Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Polymers 60 (2005) 499–505
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Cation-induced polymorphism in iota-carrageenan

Srinivas Janaswamy, Rengaswami Chandrasekaran*

Department of Food Science, Whistler Center for Carbohydrate Research, Purdue University, 745 Agriculture Mall Drive, West Lafayette, IN 47907-2009, USA

Received 19 January 2005; revised 24 February 2005; accepted 7 March 2005
Available online 3 May 2005

Abstract

Recent X-ray fiber diffraction patterns from polycrystalline and well-oriented fibers of calcium, rubidium and potassium salt forms of ι-carrageenan correspond to large trigonal unit cells. In contrast to the previously reported sodium and calcium forms that contain three half-staggered double helices, these new unit cells accommodate 4, 16 and 27 double helices, respectively. Interestingly, their basal net dimensions ranging from 23.6 to 68.2 Å can all be generated from the smallest trigonal lattice having cell edge 13.7 Å. The peripheral sulfate groups on the prism shaped ι-carrageenan helix, along with cations and water molecules, are mainly responsible for the association of helices. The existence of several cation-dependent stable crystalline states is compatible with the observed versatility in the physical properties of ι-carrageenan.

Keywords: Sulfated polysaccharides; Carrageenan; Polymorphism; Packing; Junction zone

1. Introduction

Sulfated polysaccharides with significant biological activities are found in a variety of marine animals, plants and microorganisms (Amornrut et al., 1999; Cimino, Bifulco, Casapullo, Bruno, Gomez-Paloma, & Riccio, 2001; Farias, Valente, Pereira, & Maurao, 2000; Kolender, Pujol, Damonte, Matulewicz, & Cerezo, 1997; Witvrouw & DeClercq, 1997). Among them, heparin, dextran sulfate and mannan sulfate, to name a few, have shown inhibitory activity against several viruses such as human immunodeficiency virus (HIV), herpes simplex virus (HSV) and human cytomegalovirus (HCMV) (Schaeffer & Krylov, 2000; Witvrouw, Desmyter, & DeClercq, 1994). In general, the mode of action is attributed to the blockage of virus replication cycle at the early stages. In this regard, three-dimensional structures will be of immense use in understanding the association of these polysaccharides with different proteins in regulating a variety of biological processes. Among the sulfated polysaccharides, carrageenans represent an important class possessing anticoagulant and antiherpetic activities (Carlucci, Ciancia, Matulewicz, Cerezo, & Damonte, 1999; Franz, Pauper, & Alban, 2000). These are α-(1→3) and β-(1→4) galactose linked polymers. Till date, seven carrageenans have been identified based on the presence or absence of anhydrogalactose residues and the positions of sulfate groups. In general, the use of carrageenans in food and pharmaceutical industries has increased, owing to their complex forming ability with other hydrocolloids and proteins (Therkelsen, 1993; Piculell, 1995). In particular, their applications in the food industry include bodying, gelling, thickening and emulsion stabilizing in water- and milk-based systems. However, only kappa (κ)-, iota (ι)- and lambda (λ)-carrageenans have so far been studied extensively due to their greater versatility. They carry one, two and three sulfate groups, respectively, per disaccharide repeat. Importantly, their functional properties are different. In the presence of mono and divalent cations, gelation occurs for κ- and ι-carrageenans, but λ-carrageenan does not gel at any concentration. Gels of κ-carrageenan are relatively hazy and brittle, while ι-carrageenan develops very clear and elastic gels that are free from syneresis and hysterisis. Unlike these two, λ-carrageenan is reported to have anti-HIV activity (Nakashima, Kido, Kobayashi, Motoki, Neushul, & Yamamoto, 1987).

Variations in the rheological properties must stem from differences in molecular structure, in the polymer assembly

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

0144-8617/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2005.03.013

or both. In this context, structural information at Ångstrom level is vital for describing the polymer behavior. For many decades, our research group is engaged in determining the three-dimensional structures of helix forming biopolymers using X-ray fiber diffraction data. In this regard, we have embarked on a systematic structural investigation of carrageenans in the presence of mono and divalent cations. So far, the studies on ι-carrageenan organization with Na^+ and Ca^{2+} ions have been completed (Janaswamy & Chandrasekaran, 2001, 2002). One of the important results is that direct helix–helix interactions do not exist in the crystalline lattice. Instead, cations bind the sulfate groups in such a way as to tie the helices together aided by surrounding water molecules and both crystal structures are nearly isomorphous. Recently, however, we have obtained evidence, for the first time, that K^+, Rb^+ salts and also a new Ca^{2+} form favor larger unit cells, but with similar fiber repeat. Such observations strongly imply that cations govern the associative properties of ι-carrageenan to a large extent. The relevant details are described in this article.

2. Experimental

2.1. Fiber preparation

About 15 mg of sodium ι-carrageenan (gift from FMC Corporation, USA) was dissolved in 1 ml of distilled water, and a few drops were placed in between the beaded ends of the two glass rods in a fiber puller at 75% relative humidity. Oriented fibers of 3–5 mm long were obtained after stretching the semi-dried solution for about 2 h at regular intervals. Often, adding a few NaCl (0.01 M) droplets to the drying sample helped to improve crystallinity. In a second procedure, NaCl (0.01 M) dissolved in the polysaccharide solution with constant stirring at 50 °C was used for obtaining fibers. The diffraction patterns from them indicated large crystallites and higher degree of orientation. Hence, several sodium form fibers were prepared by this method and subsequently immersed in 0.05 M potassium, rubidium and calcium chloride aqueous-isopropanol solution (10:90 v/v) for about 12 weeks at cold (4 °C) and room temperature (20 °C), respectively. The soaking experiments were intended to convert the fibers from the sodium form to the potassium, rubidium and calcium forms, respectively. Subsequent energy dispersive spectrum analysis on calcium fibers clearly indicated the complete replacement of calcium for a pair of sodium ions (Janaswamy & Chandrasekaran, 2002).

2.2. X-ray patterns

X-ray fiber diffraction patterns from the sodium and calcium forms were recorded on flat photographic films in pinhole cameras using Ni-filtered Cu Kα radiation ($\lambda = 1.5418$ Å) at room temperature. The microfocus generator was operated at 40 kV and 6 mA and exposure lasted up to 2 days. The specimen chamber was continuously flushed with a steady stream of helium gas bubbled through a saturated salt solution to minimize air scattering and maintain the fiber at the desired humidity. Fibers were dusted with calcite powder (characteristic spacing 3.035 Å) for internal calibration. Positions of reflections were obtained by using a Stoe measuring device. Diffraction patterns for the potassium and rubidium forms (fibers soaked at room temperature) were obtained at the Argonne National Laboratory (ANL), Chicago using 1 Å radiation. The data were recorded on a CCD with 2 s exposure. The ICE routine from the CCP13 suite (http://www.ccp13.ac.uk/software/software.htm) was used for processing the data and obtaining the reflection positions. All these measurements were used to calculate the pattern center, fiber rotation and tilt and finally the distance of each reflection from the origin of the reciprocal lattice (ρ). The relationship between the lateral ξ (cylindrical radius) and vertical ζ components of ρ is given by $\rho^2 = \xi^2 + \zeta^2$, where $\xi = a^*(h^2 + k^2 + hk)^{1/2}$ and $\zeta = lc^*$ for the trigonal system; a^* and c^* are the dimensions of the reciprocal unit cell and h, k, l are the Miller indices. These two components for each reflection were estimated and the unit cell parameters determined using in-house programs.

3. Results

X-ray diffraction patterns from fibers of sodium, potassium, rubidium and calcium salts of ι-carrageenan are shown in Fig. 1. Table 1 summarizes the unit cell dimensions and related X-ray parameters for each of the eight forms available to date. It is to be noted that calcium ι-carrageenan takes up three distinct forms denoted by I, II and III. All the patterns indicate clearly that the fibers are uniaxially oriented and polycrystalline and display sharp Bragg reflections extending up to 2.8 Å on layer lines 0–4. The effect of cation is readily seen from the intensity distribution in each pattern. A three-fold helical symmetry for the polymer chain is evident from the third layer line meridional reflection. While the sodium pattern (Fig. 1c) is indexable on a trigonal lattice with $a = 24.0$ Å and $c = 12.9$ Å, the calcium (II) pattern (Fig. 1a; fiber obtained by soaking in 0.05 M $CaCl_2$ aqueous-isopropanol solution at 20 °C) is similar with $a = 23.6$ Å and $c = 13.2$ Å. In both cases, the unit cell accommodates three half-staggered double helices, half-a-turn of each spanning the c-repeat. The resulting molecular structures as well as the specific interactions between the neighboring polymer helices are now known. Although the helices in the unit cell are too far apart to have any direct attractive interactions, strategically located cations and water molecules connect them via their 2-sulfate and 4-sulfate groups. Similarities, as well as differences, in the types of cation/water-mediated

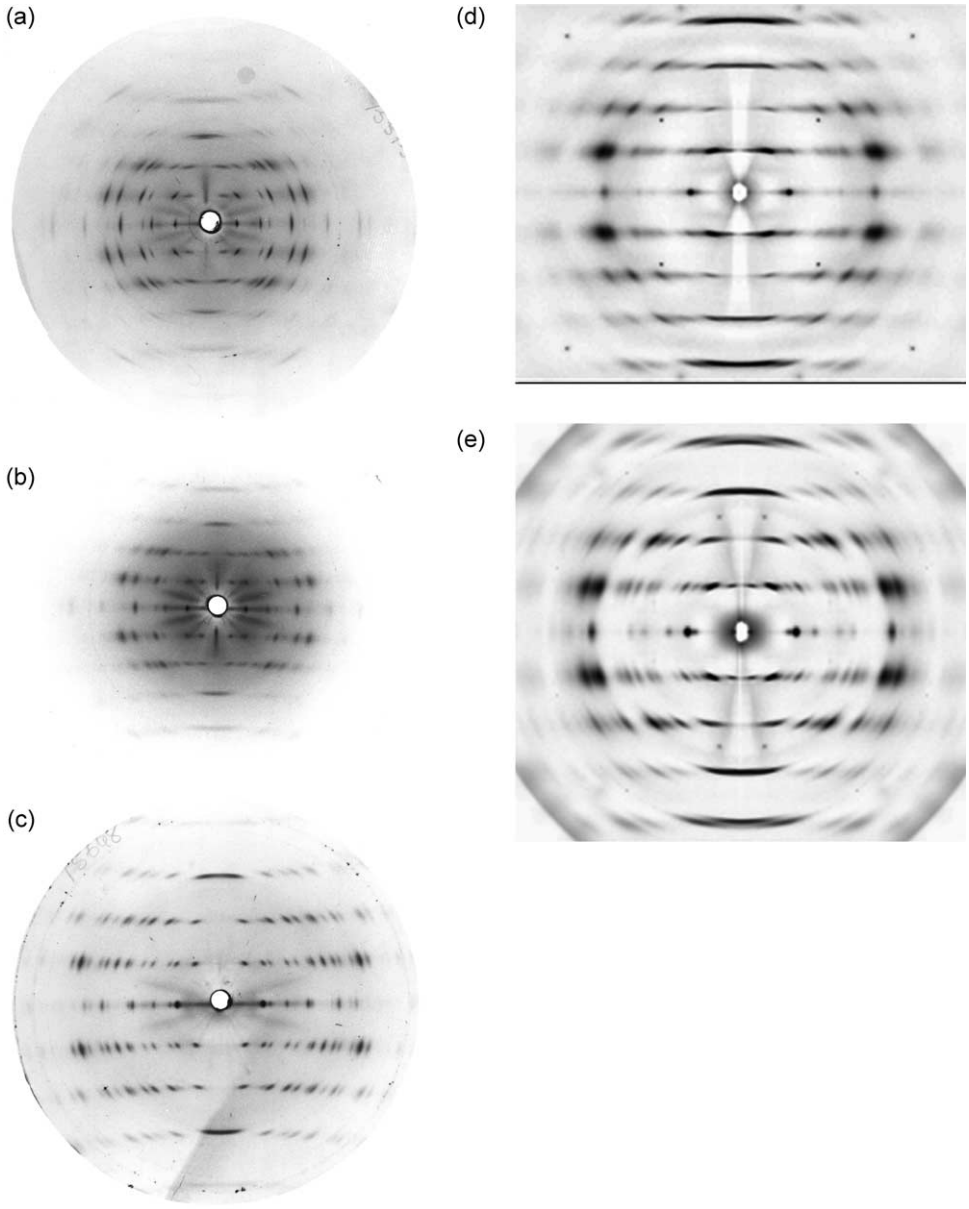

Fig. 1. X-ray diffraction patterns from well oriented and polycrystalline fibers of ι-carrageenan corresponding to (a) calcium (II), (b) calcium (III), (c) sodium, (d) rubidium and (e) potassium salt forms. The first three are from in-house generator and the last two using synchrotron radiation.

Table 1
Cation dependent trigonal unit cell and related parameters of ι-carrageenan in different allomorphs

Cation	Number of reflections	Unit cell dimensions (Å)		Z^a	Unit cell type[b]	Reference
		a	*c*			
Ca^{2+} (I)	24	13.7	13.3	1	A	Arnott et al. (1974)
Ca^{2+} (II)	29	23.6	13.2	3	B	Janaswamy and Chandrasekaran (2002)
Ca^{2+} (III)	30	27.4	13.1	4	C	Present work
Sr^{2+}	21	13.7	13.3	1	A	Arnott et al. (1974)
Mg^{2+}	14	14.7	13.0	1	A	Arnott et al. (1974)
Na^{+}	44	24.0	12.9	3	B	Janaswamy and Chandrasekaran (2001)
Rb^{+}	26	54.6	12.3	16	D	Present work
K^{+}	34	68.2	13.1	27	E	Present work

[a] Number of helices/unit cell.
[b] See Fig. 3.

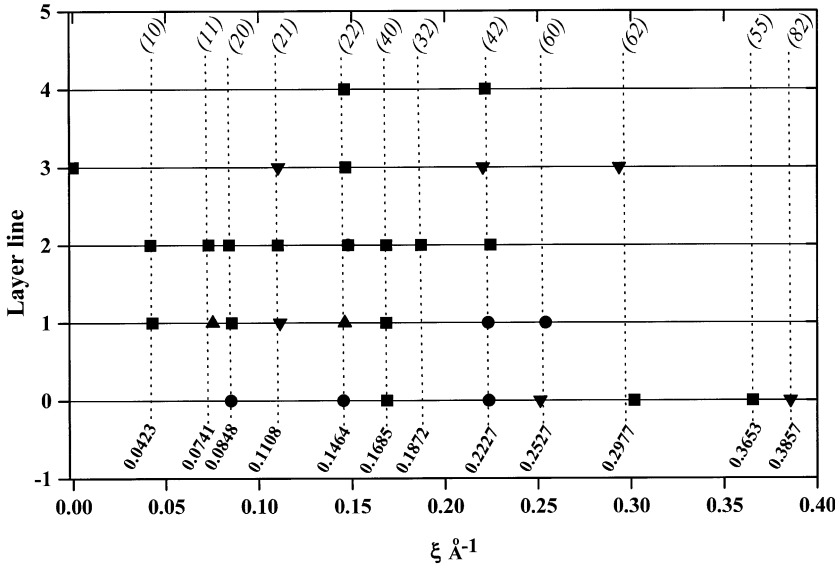

Fig. 2. Distribution of Bragg reflections as a function of ξ in each layer line in the calcium (III) ι-carrageenan diffraction pattern (● strong, ■ medium, ▲ weak and ▼ very weak intensity). The h and k values are indicated at the top and the ξ value at the bottom for each row line.

interactions in the monovalent and divalent forms diligently reflect the gelation properties (Janaswamy & Chandrasekaran, 2001, 2002).

In contrast, newer calcium (III) pattern (Fig. 1b) obtained from fibers soaked in 0.05 M $CaCl_2$ aqueous-isopropanol solution at 4 °C is diagnostic of a larger basal plane, but the c-repeat is almost the same. Fig. 2 highlights the distribution of ξ for the Bragg reflections on each layer line for this pattern. With the smallest at 0.0423 $Å^{-1}$, these ξ values do not superpose on those for the calcium (II) pattern (Janaswamy & Chandrasekaran, 2002). All the 30 reflections are consequently indexable on a larger trigonal cell with $a=27.4$ Å and $c=13.1$ Å (Table 1) that accommodates four double helices.

The rubidium pattern (Fig. 1d) requires a still larger basal plane. The first reflection on $l=1$ with $\xi=0.021$ $Å^{-1}$ results in $a=54.6$ Å and $c=12.3$ Å so that this trigonal unit cell contains as many as 16 helices. Similarly, the potassium pattern (Fig. 1e) indexes on the largest trigonal cell so far: $a=68.2$ Å and $c=13.1$ Å and there are a maximum of 27 helices in it. Thus, the unit cell for the potassium form is nine times as large as the sodium cell and the rubidium cell is four times that of the calcium (III) cell. From a historical perspective, it is known for more than 30 years that the calcium (I) form has the smallest trigonal cell with $a=13.7$ Å and $c=13.3$ Å derived from a diffraction pattern, in which Bragg reflections superposed on continuous intensities, similar to those of the Sr^{2+} and Mg^{2+} forms (Arnott, Scott, Rees, & McNab, 1974). Together, the old and new observations strongly suggest that ι-carrageenan has an intrinsic ability to crystallize in at least five distinct types of packing arrangements, while conserving its molecular geometry.

4. Discussion

4.1. Polymorphism in polysaccharide structures

Structural perturbations in biopolymers induced by water molecules and co-solutes are well documented in the literature (Sauna, Madhavarao, & Sitaramam, 2001; Timasheff & Arakawa, 1989). Firstly, these perturbations could alter the molecular symmetry (i.e. shape) and subsequently force them to crystallize in quite distinct unit cells. Among polysaccharides, dermatan sulfate, chondroitin 4-sulfate and hyaluronan, to name a few, belong to this category. Sodium ions produce three different crystalline allomorphs of dermatan sulfate (Mitra, Arnott, Atkins, & Isaac, 1983). Divalent cations, however, dramatically change the three-fold helix of monovalent chondroitin 4-sulfate to an extended two-fold helical configuration (Cael, Winter, & Arnott, 1978; Millane, Mitra, & Arnott, 1983). The effect is further pronounced in hyaluronan which displays seven types of packing arrangements arising from three different molecular structures (Chandrasekaran, 1997).

On the other hand, perturbations could be well limited to a mild nature. In such a situation, the core molecular structure will be intact, but the perturbations will be just sufficient enough to modify the orientations of peripheral carboxyl, hydroxymethyl and sulfate groups. As a result, symmetry of the packing arrangement is transformed to either a different crystal system or an altered unit cell that accommodates fewer or more molecules. In the case of cyclohexaicosaose, for example, water molecules lead to the transition from an orthorhombic to a triclinic unit cell with no accompanying change in the main chain geometry

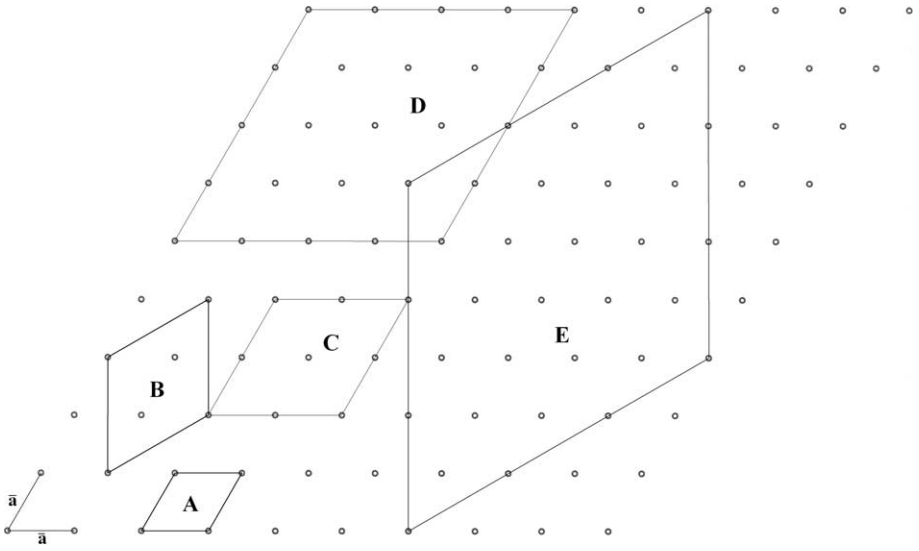

Fig. 3. Two-dimensional trigonal net with grid dimension $a = 13.7$ Å. The unit cells A–E are adopted by ι-carrageenan under different experimental conditions (see text).

(Gessler et al., 1999; Nimz, Gessler, Usón, & Saenger, 2001). Our present study demonstrates that, depending on the cation type, ι-carrageenan can crystallize in five distinct packing arrangements so far, all in trigonal unit cells.

A careful examination of these results reveals that all the five ι-carrageenan unit cells can be readily derived from the smallest calcium (I) unit cell. Since the *c*-repeat remains the same in all the cases, the differences are mainly in the basal net (i.e. *ab*-plane). A two-dimensional trigonal net in which the lattice pointes are spaced at 13.7 Å is sketched in Fig. 3, where each point represents an ι-carrageenan helix. The unit cells labeled A–E contain (see Table 1) 1, 3, 4, 16 and 27 helices, respectively. The corresponding calculated cell edge dimensions in this drawing are 13.7, 23.7, 27.4, 54.8 and 71.2 Å. The experimental dimensions (Table 1) are very close to these values. While structural details are so far available only for the polymer chains in the unit cells A (Arnott et al., 1974) and B (Janaswamy & Chandrasekaran, 2001, 2002), it is perplexing as to how the helices might be organized in the remaining unit cells.

If all the helices in the unit cell are identical and have the same polarity, orientation and positioning parameters, then the basal net will have only one lattice point as in A. This situation has not yet been trapped in oriented fibers. However, the experimental structure in unit cell A consists of an up- and a down-pointing helices of half occupancy each at the lattice point that results in spots along with streaks on the layer lines (Arnott et al., 1974). In contrast, amazingly, all the diffraction patterns representing the larger unit cells contain only sharp Bragg reflections. Therefore, the ι-carrageenan helices should exploit polarity, orientation and position as major variable parameters and even dissimilar sulfate group rotations, if needed, while crystallizing in the larger unit cells. The resulting low energy conformations might readily be stabilized by the surrounding cations and water molecules to give rise to ordered crystalline domains. However, validation of such attractive speculations must await the completion of structure analysis related to the unit cells C–E.

4.2. Hydrogen bonding interactions and polymer assembly

The two potential donors O–2H and O–6H of the galactose residue and a water molecule per disaccharide repeat are utilized in stabilizing the ι-carrageenan double helix. With both sulfate groups on the surface, the prism shaped helices are nearly separated by the helix diameter

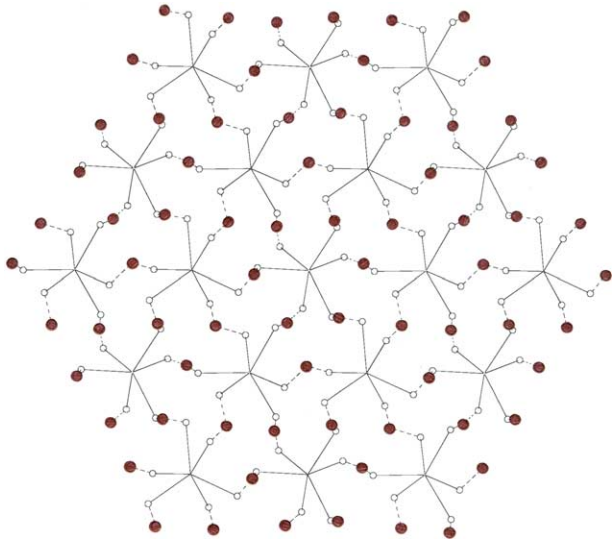

Fig. 4. Schematic *c*-axis projection of the hexagonal packing of ι-carrageenan helices in the presence of calcium ions (filled circles). In each helix (drawn as a star), the small circles at the ends of short and long arms are the 2- and 4-sulfate groups, respectively, which lead to strong sulfate–calcium–sulfate bridges.

and held merely by non-bonded contacts. Both cations and water molecules are utilized in mediating communication between helices in this setup. The sulfate groups at position O-2, as well as O-4, are active partners in this process. Fig. 4 shows a schematic representation of ι-carrageenan association (down the *c*-axis) in the presence of calcium ions, derived from the calcium (II) structure (Janaswamy & Chandrasekaran, 2002). Each calcium ion snugly binds two sulfate groups from adjacent helices. In the case of sodium ions, longer bridges involving ordered water molecules (*W*) such as –Na–*W*–Na– and –Na–*W*–*W*–*W*–Na– are needed (Janaswamy & Chandrasekaran, 2001). In projection, these cations constitute a quasi-hexagonal net that propagates to give rise to an extended lattice. In general, no two ι-carrageenan helices can come close to each other, due to charge repulsion between the sulfate groups, unless and until cation(s) are trapped to balance the charge and subsequently able to connect them. Thus, the cations have crucial dual role in the ι-carrageenan association. Unlike this scenario, the other known charged polysaccharides such as gellan (Chandrasekaran, Radha, & Thailambal, 1992), welan (Chandrasekaran, Radha, & Lee, 1994) and RMDP17 (Bian, Chandrasekaran, & Rinaudo, 2002) exhibit direct inter-helical hydrogen bonding interactions. The cations in these structures are mainly used for charge balancing and to a lesser extent in the polymer assembly. This could be a reason why no polymorphic behavior is reported for any of them. On the other hand, in the neutral polysaccharides cellulose and mannan, adjacent sheets in the unit cell are packed and stabilized only by van der Waals forces and hydrogen bonds in some cases. Thus, these polymer helices gain extra freedom giving rise to quite distinct packing arrangements, as found in cellulose I, II, III and IV, and mannan I and II (Chandrasekaran, 1997). The above examples clearly demonstrate that polysaccharide assembly in the crystalline state is to a great extent governed by the experimental conditions such as type and amount of cations and water molecules in order to display a variety of functional properties. Since such guest molecules are essential for ι-carrageenan assembly, and depending on their constellation in the lattice, there could be several stable packing arrangements or low-energy ensembles such as A–E in Fig. 3 and beyond. High-sensitivity differential scanning calorimetry studies on K^+ ι-carrageenan suggest the occurrence of double helix dimers (Grinberg, Grinberg, Usov, Shusharina, Khokhlov, & deKruif, 2001). Such large ensembles might equally require larger unit cell dimensions as observed in our study. Further structural work is needed to decipher the complex molecular details.

Acknowledgements

The Industrial Consortium of the Whistler Center for Carbohydrate Research supported this work. We thank the *FiberNet* Fiber diffraction workshop organizers and ANL staff for the synchrotron intensity data. We also appreciate Rahul Kasat's help in data collection.

References

Amornrut, C., Toida, T., Imanari, T., Woo, E.-R., Park, H., Linhardt, R., et al. (1999). A new sulfated β-galactan from clams with anti-HIV activity. *Carbohydrate Research*, *321*, 121–127.

Arnott, S., Scott, W. E., Rees, D. A., & McNab, C. G. A. (1974). ι-carrageenan: Molecular structure and packing of polysaccharide double helices in oriented fibers of divalent cations fibers. *Journal of Molecular Biology*, *90*, 253–267.

Bian, W., Chandrasekaran, R., & Rinaudo, M. (2002). Molecular structure of the rhamsan-like exocellular polysaccharide RMDP17 from *Sphingomonas paucimobilis*. *Carbohydrate Research*, *337*, 45–56.

Cael, J. J., Winter, W. T., & Arnott, S. (1978). Calcium chondroitin-4-sulfate: Molecular conformation and organization of polysaccharide chains in a proteoglycan. *Journal of Molecular Biology*, *125*, 21–42.

Carlucci, M. J., Ciancia, M., Matulewicz, M. C., Cerezo, A. S., & Damonte, E. B. (1999). Antiherpetic activity and mode of action of natural carrageenans of diverse structural types. *Antiviral Research*, *43*, 93–102.

Chandrasekaran, R. (1997). Molecular architecture of polysaccharide helices in oriented fibers. *Advances in Carbohydrate Chemistry and Biochemistry*, *52*, 311–439.

Chandrasekaran, R., Radha, A., & Lee, E. J. (1994). Structural roles of calcium ions and side chains in welan: An X-ray study. *Carbohydrate Research*, *252*, 183–207.

Chandrasekaran, R., Radha, A., & Thailambal, V. G. (1992). Roles of potassium ions, acetyl and L-glyceryl groups in native gellan double helix: An X-ray study. *Carbohydrate Research*, *224*, 1–17.

Cimino, P., Bifulco, G., Casapullo, A., Bruno, I., Gomez-Paloma, L., & Riccio, R. (2001). Isolation and NMR characterization of rosacelose, a novel sulfated polysaccharide from the sponge *Mixylla rosacea*. *Carbohydrate Research*, *334*, 39–47.

Farias, W. R. L., Valente, A., Pereira, M. S., & Mourão, P. A. S. (2000). Structure and anticoagulant activity of sulfated galactans-isolation of a unique sulfated galactan from the red algae *Botryocladia occidentalis* and comparison of its anticoagulant action with that of sulfated galactans from invertebrates. *Journal of Biological Chemistry*, *275*, 29299–29307.

Franz, G., Pauper, D., & Alban, S. (2000). Pharmacological activities of sulfated carbohydrate polymers. In B. S. Paulsen (Ed.), *Bioactive carbohydrate polymers* (pp. 47–58). Boston: Kluwer.

Gessler, K., Usón, I., Takaha, T., Krauss, N., Smith, S. M., Okada, S., et al. (1999). V-Amylose.at atomic resolution: X-ray structure of a cycloamylose with 26 glucose residues (cyclomaltohexaicosaose). *Proceedings of the National Academy of Sciences of the United States of America*, *96*, 4246–4251.

Grinberg, V. Y., Grinberg, N. V., Usov, A. I., Shusharina, N. P., Khokhlov, A. R., & deKruif, K. G. (2001). Thermodynamics of conformational ordering of ι-carrageenan in KCl solutions using high-sensitivity differential scanning calorimetry. *Biomacromolecules*, *2*, 864–873.

Janaswamy, S., & Chandrasekaran, R. (2001). Three-dimensional structure of the sodium salt of iota-carrageenan. *Carbohydrate Research*, *335*, 181–194.

Janaswamy, S., & Chandrasekaran, R. (2002). Effect of calcium ions on the organization of iota-carrageenan helices: An X-ray investigation. *Carbohydrate Research*, *337*, 523–535.

Kolender, A. A., Pujol, C. A., Damonte, E. B., Matulewicz, M. C., & Cerezo, A. S. (1997). The system of sulfated α-(1→3) linked D-mannans from the red seaweed *Nothogenia fastigiata*: Structures,

antiherpetic and anticoagulant properties. *Carbohydrate Research*, *304*, 53–60.

Millane, R. P., Mitra, A. K., & Arnott, S. (1983). Chondroitin 4-sulfate: Comparison of the structures of the potassium and sodium salts. *Journal of Molecular Biology*, *169*, 903–920.

Mitra, A. K., Arnott, S., Atkins, E. D. T., & Isaac, D. H. (1983). Dermatan sulfate: Molecular conformations and interactions in the condensed state. *Journal of Molecular Biology*, *169*, 873–901.

Nakashima, H., Kido, Y., Kobayashi, N., Motoki, Y., Neushul, M., & Yamamoto, N. (1987). Purification and characterization of an avian-myeloblastosis and human-immunodeficiency-virus reverse-transcriptase inhibitor, sulfated polysaccharides extracted from sea algae. *Antimicrobial Agents and Chemotherapy*, *31*, 1524–1528.

Nimz, O., Gessler, K., Usón, I., & Saenger, W. (2001). An orthorhombic crystal form of cyclohexaicosaose, CA26 32.59 H_2O: Comparison with the triclinic form. *Carbohydrate Research*, *336*, 141–153.

Piculell, L. (1995). Gelling carrageenans. In A. M. Stephen (Ed.), *Food polysaccharides and their applications* (pp. 205–244). New York: Marcel Dekker.

Sauna, Z. E., Madhavarao, C. N., & Sitaramam, V. (2001). Large solutes induce structural perturbations in proteins and membranes. *International Journal of Biological Macromolecules*, *29*, 5–18.

Schaeffer, D. J., & Krylov, V. S. (2000). Anti-HIV activity of extracts and compounds from algae and cyanobacteria. *Ecotoxicology and Environmental Safety*, *45*, 208–227.

Therkelsen, G. H. (1993). Carrageenan. In R. L. Whistler, & J. N. BeMiller (Eds.), *Industrial gums. Polysaccharides and their derivatives* (pp. 145–180). New York: Academic Press.

Timasheff, S. N., & Arakawa, T. (1989). Stabilization of protein structure by solvents. In T. E. Creighton (Ed.), *Protein structure: A practical approach* (pp. 311–349). New York: Oxford University Press.

Witvrouw, M., & DeClercq, E. (1997). Sulfated polysaccharides extracted from sea algae as potential antiviral drugs. *General Pharmacology*, *29*, 497–511.

Witvrouw, M., Desmyter, J., & DeClercq, E. (1994). Antiviral portrait series: 4. Polysulfates as inhibitors of HIV and other enveloped viruses. *Antiviral Chemistry and Chemotherapy*, *5*, 345–359.