Characterization of Polysaccharide Structure and Interactions by Circular Dichroism: Order–Disorder Transition in the Calcium Alginate System

By EDWIN R. MORRIS, DAVID A. REES,* and DAVID THOM
(*Unilever Research Colworth/Welwyn Laboratory, Colworth House, Sharnbrook, Bedford*)

Summary Circular dichroism evidence is given for the involvement of carboxylate *n* orbitals of contiguous α-L-guluronate residues, in specific binding of Ca^{2+} which occurs co-operatively with the sol → gel transition for alginate.

THE sign of the $n \rightarrow \pi^*$ band in the c.d. spectrum of uronic acid glycosides and their salts depends mainly on the absolute configuration at C-5.[1] We now show that the form of this band has great scope for the characterization of polysaccharide structure and behaviour.

Firstly, it provides a simple method for estimating the composition of alginates, which are built up from two such residues of opposite configuration (β-D-mannuronate and α-L-guluronate; see Figure 1). Second-order influences allow residue sequence to be determined by computer matching of observed alginate spectra with mixtures of spectra of the constituent sequences, which are[2] poly-(α-L-guluronate), poly-(β-D-mannuronate), and poly-(β-D-mannosyluronate α-L-guluronate). Finally, the controlled introduction of Ca^{2+} causes (Figure 2) the solution to gel and the spectrum to change dramatically giving rise to a mathematically gaussian difference band confined to the $n \rightarrow \pi^*$ region alone. Optical density measurements show no concomitant increase, which together with the selectivity, argues that the change is not merely due to distortion or scattering.

The difference band increases with the proportion of poly-(α-L-guluronate), and we therefore suggest that *n*-orbitals in these sequences are perturbed by the proximity of specifically bound Ca^{2+}. Physical and mathematical model building show a likely co-ordination site in the same region of symmetry space as O-4 and O-5, which normally determine the sign of the band.[1] Induction by the positive ion of a more pronounced band of opposite sign is consistent with established effects[3] for the related but better understood ketone transition, further supporting our interpretation. The magnitude of the spectral change and specific involvement of poly-(α-L-guluronate) sequences indicate that three-dimensional gel network formation with Ca^{2+} involves co-operative interactions between "blocks" of guluronate residues rather than "point cross-linking" as has often been suggested previously.[4] This interpretation accounts for observed variations in Ca^{2+} binding affinities with alginate composition.[5] Similar, but less detailed conclusions, were indicated[6] by the timescale of elastic properties and the variation in gel stiffness with guluronate content,[7] and reinterpretation of existing data for competitive binding of Ca^{2+} and Na^{+} provides further evidence of co-operativity.[8]†

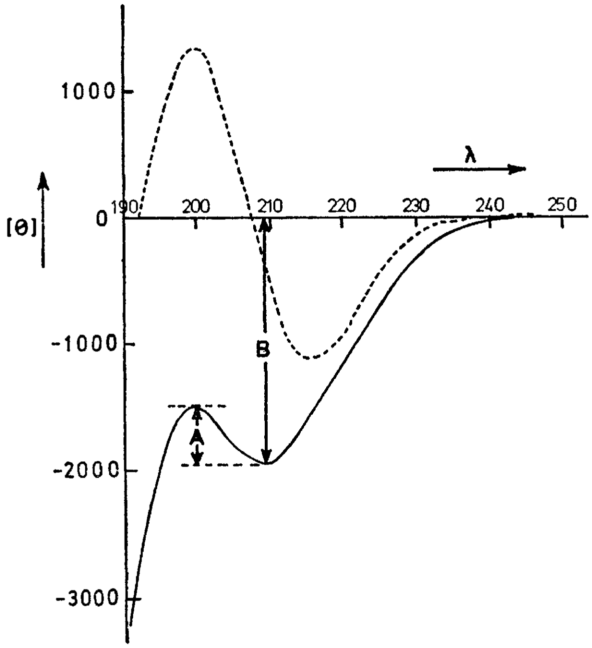

FIGURE 1. *C.d. spectra for poly-(β-D-mannuronate)* (– – – – –) *and for an alginate having mannuronate and guluronate residues in the ratio* 29:71 *as shown by chemical analysis. There is a direct correlation between the ratio of the two residues and the ratio of the increments marked as* A *and* B. $[\theta]$ = *molecular ellipticity* (degree cm^2 per dmol) *and* λ = *wavelength* (nm).

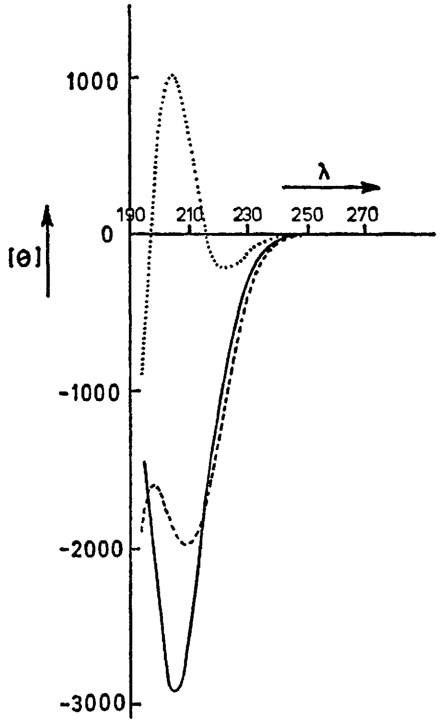

FIGURE 2. *Changes in c.d. spectrum with the diffusion of* Ca^{2+} *to a final concentration of* 6 mM *into a solution of alginate* (0·1%) *having mannuronate and guluronate residues in the ratio* 29:71. *Broken and dotted lines show spectra before and after this process and the full line shows the difference spectrum. Units of* $[\theta]$ *and* λ *as in Figure* 1.

This model explains the faster gelation[7b] of alginates of lower guluronate content, since when fewer binding regions exist they restrict each other less as the network develops. More spectacularly, it predicts the recently reported variation[9] in stability constants of Ca^{2+} complexes of uronate oligomers.

D.T. acknowledges the award of an S.R.C. Studentship, and is on leave from the University of Edinburgh.

(*Received*, 9*th February* 1973; *Com.* 181.)

---

† The details of this reinterpretation will be reported, with new measurements, when our work is published in full. However, the experimental points (though not the curve drawn through them) in Figure 5 on p. 45 of ref. 9 show a distinct stepwise increase in Ca^{2+} binding in competition with Na^{+}, when [Ca^{2+}] is held at 6 mM and the [Na^{+}] is decreased from 200 to 60 mM; this is characteristic of a co-operative mechanism.

[1] I. Listowsky, S. Englard, and G. Avigad, *Biochemistry*, 1969, **8**, 1781.

[2] A. Haug, B. Larsen, and O. Smidrød, *Acta Chem. Scand.*, 1966, **20**, 183; 1967, **21**, 691; A. Haug, S. Myklestad, B. Larsen, and O Smidsrød, *ibid.*, p. 768.

[3] L. Bartlett, D. N. Kirk, W. Klyne, S. R. Wallis, H. Erdtman, and S. Thoren, *J. Chem. Soc.* (*C*), 1970, 2678.

[4] *E.g.*, R. G. Schweiger, *Kolloid Z.*, 1964, **196**, 47.

[5] R. Kohn, I. Furda, A. Haug, and O. Smidsrød, *Acta Chem. Scand.*, 1968, **22**, 3098.

[6] D. A. Rees, *Adv. Carbohydrate Chem. Biochem.*, 1969, **24**, 267.

[7] (a) O. Smidsrød and A. Haug, *Acta Chem. Scand.*, 1972, **26**, 79; (b) J. B. Hutchins and F. W. Wood, unpublished observations.

[8] T. E. Brunelle, Ph.D. Thesis, University of Minnesota Minneapolis, Minnesota, 1968. (University Microfilms, Ann Arbor, Michigan, No. 69–11373).

[9] R. Kohn and B. Larsen, *Acta Chem. Scand.*, 1972, **26**, 2455.