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# Chain conformation in concentrated pectic gels: evidence from $^{13}C$ NMR

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**Abstract**

$^{13}C$ NMR spectroscopy is a sensitive method of exploring the conformation of polysaccharides in both solid and gel states, because the positions of the peaks for the two carbons on either side of the glycosidic linkage, C-1 and C-4 for pectins, depend on the chain conformation. This approach was used to determine the conformation of calcium pectate at gel concentrations of ca. 0.3 g $cm^{-3}$, comparable with those found in vivo. The solid acid, methyl-esterified, and sodium forms of pectate, which are known to have a right-handed, threefold helical ($3_1$) conformation, were used to define the spectral features of this conformation. These features were also present in both gel and solid calcium pectate, and were more conspicuous in the solid form. When dilute calcium pectate gels are dried they are known from CD and EXAFS evidence to undergo a conformational transition from the "egg-box" twofold helical ($2_1$) conformation to the $3_1$ conformation. Calcium guluronate does not undergo this change, nor does calcium pectate in the presence of excess of monovalent cations. Changes in the $^{13}C$ NMR spectrum marking this transition were observed on drying calcium pectate gels, were prevented by excess of $K^+$, and were not found for guluronate. This enabled the spectral features corresponding to the $2_1$ conformation to be identified. The spectral assignments were in agreement with the stereoelectronic theory of conformation-dependent chemical shifts, which also allowed a third set of peaks to be assigned to conformations dispersed between the $3_1$ and $2_1$ helices. About 70% of the pectate was visible in the gel spectra, the remaining chains being too mobile to be detected. It was concluded that the egg-box form, as dimers or larger aggregates, was the largest single component of the gels studied here, but that substantial quantities of $3_1$ and intermediate helical aggregated forms were also present. Intact, pectin-containing plant cell walls also show spectral features characteristic of these components.

*Keywords:* Pectin; Galacturonan; Calcium; Conformation; Egg-box form

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## 1. Introduction

Pectins cross-linked into the gel state with calcium ions are a crucial structural component of the cell walls of most higher plants [1]. They hold the layers of the cell wall together [2] and form the middle lamella that bridges the cells together [3–5]. Their physical properties influence the textural properties of fruit and vegetables [6], and pectic gels in situ are at least as important to the food industry as those manufactured from isolated pectins.

It is conventional to define two components in a polysaccharide gel; inter-junction segments where the polymer chains are in a solution-like state, and junction zones where parts of two or more chains are bound together [7]. The "egg-box" model for the structure of calcium pectate gels has become established in the literature [1,8–10]. In the form in which this model is popularly presented [1], the inter-junction segments are single chains and the junction zones are pairs of galacturonan chains in the $2_1$ helical conformation (two residues per turn of the helix), with calcium ions fitted between them like eggs in an egg-box. This is an over-simplification of the egg-box model as originally published [11,12], which states that when enough calcium is available the chain dimers can aggregate into larger sheets, retaining the egg-box conformation.

The egg-box model was derived from experiments on dilute pectate gels, mostly with polymer concentrations of ca. 1 mg $cm^{-3}$. In a different model published at the same time [13], the junction zones contained between three and six chains in the right-handed $3_1$ helical form, with the calcium ions fitted between them in a more complex way. This model was based on very concentrated, highly oriented gels that gave weak X-ray diffraction patterns resembling those derived from solid calcium pectate, which had previously been shown [14] to adopt the $3_1$ helical conformation.

Because they apply to calcium pectate gels differing in concentration by a factor of $> 10^2$, these two models need not be contradictory. Indeed the most convincing evidence for the egg-box model [11] came from circular dichroism (CD) spectra implying (a) that a major conformational change took place between dilute gels and solid calcium pectate, and (b) that the conformation in the dilute gels was a near mirror image of that of calcium polyguluronate, which showed no CD change on drying and was known, from crystallography, to adopt the egg-box structure in both solid and gel states [15,16]. On this basis it was argued [12] that although the $3_1$ helix was the only species with sufficient order to give identifiable diffraction patterns in the highly concentrated calcium pectate gels used for the crystallographic experiments [13], it was replaced by the $2_1$ helical egg-box structure in the junction zones of the gels at lower concentrations. More recent evidence from EXAFS studies [17] has confirmed that a conformational transition does take place between gel and solid forms of calcium pectate, and the patterns of calcium coordination were consistent with this transition being from the $2_1$ to the $3_1$ helical form.

Pectin concentrations in the primary cell walls of living dicot plants are in the range 50–200 mg $cm^{-3}$, between the concentrations to which the two models are applicable: they are too dilute for crystallographic studies, and intact cell walls are not transparent enough for CD. Thus there is no secure experimental basis for describing calcium pectate gels in vivo as having egg-box junction zones. In particular, the idea that cell

wall pectate has the simplified egg-box structure, with single-chain inter-junction segments and dimeric junction zones, is unlikely if the inter-junction segments are in a solution-like state. In solution, galacturonan chains are dimerised to a substantial extent by calcium ions even at very low polymer concentrations where no gel forms [18,19]. Since the extent of chain aggregation increases with polymer concentration [11,12] it is unlikely that many single chains remain in concentrated gels in the 50–200 mg $cm^{-3}$ region. In that concentration range a more reasonable hypothesis is that the junction zones are multi-chain aggregates, of whatever conformation, and the inter-junction segments include egg-box dimers. None of the CD, diffraction, or EXAFS experiments contradict this: they provide information on the structure of polymer aggregates, not on the question whether these are junction zones or inter-junction segments. Even if the conformation is a random coil, implying a single chain and hence an inter-junction segment, there is some uncertainty because the mean conformation of a random coil is close to that of a regular $3_1$ helix. Calcium-binding studies were used to support the presence of egg-box dimers [11], although other interpretations of these results are possible [13,20].

Thus in topological terms three network elements are possible: single chains, dimers, and larger aggregates. In principle a pectate gel could be constructed from any two or all three of these, depending on concentration, and it remains to be determined which of them are present in any gel. The conventional terminology — junction zones and inter-junction segments — would not cope with a three-element network. Instead the terms monomeric, dimeric, oligomeric, and multimeric elements will be used here. Oligomeric and multimeric elements are defined as comprising $> 1$ and $> 2$ chains, respectively.

The in vivo structure of the oligomeric elements (junction zones) themselves is uncertain because the pectic concentration in cell walls is close to that at which the conformational transition occurs. Molecular modelling, molecular dynamics, and solution-state NMR studies of methylated [21] and acid [22,23] pectic oligosaccharides have demonstrated that the allowed conformations at the glycosidic linkage range along a low-energy path from the approximate conformation of a $3_1$ helix, through the $2_1$ helical conformation to that of a left-handed $3_2$ helix. Normally only regular forms with an integral number of residues per turn of the helix can participate in ordered, multi-chain packing arrangements. However, here all of the low-energy conformations have essentially the same value, 0.44 nm, for the residue length $h$ projected along the chain axis. In principle this could allow some residues linked in intermediate conformations to participate in otherwise regular structures, provided that the counterions could still be effectively coordinated. There is no crystallographic evidence for regular packing arrangements of chains in the $3_2$ helical form, but the presence of left-handed helices cannot be excluded. Ruben and Bokelman [24] have obtained electron micrographs of single pectate chains, apparently as left-handed helices with pitch 1.3 nm, i.e., $3h$, in dried sodium pectate gels and tobacco cell walls.

Solid-state $^{13}C$ NMR spectrometry by the CP-MAS technique (cross polarisation and magic-angle spinning) can be used to determine conformations in solid polysaccharides [25–28], since the electron density at the carbon nuclei on either side of the glycosidic linkage is controlled by the anomeric, exo-anomeric, and related stereoelectronic effects

depending on the conformation of the linkage [29]. With some limitations and experimental difficulties, CP-MAS $^{13}C$ NMR can be applied to gels [27] and intact plant materials [30] as well as solids. For this purpose it has one great advantage: if two conformational isomers are present their spectra are superimposed, whereas in solution-state NMR they are averaged and in crystallography only the most ordered conformations are visible. A disadvantage is that the least rigid elements of some gels may have chain mobilities approaching that of the free polymer in solution, and if so these will not appear in spectra obtained with cross-polarisation [31]. Nevertheless solid-state NMR clearly has great potential for the elucidation of gel structures.

The objective of the experiments reported here was to use CP-MAS $^{13}C$ NMR to determine the conformation of the galacturonan chains in calcium pectate gels of similar concentration to those found in plant cell walls. It was necessary first to investigate the spectra of a number of analogous glycuronans in the solid state in order to provide the link between known conformations and the corresponding spectral features. It was also necessary to establish how much of the polymer was represented in the CP-MAS spectra of the gels, and a complementary study [32] addresses the same question for the solution-state $^{13}C$ spectra.

## 2. Results and discussion

*Galacturonans of known* $3_1$ *helical conformation.*—The sodium, calcium, acid, and methyl-esterified forms of pectate have all been shown crystallographically to adopt $3_1$ helices as the main ordered conformation in the solid state, although the chains are packed differently [13,14]. In each case the diffraction pattern was derived from ordered chains, and other conformers may have been present as well without having a high enough degree of order to diffract [33].

Figs 1–3 show the $^{13}C$ CP-MAS spectra of the solid Na-, H-, and Me/H-galacturonans, respectively. The chemical shifts of the C-2, C-3, and C-5 resonances were almost constant, while the carboxyl resonance was at 171 ppm for the protonated and methyl-esterified forms and 176 ppm for the ionised forms. In (1 → 4)-linked glycans it is principally the C-1 and C-4 resonances that are sensitive to the conformation at the glycosidic linkage [25–29]. The Na-, H-, and Me/H-galacturonans all had the C-1 resonance at 101 ppm with a small upfield shoulder. The C-4 resonance was centred on 80–81 ppm and slightly broader than the others, which were typical in width for non-crystalline polysaccharides. It may be assumed that a small degree of disorder in the glycosidic bond angles contributed to the variation in chemical shift for C-1 and C-4, in addition to the factors controlling the width of the other resonances.

The methods used to prepare solid samples for diffraction studies (e.g., [14]) are unusual, and are intended to maximise the degree of crystalline order. It is likely that the nature of the disordered components depends on the history of the sample, and that they would be present in greater quantity and different proportions in the samples used here. However, it seems reasonable to suppose that the structure of the ordered components will be relatively independent of the sample history, and thus that the dominant ordered structure in our solid Na-, H-, and Me/H-galacturonans was the $3_1$ helix. On that basis

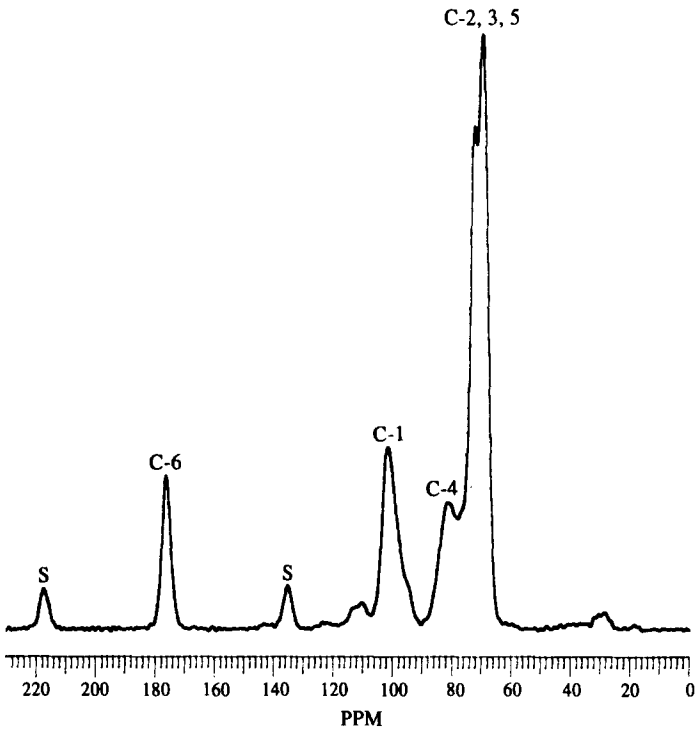


Fig. 1. CP-MAS spectrum of solid sodium pectate, showing resonance assignments. S = spinning side-bands of the carboxyl resonance.

it is concluded that the $3_1$ helix has C-1 and C-4 resonances centered on 101 and 80–81 ppm, respectively.

*Calcium pectate and polyguluronate.*—Solid calcium pectate (Fig. 4A) was used as the starting point for the study of the gel, since the solid form is known to contain $3_1$ helical chains. Although the maxima of its C-1 and C-4 resonances were similar to those of the Na-, H-, and H/Me-forms at 100 and 81 ppm, respectively, a considerable part of its C-1 intensity was distributed in a complex series of unresolved peaks decreasing in intensity to 94 ppm, and the C-4 resonance extended in a broad shoulder to ca. 76 ppm. This was not found with calcium poly-L-guluronate (Fig. 5), which is known to retain the $2_1$ conformation in both the solid and the gel state. The C-1 and C-4 resonances of calcium polyguluronate were narrower and were both slightly downfield of those of calcium pectate.

The gel–solid transition was studied in both directions, by drying pectate gels and by addition of water to solid calcium pectate (Figs 4 and 6). In each case the C-1 resonance of the gel was quite similar in form to that of the solid, while the C-4 resonance in the gel showed much less spectral intensity at 80–81 ppm than in the solid, and more at 77 ppm. There is CD evidence that in the presence of excess of monovalent cations, which suppress the further aggregation of chain dimers, the $2_1$ helix is retained even in the solid state [11]. Fig. 7 shows that under these conditions the maximum C-4 resonance

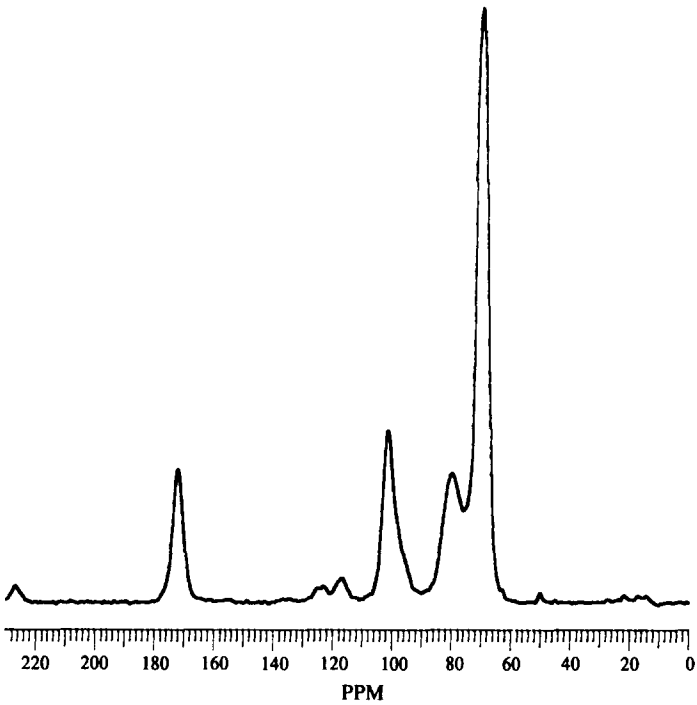


Fig. 2. CP-MAS spectrum of solid pectic acid.

intensity for the solid form was at 77 ppm, as in the gel. This confirms that the displacement of the C-4 resonance from 80–81 to 77 ppm marks a genuine conformational change, rather than perhaps the disappearance of a random-coil (80–81 ppm) component from the CP-MAS spectrum when hydration gave it enough mobility to stop cross-polarising [31].

The exact correspondence between the NMR and CD data for these conformational transitions, despite the very different methods used to prepare the gel and solid forms here and in ref. [12], implies that the same conformations were being observed by both techniques. The comparison with solid glycuronans of established conformation leads to the following assignments, ignoring for the moment the subsidiary, upfield C-1 peaks: the $3_1$ and $2_1$ helical forms both have the principal C-1 resonance at 100–101 ppm, while the C-4 resonance is at approximately 81 ppm in the $3_1$ form and 77 ppm in the $2_1$ form.

There was a large and variable loss of resonance intensity from C-6 in the galacturonan and guluronan gel forms compared with the solids. This effect has also been noted with pectins in situ in cell walls, where since the C-6 peak reappeared at long contact times [34] it was probably attributable to a less efficient cross-polarisation route for carboxyl carbons after hydration, and not relevant to the hydrated conformation.

*Stereoelectronic effects.*—The chain conformation can be described by the two glycosidic torsion angles $\psi$ and $\phi$, defined here as $\psi = (\text{O-5–C-1–O-4'–C-4'})$, $\phi = (\text{C-}$

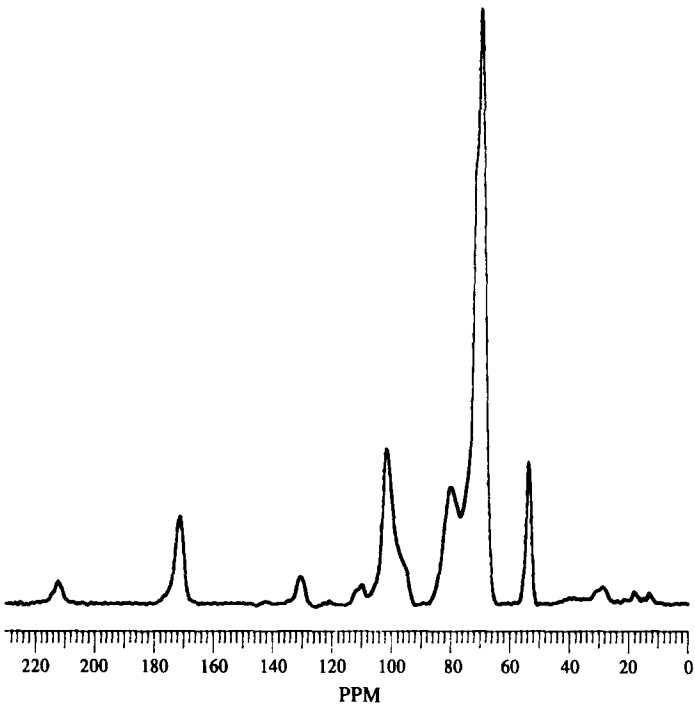


Fig. 3. CP-MAS spectrum of solid, methyl-esterified pectin, with non-esterified carboxyl groups in acid form.

5′–C-4′–O-4′–C-1). Note that a different convention was used in ref. [29] for $\psi$ and $\phi$. The relationship recently described [29] to link chemical shifts to chain conformations has three components; the anomeric effect, the exo-anomeric effect, and what was called the pseudo-anomeric effect.

In the anomeric effect, donation of electron density from a lone pair on O-5 into an antibonding $\sigma^*$ orbital of the C-1–O-4′ bond displaces the C-1 resonance upfield. The anomeric effect is evident in all $\alpha$-linked polysaccharides independently of the glycosidic conformation, but can be enhanced by anything that increases the electron-withdrawing tendency of O-4′, such as coordination of a calcium ion retaining some of its positive charge.

In the exo-anomeric effect, donation of electron density from a lone pair on O-4′ into the $\sigma^*$(C-1–O-5) orbital likewise displaces the C-1 resonance upfield. The anomeric and exo-anomeric effects are symmetrically related and enhance one another. The exo-anomeric effect is maximal at $\psi$ = ca. 60°, somewhat less than the trough of potential energy joining the regular $2_1$ and $3_1$ helical forms ($\psi$ = ca. 80°). The effect would be expected to be smaller in the $2_1$ form ($\psi$ = 100°) (Fig. 8A) than in the $3_1$ form ($\psi$ = 80°) (Fig. 8B). However the O-3′–O-5 hydrogen bond in the $2_1$ form offsets this by increasing the electron-withdrawing tendency of O-5 and possibly also allowing delocalisation of electron density between lone pairs aligned with each other on O-3′ and O-5 as the conformation at C-3′ is locked by the hydrogen bond. The O-3′–O-5

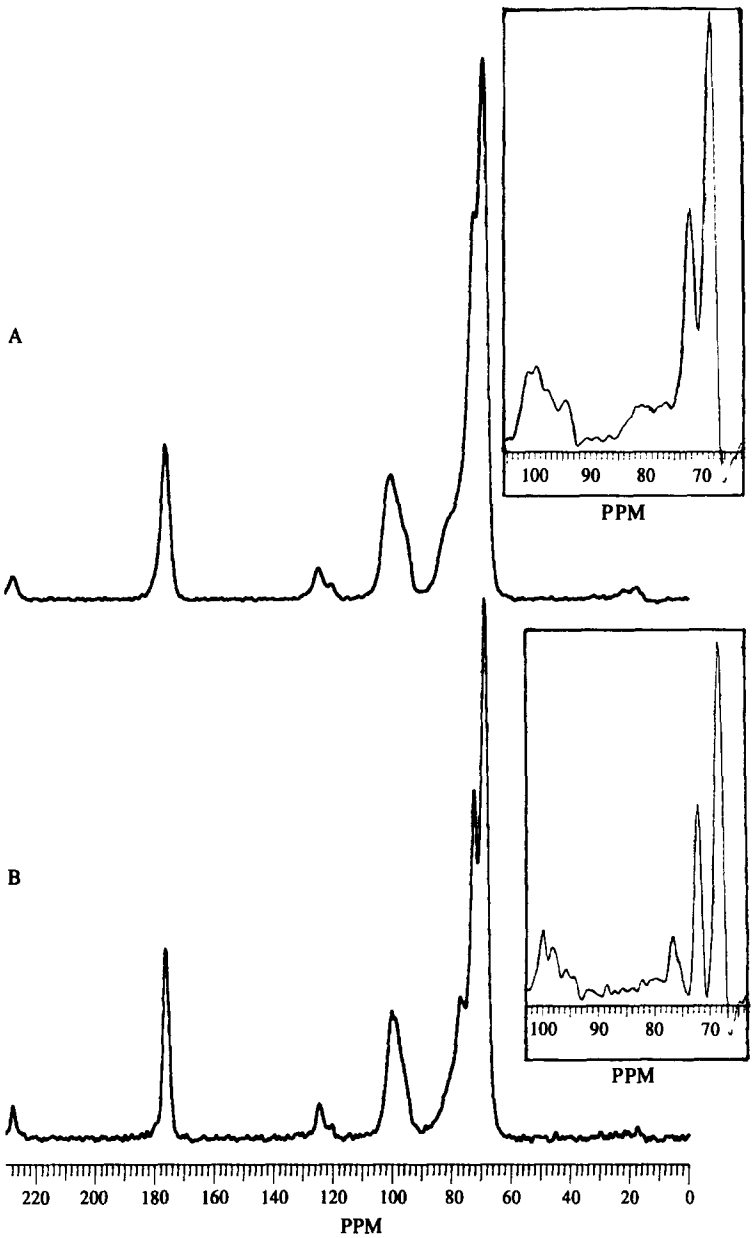


Fig. 4. CP-MAS spectra of (A) solid calcium pectate prepared by dehydration of gel, and (B) calcium pectate gel, of pectate concentration 290 g $L^{-1}$. Inset: resolution-enhanced spectra.

hydrogen bond is absent in the established $2_1$ helical form of calcium poly-L-guluronate, which is a mirror image of the $2_1$ form of calcium pectate except that O-3 is equatorial, and Fig. 5 shows that the C-1 resonance of polyguluronate is noticeably downfield of

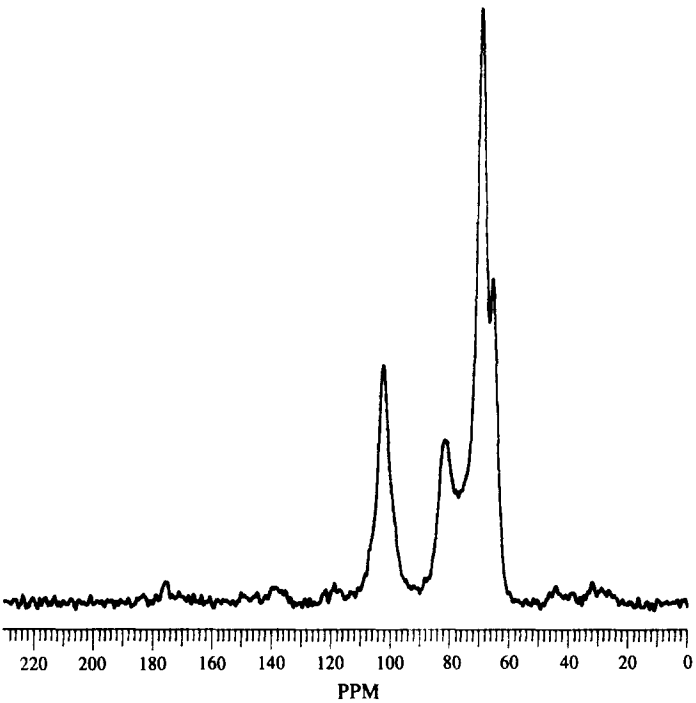


Fig. 5. CP-MAS spectrum of calcium poly-L-guluronate, hydrated solid.

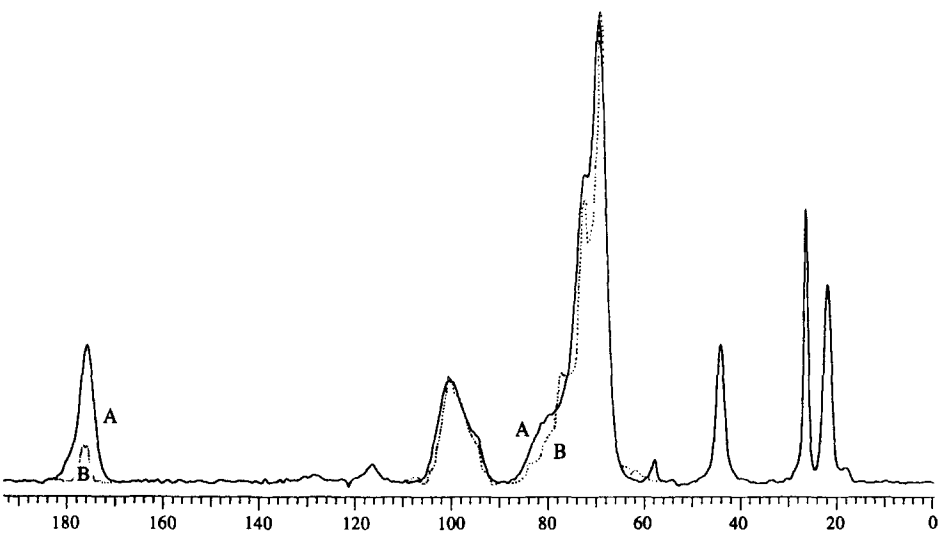


Fig. 6. CP-MAS spectrum of solid calcium pectate (A) with spectral changes on hydration to a pectate concentration of 390 g $L^{-1}$ (B: dotted line). Spectra A and B were corrected to the same mass basis using poly(propylene) as an internal standard.

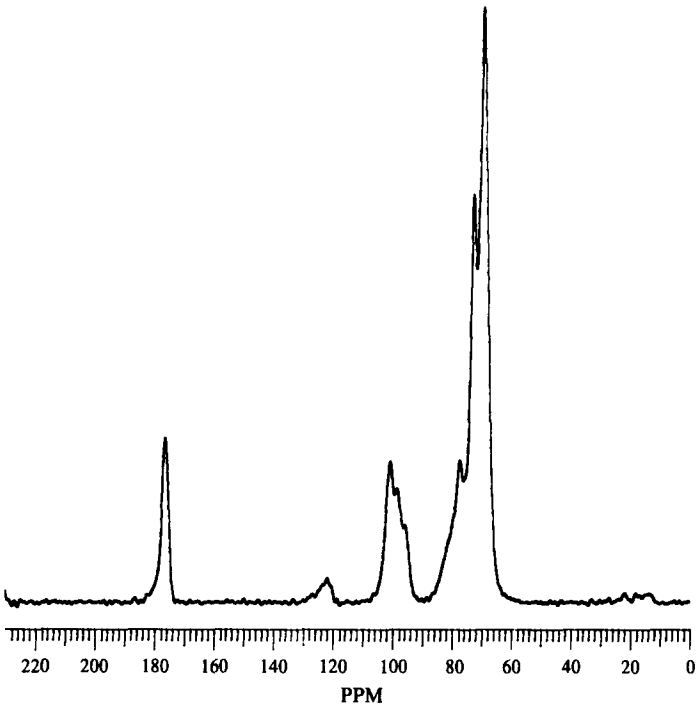


Fig. 7. CP-MAS spectrum of solid calcium pectate with 50% substitution of potassium counterions (cf. with Figs 4B and 5B).

either form of pectate. A similar influence of an O-3′–O-5 hydrogen bond, enhancing the exo-anomeric effect, may be seen in, for example, cellobiose and lactose, which being $\beta$-linked do not show the anomeric effect [29].

Insufficient data are available on crystallographically characterised carbohydrates to put this theoretical background on a fully quantitative basis, but the glucan spectra show that the total magnitudes of the anomeric and exo-anomeric effects are comparable, and that together they are sufficient to explain most of the range of conformation-dependent chemical shifts observed. The effect of the hydrogen bond in the $2_1$ form and the larger exo-anomeric effect in the $3_1$ form of calcium pectate appear to balance out, since the primary C-1 resonances of both forms are at nearly the same chemical shift.

The distinctive group of subsidiary peaks between 100 and 94 ppm was prominent and rather similar in both the solid and the gel form of the Ca-galacturonan, but not in any of the other galacturonan preparations, nor in polyguluronate. Digital resolution enhancement (Fig. 4) suggested minor differences between the solid and the gel but the resolution was inadequate for reproducible definition of their constituent peaks. In the region of $(\psi,\phi) = (75°, 120°)$, between the regular $2_1$ and $3_1$ helical forms, $\psi$ is as well suited to the anomeric effect as in the $3_1$ form but hydrogen bonding between O-3′ and O-5 is still possible. A C-1 resonance further upfield than either regular form would therefore be predicted, and it is suggested that the series of signals in the 94–100 ppm

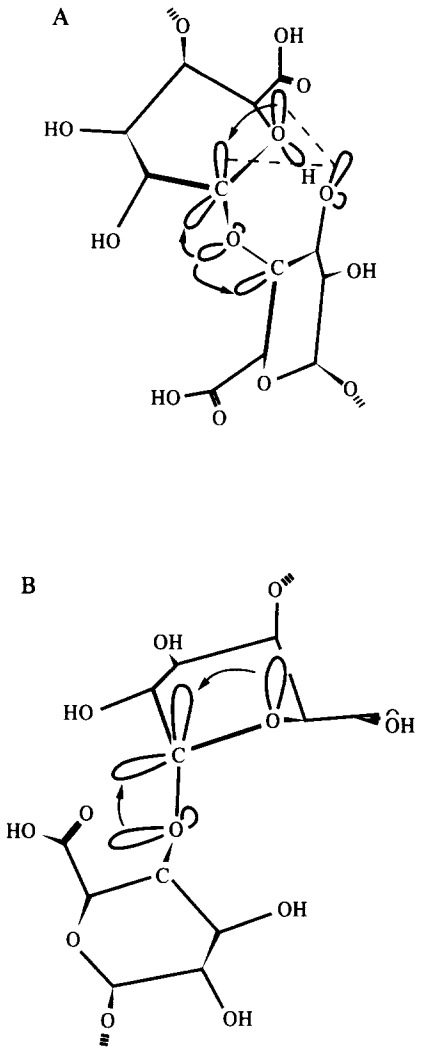


Fig. 8. Stereochemistry and inter-orbital interactions in the $2_1$ (egg-box) (A) and $3_1$ (right-handed, threefold helical); (B) conformations of pectate. There is a hydrogen bond between O-5 and O-3′ in the $2_1$ helical form. Coordination to calcium ions [17] is not shown.

region arises from a range of more or less irregular conformations in this area. Enhancement of the anomeric and exo-anomeric effects by coordination of calcium ions to O-4′ and O-5, respectively [17], is another possible contributory factor. Anomeric and related effects stabilise conformations that permit them and this stabilisation may indeed be one reason why intermediate conformations are found at all in calcium pectate, yet apparently not in polyguluronate.

The spectra (Figs 4 and 6) suggest that the range of intermediate conformations may

not be entirely continuous, perhaps because association with regular helical chains of either form favours particular coordination patterns for calcium [17] and the associated conformations. If that is correct it is evidence against delocalisation of the calcium ion along the chains as suggested for heparin [35].

The left-handed $3_2$ helical form and intermediates between it and the $2_1$ form allow none of these mechanisms for moving the C-1 resonance upfield and this resonance would be predicted to lie downfield of its position in either the $2_1$ or the $3_1$ form. In the spectrum of the gel the C-1 resonance does spread 1–2 ppm further downfield than in that of the solid, and this may represent a small proportion of chains with a slight left-handed twist, but the regular $3_2$ form does not seem to be a major component of any of the preparations of calcium pectate examined here.

In the $2_1$ helical form the value of $\phi$ permits a pseudo-anomeric effect in which electron density is donated from one of the lone pairs on O-4′ into the $\sigma^*$(C-4′–C-3′) orbital, moving the C-4′ resonance upfield relative to its position in the spectrum of the $3_1$ form, in which this interaction is not possible. Like the exo-anomeric effect, the pseudo-anomeric effect is enhanced by delocalisation into the lone pairs on O-5 and O-3′ in the $2_1$ form. Thus the relative chemical shifts observed for C-4′ in the $2_1$ and $3_1$ forms, 77 and 80 ppm, respectively, are as predicted, and it is clear that considerable quantities of material of intermediate conformation are present in both the solid and the gel states of calcium pectate. The delocalisation interaction is not possible in the $2_1$ form of calcium polyguluronate because of the difference in orientation of O-3′, and Fig. 5 shows that its C-4′ resonance is a little downfield from those assigned to C-4′ in the $2_1$ form of pectate, as predicted.

The correspondence between electronic theory and observation confirms the evidence from comparison with crystallographically established solid forms that the resonance assignments are correct, as well as explaining the anomalous resonances at 94–100 ppm. Thus at the polymer concentrations studied here the calcium pectate gel — or that part of it which was rigid enough to appear in the CP-MAS spectrum — contained chain segments in the $2_1$, $3_1$, and intermediate conformations, with the ratio of $3_1$ to $2_1$ helical chain segments increasing on drying unless excess of monovalent cations were present.

It may be assumed that other, more mobile chains were present in the gel but invisible in the CP-MAS spectra. An inert internal standard [poly(propylene)] was used to quantify the loss in resonance intensity from the spectrum on hydration. The anomalous C-6 resonance (see below) was excluded. The loss amounted to ca. 30% [32], an indication of the proportion of more mobile segments. Fig. 6B, the spectrum of the hydrated calcium pectate, is adjusted accordingly to 70% of the total resonance intensity of the dehydrated sample. It shows that the absolute intensity of the 77 ppm peak increased as the 80–81 ppm peak diminished on hydration, confirming that the conformational transition was genuine. In sodium pectate gels of 0.04–0.1 g $cm^{-3}$ concentration, only ca. 5% of the pectic residues were mobile enough to be detectable by solution-state NMR [32], and the proportion may be assumed to be smaller still in the calcium gels examined here. Thus most of the 30% of the residues "invisible" in CP-MAS experiments are also "invisible" in solution-state NMR. This fraction is likely to include any single-chain, random-coil segments that are present.

Comparison with the CP-MAS spectra of intact primary cell walls shows that many of the features observed here are also characteristic of pectins in situ. The resonance at 80 ppm assigned to C-4 in the $3_1$ conformation was visible in the spectra of dried mung-bean hypocotyl cell walls [36] and hydrated celery collenchyma [30], and was confirmed to show the relaxation kinetics typical of pectins in partially hydrated tomato cell walls [37]. The partially resolved resonances between 94 and 98 ppm, assigned to C-1 of conformations intermediate between the $3_1$ and $2_1$ helices, were apparent in dried cell walls but not after hydration, possibly due to methyl-esterification.

It is concluded that both solid calcium pectate, and gels corresponding in concentration to those of the plant cell wall, have more complex and less ordered architecture than was previously envisaged. The solid contained almost as much $2_1$ as $3_1$ helix, and the 70% of the gel "visible" in CP-MAS NMR contained a significant proportion of $3_1$ helix as well as $2_1$ helix—although this proportion may well have been lower or zero in the much more dilute gels on which the original egg-box model was based [11,12]. Both the solid and the gel contained substantial quantities of chains dispersed between the conformational parameters of the $2_1$ and $3_1$ forms, but few, if any, left-handed helical chains were present.

The complex multimeric elements may have been linked together by either egg-box dimers or random-coil monomeric chains, or both. The question whether egg-box dimers function as junction zones, or link multimeric aggregates together, may be answered by conformational studies of the "invisible" fraction by mobility-resolved NMR [38]. In vivo there are still more possibilities since methylated, branched, or acetylated pectic chains are alternative possibilities for the monomeric elements. This complexity, remarkable for such a simple polysaccharide, offers considerable flexibility and potential for the interaction of physical and mechanical properties with ionic composition.

## 3. Methods

Calcium pectate gels and solids were prepared by suspending 2.5 g of polygalacturonic acid from orange peel (Sigma) in 80 $cm^3$ of deionised water and adding 10 $cm^3$ of 1 M KOH. An excess of 2.5 M $Ca(NO_3)_2$ was added with very rapid mixing and the gelatinous precipitate homogenised in a Waring Blendor and washed with 8 L of deionised water, then collected on a 53-$\mu$m seive and rotary-evaporated to the specified moisture content. Sodium pectate was prepared by similarly suspending polygalacturonic acid in deionised water, adding an equimolar quantity of 1 M NaOH, rotary-evaporating to a film, and air-drying. The calcium/potassium form was prepared by rapid mixing of 1-$cm^3$ portions of 50 g $L^{-1}$ potassium polygalacturonate and 0.1 M $Ca(NO_3)_2$. The free acid form was washed with 0.05 M methanolic HCl and MeOH before use. The acid methyl-esterified form was prepared by dissolving 1 g of citrus pectin (Sigma) in 50 $cm^3$ of hot water and pouring into 0.5 L of aq 90% EtOH containing 10 mM HCl. The resulting gelatinous precipitate was crushed through a 2-mm sieve and stirred with ethanolic HCl and EtOH, then air-dried.

Sodium poly-L-guluronate of ca. 90% purity was dissolved in deionised water (0.2 g $L^{-1}$) and precipitated with a 4 molar excess of 1 M $Ca(NO_3)_2$. The resulting gel was washed with 1.5 mM ethanolic $Ca(NO_3)_2$ and ethanol, and air-dried.

Gels of less than 1:3 solid–water ratio lost excessive amounts of water due to the centrifugal force generated by magic-angle spinning, and also absorbed too much decoupler energy for spectra to be recorded. This unfortunately prevents a direct comparison with the experiments [11,12], on much more dilute gels, that were used to extend the "egg-box" model to calcium pectate. However the gel concentrations used here were comparable with those in plant cell walls. The gels were weighed before and after the spectra were recorded, and concentrations corrected for any loss of moisture that occurred.

$^{13}C$ CP-MAS spectra were recorded at 75.4 MHz on the 300-MHz Varian spectrometer at Durham. Details were as published [30], except that a ceramic probe insert was used to reduce moisture loss from the samples in some of the experiments. Contact time was 1 ms and relaxation delay normally 0.5 s. Spin rates were 2–4 kHz; spinning sidebands, located by varying the spin rate, were evident only for the carboxyl resonances.

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