# Spectroscopic and Stoicheiometric Characterisation of the Calcium-mediated Association of Pectate Chains in Gels and in the Solid State

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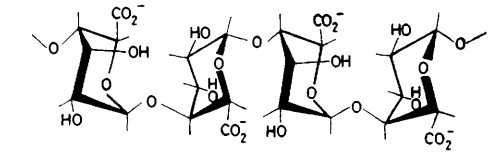
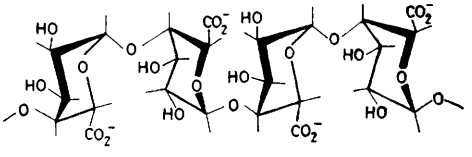
*Summary* Circular dichroism and stoicheiometric evidence is presented, which indicates that the calcium-mediated association of pectate chains under hydrated conditions occurs by a pairwise mechanism in which the chains adopt two fold symmetry; this contrasts with the known three-fold conformation into which the chains convert in the solid state, and represents an unusual example of an ordered polysaccharide tertiary structure which is stabilised by high levels of hydration.

THE primary mode of interchain association in calcium alginate gels is by dimerisation[1] of poly-L-guluronate chain sequences (**1**) in a regular, buckled, two-fold conformation, with interchain chelation of cations on specific binding sites along each chain. This is illustrated schematically in structure (**3**), and for obvious reasons is known as the 'egg-box' model.[2] The basic poly-D-galacturonate structure of pectin (**2**) shows close structural analogy to (**1**), being the exact mirror image other than at C(3), and we might therefore expect similar cation binding behaviour. The *X*-ray diffraction evidence for various pectin derivatives[3–5] including recent evidence for the calcium salt,[6] however, has always shown three-fold symmetry for the condensed

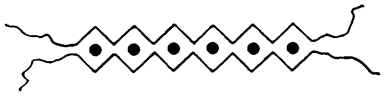


(1)

Mirror plane



(2)



(3)

phase. The crystal structure of a model disaccharide[7] also suggests that the conformation angles in the three-fold form are stable in the solid state. All this is in contrast with the two-fold symmetry of poly-L-guluronic acid[8] and many of its salts,[9] in the solid state as well as in calcium gels. We have therefore examined the mechanism of calcium-mediated chain association in pectate gels, to see whether two-fold or three-fold symmetry is found.

Stoicheiometry of calcium chelation can be conveniently characterised by equilibrium dialysis studies,[1] in which less specifically bound calcium ions are displaced by swamping concentrations of a monovalent cation. As shown in Figure 1, the level of calcium ions which resist

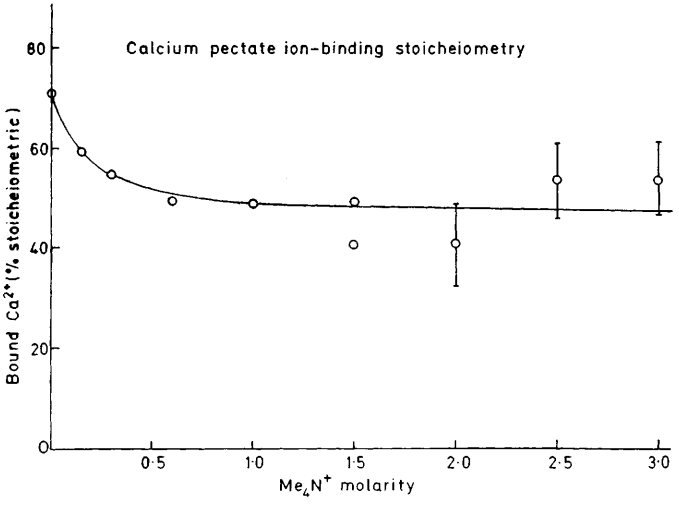



FIGURE 1. Equilibrium dialysis study of the co-operative binding of calcium to non-esterified pectate chains. A constant $Ca^{2+}$ concentration of 6 mM was used, in competition with varying concentrations of tetramethylammonium chloride, as shown. The level of bound calcium not available for equilibration across the dialysis membrane is expressed as a percentage of the stoicheiometric requirement of the pectate present.

competitive displacement corresponds to half the total pectate stoicheiometric requirement. This is entirely consistent with a two-fold dimerisation mechanism as for alginate (since only one face of each two-fold chain is involved in site binding of cations), but is difficult to reconcile with three-fold chain symmetry. To explore this further we have monitored cation binding by circular dichroism, which is established[10,11] as a sensitive probe of the local environment of uronic acid carboxy chromophores. As shown in Figure 2, calcium gelation of sodium pectate solutions is accompanied by a large reduction in c.d. ellipticity. The spectral change is similar in position and magnitude to that previously observed[11,12] for alginate, but is of opposite sign, consistent[13] with the near mirror image relationship of (**1**) and (**2**). Calcium alginate gels show little further spectral change when dried down to solid films.[14] Calcium pectate films, by contrast, show a complete reversal of sign (Figure 2), indicative of a large change in molecular organisation. Partially hydrated films (*ca.* 50% moisture) show c.d. spectra similar to that of the gel. Dry films cast from solutions containing exactly half the total stoicheiometric calcium requirement also show 'gel-like' c.d. behaviour.

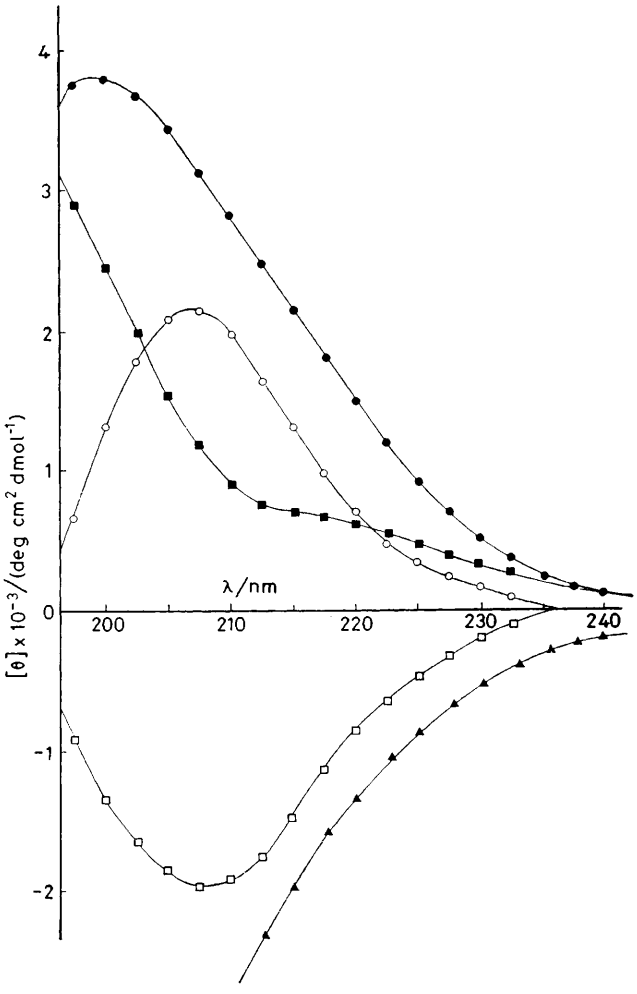



FIGURE 2. Changes in polyuronate c.d. behaviour on cation chelation. Spectral changes (○) between $Na^+$ solution (●) and $Ca^{2+}$ gel (■) for non-esterified pectate chains are similar in form and magnitude but opposite in sign to those observed for alginate poly-L-guluronate sequences (□). On drying down to a solid film (▲) calcium pectate shows large changes in c.d. which are not observed for alginate.

On the basis of this evidence we therefore conclude that, as in the case of alginate, the primary event in calcium gelation of pectin is the formation of two-fold dimers, which in the absence of further divalent cations may persist in the condensed phase. At higher $Ca^{2+}$ levels (stoicheiometric or greater), however, this form is stable only when hydrated, and converts on drying into an

alternative form in which the chains are packed with three-fold symmetry. This is the first example of a change in polysaccharide junction zone conformation between the gel and the solid state.

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