Carbohydrate Polymers **12** (1990) 219–224

Research Note

The Vacuum Ultraviolet Circular Dichroism of Carrageenans

ABSTRACT

The vacuum ultraviolet CD of ι- and κ-carrageenan reveals a negative band at 180 nm in solid films and in aqueous solution, with and without 3 mM KCl. In the solid (film) state a positive band at 148–150 nm is also observed. The sign of the low energy (180 nm) band is consistent with a recently proposed quadrant rule.

INTRODUCTION

We have been developing means of obtaining absolute structural information for polysaccharides and monitoring their conformational interconversions using measurements of circular dichroism (CD) extended into the vacuum UV region (Stevens *et al.*, 1985; Cziner *et al.*, 1986; Morris *et al.*, 1986; Stevens, 1987). Here we report results on carrageenans which, together with agar, furcellaran and alginate, are the most commercially significant marine polysaccharides.

The carrageenans, ι- and κ- (Fig. 1), are sulfated polysaccharides based on a disaccharide repeat unit containing (1→3)-linked β-D-galactose-4-sulfate and (1→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 variations in structure include incomplete sulfation and occasional replacement of the anhydro moiety with D-galactose sulfate or disulfate, e.g. typically near 10% in ι-carrageenan. They form thermoreversible gels at relatively low concentration (~1%). The indication by optical rotation of a cooperative conformational transition (McKinnon *et al.*, 1969; Rees *et al.*, 1970; Dea *et al.*, 1972) and the observation of helical forms by fiber diffraction (Anderson *et al.*, 1969; Arnott *et al.*, 1974) have implicated helix formation as a step in the gelling mechanism. Cation and anion effects have been interpreted in terms of the further association of helices to

Carbohydrate Polymers 0144-8617/90/$03.50

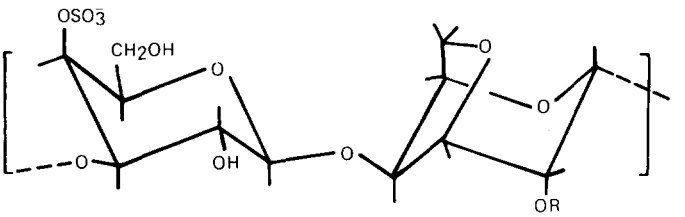

Fig. 1. Dominant repeating disaccharide unit in ι-carrageenan ($R = SO_3^-$) and κ-carrageenan ($R = H$).

form the gel network (Morris *et al.*, 1980; Smidsrød *et al.*, 1980; Smidsrød and Grasdalen, 1982; Norton *et al.*, 1984).

The present work is directed at the nature of the ordering of the polymer structure *prior* to network formation. The experimental data are analyzed in terms of a recently proposed quadrant rule (Cziner *et al.*, 1986).

EXPERIMENTAL

Materials

ι-Carrageenan (*Eucheuma spinosa*) and κ-carrageenan (*Eucheuma cottonii*) were obtained from Sigma. From carbon and sulfur analyses, the moles of sulfur per disaccharide were obtained (1·15 for κ-; 2·30 for ι-), from which average disaccharide molecular weights were calculated (397 for κ-; 488 for ι-). These values, together with the cation analyses provided by Sigma, allowed the calculation of H_2O content of the samples (19·1% for κ-; 20·5% for ι-). The total hydrogen content calculated from such an analysis agreed with the measured hydrogen (5·36% calc., 5·39% obs. for κ-; 4·59% calc., 4·46% obs. for ι-).

Solutions (1·0–4·4 mg/ml) were prepared using either D_2O or 3·0 mM KCl. Cell pathlengths were 0·100 mm or 0·050 mm. Films were made by evaporation of solutions onto CaF_2 discs in a desiccator.

Vacuum UV CD spectra

The vacuum UV CD spectrometer, described previously (Pysh, 1976), was operated with a spectral resolution of 3·2 nm, scan rate 1·0 nm/min, and time constant 100 s. The instrument was calibrated with camphor-sulfonic acid, and disaccharide molar ellipticities are reported. The

experimental uncertainty in the solution spectra is estimated to be ± 5%. Films were rotated in the light beam by increments of 90° to detect any polymer orientation in the plane perpendicular to the light beam. Variation was within the experimental uncertainty in the film spectra of ± 10% at 180 nm and ± 20% at 150 nm.

Spectra were also obtained on a CD instrument at the US National Synchrotron Light Source at Brookhaven National Laboratory, in collaboration with Dr John C. Sutherland (Sutherland *et al.*, 1986). The two instruments gave similar spectra; the signal-to-noise ratio is better with the synchrotron light source.

RESULTS

The CD spectrum of κ-carrageenan in D_2O, pD 7·0, is shown in Fig. 2. In solution a negative band centered near 180 nm appears with a molar ellipticity of $[\theta] = -7{\cdot}0 \times 10^3$ deg cm^2 dmol^{-1}. The film spectrum could be measured to 140 nm, revealing a large positive band centered near 148 nm. The ratio of the positive band to negative band intensities is approximately 3:1. Inhomogenities in film thicknesses made absolute intensity measurements impossible; the negative band intensity (Fig. 2a) was scaled to that in solution (Fig. 2b). CD spectra of κ-carrageenan obtained in 3 mM KCl solution and in films cast from that solution were qualitatively the same as those shown in Fig. 2. The results for both carrageenans are summarized in Table 1. The lower intensity of the 180 nm band in ι-carrageenan is significant but not large.

DISCUSSION

The vacuum UV CD of ι-carrageenan was reported earlier in the first application of the technique to polysaccharides (Balcerski *et al.*, 1975).

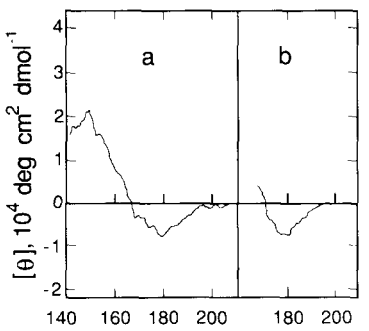

Fig. 2. Circular dichroism of κ-carrageenan: (a) film; (b) D_2O, 4·4 mg/ml, 0·100 mm pathlength. Molar ellipticity, $[\theta]$, is given per disaccharide.

Since that time, instrumentation has been improved, allowing the present measurement of solution spectra to 170 nm. The CD of the two carrageenans are qualitatively similar (Table 1), as expected from the similarity in primary structure (Fig. 1). The sign of the 180 nm band is negative, consistent with the quadrant rule proposed for that band (Cziner *et al.*, 1986).

In 3 mM KCl κ-carrageenan is only partially ordered. At ambient temperature, a higher KCl concentration results in the onset of gelation (Rinaudo and Rochas, 1981), which we aimed to avoid, and current instrumentation does not allow temperature variation. Within the uncertainty of the measurement, 3 mM KCl produced no significant intensity change in the 180 nm band. The ordered forms which exist in the films are similar by CD criteria (Table 1), in that the intensity of the 148 nm band relative to that of the 180 nm band is the same whether or not the casting solution contains 3 mM KCl.

The quadrant rule for the 180 nm band can be applied to account for the difference in intensity of that band in the two carrageenans, when both are in the random coil form (Table 1). There is no evidence that sulfate groups have any electronic effect on carbohydrate CD; e.g. chondroitin and chondroitin-6-sulfate display similar CD (Stipanovic and Stevens, 1981). However, the sulfate group at the 2-position of the anhydrogalactose residue in ι-carrageenan will have an effect on the average values of the rotation angles at the (1→3) linkage (ϕ_1, ψ_1). According to the quadrant rule these angles are important determinants of CD intensity at 180 nm.

Specifically, the perturbation of the (1→3) linkage oxygen atom by the axial O-2 atom and the ring oxygen atom of the anhydrogalactose

TABLE 1
Vacuum UV Circular Dichroism of Carrageenans[a]

Compound		λ_2 *(nm)*	$[\theta]^0_2$	λ_1 *(nm)*	$[\theta]^0_1$
ι-Carrageenan	D_2O	—	—	180	−6·4
	Film (D_2O)	148	18·3	180	−6·4[b]
κ-Carrageenan	D_2O	—	—	180	−7·0
	3 mM KCl	—	—	180	−7·3
	Film (D_2O)	148	21·8	180	−7·0[b]
	Film (3 mM KCl)	150	22·6	180	−7·3[b]

[a] $[\theta]^0$ in units of 10^3 deg cm^2 dmol^{-1}.
[b] The CD intensity of the films at 180 nm was scaled to that observed in solution.

residue contributes negative dichroism, and the magnitude of the contribution depends on ϕ_1, being maximal when ϕ_1 is approximately $-60°$. The perturbation of the $(1\rightarrow3)$ linkage oxygen atom by the axial O-4 atom of the β-D-galactose residue also contributes negative dichroism. The magnitude of that contribution depends on ψ_1 and is maximal when ψ_1 is near 0°. The 180 nm CD intensity thereby indicates that the random coil conformations of the two carrageenans have small but measurable differences in the average values of ϕ_1 and/or ψ_1 describing the $(1\rightarrow3)$ linkage. In κ-carrageenan ϕ_1 is closer to $-60°$ and/or ψ_1 is closer to 0° than in ι-carrageenan.

As locally ordered conformations become stabilized prior to the onset of gelation, either through addition of salt or upon cooling, it is possible that this difference in $(1\rightarrow3)$ linkage geometry continues to play a determining role, and may be relevant to the origin of the small but measurable difference in repeat distance in the double helices observed in fibers, i.e. 2·60 nm in ι- and 2·46 nm in κ-carrageenan (Anderson *et al.*, 1969).

ACKNOWLEDGEMENT

This work was partially supported by NIH grant GM24862. The National Synchrotron Light Source, BNL, is supported by the US Department of Energy, Division of Materials Sciences and Division of Chemical Sciences, under contract DE-AC02-76CH00016.

REFERENCES

Anderson, N. S., Dolan, T. C. S. & Rees, D. A. (1968). *J. Chem. Soc. C*, 596.

Anderson, N. S., Campbell, J. W., Harding, M. M., Rees, D. A. & Samuel, J. W. B. (1969). *J. Mol. Biol.*, **45**, 85.

Anderson, N. S., Dolan, T. C. S. & Rees, D. A. (1973). *J. Chem. Soc., Perkin Trans. 1*, 2173.

Arnott, S., Scott, W. E., Rees, D. A. & McNab, C. G. A. (1974). *J. Mol. Biol.*, **90**, 253.

Balcerski, J., Pysh (Stevens), E. S., Chen, G. C. & Yang, J. T. (1975). *J. Am. Chem. Soc.*, **97**, 6274.

Cziner, D. G., Stevens, E. S., Morris, E. R. & Rees, D. A. (1986). *J. Am. Chem. Soc.*, **107**, 2982.

Dea, I. C. M., McKinnon, A. A. & Rees, D. A. (1972). *J. Mol. Biol.*, **68**, 153.

McKinnon, A. A., Rees, D. A. & Williamson, F. B. (1969). *Chem. Commun.*, 701.

Morris, E. R., Rees, D. A. & Robinson, G. (1980). *J. Mol. Biol.*, **138**, 349.
Morris, E. R., Stevens, E. S., Frangou, S. A. & Rees, D. A. (1986). *Biopolymers*, **25**, 959.
Norton, I. T., Morris, E. R. & Rees, D. A. (1984). *Carbohydr. Res.*, **134**, 89.
Pysh (Stevens), E. S. (1976). *Ann. Rev. Biophys. Bioeng.*, **5**, 63.
Rees, D. A., Scott, W. E. & Williamson, F. B. (1970). *Nature*, **227**, 390.
Rinaudo, M. & Rochas, C. (1981). Investigation on aqueous solution properties of κ-carrageenans. In *Solution Properties of Polysaccharides*, ACS Symposium Series 150, ed. D. A. Brant. American Chemical Society, Washington, DC, chapter 25, p. 367.
Smidsrød, O. & Grasdalen, H. (1982). *Carbohydr. Polym.*, **2**, 270.
Smidsrød, O., Andersen, I.-L., Grasdalen, H., Larsen, B. & Painter, T. (1980). *Carbohydr. Res.*, **80**, C11.
Stevens, E. S. (1987). Vacuum UV circular dichroism. In *Industrial Polysaccharides*, ed. S. S. Stivala, V. Crescenzi & I. C. M. Dea. Gordon and Breach, New York, p. 255.
Stevens, E. S., Morris, E. R., Rees, D. A. & Sutherland, J. C. (1985). *J. Am. Chem. Soc.*, **107**, 2982.
Stipanovic, A. J. & Stevens, E. S. (1981). *Bipolymers*, **20**, 1565.
Sutherland, J. C., Desmond, E. J. & Takacs, P. Z. (1986). *Nucl. Instrum. Methods*, **172**, 195.

Eugene S. Stevens
Department of Chemistry,
State University of New York at Binghamton,
Binghamton, NY 13901, USA

&
Edwin R. Morris
Department of Food Research & Technology,
Cranfield Institute of Technology,
Silsoe College,
Silsoe, Bedford MK45 4DT, UK

(Received 4 October 1988; revised version received 22 January 1989; accepted 27 January 1989)