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Anhydro sugar and linkage contributions to circular dichroism of agarose and carrageenan, with conformational implications

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Abstract

The geometry-dependent linkage contributions to the circular dichroism (CD) of agarose and carrageenan were determined by subtracting the monomeric CD from the CD of the polymers. For this purpose, the CD of methyl 3,6-anhydro-α-D-galactopyranoside, and of other anhydro sugars, was measured. Application of empirical quadrant rules indicates the observed CD to be that expected for the helical conformations of agarose and carrageenan as derived from diffraction data and modeling calculations. The difference in CD sign in the two polymers arises from a translocation of the β-D-galactose O-2 atom from one quadrant to the neighboring quadrant of the C-5–O-5–C-1 ether chromophore of the preceding anhydro sugar residue, demonstrating the unusually high spatial resolution of conformational analysis that can be achieved with CD under favorable circumstances. © 1997 Elsevier Science Ltd.

Keywords: Circular dichroism; Agarose; Carrageenan; Anhydro sugars

1. Introduction

Carbohydrate conformational analysis based on circular dichroism (CD) has particular strengths, which complement other methods [1–5]. CD can be measured with both solution and solid (film) samples, which allows reference to solid-state structures determined by diffraction methods, and allows conformational differences in the two phases to be displayed. The time scale of CD is fast compared to molecular motion, so that conformational information need not be deconvoluted from dynamic contributions as is the case with NMR. CD is equally applicable to polysaccharides and monosaccharides, allowing the observed linkage contributions (defined as the difference between polymer and monomer CD) to be interpreted in terms of empirical sector rules.

In the present work we have determined linkage CD contributions in two algal polysaccharides, agarose and carrageenan, [6–8] (Fig. 1), by measuring the CD of a constituent monomer, 3,6-anhydro-α-D-galactopyranose. The CD of the alternate monomer, β-D-galactopyranose, and of the polymers have been reported previously [3,9–12]. Analysis of the linkage CD contributions reveals distinct conformational preferences in the chains of both algal polysaccharides. For comparison, we also measured the CD of 1,6-anhydro-β-D-glucopyranose and 1,6-anhydro-β-D-mannopyranose. The anhydro bridge imparts rigid-

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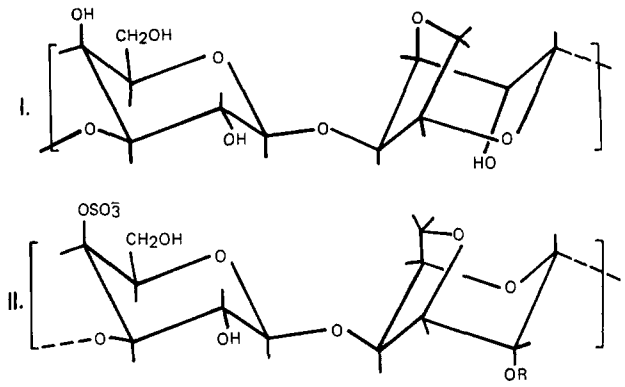

Fig. 1. I. Agarose [→ 3)-β-D-Gal*p*-(1 → 4)-3,6-anhydro-α-L-Gal*p*-(1 →]. II. Carrageenan [→ 3)-β-D-Gal*p*-4-OSO_3^--(1 → 4)-3,6-anhydro-α-D-Gal*p*-(1 →]. R = H in κ-carrageenan, SO_3^- in ι-carrageenan.

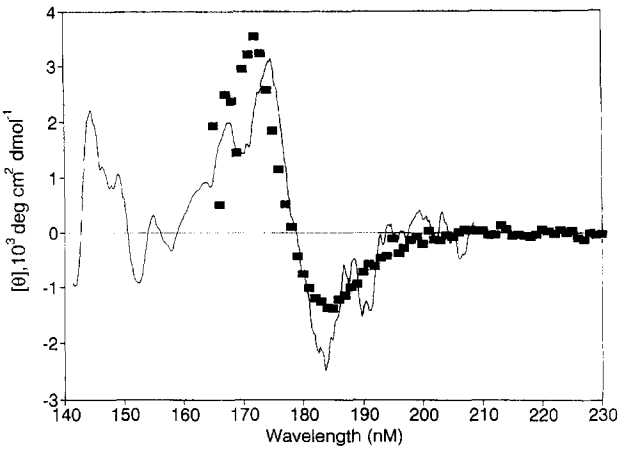

Fig. 3. CD of 1,6-anhydro-β-D-glucopyranose solution (dark curve) and film (light curve).

ity to the sugar ring, which allows a rigorous determination of solution–solid structural equivalence.

2. Results

Figs. 2–4 show the solution CD (dark curves) and film CD (light curves) of methyl 3,6-anhydro-α-D-galactopyranoside, 1,6-anhydro-β-D-glucopyranose, and 1,6-anhydro-β-D-mannopyranose, respectively. In all cases the solution spectra display larger signal-to-noise ratios than the film spectra in the overlapping wavelength region, because of the much smaller effective pathlengths in the film samples (see Experimental section). Thicker film samples, which would have resulted in larger signal-to-noise ratios in the long-wavelength region, consistently displayed birefringence artefacts. Because of the short effective pathlength of the films, and the resulting low signal-to-noise ratios, significance cannot be attributed to the variations in the film CD that occur over a wavelength range less than ~ 10 nm. That variation must be ascribed to random noise fluctuations.

The solution spectrum of 1,6-anhydro-β-D-glucopyranose obtained with synchrotron radiation (Fig. 3, dark curve) shows a particularly large signal-to-noise ratio, a result of the intensity of the synchrotron light source. In that case it was also possible to record the CD to a shorter wavelength (165 nm) than achieved with a conventional deuterium lamp (173 nm). Within the limits set by noise considerations, the film CD and solution CD appear to be similar for all three anhydro sugars. The similarity is significant particularly in light of the qualitative differences in CD arising from the variation in chemical structure.

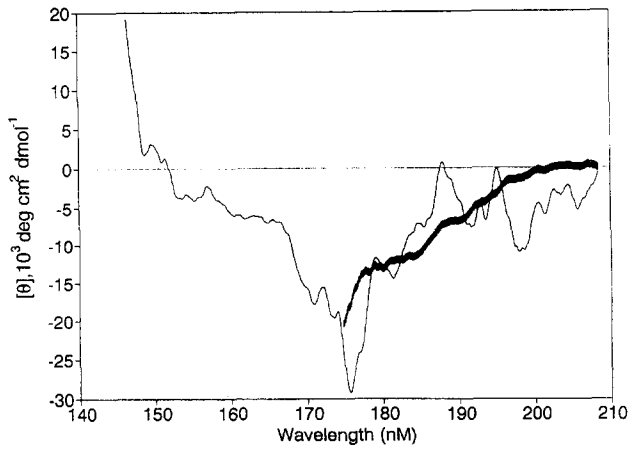

Fig. 2. CD of methyl 3,6-anhydro-α-D-galactopyranoside solution (dark curve) and film (light curve).

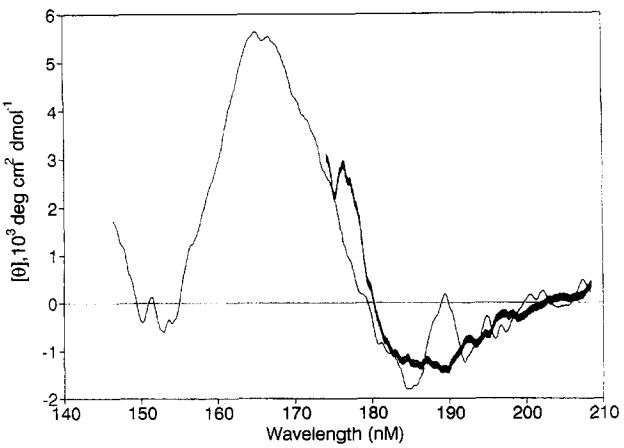

Fig. 4. CD of 1,6-anhydro-β-D-mannopyranose solution (dark curve) and film (light curve).

3. Discussion

Agarose.—The CD of 3,6-anhydro-α-L-galactopyranose (the mirror image of the CD shown in Fig. 2) can be combined with the CD of methyl β-D-galactopyranoside [3] to produce the CD expected for agarose in a 'noninteracting residue model'. Any difference between such a summed spectrum and the observed polysaccharide spectrum represents additional CD contributions arising from the geometry-dependent linkage contributions. Small linkage CD occurs when contributions average to nearly zero, as in particularly flexible chains. They are also small in preferred linkage geometries, if those linkage geometries do not have perturbing groups which break the local planar symmetry of the C–O–C ether chromophores responsible for simple saccharide CD above 150 nm [4]. Highly extended chains may, for example, have small linkage contributions.

Fig. 5 shows the CD of agarose expected in the 'noninteracting residue model' (dark solid curve). The CD of agarose observed in solution at high temperatures, and in dried solutions, is also shown (light curve) as well as the CD of dried agarose gels (■) [3,9,10]. The solution CD is identical within experimental error to the summed monomer CD with respect to shape and intensity; there is no evidence of a significant linkage CD contribution.

The gel CD, on the other hand, displays a large negative CD band near 155 nm, not expected on the basis of the simple summation of monomer spectra. The magnitude of the negative linkage CD contribution in the agarose gel is $\sim 1.3 \times 10^4$ deg cm^2 dmol^{-1}. This large difference between the linkage contribution in the agarose gel, and that in the agarose solution can be attributed to a difference in the local linkage geometries.

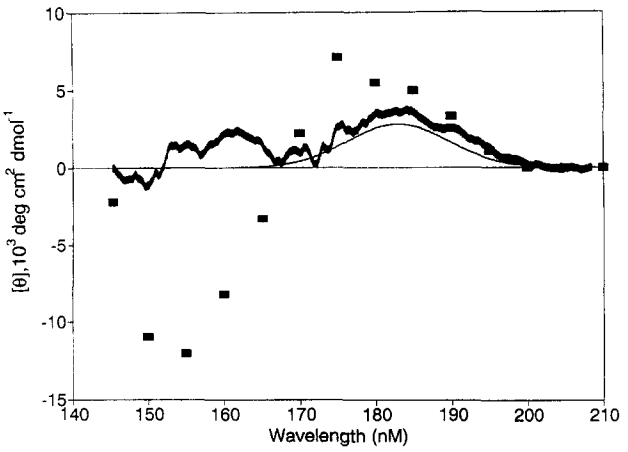

Fig. 5. Agarose CD observed in the gel (■) and in solution (light curve) [1,9,10], and the simple sum of monomer CD (dark solid curve). The gel, but not the solution, shows large linkage CD contributions (see text).

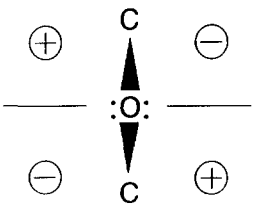

Fig. 6. CD quadrant rule for the 150–155 nm region in simple sugars [2–4].

In order to relate the observed linkage CD contribution to a specific linkage geometry, putative geometries can be examined with the aid of CD quadrant rules. Fig. 6 illustrates an empirical quadrant rule operative in the 155-nm region of the CD spectrum [3,4]. If a perturbing group lies in one or another of the four quadrants defined by the two symmetry planes of the linkage C–O–C chromophore, it contributes CD of the indicated sign. The quadrant rule is also applied, in separate examinations, to the ring C–O–C chromophores.

In the case of agarose, a wide helix of short extension, capable of intertwining to form double helices, has been observed in modeling studies to be energetically stable (helix 3, Table 5, ref. [13]) and also to satisfy the geometrical requirements determined from X-ray diffraction studies of oriented fibers [14–16]. That proposed helix geometry is compatible with the CD of the gel (Fig. 5, ■). Specifically, O-2 of the galactose residue breaks the symmetry of the ring ether chromophore of the preceding anhydro sugar residue, across the (1 → 3) linkage, and lies in a negative quadrant of that chromophore.

The much smaller linkage CD contribution in solutions and dried solutions (Fig. 5, light curve) may be the simple consequence of the highly extended chain geometries observed in agarose solutions with X-ray diffraction [14] and small-angle neutron scattering [17,18].

Carrageenan.—Fig. 7 shows the CD of 'noninteracting' carrageenan residues (dark solid curve), i.e. the summation of the CD of 3,6-anhydro-α-D-galactopyranose (Fig. 2) and that of methyl β-D-galactopyranoside [3]. The CD spectra of partially ordered carrageenan in solution (▲) and fully ordered as a film (■) [11,12], which are similar to one another, are also shown. The polymer CD contains a

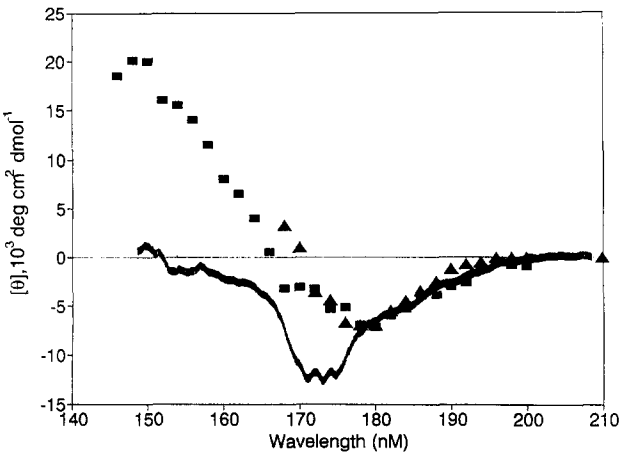

Fig. 7. Carrageenan CD observed in the gel (■) (fully ordered) and in solution (▲) (partially ordered) [11,12], and the simple sum of monomer CD (dark solid curve). Ordered carrageenan shows large linkage contributions.

large positive linkage contribution below 180 nm. In the region near 150 nm the linkage CD is $\sim 2.0 \times 10^4$ deg cm^2 dmol^{-1}. The similarity of the solution CD to the film CD suggests that the same conformation predominates in both phases, under the specified experimental conditions.

The large linkage CD contribution arises, as in agarose, from perturbations which break the symmetry of ether chromophores. In carrageenan, a helical structure is observed in X-ray diffraction studies of films [19], and it has been proposed that the same or a similar helical structure persists in solution. Modeling studies have demonstrated the stability of such conformations [20]. The X-ray structure, examined in terms of the CD quadrant rule (Fig. 6) is found to be compatible with the observed linkage CD. Specifically, O-2 of the galactose residue breaks the symmetry of the preceding anhydro sugar's ring ether chromophore across the (1 → 3) linkage, as in agarose, but in carrageenan the atom lies in a positive quadrant of that chromophore.

The helical structures derived from diffraction studies of agarose and carrageenan have been compared previously [16]. The occurrence of the anhydro L-sugar in agarose and anhydro D-sugar in carrageenan does not mean, of course, that their helices are mirror images. Nor are their disaccharide repeat-distances along the helix axis similar; the carrageenan helix is much more extended than the agarose helix. Nevertheless, the helix sense is opposite in the two, left in agarose and right in carrageenan. The present work indicates that the difference in helix sense is sufficient to cause their CD spectra to be approximate inversions of each other; with the left-handed helix of agarose displaying predominantly negative CD and the right-handed helix of carrageenan displaying predominantly positive CD.

Anhydro sugars.—CD spectra of anhydro sugars have not been previously reported. Several features displayed in Figs. 2–4, therefore, are worth noting. For the three anhydro sugars, the film spectrum uniformly covers that of the solution over the entire overlapping wavelength range, to within experimental uncertainty. Solid-state–solution conformational equivalence in anhydro sugars is supported by a large amount of theoretical and experimental data (e.g., refs [21,22]) and is not unexpected on the basis of chemical structure. What is newly noteworthy is that conformational equivalence in the two phases is clearly reflected in CD equivalence.

In the 150–200 nm region, the anhydro sugar CD bears some resemblance to the CD of the corresponding methyl pyranoside. Superposition of the CD of 1,6-anhydro-β-D-glucopyranose (Fig. 3) with that of methyl β-D-glucopyranoside film [3] (not shown) reveals the resemblance clearly; with a 7.5-nm redshift of the methyl pyranoside CD the similarity becomes more striking (Fig. 8). The CD of methyl α-D-galactopyranoside [3], like that of methyl 3,6-anhydro-α-D-galactopyranoside (Fig. 2) is predominantly negative, with further suggestion of a redshift in the anhydro sugar CD. In heat-dried agarose gels [1] the CD is well represented by a single gaussian band centered at 189 nm, which does not match the wavelength associated with the lowest-energy CD band in simple pyranosides. The present work shows its origin to be the anhydro sugar residue.

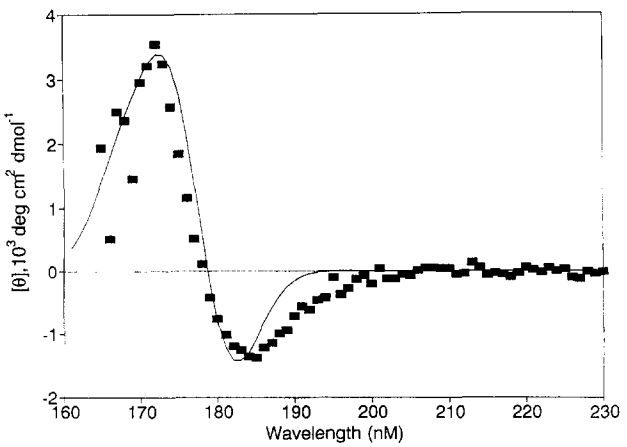

Fig. 8. CD of 1,6-anhydro-β-D-glucopyranose solution (Brookhaven data), with fit spectrum of methyl β-D-glucopyranoside film [3], redshifted 7.5 nm (see text).

4. Experimental

Methyl 3,6-anhydro-α-D-galactopyranoside (V-labs, Inc., Covington, LA 70433) solns were prepared in D_2O (99.9 atom%, Aldrich Chemical, Milwaukee, WI 53223) at a concn of 5.08 mg/mL. 1,6-Anhydro-β-D-glucopyranose (Toronto Research Chemicals, Inc., Downsview, Ontario, Canada M3J 2C3; lot #156-01-c3-9207) solns were prepared at concns of 7.55 and 4.98 mg/mL. 1,6-Anhydro-β-D-mannopyranose (Toronto Research Chemicals, Inc.; lot #15-IG-146) solns were prepared at a concn of 5.71 mg/mL.

Commercial quartz cells (Hellma) of 24-, 50-, 100-, and 200-μm thickness were used for the soln spectra. The thicker cells were used until solvent absorption required a smaller pathlength. This procedure optimizes the signal-to-noise ratio in the long-wavelength region. Results are reported in units of molar ellipticity.

Films were cast on CaF_2 from 4.0 mg/mL trifluorethanol (Aldrich Chemical Co., 99.5%) solns using techniques described previously [3]. They were screened against birefringence by noting invariance of CD upon a 90° rotation of the sample.

The difficulty in obtaining birefringence-free films precluded the use of a series of films of variable thickness, a procedure which would have allowed optimization of the signal-to-noise ratio in the long-wavelength region (as with solns). Film data were therefore obtained only on very thin films, which allowed measurements to approach the instrument wavelength limit. For this reason, film spectra display a much smaller signal-to-noise ratio in the long-wavelength region than do the soln spectra. The difference between the signal-to-noise ratios in the soln spectra and those in the film spectra does not reflect a difference in ellipticity-to-absorption ratio; there was no evidence of a difference in that ratio between soln and film samples. The ellipticities of the films were ~ 80% of the ellipticities of 4 mg/mL solns measured with 24-μm cells, i.e. ~ 3–4 mdeg.

Moreover, because of uncontrollable film thickness inhomogeneities, it was not possible to determine film pathlengths. However, the film spectra had band shapes identical to the corresponding soln spectra in the overlapping wavelength region. Film CD intensities were therefore scaled to the soln CD, using the entire range of overlapping wavelengths to determine the appropriate scale factor. Scaling the film spectra to overlay the soln spectra required a scale factor of ~ 1.2, further indication that the ellipticity to absorption ratio is not much different in the two phases.

The vacuum ultraviolet CD spectrometer has been previously described in detail [23]. It was used with a deuterium lamp, a spectral resolution of 3.2 nm, a scan rate of 0.5 nm/min, and a time constant of 300 s. Calibration was carried out with (+)-10-camphorsulfonic acid. The estimated uncertainty is 0.5 mdeg. Spectra were recorded with a Keithly model 575 digitizer controlled with a PC. Up to 11 spectra were averaged, then further smoothed using a 1.5-nm interval to produce the reported spectra. Some spectra reported earlier [3] were averaged over a 3-nm interval. The difference is only one of presentation.

The CD of a 1,6-anhydro-β-D-glucopyranose soln (7.55 mg/mL, 0.050 mm pathlength) was also measured at port U9B of the National Synchrotron Light Source, Brookhaven National Laboratory, with the cooperation of Dr. John Sutherland. The intense light source leads to improved signal-to-noise ratios.

Acknowledgements

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