BIOLOGICAL INTERACTIONS BETWEEN POLYSACCHARIDES AND DIVALENT CATIONS: THE EGG-BOX MODEL

Gregor T. GRANT *, Edwin R. MORRIS, David A. REES, Peter J.C. SMITH * and David THOM *

Unilever Research Colworth/Welwyn Laboratory, Colworth House, Sharnbrook, Bedford, MK44 1LQ, UK

Received 14 February 1973

We have shown that specific binding of divalent cations to a polysaccharide polyelectrolyte, leading firm cohesion between the chains, can cause characteristic effects in the circular dichroism spectrum which are understandable in terms of modern theory [1]. For alginate, this binding is a co-operative process that predominantly involves consecutive guluronate residues. Some other systems have now been investigated in an attempt to formulate a general interpretation of biological phenomena of this type. The known strength and specificity of complexation are explained in terms of an "egg-box model" which is derived from our measurements, the known coordination geometries in model compounds, and the requirements for cooperativity.

In pectin, the methyl ester of poly(galacturonic acid), the interactions with cations is relatively weak because the chains are uncharged. In contrast to the behaviour of alginates, in which the amplitude diminishes with gelation [1], the broad, positive, $n \rightarrow \pi^*$ band in the circular dichroism spectrum of the sol increases in amplitude when chains associate to form a gel (fig. 1). No such large change occurs in solutions of the polysaccharide which do not gel over this temperature range. Our interpretation is therefore that, in the sol, the methyloxycarbonyl substituent is distributed between a number of rotational states about C(5)–C(6) which, due to the different orientations of the carboxyl chromophore in the asymmetric environment of the sugar ring, have $n \rightarrow \pi^*$ bands of varying amplitude, some perhaps even having negative amplitude. As in the alginate system [1,2] this equilibrium is shifted to a much narrower distribution in the observed association that forms. This causes an increase in optical activity in the manner frequently associated with locking of conformation [3]. Cations are absent, however, and their characteristic influence on the π orbitals is therefore not seen.

After saponification, the polysaccharide binds Ca^{2+} with gel formation and with a large decrease in amplitude of the $n \rightarrow \pi^*$ band (fig. 1) which shows a gaussian difference spectrum centered on 208 nm. This is therefore [1] explained as a specific binding of most of the uronate residues to Ca^{2+} which tends to reverse the sign of the $n \rightarrow \pi^*$ band. Derivatives that are only partly saponified, as in "low methoxy pectins" used industrially and present in biological systems, show similar spectroscopic changes during gelation with Ca^{2+}, except that an additional peak remains with the position (210 nm) and amplitude expected for methyl galacturonate residues. Being uncharged these groups would not participate directly in ion binding, and the residual peak therefore confirms our view that the extreme spectral changes arise from specific perturbation of bound residues by proximity of the ionic charge.

Polymannuronate as well as polyguluronate and polygalacturonate sequences can be perturbed spectroscopically by Ca^{2+} binding, as shown by comparison of circular dichroism at various time intervals during the diffusion of Ca^{2+} into a solution of alginate that is rich in mannuronate residues. The $n \rightarrow \pi^*$ maxi-

* On leave of absence from the University of Edinburgh.

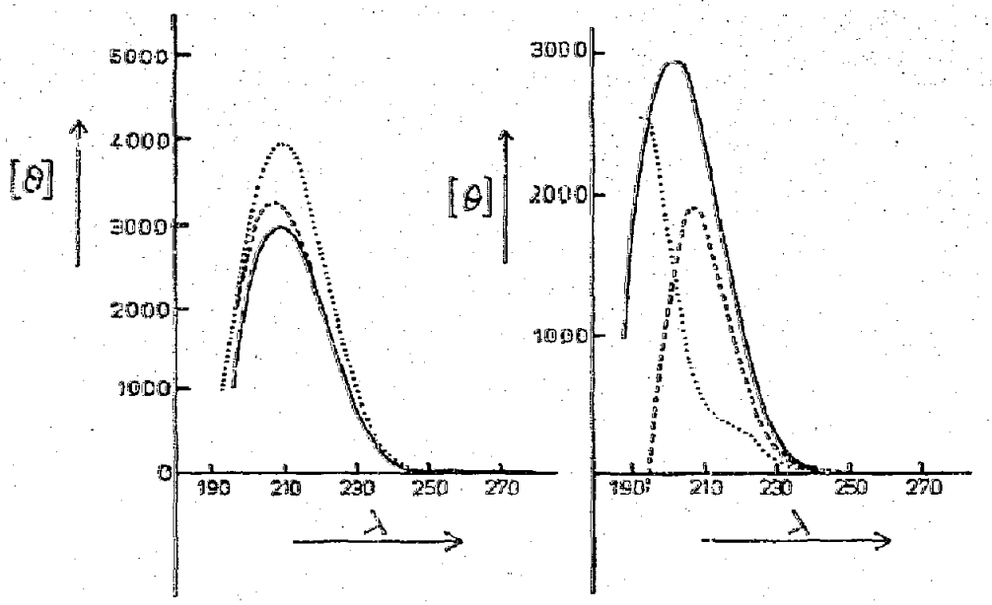


Fig. 1. Changes in circular dichroism spectra in solutions and gels of pectins. Left: poly(methyl galacturonate) in solution (0.5%) in aqueous ethylene glycol (3:7, v/v) at 90° (——) and in a gel in the same solvent but at 25° (·····). When a solution in water having the same amplitude as the former at 90° is cooled to 25°, the spectrum is as shown by the broken line. Right: sodium polygalacturonate (0.5%) in water, before (——) and after (·····) diffusion of Ca^{2+} to a concentration of 12 mM; the difference spectrum is also shown (- - - - -). [θ] = molecular ellipticity (degree-cm^2 per decimole) and λ = wavelength.

mum is initially negative because polyguluronate blocks dominate the spectrum, but this changes until it is eventually positive as a result of binding by these blocks [1]. In a subsequent stage, however, the spectrum swings back to become less positive (fig. 2). Our interpretation is that the binding of Ca^{2+} by polyguluronate proceeds with the characteristic spectroscopic change until the available binding sites are saturated and the concentration of Ca^{2+} is able to rise. The threshold is then passed for binding by polymannuronate to cause a shift in the negative direction, which stops when chain-association is constrained by the network. Similar effects are observed when Sr^{2+} is used instead of Ca^{2+} but the swing proceeds further in the positive direction before it reverses, as expected from the higher selectivity of polyguluronate for Sr^{2+} [4]. Although the interactions of alginate with Ca^{2+} are indeed dominated by polyguluronate sequences [1], it would appear that the polymannuronate sequences can also play a role.

Three main factors must be considered in attempting to understand the binding of cations by these polyuronates [5], namely the geometry of the ligand (i.e., the intramolecular stereochemistry), the separation between unit charges on the chain (i.e., the polyelectrolyte effect), and when chain-association is part of

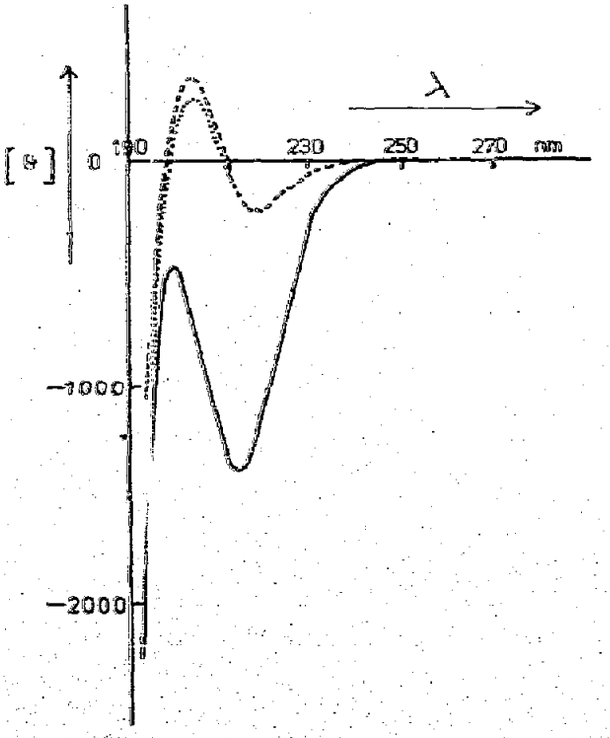
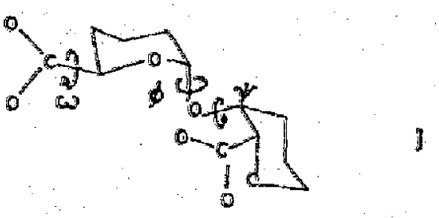


Fig. 1. Changes in circular dichroism spectrum with the diffusion of Ca^{2+} to a final conc. of 6 mM into a solution of alginate (0.1%) having mannuronate and guluronate residues in the ratio 57:43. The spectrum is shown for the solution (——) and for the gel at intermediate (- - -) and final (· · ·) stages of formation. Units of [θ] and λ as for fig. 1.

the binding process, the ease with which the polysaccharide chains can pack. It has now been confirmed that the stability constants of the Ca^{2+} complexes increase with chain length in all three series – oligogalacturonate, oligoguluronate, and oligomannuronate [6,7], and that the effect is more pronounced for the first two. This was expected because the electrostatic free energy must increase with the number of residues and be greater for the two axial–axial linked series which have a smaller separation between charges. In the guluronate series there is a second sharp increase between chain length 18–26 which gives the graph a two-step appearance. This "second step" must represent the onset of the process that we have seen by circular dichroism spectroscopy, namely an enhanced binding when the chain length is long enough for the cooperative mechanism with chain-association. A similar pattern has recently been shown for the galacturonate series [8], confirming expectations based on the close similarity between stability constants for long [4] and short [6,7] chains in the two series. There is no evidence from these measurements of such cooperative association in the mannuronate case, but our observations by circular dichroism would suggest that under conditions of high Ca^{2+} concentration a "second step" would be observed. We have no evidence, however, of similar behaviour in the oligo(β-D-mannuronosyluronate-α-L-guluronate) series.

Using the criterion for optimum binding that a Ca–O(6) distance of 2.6 ± 0.2 Å is required, with as many as possible other Ca–O distances of 2.7 ± 0.3 Å, we have attempted to further interpret this ion-binding by computer model building. The Reeves C1 ring form was used in the calculations for D-mannuronic and D-galacturonic acids, and the corresponding 1C ring form for L-guluronic residues [9]. The angles φ, ψ and ω (see I) were varied systematically over reasonable ranges, and at each stage the likelihood of ion-binding was assessed in terms of the above criterion. Since we have demonstrated that ordered chain associations are involved, only those conformations having integral screw symmetry are relevant to this binding. In this way, polyguluronate was shown to form chains with two- or three-fold screw symmetry which offered four-oxygen coordination involving O(6) and in most cases O(5), with O(2) and O(3) on the next residue in the "non-reducing" direction. This is in agreement with the experimental observation that the crystal con-

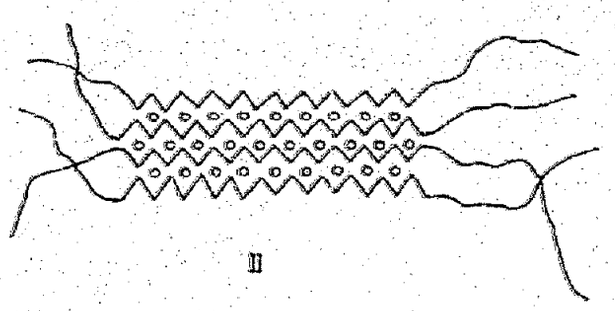


I

formation of calcium polyguluronate is two-fold [10]. There are fewer coordination possibilities for polygalacturonate, most of which involve only three oxygens, and two-fold symmetry would seem to be preferred. Therefore we would expect polyguluronate to form the stronger complexes, and indeed strontium polyguluronate is much more stable than any known complex of polygalacturonate [4]. Moreover, the calculations show that coordinating oxygen atoms are more widely spaced for polyguluronate (typically 3.8 Å rather than 3.4 Å); we suggest that this explains why polyguluronate shows strong preference for the large Sr^{2+} ion rather than Ca^{2+}, whereas polygalacturonate does not [4].

Polymannuronate chains offer sites with both two- and three-fold symmetry and in fact calcium polymannuronate crystallizes with three-fold screw symmetry [10]. Inspection of models, however, shows that the eq–eq linkage, as opposed to the ax–ax linkages in polyguluronate and polygalacturonate, leads to a much flatter structure with more shallow 'nests' for the cations to occupy. This would explain the inability of such chains to complex except at higher ion concentrations. The chains of alternating mannuronate and guluronate having integral screw symmetry (mainly four-fold) did not offer suitable sites for complexation.

Our conclusions and proposals are therefore summarised in terms of an "egg-box model" for the cooperative mechanism of binding involving two or more chains, as shown schematically in II. The buckled chain is shown as a two-dimensional analogue of a corrugated egg-box with interstices in which the cations may pack and be coordinated. When the chain has three-fold screw symmetry, assembly is of course likely in three dimensions rather than two as in our simplified model. Chains with two-fold screw symmetry could conceivably form sheet-like aggregates but may well also associate in the third dimension. The analogy is that the strength and selectivity of cooperative bind-



II

ing is determined by the comfort with which "eggs" of the particular size may pack in the "box", and with which the layers of the box pack with each other around the eggs. The nest-like sites are also likely to be relevant to the statistical ion-binding that occurs below the threshold for the cooperative process but we do not suggest that their geometry is then of overriding importance.

Acknowledgements

Two of us (G.T. Grant and P.J.C. Smith) would like to thank Unilever Research for the provision of research grants and one of us (D. Thom) acknowledges the award of an SCR Studentship. We thank Dr. R. Kohn and Dr. R.J.P. Williams, FRS for helpful discussions.

References

[1] E.R. Morris, D.A. Rees and D. Thom, Chem. Commun. (1973) 245.
[2] D.A. Rees, Advan. Carbohyd. Chem. Biochem. 24 (1969) 267.
[3] W. Kauzmann and H. Eyring, J. Chem. Phys. 9 (1941) 41;
W. Kauzmann, F.B. Clough and I. Tobias, Tetrahedron 13 (1961) 57.
[4] A. Haug and O. Smidsrød, Acta Chem. Scand. 24 (1970) 843.
[5] R. Kohn and I. Furda, Collect. Czech. Chem. Commun. 33 (1968) 2217;
D.A. Rees, Biochem. J. 126 (1972) 257.
[6] R. Kohn and B. Larsen, Acta Chem. Scand. 26 (1972) 2455.
[7] R. Kohn, Carbohyd. Res. 20 (1971) 351.
[8] R. Kohn, personal communication.
[9] E.D.T. Atkins, W. Mackie and E.F. Smolko, J. Polymer Sci. Part B. 9 (1971) 311;
A. Penman and G.R. Sanderson, Carbohyd. Res. 25 (1972) 273;
D.A. Rees and A.W. Wight, J. Chem. Soc. B. (1971) 1366.
[10] W. Mackie, Biochem. J. 125 (1971) 89P.
[11] A. Haug and O. Smidsrød, Acta Chem. Scand. 24 (1970) 843.