structure of the sodium salt of iota-carrageenan

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Abstract

The three-dimensional structure of the sodium salt of ι-carrageenan has been determined by using X-ray diffraction data collected from its polycrystalline and oriented fibers. The molecule forms a half-staggered, parallel, threefold, right-handed double helix that is stabilized by interchain hydrogen bonds from 2- and 6-hydroxyl groups in the galactosyl units. Three helices are organized in a trigonal unit cell, of dimensions $a = 24.02$ and $c = 12.93$ Å, with a lateral separation of 13.9 Å for each pair. Both 2- and 4-sulfate groups are essential in helix–helix interactions that are mediated only by sodium ions and water molecules.

Keywords: Iota-carrageenan; X-ray diffraction; Three-dimensional structure; Junction zone

1. Introduction

Carrageenans constitute a group of sulfated galactans originating from red marine algae *Rhodophycea*, and are well known to the food industry for their cation dependent thickening and gelling properties. The disaccharide →3)-β-D-Gal*p*-(1→4)-α-D-Gal*p*-(1→ is the basic repeat in these linear polysaccharides. By enzymatic reaction or chemical treatment with alkali during processing of carrageenans, the α-galactopyranosyl residue forms a 3,6-anhydro ring. Depending on the source, location and degree of sulfate substitution, seven different carrageenans have so far been identified. However, only three of them—iota, kappa, and lambda—are prominent as they are more versatile than the rest. The former two form excellent thermally reversible gels, while the latter is a good viscosity builder.[1]

The idealized chemical repeat of ι-carrageenan is →3)-β-D-Gal-4-SO_3^--(1→4)-3,6-anhydro-α-D-Gal-2-SO_3^--(1→ and may be written as →3)-*A*-(1→4)-*B*-(1→ for simplicity. The same is also true for κ-carrageenan except that it does not have any sulfate substitution on the α-galactosyl unit. The major impact of this chemical difference is on the gel nature. For example, clear and elastic gels of ι-carrageenan are resistant to syneresis and hysteresis. In contrast, those of κ-carrageenan are cloudy and hazy and prone to syneresis. It was proposed, nearly three decades ago, that these polysaccharides would be double helical in solution[2] and in the gel state.[3] An X-ray fiber diffraction analysis subsequently showed that, in the presence of divalent ions, ι-carrageenan forms a threefold, right-handed, half-staggered, parallel double helix of pitch

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26.6 Å.[4] Although the general molecular structure could be visualized, the details on the packing arrangement of helices in the unit cell have remained obscure owing to statistical disorder. It has been reported that κ-carrageenan is also double helical.[5] Unfortunately, the details are sketchy and no information on the lateral organization of helices is available due to the non-crystalline nature of the sample.

In order to determine and compare the structural details of the junction zones in different carrageenan gels as influenced by cations, we have embarked on a systematic X-ray investigation of the monovalent and divalent forms of both ι- and κ-carrageenans. In this paper, we report the results on the three-dimensional structure of the sodium salt of ι-carrageenan.

2. Experimental

Preparation of fibers.—A generous amount of ι-carrageenan in the sodium form was provided by FMC Corporation, USA. A concentrated polysaccharide solution was prepared in a vial containing about 15 mg of the polymer, and 6 mg of NaCl, in 1 mL of distilled water ensuring that at least three cations were present in the medium for every disaccharide repeat. We used an excess of ions to promote crystallinity, as is our experience with nucleic acid[6] and other anionic polysaccharide[7] fibers. The vial was kept in a hot water bath at 50 °C, with magnetic stirring for a few hours, and then cooled. A few drops of the solution were placed between the glass rods in a fiber puller[8] and allowed to dry slowly at reduced humidity. Upon reaching a semi-solid state, the material was stretched little by little periodically, at about 75% relative humidity until it reached two-to-three times the original length giving an oriented fiber. Some of the fibers prepared by this process were chosen for density measurement by the flotation technique using a mixture of CCl_4 and $CHBr_3$.

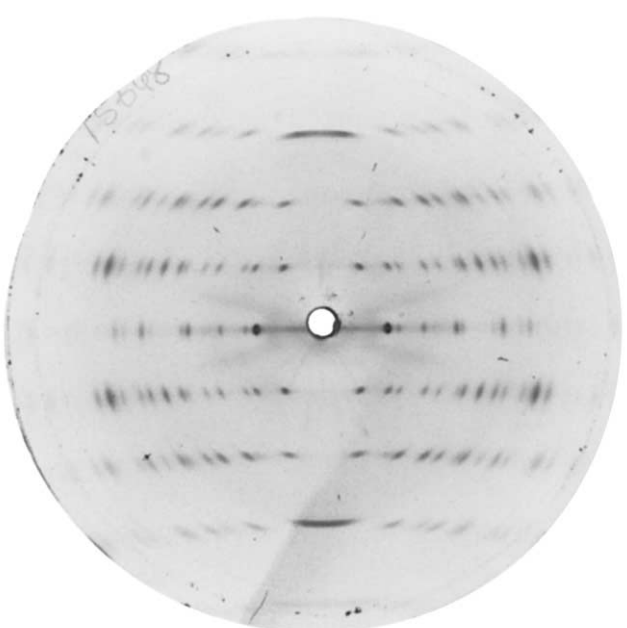

Fig. 1. Diffraction pattern from a well oriented and polycrystalline fiber of sodium ι-carrageenan that is almost perpendicular to the incident X-ray beam.

X-ray pattern and unit-cell dimensions.—Diffraction patterns were recorded on photographic film in pinhole cameras using nickel-filtered CuKα radiation of wavelength 1.5418 Å from microfocus generators operated at about 40 kV and 6 mA. During exposure to X-rays, which lasted up to 2 days, the fiber chamber was continuously flushed with a gentle stream of He gas previously bubbled through a saturated salt solution. This reduced air scatter and maintained the specimen at a desired hydrated state. Some selected samples were calibrated with calcite powder.

The carrageenan fibers were always uniaxially oriented and polycrystalline. They diffracted nicely, as judged by the sharp Bragg reflections extending out to 2.9 Å resolution on layer lines 0 through 4. The pattern shown in Fig. 1 was selected for structure analysis. All its spots could be unambiguously indexed with a trigonal unit cell of dimensions $a = b =$ 24.02(2) and c (fiber axis) = 12.93(1) Å. The meridional reflection on the third layer line indicates that the polysaccharide chain has threefold helical symmetry. According to the measured fiber density (1.67 g/cc), the unit cell can accommodate nine disaccharides, as well as 18 sodium ions and 108 water molecules.

Intensity data.—A Joyce Loebl Mark III microdensitometer was used to record the radial trace of the optical density of each reflection in the pattern. A base line was drawn properly in the trace to remove the back-

ground. The trace area above this line gave the intensity of the reflection and it was averaged over all the quadrants in the pattern. The Lorentz and polarization corrections were applied prior to getting the observed structure amplitudes F_o. Out of a total of 68 possible reflections within the field of view, 22 were too weak to be seen. The lowest measured intensity was assigned as the threshold value for each of them. These weak reflections were included in structure refinement only when their F_c was greater than the threshold F_o, and omitted otherwise.

3. Structure analysis

Molecular modeling and refinement.—The uniformly sharp and small-sized Bragg reflections in the diffraction pattern (Fig. 1) attest to the long-range lateral ordering of carrageenan helices in oriented fibers. The overall intensity distribution, including the meridional reflection on the third layer line, is reminiscent of that from the divalent form.[4] The inference is that the previously reported geometry of the ι-carrageenan helix prevails in this case, but the mode of crystal packing does not.

Following the earlier analysis,[4] we adopted the favored $^4C_1$ and $^1C_4$ chair conformations for units *A* and *B*, respectively. The linked-atom least-squares (LALS) method[9] was employed to construct a threefold, right-handed, half-staggered double helix of pitch $2c = 25.86$ Å. Of the nine major conformation angles varied (see Table 3), the helical parameters were controlled by four of them: (ϕ_1, ψ_1) and (ϕ_2, ψ_2) across the ($1\rightarrow4$) and ($1\rightarrow3$) linkages, respectively. Orientations of the sulfate groups were changed by varying (θ_1, θ_2) in *A* and (θ_3, θ_4) in *B*, and that of the hydroxymethyl group by χ; together, these five angles defined the peripheral shape of the helix. The bond angles τ_1 and τ_2 at the glycosidic bridge oxygen atoms O-4 and O-3, respectively, were held at 116.5°.

The nine disaccharides in the unit cell correspond to half a pitch length of three double helices. Analogous to the packing scheme reported for calcium welan,[10] a member of the gellan family of polysaccharides, these helices (I, II and III) were positioned at fractional coordinates (u, v) equal to (2/3, 1/3), (1/3, 2/3) and (0, 0) and set sequentially up, down, and up-pointing so that II was aligned antiparallel to both I and III. Therefore, additional parameters to be determined were (μ_1, w_1), (μ_2, w_2) and (μ_3, w_3), respectively, for I, II, and III. While μ defines helix orientation relative to the a*-axis, w (expressed in fraction of c) is its translation relative to the ab-plane. By setting $w_1 = 0$ (arbitrarily), the resulting packing arrangement conformed to space group $P3_2$.

In the final stages of structure analysis, the endocyclic conformation and bond angles in both galactopyranosyl units were allowed to vary. The details of the joint X-ray and contact refinement by the LALS method are the same as reported in the literature.[11] Sections of three-dimensional difference electron-density maps for the crystallographic asymmetric unit ($z = 0$ to $c/3$) were computed and studied in detail for two purposes. The first was to verify and amend, if necessary, the helix geometry, and also μ and w of the three helices. The second was to identify potential guest molecules from the several new peaks of positive density in the interstices. Although the choice of sodium (Na^+) instead of water (O) in the vicinity of a sulfate group was sometimes circumstantial, it was always confirmed by comparing the two in terms of the relative drop in the crystallographic R-value ($= \Sigma||F_o| - |F_c||/\Sigma|F_o|$). Unless otherwise specified, all R-values quoted are from structure factor calculations using water smeared atomic scattering factors.[12]

4. Results

Molecular structure and unit-cell contents.—The starting model for the carrageenan helix was based on the published structure.[4] While generating a revised threefold, right-handed, half-staggered double helix whose pitch is now reduced from the older 26.56 to 25.86 Å, the main chain conformation angles changed by a few degrees. The hydroxymethyl and the sulfate group conformations also changed nominally. The interchain hydrogen bond proposed before[4] between atoms O-2 and O-6 of the

galactosyl units remained intact. There were no short contacts among non-bonded atoms within the helix.

Guest molecules in the crystal structure.—Keeping this helix as a rigid body with atom O-1 of *B* defining the *x*-axis ($\mu = 0$, $w = 0$) of the helix, the next task was to determine the most probable values of the five packing parameters for the three helices, positioned in the unit cell as mentioned in the previous section. *R*-values, as well as contacts between helices in the five-dimensional space, were examined at small intervals for μ and w. Several local minima having low *R*-values (~ 0.32) were found but none could be preferred from the contacts data. As the helix diameter is almost the same as the 'helix–helix' separation (13.9 Å), the helices do not interdigitate. When some of these minima were examined by using difference Fourier maps, a few guest molecules connecting the helices could be identified. In every case, during refinement of the augmented crystal structure, the positions of many guest atoms were unstable and there was hardly any improvement in the X-ray fit. This approach was therefore abandoned.

What turned out later to be a successful alternative was to adjust the μ and w values for each helix carefully until the sulfate groups of neighboring helices came close enough to interact via one or more guest molecules. After scrutinizing a few options, we focused on one of them for further analysis. The *R*-value for this initial crystal structure was 0.33.

Examination of a difference Fourier map for this structure revealed that the packing arrangement chosen was very reasonable. In addition to the six galactosyl sulfate units (namely, two from each helix), the crystallographic asymmetric unit could accommodate six sodium ions and 36 water molecules. The map contained a number of positive peaks between the three helices. Twenty peaks were selected and initially named water, the criterion being that a guest must be less than 3.2 Å from two or more oxygen atoms for structural reinforcement. After augmenting the crystal structure with these water molecules one at a time, their atomic positions were refined, and found to be very stable. The *R*-value dropped significantly to 0.219. With improved phasing, a second difference map at this stage showed clearly five additional guest peaks. Also named water, their inclusion helped to enhance the X-ray fit ($R = 0.206$). When six of these guests were renamed as sodium ions (one per sulfate group) and then refinement was continued, there was a further reduction in *R* ($= 0.195$). The amended crystal structure was then refined with flexible sugar rings. The convergence was smooth as inferred from the extremely small shifts in parameters; there were no steric anomalies and at the conclusion of refinement, the *R*-value stabilized at 0.187. This X-ray fit includes 46 observed and 15 out of 21 unobserved reflections. For the observed data only, $R = 0.172$. The atomic coordinates of the final model are listed in Table 1. The observed and calculated structure amplitudes are compared in Table 2.

Morphological features.—Two distinct views of the carrageenan double helix are shown in Fig. 2. For a disaccharide repeat *A*–*B*, the turn angle is 120° and the axial rise is 8.62 Å. Using the ϕ and *z* coordinates of the bridge oxygen atoms as reference (Table 1), when dissected into individual components, these parameters are: 54° and 4.1 Å for *A*; 66° and 4.5 Å for *B*. Both pairs are surprisingly close to the mean values 60° and 4.3 Å. This means that despite having two distinct monomers connected by quite distinct $(1\rightarrow4)$ and $(1\rightarrow3)$ linkages, the bridge oxygen atoms in the polymer chain may be described by a slim helix of radius about 2.7 Å. Apparently, this is a consequence of the alternating $^4C_1$, $^1C_4$ sugar-ring conformations. The prominent structural parameters are given in Table 3, along with those of the old model[4] for comparison. Some of the main chain conformation angles reported now differ up to 15°, and the 4-sulfate group is rotated by about $-50°$, from the other.

The sulfate groups describe the periphery of the carrageenan molecule whose outer and inner diameters are 13.8 and 1.5 Å, respectively. As seen in Table 1, an oxygen atom in the 4-sulfate group is the farthest from the helix axis at 6.9 Å, and one in the 2-sulfate oxygen atoms is a little closer at 5.5 Å. These charged groups in each chain are unevenly positioned on the helix surface such that the

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

Group	Atom	x (Å)	y (Å)	z (Å)	r (Å)	ϕ (°)
Gal-4-SO_3^- (A)	C-1	−0.144	1.830	5.145	1.836	94.49
	C-2	−0.113	2.277	6.603	2.280	92.85
	C-3	−1.420	1.922	7.299	2.390	126.45
	C-4	−2.603	2.467	6.510	3.587	136.54
	C-5	−2.513	2.026	5.054	3.228	141.12
	C-6	−3.602	2.630	4.192	4.460	143.86
	O-1	1.006	2.292	4.520	2.503	66.30
	O-2	0.992	1.667	7.258	1.940	59.24
	O-3	−1.427	2.471	8.620	2.853	120.00
	O-4	−2.617	3.892	6.554	4.690	123.92
	O-5	−1.259	2.435	4.488	2.741	117.33
	O-6	−3.601	2.057	2.884	4.147	150.26
	S	−3.863	4.559	7.303	5.976	130.28
	O-S1	−4.301	3.693	8.380	5.669	139.35
	O-S2	−3.469	5.844	7.847	6.797	120.69
	O-S3	−4.950	4.746	6.361	6.857	136.21
	H-1	−0.242	0.735	5.101	0.774	108.25
	H-2	0.040	3.365	6.650	3.365	89.31
	H-3	−1.522	0.828	7.355	1.733	151.45
	H-4	−3.540	2.092	6.949	4.112	149.42
	H-5	−2.568	0.929	4.999	2.731	160.11
	H-61	−3.450	3.717	4.117	5.071	132.87
	H-62	−4.580	2.461	4.666	5.200	151.75
3,6-Anhydro-Gal-2-SO_3^- (B)	C-1	2.837	1.051	0.883	3.025	20.33
	C-2	3.435	0.563	2.210	3.481	9.31
	C-3	3.000	1.514	3.332	3.360	26.78
	C-4	1.469	1.540	3.402	2.129	46.36
	C-5	1.220	2.358	2.138	2.655	62.64
	C-6	2.266	3.454	2.261	4.130	56.73
	O-1	2.853	0.000	0.000	2.853	0.00
	O-2	2.967	−0.747	2.552	3.059	−14.12
	O-3	3.374	2.845	2.967	4.414	40.14
	O-4	1.006	2.292	4.520	2.503	66.30
	O-5	1.458	1.478	1.012	2.076	45.39
	S	3.958	−1.957	2.214	4.415	−26.30
	O-S1	5.177	−1.814	2.987	5.485	−19.31
	O-S2	3.318	−3.214	2.550	4.619	−44.08
	O-S3	4.278	−1.939	0.800	4.697	−24.39
	H-1	3.443	1.881	0.492	3.924	28.65
	H-2	4.532	0.557	2.137	4.567	7.01
	H-3	3.406	1.160	4.291	3.599	18.81
	H-4	0.761	0.726	3.190	1.052	43.65
	H-5	0.215	2.804	2.181	2.812	85.60
	H-61	2.591	3.764	1.258	4.570	55.45
	H-62	1.861	4.281	2.862	4.668	66.51
Cation	Na-1	10.688	4.568	0.611	11.623	23.14
	Na-2	6.000	2.316	1.420	6.431	21.11
	Na-3	−1.110	7.600	2.624	7.680	98.31
	Na-4	−5.174	4.369	5.572	6.772	139.82
	Na-5	0.040	5.147	4.511	5.147	89.56
	Na-6	3.302	5.747	−1.559	6.628	60.12
Water	W-1	0.519	8.501	−2.540	8.517	86.50
	W-2	−6.780	4.672	1.368	8.234	145.43

Table 1 (*Continued*)

Group	Atom	x (Å)	y (Å)	z (Å)	r (Å)	ϕ (°)
	W-3	7.624	5.036	1.454	9.137	33.45
	W-4	−6.254	−5.232	0.961	8.155	−140.09
	W-5	−0.016	9.137	0.065	9.137	90.10
	W-6	1.226	−7.919	0.835	8.014	−81.20
	W-7	9.938	−3.400	1.416	10.503	−18.89
	W-8	6.569	−7.301	1.002	9.822	−48.02
	W-9	6.809	7.560	3.200	10.174	47.99
	W-10	5.380	4.787	4.279	7.202	41.66
	W-11	−5.632	−2.012	2.611	5.981	−160.34
	W-12	−5.937	−4.752	4.116	7.605	−141.33
	W-13	−3.357	−8.490	5.157	9.130	−111.57
	W-14	−0.109	−7.724	3.539	7.724	−90.81
	W-15	−5.756	−1.959	5.361	6.080	−161.21
	W-16	10.962	−3.285	4.267	11.444	−16.68
	W-17	8.733	−4.925	−0.468	10.026	−29.42
	W-18	−8.300	5.723	−0.651	10.082	145.41
	W-19	1.161	−4.183	3.136	4.341	−74.49

The cylindrical coordinates of the next two disaccharides 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	45.0	2/3	1/3	0.0
Helix II	down	−29.0	1/3	2/3	−0.0946
Helix III	up	101.4	0.0	0.0	−0.0872
Na and water		0.0	0.0	0.0	0.0

virtual bond connecting adjacent sulfur atoms across the (1→4) linkage is 11.4 Å, while the one across the (1→3) linkage is only 5.0 Å. However, the half-staggering relationship between the two chains brings the 2-sulfate of one chain to almost the same z-level as the 4-sulfate of the other in the double helix. The resulting 4-S···2-S separation (7.1 Å) is the shortest between the chains in this structure.

Despite two hydroxyl groups (O-2H and O-6H) present per chemical repeat, both in the galactosyl units, no hydrogen bonds are detected within the polysaccharide chain. These groups are oriented so as to form interchain O-2H···O-5 and O-6H···O-2 hydrogen bonds. These are labeled ***1*** and ***2*** in Table 4 that contains the stereochemical details of the significant attractive interactions (distances between functional groups ranging from 2.3 to 3.3 Å) providing stability to the three-dimensional structure. It can be inferred from Fig. 2 that the O-6H···O-2 bonds are almost perpendicular to the helix axis.

Carrageenan–carrageenan interactions.—The three, two up and one down-pointing, conformationally identical carrageenan helices in the unit cell are spaced 13.9 Å apart laterally as shown in Fig. 3. In the absence of symmetry between the helices, their surroundings are unrelated. Since the hydroxyl groups are in the interior, there are no direct 'helix–helix' hydrogen bonds. However, the helices are glued together, by several strategically located guest molecules, by a series of distinct interactions (Table 4). The details of 'helix–guest–helix' communication (Fig. 4) are described below.

Sodium ions and water molecules.—We located two sodium ions, and only six out of 12 water molecules, per disaccharide repeat. The missing six are perhaps disordered or amorphous. A sodium ion is present near one or

Table 2
Observed and calculated structure amplitudes for sodium iota-carrageenan

h k	$l=0$	1	2	3	4
0 0	M	N	N	M	N
	[1511]	[0]	[0]	[119]	[0]
1 0	(23)	180	201	301	[65]
	(69)	*177*	*118*	*267*	[183]
1 1	386	217	169	(73)	
	196	*164*	*115*	*(99)*	170
2 0	180	178	327	257	*261*
	184	*213*	*264*	*244*	
2 1	216	233	383	203	
	223	*256*	*402*	*194*	163
3 0	(70)	214	312	279	*281*
	(126)	*176*	*307*	*212*	
2 2	365				
	302	404	487	361	153
3 1	283	*452*	*504*	*342*	*231*
	213				
4 0	[95]	470	203	(127)	[146]
	[77]	*334*	*221*	*(156)*	*[143]*
3 2	183	365	306	257	
	132	*498*	*367*	*230*	
4 1	471	342	280	264	
	450	*364*	*337*	*196*	
5 0	205	361	(137)	[153]	
	210	*399*	*(150)*	*[137]*	
3 3	377	771	421	243	
4 2	*381*	*610*	*417*	*261*	
5 1	(138)	566	346	(168)	
	(232)	*395*	*305*	*(212)*	
6 0	(150)	[154]	[165]		
	(163)	*[115]*	*[160]*		
4 3	(153)	(157)			
	(162)	*(245)*			
5 2	(158)	(161)			
	(182)	*(224)*			
6 1	(167)	265			
	(191)	*306*			
4 4	368	184			
7 0	*345*	*263*			
6 2	(188)	(192)			
	(209)	*(252)*			

In each reflection box, the observed amplitude is given in the first line and that calculated (italics) 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 meridional and systematically absent reflections, respectively. The calculated structure amplitudes include a temperature factor with $B = 6.5$ Å^2.

more oxygen atoms of every sulfate group and there are six of them in the asymmetric unit, Na-1 through Na-6, so that the net charge becomes zero. The interactions grouped under ***3***–***8*** are of particular relevance. Both Na-2 and Na-5 are confined to only one helix III but attached to different 4- and 2-sulfates, respectively. Na-3 has the unique ability of holding tightly all the three helices together (***5***). The remaining sodium ions are able to interact with the sulfate groups from two helices. These features illustrate that beyond balancing the charge on a sulfate group, each sodium ion serves as a linker between neighboring functional groups, such as two oxygen atoms within a sulfate group (***4***), or two sulfate groups from different helices (***3***, ***6***, ***8***) as shown in Fig. 4. Up to three water molecules are additional ligands for an ion in every case. When bridging occurs from a 2-sulfate group to another 2-sulfate (***8***) or 4-sulfate (***3***, ***5***), or between two different 4-sulfate groups (***5***), the helices are aligned antiparallel.

The asymmetric unit has 19 water molecules, *W*-1 through *W*-19, and 11 of them interact with the sulfate groups at least once. Notable are *W*-9, *W*-16 and *W*-19 as active participants in ***9***, ***10*** and ***11*** (Table 4). Exclusive to helices II, I and III, respectively, they have the same important structural role, viz., to stabilize the interchain hydrogen bond O-6H···O-2 by themselves hydrogen bonding with O-6 and O-2 and receiving two of the 2-sulfate oxygen atoms to complete a distorted tetrahedral coordination at the water oxygen (Fig. 2). They are thus to be regarded as an integral part of the carrageenan double helix similar to that found in potassium gellan.[7]

There are four water molecules, each bridging a pair of helices. For example, *W*-4 connects antiparallel helices I and II (Fig. 4(a)) at their 4-sulfate oxygen atoms (***15***); *W*-13 connects parallel helices III and I (Fig. 4(c)) at 4- and 2-sulfate oxygen atoms, respectively (***21***). On the other hand, *W*-7 links the galactosyl unit of helix I with the anhydrogalactosyl unit of its translation mate (***16***). *W*-12 hydrogen bonds with the 4-sulfate group of helix III, as

Table 3
Major conformation angles (and e.s.d) in degrees in ι-carrageenan helix (a) sodium salt (this study) (b) calcium salt[4]

Parameter	Location	*a*	*b*
ϕ_1(O-5–C-1–O-4–C-4)	β-(1→4)	−89(1)	−87
ψ_1(C-1–O-4–C-4–C-5)	β-(1→4)	109(1)	94
ϕ_2(O-5–C-1–O-3–C-3)	α-(1→3)	60(1)	75
ψ_2(C-1–O-3–C-3–C-4)	α-(1→3)	77(1)	79
χ(C-4–C-5–C-6–O-6)	hydroxymethyl	−172(3)	176
θ_1(C-3–C-4–O-4–S)	4-sulfate	−115(2)	−124
θ_2(C-4–O-4–S–O-S-1)	4-sulfate	32(3)	81
θ_3(C-1–C-2–O-2–S)	2-sulfate	99(3)	101
θ_4(C-2–O-2–S–O-S-1)	2-sulfate	63(2)	75

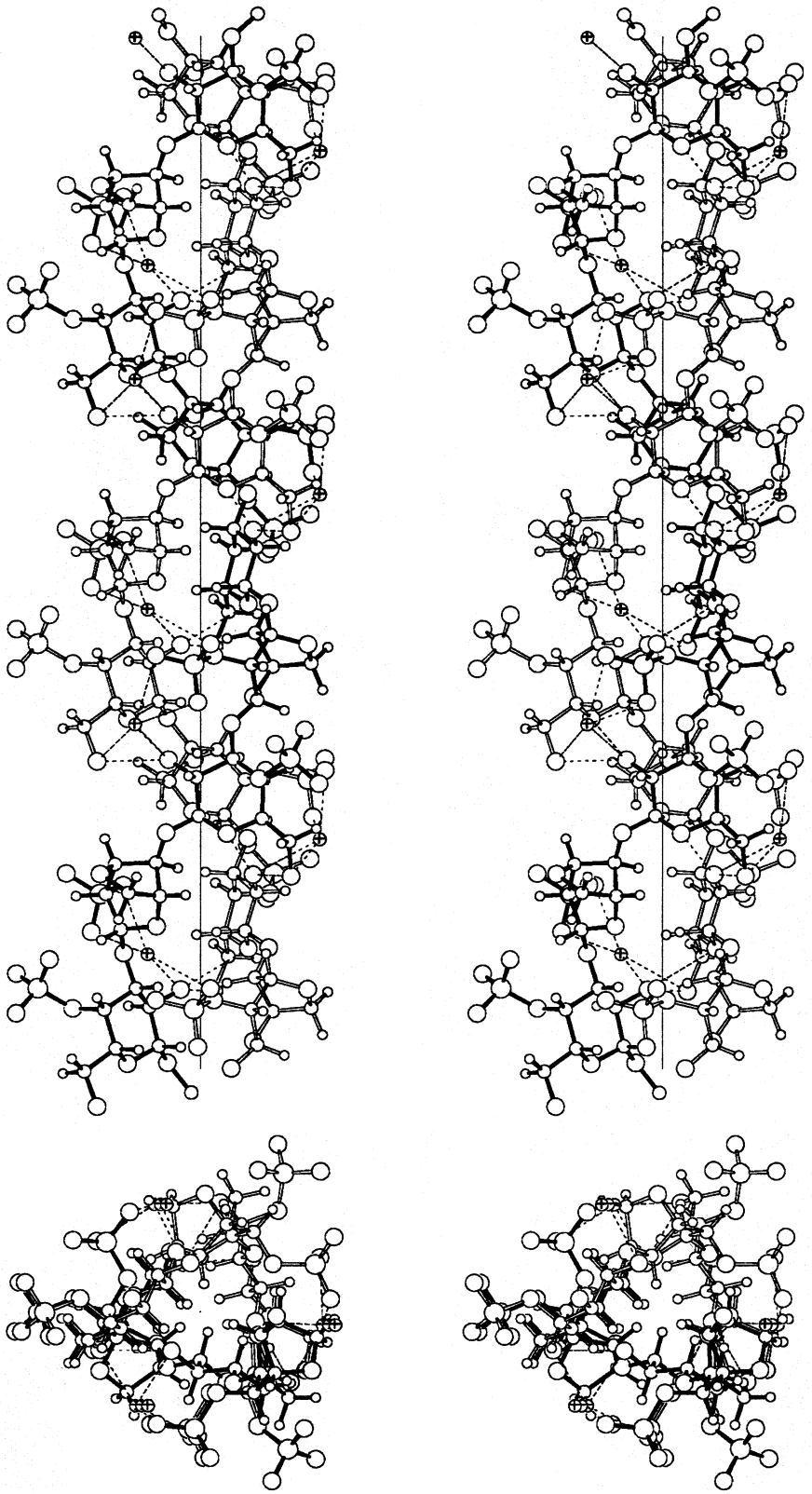

Fig. 2. Stereo drawing of two mutually perpendicular views of the ι-carrageenan double helix stabilized by interchain O-2H···O-5 and O-6H···O-2 hydrogen bonds (dashed line). Water molecules (crossed circle) enhance structural stability by connecting sulfate groups to neighboring hydroxymethyl groups. All the hydrogen atoms are included only in this drawing. For distinction, the bonds in one chain are filled. The vertical line (top) is the helix axis.

Table 4
Attractive interactions among iota-carrageenan helices, sodium ions and water molecules

Interaction	Atom *X*	Atom *Y*	*X*···*Y* (Å)	Precursor *P*	*P*–*X*···*Y* (°)	Bridge
1	O-2*A*(1)	O-5*A*(2)	2.94	C-2*A*	165	
2	O-6*A*(2)	O-2*A*(1)	2.62	C-6*A*	107	
3	O-S3*A*(II,1)	Na-1	2.84	S-*A*	122	4-S–Na–2-S
	O-S1*B*(I,2,−10)	Na-1	2.79	S-*B*	92	
	O-S2*B*(I,2,−10)	Na-1	2.58	S-*B*	100	
	W-3	Na-1	3.21			
4	O-S1*A*(III,2)	Na-2	2.73	S-*A*	95	
	O-S3*A*(III,2)	Na-2	2.77	S-*A*	94	
	W-3	Na-2	3.17			
	W-13	Na-2	3.24			
	W-14	Na-2	2.73			
5	O-S1*A*(I,2,11)	Na-3	3.00	S-*A*	94	4-S–Na–4-S
	O-S3*A*(I,2,11)	Na-3	2.75	S-*A*	105	
	O-S2*A*(II,2)	Na-3	2.69	S-*A*	131	
	O-S1*B*(III,1)	Na-3	2.95	S-*B*	153	4-S–Na–2-S
	W-4	Na-3	2.70			
	W-5	Na-3	3.18			
6	O-S2*A*(I,2,−10)	Na-4	2.31	S-*A*	136	4-S–Na–O6
	O-6*A*(III,2)	Na-4	2.77	C-6*A*	96	
	W-6	Na-4	2.76			
	W-14	Na-4	2.74			
7	O-S1*B*(III,1)	Na-5	2.75	S-*B*	120	
	W-11	Na-5	2.72			
8	O-S1*B*(II,2)	Na-6	2.63	S-*B*	130	2-S–Na–2-S
	O-S3*B*(III,1)	Na-6	2.83	S-*B*	118	
	W-15	Na-6	2.73			
	W-19	Na-6	2.69			
9	O-2*A*(II,2)	*W*-9	2.81	C-2*A*	98	
	O-S2*B*(II,2)	*W*-9	2.86	S-*B*	95	
	O-S3*B*(II,2)	*W*-9	2.76	S-*B*	100	
	O-6*A*(II,1)	*W*-9	2.76	C-6*A*	84	
10	O-2*A*(I,2)	*W*-16	2.79	C-2*A*	98	
	O-S2*B*(I,2)	*W*-16	2.78	S-*B*	94	
	O-S3*B*(I,2)	*W*-16	2.67	S-*B*	98	
	O-6*A*(I,1)	*W*-16	2.81	C-6*A*	83	
11	O-2*A*(III,2)	*W*-19	2.74	C-2*A*	97	
	O-S2*B*(III,2)	*W*-19	2.78	S-*B*	93	
	O-S3*B*(III,2)	*W*-19	2.63	S-*B*	99	
	O-6*A*(III,1)	*W*-19	2.83	C-6*A*	84	
12	O-S2*A*(III,1)	*W*-1	2.77	S-*A*	143	
	W-5	*W*-1	2.73			
	W-7	*W*-1	3.14			
	W-8	*W*-1	2.76			
	W-12	*W*-1	2.76			
	W-15	*W*-1	2.72			
	W-17	*W*-1	2.78			
13	O-S2*A*(I,2,−10)	*W*-2	2.86	S-*A*	103	
	W-10	*W*-2	2.78			
	W-18	*W*-2	2.74			
14	*W*-9	*W*-3	3.17			
	W-14	*W*-3	2.71			
	W-18	*W*-3	2.75			
15	O-S1*A*(I,1,−1−1)	*W*-4	2.71	S-*A*	147	4-S–*W*–4-S
	O-S2*A*(II,1,−1−1)	*W*-4	2.90	S-*A*	99	
	O-S3*A*(II,1−1−1)	*W*-4	2.80	S-*A*	103	
	O-3*B*(I,1,−1−1)	*W*-4	3.13	C-3*B*	140	
	W-12	*W*-4	3.21			

Table 4 (*Continued*)

Interaction	Atom *X*	Atom *Y*	*X*···*Y* (Å)	Precursor *P*	*P*–*X*···*Y* (°)	Bridge
16	O-6*A*(I,1)	*W*-7	2.73	C-6*A*	138	
	W-16	*W*-7	3.03			
	W-17	*W*-7	2.71			
	O4*B*(I,1,−1−1)	*W*-7	3.03	C-1*A*	126	
	O3*B*(I,1,−1−1)	*W*-7	3.15	C-3*B*	100	
17	O-6*A*(II,2,−10)	*W*-8	2.74	C-6*A*	87	
	O5*A*(II,1)	*W*-8	3.11	C-5*A*	103	
18	*W*-9	*W*-10	3.30			
19	*W*-12	*W*-11	3.14			
	W-15	*W*-11	2.75			
20	O-S2*A*(III,1)	*W*-12	2.77	S-*A*	87	
	O-S3*A*(III,1)	*W*-12	2.74	S-*A*	88	
	O-3*B*(I,1,−1−1)	*W*-12	3.15	C-3*B*	125	
	W-15	*W*-12	3.06			
	W-17	*W*-12	2.73			
21	O-S3*A*(III,1)	*W*-13	2.71	S-*A*	141	4-S–*W*–2-S
	O-S1*B*(I,1,−1−1)	*W*-13	2.80	S-*B*	115	
22	O-S1*B*(I,1,−1−1)	*W*-14	2.73	S-*B*	159	
	W-6	*W*-14	3.02			
23	O-S2*A*(III,1)	*W*-15	3.03	S-*A*	97	
	O4*A*(III,2)	*W*-15	2.83	C-4*A*	139	
24	O-S3*A*(II,2,−10)	*W*-17	2.84	S-*A*	137	

I, II, and III, or 1 and 2, in parentheses after the atom name, refer to the three helices in the unit cell, or the two chains in the helix; the third and fourth digits, if any, refer to *a*- and *b*-translations. In the last column, 2-S and 4-S indicate 2- and 4-sulfate groups, respectively.

well as the anhydrogalactosyl unit of its parallel neighbor helix I (***20***). In every case, the interactions are further strengthened by hydrogen bonds from nearby molecules as listed in Table 4.

Six more water molecules *W*-1, *W*-2, *W*-8, *W*-14, *W*-15 and *W*-17 belong to another category in which each is hydrogen bonded to only one helix and to some other water molecules. With the 4-sulfate group as acceptor, as in ***12*** and ***23***, both *W*-1 and *W*-15 are associated with helix III and, similarly, *W*-2 with helix I (***13***) and *W*-17 with helix II (***24***). Instead, with 2-sulfate as acceptor, *W*-14 is connected to helix I (***22***). *W*-8 interacts with two galactosyl oxygen atoms in helix II (***17***). Finally, the surroundings of *W*-3 (***14***), *W*-10 (***18***) and *W*-11 (***19***) confirm that they form the second shell of hydration in the unit cell.

The foregoing description pertains only to the immediate vicinity of the guest molecules. However, they are aptly positioned so as to join together to form spacers (bridges) of appropriate dimensions that are able to span the peripheral sulfate groups of adjacent helices in order to produce tertiary interactions. We find that the spacer size ranges from one to four guest molecules. Some of the main features are seen in Fig. 4. In one *c*-repeat, taken as reference, there are three bridges connecting the antiparallel pair of helices I and II: 4-S–

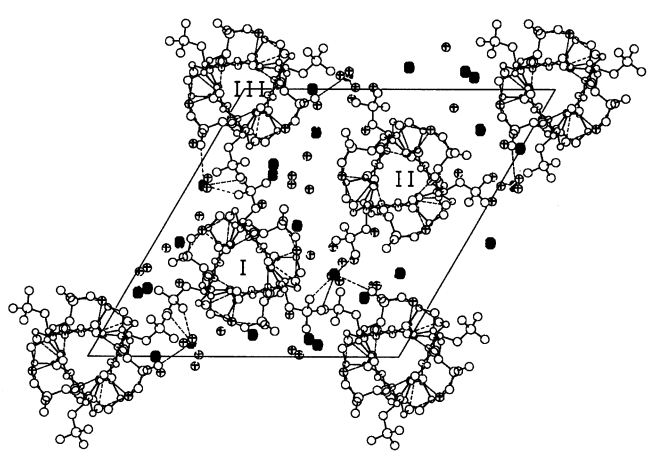

Fig. 3. A projection of the trigonal unit cell down the *c*-axis. Helices I, II, and III, are respectively pointing up, down, and up. Due to lack of nestling between them, their peripheral sulfate groups communicate only via sodium ions (filled circle) and water molecules.

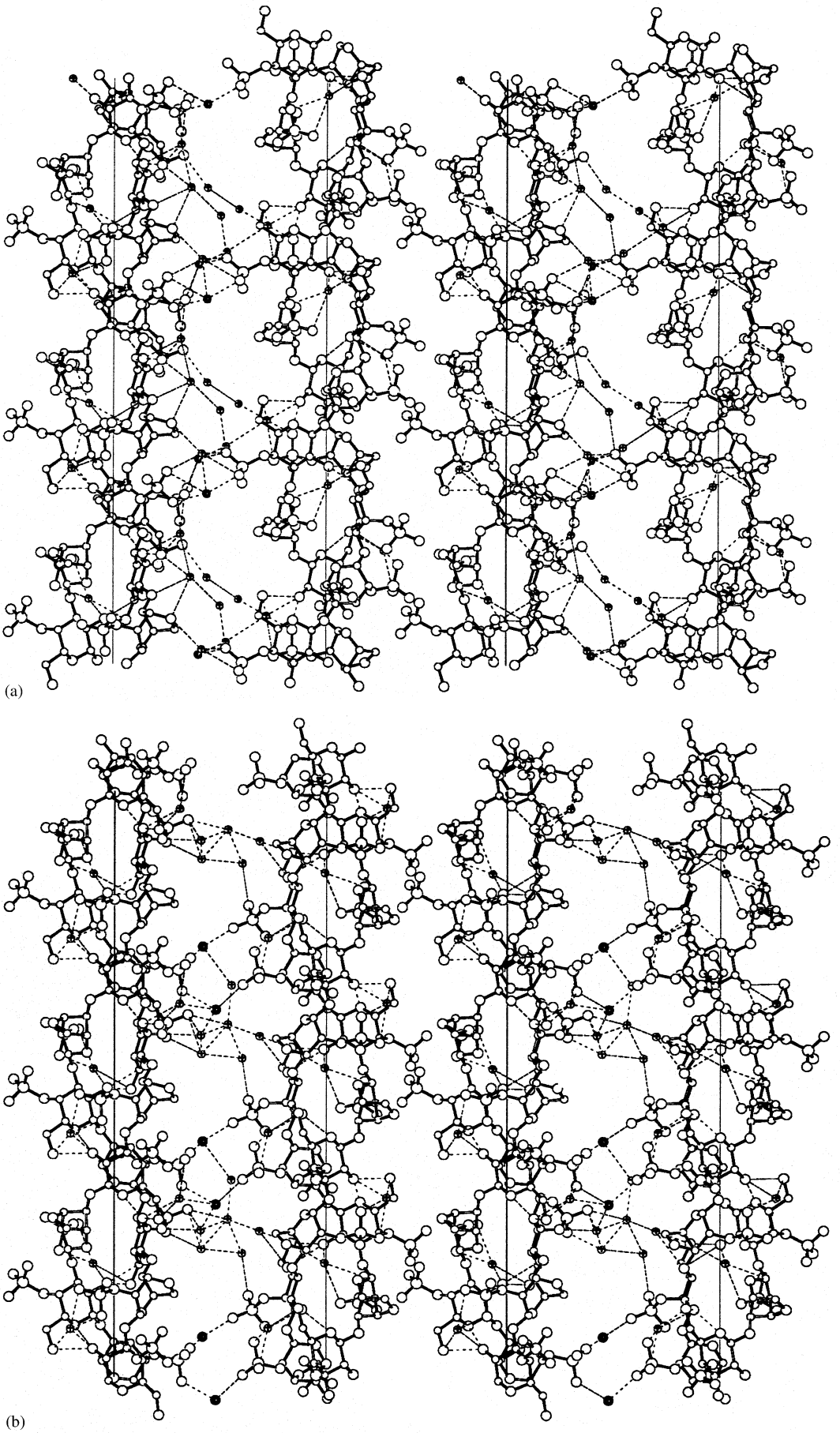

Fig. 4.

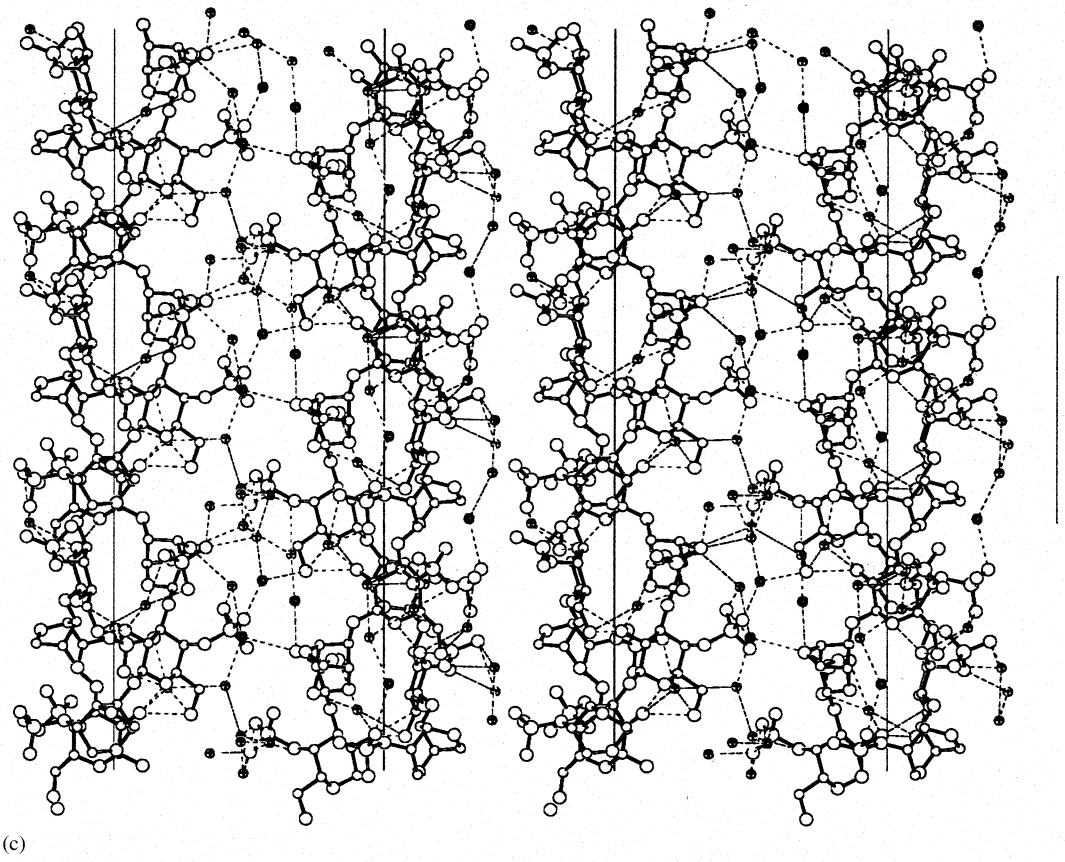

Fig. 4. Stereo drawings showing pairwise interactions between ι-carrageenan helices in the unit cell viewed normal to helix–helix separation (13.9 Å). Hydrogen bonds and coordination bonds for sodium ions are in dashed lines: (a) I (up) and II (down); (b) III (up) and II (down); and (c) I (up) and III (up). The height of the bar on the right is *c*.

W/Na–4-S, O-6–*W*–*W*–4-S and 2-S–*W*–*W*–*W*–4-S. In this notation, 4-S is the surrogate for an oxygen atom in the 4-sulfate group and similarly 2-S for 2-sulfate; the three bridges correspond to a monomer, dimer and trimer, respectively. As shown in Fig. 4(a), the sodium (as well as water) bridge connects the 4-sulfate groups of I and II; the dimeric water bridge connects atom O-6 of I to 4-sulfate of II; and the trimeric water bridge connects the 2-sulfate of I to the 4-sulfate of II. In the second antiparallel pair III and II, five bridges are present (Fig. 4(b)): 2-S–Na–2-S, 2-S–Na–4-S, 2-S–Na–*W*–2-S, 4-S–*W*–*W*–4-S and 4-S–Na–*W*–*W*–*W*–4-S. When the helices are parallel, as in I and III, there are four bridges as seen in Fig. 4(c). They are 4-S–Na–O-6, 2-S–*W*–Na–4-S, 4-S–Na–*W*–Na–4-S, and 2-S–*W*–*W*–*W*–4-S. These results emphasize that both 2- and 4-sulfate groups are required for inter-helical communication in the lattice. The ionic links from sodium ions to the polysaccharide chains and hydrogen bonds from the latter to water molecules are crucial for the lateral organization of ι-carrageenan helices in oriented fibers. These interactions are extremely similar to those observed in nucleic acid duplexes where phosphates are involved instead of sulfate groups.[13,14]

5. Discussion

More than three decades ago, based on a high quality diffraction pattern from potassium ι-carrageenan, Anderson et al. suggested that three double helices of pitch 26 Å pass through a trigonal net of side 22.6 Å.[3] Our diffraction pattern from sodium ι-carrageenan (Fig. 1) belongs to the same category. We have now determined the final structural details due to the availability of better computational facilities and more efficient technical

approaches for solving fiber structures: the LALS refinement program exploits X-ray data in conjunction with observations on non-bonded contacts and hydrogen bonds so that models are optimized for both X-ray fit and minimum energy; electron density maps are routinely used for adjudication of current structure and location of guest molecules.

The final molecular structure, threefold, right-handed, half-staggered, parallel, double helix shown in Fig. 2 has vindicated the Arnott et al. model.[4] In the absence of additional crystallographic symmetry relating them, our assumption that two of the carrageenan helices are antiparallel to the third in the unit cell is in accord with our correct judgment on calcium welan.[10] The subsequent structure analysis proceeded smoothly and the final *R*-value (0.19 for 61 reflections) is very low and comparable to similarly well defined polysaccharide structures such as potassium gellan[7] (0.18 for 51 reflections) and calcium welan[10] (0.22 for 102 reflections).

Only one interchain hydrogen bond between O-2 and O-6 was initially proposed and there was some ambiguity about which oxygen atom was the donor. By comparing the bond angles C-2–O-2···O-6 (135°) and C-6–O-6···O-2 (105°), it was concluded that O-6 was more likely to be the hydrogen donor.[4] Having established a second interchain O-2H···O-5 hydrogen bond in the carrageenan helix (Table 4, Fig. 2), we are able to confirm that O-6 is the donor. Further reinforcement of the O-6H···O-2 bond by a water molecule linking with both O-6 and O-2 along with sulfate oxygen atoms of the anhydro galactosyl unit (***9***, ***10***, ***11***) makes the double helix even more robust than thought earlier.

Iota-carrageenan initially forms highly ordered structures in solution prior to becoming junction zones in the gel state. These ordered states have been the subject of several physicochemical investigations in search of the details. Results from small angle neutron scattering of sodium ι-carrageenan were interpreted to suggest aggregation of chains.[15] Some reports suggest that, under appropriate experimental conditions, both double and single stranded carrageenan structures exist. For example, analysis of the order–disorder transition by size-exclusion chromatography combined with low-angle laser light scattering suggests that the ordered conformation of ι-carrageenan has either double or multiple strands.[16] On the other hand, at sufficiently low dilution, from a distribution of molecular weight and radius of gyration using multi-angle laser light scattering coupled with gel chromatography, single stranded structures for both ι- and κ-carrageen have been detected.[17] There is strong evidence from scanning-tunneling microscopy in support of the double helical form,[18] but non-contact atomic force microscopy of ι-carrageenan suggests that the structure might be single stranded under very low salt conditions and a duplex of some sort in the presence of more salt.[19] A single helix might be an intermediate in sol–gel transition according to a conformational analysis.[20] The debate still goes on. Our X-ray results are confined to the double helical species.

On the basis of our fiber structure, the junction zone in ι-carrageenan gel may be achieved in theory from just a pair of double helices whose molecular axes are set 13.9 Å apart. Regardless of whether the helices are antiparallel as in Fig. 4(a) or (b), or parallel as in Fig. 4(c), cations and structured water molecules can interact with both helices for providing structural stability. However, in practice, three or more helices might be needed to form a strong junction zone that mimics the trigonal packing arrangement in the solid state. While a monovalent ion binds a sulfate group, a divalent ion will bind two sulfate groups either within or between helices. In the latter case, there will be a direct cation-mediated inter helical connection that could lead to stronger gels.

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