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The optical rotation of ordered carrageenans

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The optical rotation of carrageenan is modeled as a function of chain conformation, by application of the MOLROT algorithm. As with agarose, previously studied, the optical rotation observed for gels is adequately accounted for by a variant of the chain geometry found in the fiber x-ray diffraction based model, albeit one with a smaller chain extension. Also as with agarose, the coil rotation indicates the presence of chain segments which are locally almost fully extended.

INTRODUCTION

Carrageenans are linear sulfated galactan polysaccharides extracted from red seaweeds (Mackie & Preston, 1974). They have commercial importance in the food and other industries, on account of their gelling and thickening properties, and studies on them have recently been reviewed (Therkelsen, 1993). The *ι*- and *κ*-carrageenans are alternating copolymers based on a disaccharide repeat unit containing 3-linked β-D-galactose-4-sulfate and 4-linked 3,6-anhydro-α-D-galactose, the latter of which is sulfated at the 2-position in *ι*- but not *κ*-carrageenan (Anderson *et al.*, 1968, 1973). The major variants in structure include incomplete sulfation and occasional replacement of the anhydro moiety with D-galactose sulfate or disulfate, typically near 10% in *ι*-carrageenan. They form thermoreversible gels at relatively low concentration (< 1%).

X-ray diffraction patterns indicate the presence of three-fold right-handed double helices in fibers for both *ι*- and *κ*-carrageenan, with particularly sharp patterns occurring in the case of *ι*-carrageenan (Anderson *et al.*, 1969; Arnott *et al.*, 1974; Millane *et al.*, 1988) (Fig. 1).

Thermally induced optical rotation changes in solution indicate an order–disorder transition, which led to the proposal that double helices constitute a major structural element of carrageenan gels (Anderson *et al.*, 1969; McKinnon *et al.*, 1969; Rees *et al.*, 1969; Dea *et al.*, 1972; Bryce *et al.*, 1974; Norton *et al.*, 1978, 1979), with additional associative crosslinks leading to a three-dimensional network (Morris *et al* 1980; Smidsrød *et al.*, 1980; Bryce *et al.*, 1982; Smidsrød & Grasdalen, 1982; Norton *et al.*, 1984).

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Corroborative evidence supporting the double helical gel model came from an early empirical interpretive model of saccharide optical activity (Rees *et al.*, 1970). The purpose of the present work was to confirm the compatibility of the double helix chain geometry derived from x-ray diffraction and the observed optical rotation through application of a less empirical, more detailed calculational model and its accompanying algorithm (MOLROT) (Stevens & Sathyanarayana, 1987, 1989; Duda & Stevens, 1990, 1993; Schafer & Stevens, 1995).

Molecular modeling methods have now been applied to carrageenan structures which go beyond the hard-sphere calculations originally applied in the course of x-ray diffraction pattern analyses (Anderson *et al.*, 1969; Arnott *et al.*, 1974; Millane *et al.*, 1988). Wider searches for energetically stable conformations have been carried out, and disaccharide potential energy surfaces have been calculated for the two pairs of linkage dihedral angles, in spite of the impediments caused by the presence of sulfate groups (Lambda *et al.*, 1990; Urbani *et al.*, 1993; Ferro *et al.*, 1995; Le Questel *et al.*, 1995). The (1→3)-linkage conformations found from fiber x-ray diffraction studies of *ι*-carrageenan and *κ*-carrageenan usually appear in that low energy region of calculated potential energy surfaces containing the global energy minimum. The (1→4)-linkage conformations derived from the x-ray studies similarly occur in calculated low energy regions, but usually not the one containing the calculated global energy minimum; the (1→4)-linkage appears to have more low lying conformations potentially accessible.

The molecular modeling results have provided a set of chain conformations with which to explore the dependence of optical rotation on linkage geometry for conformations having the same chain extension as

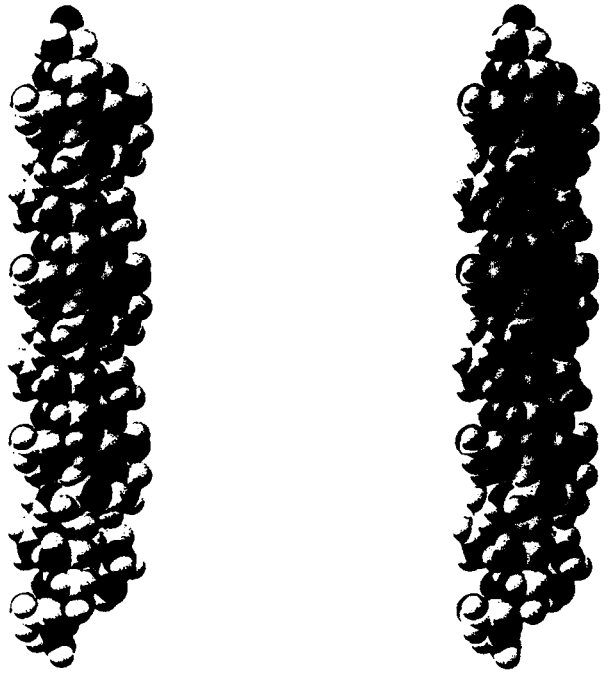

Fig. 1. Stereo view, in cross-viewing mode, of the ι-carrageenan double-helix structure derived from x-ray diffraction (Arnott *et al.*, 1974).

determined from x-ray studies, as well as its dependence on chain extension.

METHODS

We first applied the MOLROT algorithm to the chain geometry derived from the fiber x-ray diffraction pattern of ι-carrageenan (XI in Table 1). In order to explore the conformation dependence of calculated optical rotation within the range of energetically favorable conformations having the same chain extension as observed in the x-ray structure, we also examined chain structures originally reported by Anderson *et al.* (1969). (V1–V6 in Table 1). The molecular modeling calculations of Le Questel *et al.* (1995) provided coordinates for a three-fold right-handed ι-carrageenan helix having a chain extension significantly greater than observed in fibers (S in Table 1). Finally, we generated chain coordinates from the pair of energy minimized unsulfated disaccharide linkage conformations obtained by applying the molecular mechanics package SYBYL (Tripos Assoc., Inc.) to the starting geometries reflected in the x-ray structure (G in Table 1). In all examined structures, the linkage geometries represent a common pair of low energy regions of conformational energy maps. Figure 2 illustrates the S, XI and G helices.

In Table 1 the (1→4) carrabiose linkage angles are denoted as $\phi_{1\text{-}4}$ = H1—C1—O1—C4′, $\psi_{1\text{-}4}$ = C1—O1—C4′—H4′ and the (1→3) neocarrabiose linkage angles as $\phi_{1\text{-}3}$ = H1′—C1′—O1′—C3, $\psi_{1\text{-}3}$ = C1′—

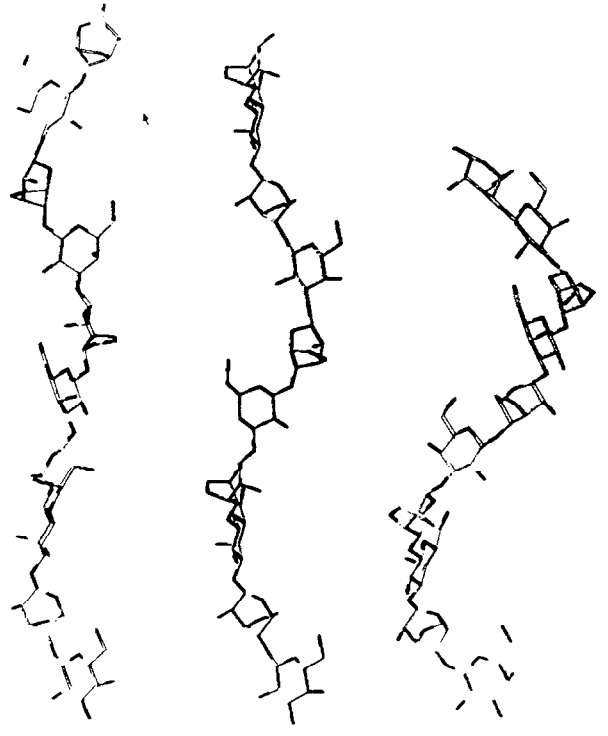

Fig. 2. Single chain representations of (unsulfated) carrageenan in the x-ray diffraction derived structure, XI (middle), the more extended helix, S (left), and the more compact helix, G (right).

Table 1. Calculated optical rotation for carrageenan conformations, per mole of disaccharide. In the gel $[M]_D^{obs} = 333°$ cm^2 dmol^{-1} (McKinnon *et al.*, 1969; Rees *et al.*, 1970)

Conformation	Chain extension (Å)	$\phi_{1\text{-}4}$ (deg)	$\Psi_{1\text{-}4}$ (deg)	$\phi_{1\text{-}3}$ (deg)	$\Psi_{1\text{-}3}$ (deg)	$[M]_D$ (°cm^2 dmol^{-1})	
						Single chain	Duplex
S	9.40	60	−30	−52	−36	3	4
XI	8.85	33	−39	−43	−41	142	178
V1	8.85	20	−58	−20	−36	124	155
V2	8.85	0	−42	−9	−44	132	165
V3	8.85	28	−40	−40	−42	136	170
V4	8.85	0	−27	−20	−50	140	175
V5	8.85	40	−29	−58	−46	152	190
V6	8.85	23	−20	−50	−49	156	195
G	7.20	50	−60	−70	−60	237	294

O1′—C3—H3. Primed atoms refer to the anhydrogalactose residue, values of zero correspond to the eclipsed conformations, and positive values indicate clockwise rotation.

Additional details of the optical rotation calculation are described in our recent, parallel study of agarose (Schafer & Stevens, 1995). As in that work, we replaced the calculated 3,6-anhydrosugar residue contribution with the observed residue contribution; MOLROT is not yet properly parameterized for the bridging C—O bonds of 3,6-anhydrosugars (Schafer *et al.*, 1995). As with agarose, a significant chain–chain contribution to rotation, resulting from the double helix, was found with carrageenan. The calculated contribution for the XI conformation is 25% (as opposed to 35% for agarose), and we use the same value for all conformations. Results are shown in Table 1.

RESULTS

Two significant observations can be made from the results of Table 1. First, for the family of conformations having the same chain extension as observed in fibers, but differing in specific linkage geometries (XI, V1–V6 in Table 1) there is a relatively small variation in calculated optical rotations. The median value (and mean) of 175° cm^2 $dmol^{-1}$ differs from the extremes no more than the estimated uncertainty in the calculation. Apparently, as long as the chain geometry is constrained to a constant chain extension, the net result of the interactions among σ–σ^* transitions along the chain do not differ in a major way.

Secondly, in contrast, there is a strong dependence of rotation on chain extension, even though the same pair of local energy minimum regions of the disaccharide energy maps is being sampled. The chain with a larger chain extension than observed in fibers (S in Table 1) displays a small rotation; the more tightly wound helix (G in Table 1) displays an increased rotation, which approaches the 333° cm^2 $dmol^{-1}$ value observed in hydrated gels (McKinnon *et al.*, 1969; Rees *et al.*, 1970). In Fig. 2, the decreased chain extension, indicated from left to right, is associated with increased optical rotation.

DISCUSSION

According to the present results, the observed gel rotation, of 333° cm^2 $dmol^{-1}$ (McKinnon *et al.*, 1969; Rees *et al.*, 1970), requires an average chain extension (7.20 Å) somewhat shorter than that found in the fibers of the x-ray experiments (8.85 Å for ι-carrageenan). A picture in which increased involvement of solvent molecules leads to reduced chain extension has support in the relative flatness of the low energy regions of calculated conformational energy surfaces, i.e., a strong solvent dependence is compatible with that flatness. A carrageenan chain extension of 7.20 Å per disaccharide is significantly larger than the 6.3 Å repeat distance of agarose gels. Indeed, while the 'grooves' of the carrageenan duplex may provide a stable environment for water molecules, the water molecules do not achieve as particularly stable an environment as in the 'core' that exists in the agarose double helix. In agarose, evidence for a tightly bound water component has been observed as a reduced uv transmission in gel films, relative to dried sols or hot-dried gels (Arndt & Stevens, 1994). In contrast, there is no evidence of such a water fraction in carrageenan films (Stevens & Morris, 1990). Nevertheless, the grooves in the carrageenan duplex are more marked at an extension of 7.20 Å than at 8.85 Å, and are, perhaps, describable as incipient cores. A reduced chain extension is thereby pictured as arising from energetically favorable water–groove interactions.

An uncertainty in the present results is the absence in MOLROT of rotation contributions from sulfate groups. Although sulfation affects the observed rotation of simple saccharides (Parra *et al.*, 1990) it is not known what part of that effect results from conformational perturbations, as distinct from electronic perturbations. Moreover, the model compound data do not support a sulfate contribution of over 150° cm^2 $dmol^{-1}$. To the extent that sulfate groups contribute to the observed rotation, it would be accounted for with chains less reduced in extension than the G conformation. Nevertheless, the large observed rotation strongly suggests that the chain conformations in the gel, while resembling those in fiber samples, have a somewhat reduced extension (Fig. 2).

The observed rotation of κ-carrageenan gels is larger in magnitude than that of ι-carrageenan gels in spite of being less sulfated. The decreased sulfation of κ-carrageenan, relative to ι-carrageenan, which results in a smaller chain extension in fibers (8.33 Å) (Millane *et al.*, 1988), similarly results, in the present interpretation, in a smaller chain extension in gels, which is directly manifested in its optical rotation.

Carrageenans display reduced optical rotation when their gels become disordered, a result which is most naturally understood in terms of an increased population of linkage geometries associated with extended chains, similar to the S conformation in Table 1, in a manner analogous to agarose (Schafer & Stevens, 1995). The present results demonstrate that the change in predominant chain conformations that occurs during the sol–gel transition in carrageenan can be approximated by the conformational conversion S → G (Table 1, Fig. 2).

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