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Conformation and mobility of the arabinan and galactan side-chains of pectin

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Abstract

The function of the arabinan and galactan side-chains of pectin remains unknown. We describe ^{13}C NMR experiments designed to yield spectra from the most mobile polymer components of hydrated cell walls isolated from a range of plant species. In pectin-rich cell walls, these corresponded to the pectic side-chains. The arabinan side-chains were in general more mobile than the galactans, but the long galactan side-chains of potato pectin showed high mobility. Due to motional line-narrowing effects these arabinan and galactan chains gave ^{13}C NMR spectra of higher resolution than has previously been observed from 'solid' biopolymers. These spectra were similar to those reported for the arabinan and galactan polymers in the solution state, implying time-averaged conformations resembling those found in solution. The mobility of the highly esterified galacturonan in citrus cell walls overlapped with the lower end of the mobility range characteristic of the pectic side-chains. The cellulose-rich cell walls of flax phloem fibres gave spectra of low intensity corresponding to mobile type II arabinogalactans. Cell walls from oat coleoptiles appeared to contain no polymers as mobile as the pectic arabinans and galactans in primary cell walls of the other species examined. These properties of the pectic side-chains suggest a role in interacting with water.

Keywords: *Allium cepa*; *Avena sativa*; *Citrus* sp.; *Linum usitatissimum*; *Lupinus albus*; *Solanum tuberosum*; Cell walls; Pectin; Arabinan; Galactan; Mobility; Solid-state NMR

1. Introduction

Pectins are the most complex polysaccharides in the cell walls of non-woody higher plants (Voragen et al., 1995). The complexity of their structure is probably linked to their mechanical function, but the details of the connection are not known. There is evidence (Jarvis, 1992, 1998) that one function of pectins is to withstand the cell separation stresses, transverse to the cell wall, that are imposed indirectly by turgor pressure. Characteristic low-ester pectic galacturonans (Knox et al., 1990; Roy et al., 1992; Liners and Van Cutsem, 1992; Willats et al., 2001) and bound calcium ions (Roy et al., 1994; Huxham et al., 1999) are located at junctions between cell walls, where these stresses are greatest.

It is reasonable to suppose that pectins also contribute to the mechanical properties of the cell wall under stress in its own plane: that is, the ability of the cell wall to withstand the direct stress imposed by turgor pressure, but also to expand laterally under

Abbreviations: NMR, nuclear magnetic resonance; CP, cross-polarisation; DP, direct polarisation; MAS, magic-angle spinning; RGI, rhamnogalacturonan I.

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that stress so that the cell can grow. Tomato cells adapted to tolerate the herbicide dichlorobenzonitrile retained a coherent cell wall deficient in polysaccharides other than pectin but still able to resist turgor (Wells et al., 1994). Borate diesters cross-linking pectic rhamnogalacturonan II (RGII) are necessary for normal growth and to prevent tip-growing cells from bursting (Findeklee and Goldbach, 1996; Fleischer et al., 1998, 1999).

A correlation between capacity for rapid growth and high content of the neutral monosaccharides rhamnose and arabinose or galactose, characteristic of rhamnogalacturonan I (RGI), was long ago noted in dicot seedlings (Rees and Wight, 1969). There was at that time no explanation for the widely varying ratios of pectic arabinose to galactose in different plant tissues (Jarvis, 1984). Recently it has been suggested that arabinan-rich pectins are characteristic of tissues in which cell division is prolific, and of cell walls originally laid down in such tissues and subjected to little modification thereafter; whereas galactan-rich pectins are more abundant in cell walls composed mainly of material deposited during cell expansion (Willats et al., 1999; McCartney et al., 2000; Bush et al., 2001). This evidence is suggestive of different mechanical roles for the arabinan and galactan chains (McCartney et al., 2000), but there is little basis for hypotheses on what these roles might be. Transgenic potato plants with shortened galactan chains showed no visible phenotype (Sorensen et al., 2000).

The contribution of each cell-wall polymer to the mechanical properties of the intact cell wall depends on the polymer's own rigidity and on how it is linked to other polymers (Jarvis, 2002). Provided that water is present, the pectic arabinan and galactan chains have considerable thermal mobility. This mobility can be monitored by solid-state NMR methods (Foster et al., 1996; Ha et al., 1997; Renard and Jarvis, 1999), and controls the potential of these chains to participate in non-covalent interactions with other polymers as well as their contribution to the mechanical properties of polymer networks in which they participate. Here we explore the mobility and conformational properties of arabinan and galactan RGI side-chains in a variety of cell-wall systems (Table 1).

2. Results

2.1. Mobile polysaccharides showing slow cross-polarisation

We have shown (Ha et al., 1996, 1997) that hydration of onion cell walls mobilises some of the pectic polysaccharides and thus reduces the rate of Hartmann–Hahn cross-polarisation (CP) in a CP-MAS ^{13}C NMR experiment. In these experiments β(1,4)-D-galactan chains showed notably slow CP, indicating considerable thermal mobility. There was some degree of averaging of CP rate between spatially adjacent polymers by proton spin diffusion, but it was minimal for the most mobile polymers because the proton spin diffusion rate and the time required for CP both depend on the time constant ^{1}H T_2 for proton spin–spin relaxation (Ha et al., 1996).

The experimental method of Ha et al. (1997) was used to obtain ^{13}C NMR difference spectra from relatively mobile polymers requiring significantly more than the general optimum of 1 ms for CP in hydrated onion, potato and *Citrus* cell walls. These difference spectra are shown in Fig. 1. The slow-CP difference spectrum for potato cell walls was dominated by signals from β(1,4)-D-galactan, which is abundant in this plant material (Jarvis et al., 1981a). The slow-CP difference spectrum for onion cell walls was similar but also included a distinctive signal at 54 ppm corresponding to pectic methoxyl groups. Onion pectins are more highly methyl-esterified than those from potato cell walls, as can be seen from the conventional CP-MAS spectra. The methoxyl group in high-methoxyl pectins is free to rotate independently of motion in the pectic galacturonan chain itself (Ha et al., 1996). The ^{13}C NMR signal from the methoxyl carbon is therefore expected to show more evidence of mobility than the signals from the other carbon atoms in the galacturonan. However these galacturonan signals can be clearly discerned at low intensity, in addition to a strong methoxyl signal, in the slow-CP difference spectrum for *Citrus* cell walls where the pectins have a much higher degree of methyl-esterification than in onion or potato cell walls (Voragen et al., 1995).

Table 1
Cell-wall systems used

Source of cell walls	Nature of pectins
Onion bulbs	Abundant, galactan-rich (Redgwell and Selvendran, 1986)
Potato tubers	Abundant, galactan-rich (Jarvis et al., 1981a)
Citrus (orange) peel	Abundant, galactan-rich, highly methoxylated (Ralet and Thibault, 1994)
Lupin seed cotyledons	Cotyledons used intact: pectic galactans comprise a large part of the dry matter (Cheetham et al., 1993)
Oat coleoptiles	Low pectic content, typical of grasses and cereals (Carpita and Gibeaut, 1993)
Flax phloem fibres	Small amounts of Type I (pectic, 1,4′-linked) and Type II (1,3′/1,6′-linked) galactans (Goubet et al., 1995)

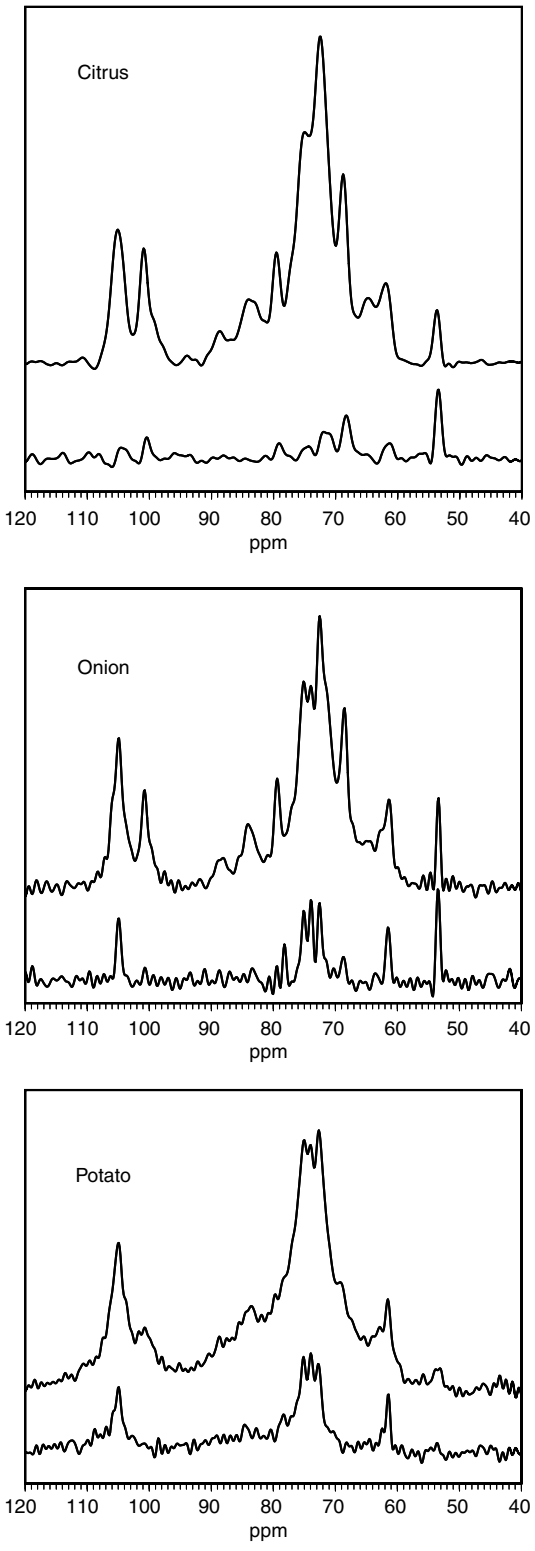

Fig. 1. Slow-CP ^{13}C NMR spectra of mobile polysaccharides in hydrated citrus, onion and potato cell walls. The upper spectrum in each case is from a standard CP-MAS experiment and is derived from rigid polymers within the cell wall. The lower spectrum is a difference spectrum derived from polymers of sufficiently high mobility to cross-polarise more slowly that fully rigid solids. For signal assignments see Table 2.

In onion and *Citrus* cell walls, therefore, the slow-CP experiment showed that the pectic galactan chains were more mobile than most of the other polymers, but their range of mobility overlapped with the most highly methyl-esterified (and thus least rigid) galacturonan chains in *Citrus* cell walls (see Table 2).

Linewidths in these slow-CP difference spectra were similar to, or slightly less than, the linewidths in the conventional CP-MAS spectra of the same cell walls (Fig. 1). Linewidths in the CP-MAS spectra of carbohydrates are normally broad due to a spread of conformational environments for the carbon nuclei (Jarvis et al., 1999). Narrower linewidths can occur for either of two reasons: if the degree of crystalline order is high so that each carbon of a given type is in an identical conformational environment, or if the molecules have sufficient thermal mobility to average the different conformational environments on the NMR timescale. Reported proton spin–spin relaxation times of 0.1 ms or more, for the galactan chains in these materials, indicate that the line-narrowing is due to mobility and not to crystalline order (Ha et al., 1996; Jarvis et al., 1996).

Signals from pectic arabinans were not observed in either the standard CP-MAS or the slow-CP NMR spectra of these materials. All three species concerned have a low ratio of arabinan to galactan in their pectins (Ralet and Thibault, 1994; Redgwell and Selvendran, 1986; Jarvis et al., 1981b), but the complete absence of arabinan signals suggests that these are not efficiently detected in CP experiments on hydrated cell walls.

Table 2
Peak assignments in ^{13}C NMR spectra of pectic polymers

Polymer	Carbon	ppm
Galacturonan	C-6 (free)	175.4
Galacturonan	C-6 (ester)	171.4
Arabinan	C-1 (t-)	109.3
Arabinan	C-1 (5-)	108.2
Arabinan	C-1 (3,5-)	107.8
Galactan	C-1	105.1
Galacturonan	C-1	100.8
Arabinan	C-4 (t-)	84.7
Arabinan	C-4 (5-/3,5-)	83.0
Arabinan	C-2 (5-/3,5-)	82.1
Arabinan	C-2 (t-)	81.6
Arabinan	C-3 (3,5-)	80.0
Galactan	C-4	78.4
Arabinan	C-3 (t-, 5-)	77.4
Galactan		75.2
Galactan		74.0
Galactan		72.6
Arabinan	C-5 (5-)	67.7
Arabinan	C-5 (3,5-)	67.2
Arabinan	C-5 (t-)	62.0
Galactan	C-6	61.5
Galacturonan	OCH_3	53.6
Rhamnose	CH_3	17.6

2.2. Polysaccharides identified in short-recycle direct-polarisation experiments

Polysaccharide components that are too mobile to cross-polarise readily can be identified in direct-polarisation ^{13}C NMR experiments. Such experiments can indeed be used to obtain spectra from all the polymer components present in cell walls, but very long recycle times are needed between repetitions so that rigid components, with ^{13}C spin-lattice relaxation times of the order of 1 min, can return to magnetic equilibrium. If the recycle time is restricted these rigid components are saturated and only mobile components, with ^{13}C spin-lattice relaxation times (^{13}C T_1) shorter than the recycle time, are observed. This principle was used as the basis of a spectral editing procedure by Foster et al. (1996). Proton decoupling is needed during spectral acquisition in these experiments and the components observed depend on how this is done. With high-power proton decoupling as for a CP-MAS NMR experiment, more rigid polymers are observed than with decoupling procedures designed for solution-state NMR (Foster et al., 1996; Viëtor et al., 2002). We found that high-resolution spectra could be obtained from the arabinan components of beet cell walls using a direct-polarisation MAS ^{13}C NMR experiment with the WALTZ-16 multiple-pulse decoupling sequence, which is designed for molecules in the solution state but of slightly restricted mobility (Renard and Jarvis, 1999).

When applied to potato cell walls, the DP-MAS WALTZ-16 experiment gave the spectrum shown in Fig. 2. As observed by Renard and Jarvis (1999) the spectral resolution was much higher than is normally found in solid-state NMR experiments on biological materials, with linewidths of about 0.4 ppm (30 Hz) for the galactan component. This indicates that the population of galactan chain segments observed in the DP-MAS/WALTZ-16 experiment was more mobile than those observed in the slow-CP experiment (Fig. 1). Equally narrow signals from the arabinan chains were present at lower intensity.

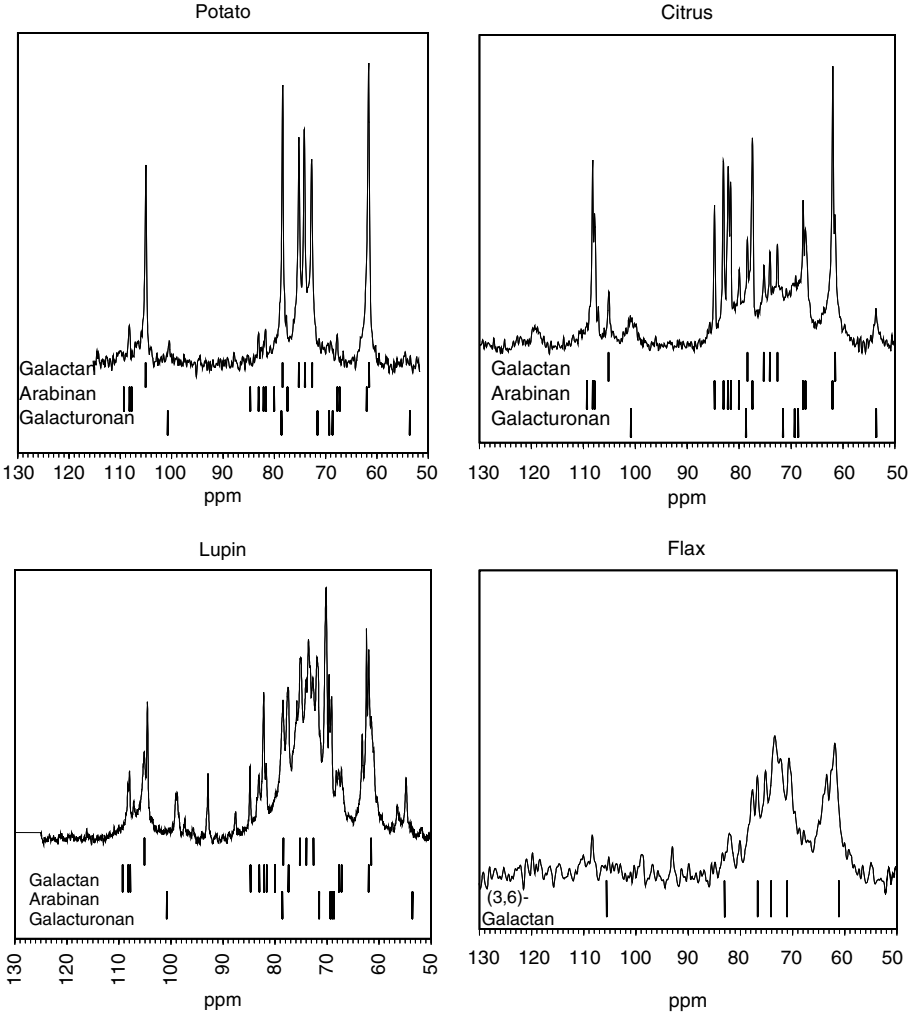

Fig. 2. DP-MAS WALTZ-16 ^{13}C NMR spectra of highly mobile components of potato, citrus, flax and lupin cell walls. The line diagrams below the potato, citrus and lupin cell-wall spectra correspond to the chemical shifts assigned to the pectic arabinan, galactan and galacturonan signals.

When applied to citrus cell walls and lupin seed meal (which consists largely of galactan-rich cell walls and protein), the DP-MAS/WALTZ-16 experiment gave spectra with even narrower lines (Fig. 2). On the basis of linewidth, it may be inferred that the standard CP-MAS experiment, the slow-CP experiment and the DP-MAS/WALTZ-16 experiment respectively detect hydrated polymer chains of increasing mobility, with limited overlap. The ability of the two latter experiments to detect mobile polymers depends on somewhat different kinds of mobility: motions in the kHz frequency range control ^{1}H T_2 and hence the response in the slow-CP experiment, whereas the DP-MAS response depends on the ^{13}C T_1 which requires MHz motions. Generally the higher the frequency, the smaller the parts of the molecule that are in motion. Linewidth, however, reflects the ^{1}H T_2 and is therefore a suitable basis for comparing the kHz mobility of the polymers detected in the two experiments.

The DP-MAS/WALTZ-16 spectrum from *Citrus* walls (Fig. 2) was dominated by very narrow arabinan signals, unlike the standard-CP and slow-CP spectra where galactan signals predominated (Fig. 1C). This indicates that the arabinan chains were even more mobile than the galactan chains.

No spectra were obtained from lignified cell walls of Arabidopsis by the DP-MAS/WALTZ-16 experiment (vascular cell walls isolated from wild-type flowering stems: data not shown). However the non-lignified but secondarily thickened cell walls from flax phloem fibres gave a spectrum of low intensity (Fig. 2), comprising narrow arabinan signals together with others apparently derived from type II arabinogalactans. Signals from the type I galactans, as observed in the other plant materials studied here, were not detected although both types are known to be present at low abundance in flax cell walls (Goubet et al., 1995).

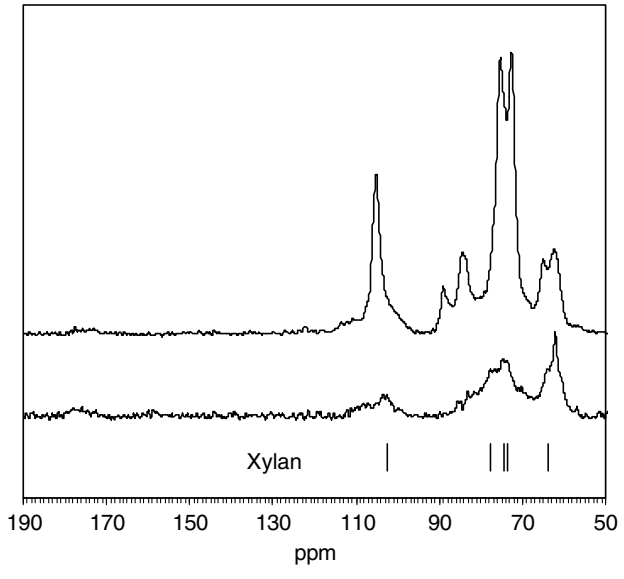

Fig. 3. ^{13}C NMR spectra of hydrated oat coleoptile cell walls. The upper spectrum is from a standard CP-MAS experiment and the lower spectrum is from a DP-MAS WALTZ-16 experiment, showing the most mobile cell-wall components which can be assigned as arabinoxylans. Because of the different nature of these experiments the relative intensity of the two spectra cannot be compared.

The primary cell walls of the Poaceae and their near relatives (Type II cell walls) fulfil the same mechanical functions as those of other plants but differ in structure, with simpler pectins in relatively low abundance (Carpita and Gibeaut, 1993). The DP-MAS/WALTZ-16 spectrum of cell walls from oat coleoptiles, typical of type II cell walls, was of very low intensity (Fig. 3): it included signals assignable to acetylated (glucurono)arabinoxylans but with linewidths greatly in excess of those in Fig. 2. In addition to the xylan signals a broad signal from C-1 of arabinosyl side-chains was evident at 109 ppm, but signals from feruloyl and glucuronoyl substituents were not discernible above the noise level. These results indicate that oat coleoptile cell walls contain no polymer chains in the same mobility range as the pectic arabinan side-chains of type I cell walls.

3. Discussion

The DP-MAS/WALTZ-16 ^{13}C spectra of pectic polysaccharides in Fig. 2 are of higher resolution than has previously been recorded from biopolymers by solid-state NMR methods. This high resolution was also observed for signals assigned to proteins (below 50 ppm, not shown in Fig. 2) in lupin seed meal.

The NMR experiments showed that the arabinan and galactan side-chains of pectin are the most mobile polymers of the hydrated cell walls of non-graminaceous plants, but that their mobility is not equal. Except in the case of potato cell walls, the galactan signals were largely confined to the slow-CP spectra, and the arabinan signals were most intense in the DP-MAS/WALTZ-16 spectra even where the galactan components were known to be quantitatively more abundant, as in citrus cell walls. Linewidths indicate that the arabinans were in general more mobile than the galactans.

Since the arabinan and galactan side-chains are glycosidically linked to the rigid rhamnogalacturonan core chain, the local amount of motion may be expected to increase along each chain with distance from the point of attachment. Our data therefore show that, except in potato cell walls, a much larger fraction of the length of the arabinan chains is sufficiently removed from the influence of the attachment point to bring it within the high mobility range probed by the DP-MAS/WALTZ-16 experiment. The greater mobility of the arabinans is consistent with the stereochemistry of the α(1,5)-L-arabinofuranosyl and β(1,4)-D-galactopyranosyl linkages (Pérez et al., 2000). Both allow some conformational

flexibility but the arabinosyl linkage, with freedom of rotation around the three bonds separating successive monosaccharide rings, is more flexible than the two-bond galactosyl linkage.

The spectra derived from the mobile components of potato cell walls were unusual in being dominated by galactan signals. Much of the potato galactan showed mobility comparable with that of the arabinan chains in other cell walls, with linewidths only a little greater than the arabinans. Potato pectins are galactan-rich, and it is known that this is because their galactan chains are very long, with a degree of polymerisation approximating to 40 (Jarvis et al., 1981b). This is consistent with their observed mobility since it allows a substantial fraction of the length of each chain to be far enough from the influence of the attachment point to reorient continually on the NMR timescale. The fraction of the chain length that can reorient rapidly in this way relates to the ratio of the degree of polymerisation of the chain and its persistence length, a measure of the length for which its intrinsic stiffness keeps it approximately straight (Jarvis, 2002).

The DP-MAS spectra assigned to the arabinan chains were closely similar for all the arabinan-containing plant species included in this study and with the beet arabinans examined by Renard and Jarvis (1999), showing that the degree and type of branching of the arabinans is conserved and that they adopt similar time-averaged conformations, even though their abundance varies greatly. This would be consistent with, but does not prove, conservation of function. The rapidly reorienting segments of the galactan chains also appear to adopt a similar time-averaged conformation to that found for the isolated galactan in solution.

The mobilisation of the arabinan and galactan chains by hydration, demonstrated here and examined in more detail for galactans by Ha et al. (1997) and Tang et al. (1999), is evidence of the close association of these polymers with water when in their native state in the living plant (Ryden et al., 2000). All cell-wall polymers are then hydrated, but while hydration of cellulose microfibrils is a surface phenomenon, the pectic side-chains are more like tethered polymer solutes. This association with water may influence the potential for aqueous phase separation within the hydrated cell wall (MacDougall et al., 1997). However no polymer abundant in the type II cell walls of oat coleoptiles seems likely to have any similar function related to chain mobility.

Molecular entanglement of pectic side-chains has been suggested to contribute to the mechanical integrity of the cell wall (Hwang and Kokini, 1992). The lifetime of such entanglements is likely to be short, limited by the mobility observed in our experiments. Their mechanical contribution will then be observable only during fast deformations of the cell wall. The high mobility of the arabinan and galactan segments observed here also makes it unlikely that they could cross-link pectic macromolecules through hydrogen-bonded chain aggregation.

In the Chenopodiaceae covalent cross-linking is possible through pectic side-chains terminated at the outer end by feruloyl esters that can dimerise (Fry, 1986; Rombouts and Thibault, 1986; Colquhoun et al., 1994). The arabinan side-chains, with their potential for entropic elasticity (Marszalek et al., 1998; Tibbits et al., 1998), thus contribute to cell-wall and intercellular cohesion in beet (Waldron et al., 1997). In most dicot families phenolic cross-linking agents are probably not abundant enough (Parr et al., 1997) to allow this form of cross-linking on a significant scale. Arabinan and galactan chains attached only by the glycosidic linkage to rhamnose at the inner end, as must be the case for pectins extractable in chelator solutions, cannot carry mechanical loads in this way. Pectic molecules that resist chelator extraction, but are released by alkaline hydrolysis, tend to be rich in arabinan or galactan chains (Jarvis, 1982). It has been suggested that these pectins are cross-linked by galacturonoyl esters (Kim and Carpita, 1992; MacKinnon et al., 2002), but the galacturonoyl moiety could be esterified to a hydroxyl group at any location on RG I or an adjacent galacturonan chain.

We conclude that the physical properties of the arabinan and galactan side-chains of pectin are consistent with a role in interaction with water, although that is not necessarily their only role.

4. Experimental

4.1. Cell walls

The preparation of the onion, potato, orange and flax cell walls is described elsewhere (Ha et al., 1996; Bush et al., 2001; Jarvis et al., 1996; Viëtor et al., 2002). The use of calcium-containing buffers throughout the cell-wall preparation sequence ensured a controlled calcium saturation level in the cell walls as examined. Cell walls were prepared from frozen oat (*Avena sativa* L.) coleoptiles by the same procedure as for citrus cell walls (Jarvis et al., 1996). The structures of the pectins from citrus peel (Ralet and Thibault, 1994), onion bulbs (Redgwell and Selvendran, 1986) and potato tubers (Jarvis et al., 1981a) and the galactans of flax phloem fibres (Goubet et al., 1995) have been described.

4.2. NMR methods

CP-MAS ^{13}C NMR experiments were carried out on a Varian VXR-300 spectrometer and DP-MAS experiments on a Varian Unity Plus, in both cases at 75 MHz for ^{13}C with MAS rates of 2–3 kHz. Standard 4 mm Kel-F MAS rotors were fitted with teflon seals

on the end-caps to retain water. Cell walls were hydrated as evenly as possible with 30–50% of H_2O immediately prior to packing into the NMR rotor. The proton decoupling field strength was set at ca. 40 kHz and the Hartmann–Hahn matching condition was optimised as described by Jarvis et al. (1999). The same proton decoupling field strength was maintained during the contact and acquisition phases of the pulse sequence. Long-contact experiments to obtain CP-MAS difference spectra from onion polymers showing slow CP were carried out as described by Ha et al. (1996). Similar spectra were also obtained from potato and orange cell walls by a simpler variable-contact experiment, using linear combinations of spectra at short and long contact times. For potato cell walls the short-contact spectrum used was the mean of spectra recorded at contact times of 200, 400 and 1200 μs and the long-contact spectrum used was the mean of spectra recorded at contact times of 5, 6, 7 and 9 ms. For orange cell walls the short-contact spectrum used was the mean of spectra recorded at contact times of 400, 500, 600 and 800 μs and the long-contact spectrum used was the mean of spectra recorded at contact times of 2, 3, 5 and 7 ms. These experiments are sensitive to details of the probe design (Hediger et al., 1999). Radiofrequency absorption by water in the outer layers of the sample means that mismatching of the Hartmann–Hahn condition and loss of proton decoupling power are considerable, and are a complex function of position within the rotor. It appears that slow but measurable CP from mobile polysaccharides, as observed here, requires rather specific conditions of Hartmann–Hahn mismatch.

DP-MAS spectra with WALTZ-16 multiple-pulse proton decoupling were obtained as described by Renard and Jarvis (1999). Resonance assignments are based on published data (Jarvis, 1990; Ha et al., 1996; Renard and Jarvis, 1999; Dinand and Vignon, 2001). Chemical shifts are referenced externally against TMS.

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