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Carbohydrate Research 337 (2002) 587–593

CARBOHYDRATE RESEARCH

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Molecular ordering of cellulose after extraction of polysaccharides from primary cell walls of *Arabidopsis thaliana*: a solid-state CP/MAS ^{13}C NMR study

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Received 31 October 2001; accepted 6 February 2002

Abstract

Solid-state CP/MAS ^{13}C NMR spectroscopy was used to determine the effects of three different sequential extraction procedures, used to remove non-cellulosic polysaccharides, on the molecular ordering of cellulose in a cell-wall preparation containing mostly primary cell walls obtained from the leaves of the model dicotyledon, *Arabidopsis thaliana*. The extractions were 50 mM *trans*-1,2-diaminocyclohexane *N*,*N*,*N*′,*N*′-tetraacetic acid (CDTA) and 50 mM sodium carbonate (giving Residue 1); 50 mM CDTA, 50 mM sodium carbonate and 1 M KOH (giving Residue 2); and 50 mM CDTA, 50 mM sodium carbonate and 4 M KOH (giving Residue 3). The molecular ordering of cellulose in Residue 1 was similar to that in unextracted walls: the cellulose was almost all crystalline, with 43% of molecules contained in crystallite interiors and similar proportions of the triclinic (I_α) and monoclinic (I_β) crystal forms. Residue 2 was partly decrystallized and the remaining crystallites were mostly in the I_β form. Residue 3 was a mixture of cellulose II, cellulose I and amorphous cellulose. The presence of signals at 100.0 and 102.3 ppm in the spectra of Residues 1 and 2, but not of unextracted cell walls, suggested that the extractions giving these residues caused some of the non-cellulosic polysaccharides, possibly xyloglucans and galactoglucomannans, to become relatively well ordered, for example through interactions with cellulose crystallite surfaces.

Keywords: *Arabidopsis thaliana*; Cellulose I and II; Cell-wall extraction; Primary plant cell walls; Solid-state CP/MAS ^{13}C NMR spectroscopy

1. Introduction

Unlignified, primary cell walls of flowering plants contain cellulose microfibrils embedded in a matrix of mostly non-cellulosic polysaccharides.[1,2] In dicotyledon primary cell walls, the predominant non-cellulosic polysaccharides are typically pectic polysaccharides, together with smaller amounts of xyloglucans, proteins and glycoproteins. An approach commonly used to investigate the polysaccharides in preparations of such cell walls is to extract them successively, first with aqueous solutions of chelating agents, such as *trans*-1,2-diaminocyclohexane *N*,*N*,*N*′,*N*′-tetraacetic acid (CDTA), followed by a dilute solution of sodium carbonate to remove much of the pectic polysaccharides, and then with aqueous solutions of KOH or NaOH of increasing strengths to remove small amounts of additional pectic polysaccharides and other non-cellulosic polysaccharides such as xyloglucans.[3] In the past, a number of primary cell-wall preparations have been extracted using the following protocol: 50 mM CDTA, 50 mM sodium carbonate, 0.5 M KOH, 1 M KOH and 4 M KOH.[3] These extraction procedures are known to modify the non-cellulosic polysaccharides in various ways.[4] For example, cold, weak solutions of alkalis saponify ester links and thus remove acetyl groups, methyl ester groups and ester-linked hydroxycinnamic acids. Polysaccharides can also undergo alkali-catalyzed β-elimination reactions. However, little is known about the effects of such extraction procedures on the molecular ordering of cellulose in plant cell walls.

Solid-state CP/MAS ^{13}C NMR spectroscopy is a useful tool for studying the molecular ordering of cellu-

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0008-6215/02/$ - see front matter
PII: S0008-6215(02)00038-1

lose in plant cell-wall preparations containing mostly primary cell walls. This technique has been used to study the cellulose in the unextracted, primary cell walls of a range of angiosperm species,[5–7] including the model dicotyledon *Arabidopsis thaliana*.[8] These studies showed that the cellulose was all in a crystalline state in the form of cellulose I. The triclinic (I_α) and monoclinic (I_β) crystal forms of cellulose were present in similar proportions, and the calculated cross-sectional dimensions of the cellulose crystallites were in the range of 2–3 nm. Little evidence was found for cellulose II, which can easily be identified by a signal from C-1 at 107.7 pm. However, it is known that in cellulose preparations cellulose I is converted to cellulose II by treatment with strong alkali followed by washing.[9] Treatment with alkali also affects the proportions of crystalline and noncrystalline cellulose, sometimes raising the degree of crystallinity, but more often lowering it.[10–12] However, little is known about the effects of commonly used extraction procedures on the cellulose in primary cell walls.

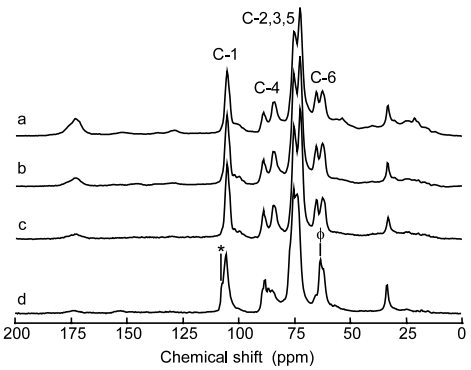

Fig. 1. Solid-state CP/MAS ^{13}C NMR spectra of *A. thaliana* cell walls: (a) unextracted; (b) extracted with CDTA and Na_2CO_3 (Residue 1); (c) extracted with CDTA, Na_2CO_3 and 1 M KOH (Residue 2); and (d) extracted with CDTA, Na_2CO_3, and 4 M KOH (Residue 3). Peaks at 63.6 and 107.8 ppm are marked ϕ and *, respectively. The numbers refer to the carbon atoms of the glucosyl residues in cellulose.

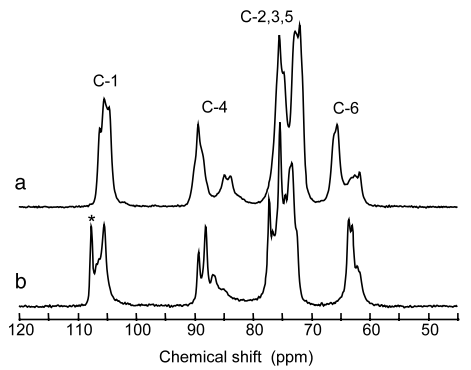

Fig. 2. Solid-state CP/MAS ^{13}C NMR spectra of microcrystalline cellulose (Avicel): (a) as cellulose I, (b) mercerized to cellulose II. See Fig. 1 for symbols.

In the present study, we used solid-state CP/MAS ^{13}C NMR spectroscopy to examine the effects of three different extraction procedures on the molecular ordering of cellulose in a cell-wall preparation containing mostly primary cell walls obtained from the leaves of the model dicotyledon, *A. thaliana*. The primary cell walls of this species have a composition similar to those of other dicotyledons.[13] The extraction procedures we used were 50 mM CDTA and 50 mM sodium carbonate (giving Residue 1); 50 mM CDTA, 50 mM sodium carbonate and 1 M KOH (giving Residue 2); and 50 mM CDTA, 50 mM sodium carbonate and 4 M KOH (giving Residue 3).

2. Results

Crystalline cellulose.—The spectra of the unextracted *A. thaliana* leaf cell walls and of the residues obtained after successively extracting these cell walls are shown in Fig. 1. These spectra are dominated by peaks and shoulders assigned to cellulose and were interpreted in terms of the chemical shifts found in the spectra of microcrystalline cellulose (Avicel) (Fig. 2(a)) or its mercerized product (Fig. 2(b)). The chemical shifts in these reference spectra were in turn assigned, as shown in Table 2, by comparison with literature data for cellulose I_α, cellulose I_β and cellulose II.[12,14,15]

A signal at 107.7 ppm (assigned to C-1 of cellulose II)[14] provides a sensitive test for cellulose II. In the unextracted cell walls, the very weak and relatively broad peak at this chemical shift (Fig. 4) may be associated with C-1 of arabinose.[16] Whatever its origin, the fact that it is weak indicates that there is little, if any, cellulose II in these cell walls. Furthermore, successive extraction of the cell walls with CDTA and sodium carbonate (giving Residue 1) or with CDTA, sodium carbonate and 1 M KOH (giving Residue 2) did not cause an increase in the size of this peak, indicating that no detectable conversion of cellulose I to cellulose II had occurred (Figs. 1, 4–6). However, successive extraction of the cell walls with CDTA, sodium carbonate and 4 M KOH (giving Residue 3) caused a pronounced increase in the size of this peak, indicating that this treatment was adequate to cause at least partial conversion of cellulose I to cellulose II (Figs. 1 and 7).

The strength of signals in the range of 65–66 ppm, assigned to C-6 of cellulose I, provides a sensitive test for residual cellulose I since signals from C-6 in cellulose II are confined to lower chemical shifts (Table 2). The spectrum of Residue 3 shows only weak signals in this range compared with the other residues, indicating that successive extraction of the cell walls with CDTA, sodium carbonate and 4 M KOH leaves only a small fraction of the cellulose in the cellulose I crystal form (Figs. 1 and 7).

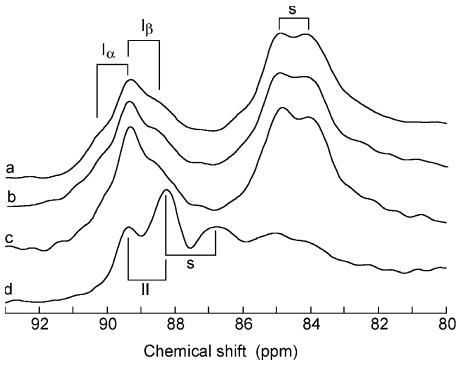

Fig. 3. Expansion of portions of solid-state CP/MAS ^{13}C NMR spectra of (a) untreated cell wall; (b) cell walls treated with CDTA and Na_2CO_3 (Residue 1); (c) cell walls treated with CDTA, Na_2CO_3, and 1 M KOH (Residue 2); and (d) cell walls treated with CDTA, Na_2CO_3 and 4 M KOH (Residue 3). The chemical-shift range covers signals assigned to C-4 in the interiors of cellulose I_α, I_β and II crystallites or on the surfaces (*s*) of those crystal forms.

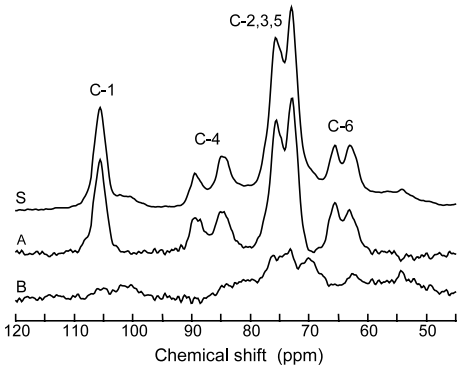

Fig. 4. Solid-state CP/MAS ^{13}C NMR spectrum of unextracted cell walls (**S**), and subspectra associated with relatively long (**A**) or short (**B**) values of $T_{1\rho}$(H).

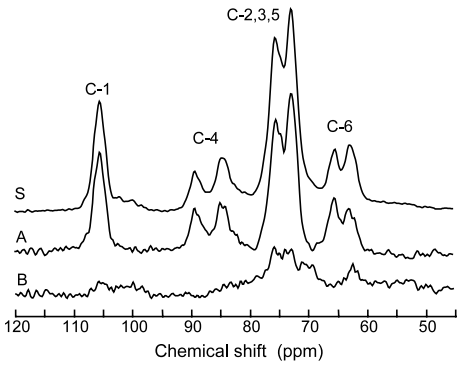

Fig. 5. Solid-state CP/MAS ^{13}C NMR spectrum of cell walls treated with CDTA and Na_2CO_3 (Residue 1) (**S**), and subspectra associated with relatively long (**A**) or short (**B**) values of $T_{1\rho}$(H).

Successive extraction of the cell walls with CDTA and sodium carbonate (giving Residue 1) did not alter the relative proportions of the cellulose I_α and I_β crystal forms, which were similar, as indicated by the relative heights of shoulders at 90.2 ppm (I_α) and 88.5 ppm (I_β) (Fig. 3(a,b)). The relevant signals were too poorly resolved to warrant quantitative estimates of the crystal forms, but previous studies of other poorly-resolved spectra have indicated that the center of gravity of the band at 89 ppm is influenced by the relative proportions of I_α and I_β through changes in the relative heights of the two shoulders.[17] Extracting the cell walls with 1 M KOH after first extracting them with CDTA followed by sodium carbonate, giving Residue 2, resulted in a smaller shoulder at 90.2 ppm (Fig. 3(c)), which indicated that the crystallites were mostly in the I_β form.

Crystallite dimensions.—PSRE NMR spectra obtained from unextracted *A. thaliana* leaf cell walls and from Residues 1, 2 and 3 are shown in Figs. 4–7. Crystallite dimensions were characterized by comparing the areas of signals at 89 and 84 ppm in subspectrum **A**, assigned to C-4 of glucosyl residues in molecules in crystallite interiors and on crystallite surfaces, respectively. It was estimated that 43% of the cellulose

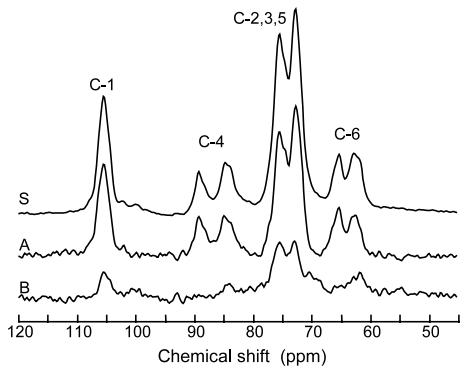

Fig. 6. Solid-state CP/MAS ^{13}C NMR spectrum of cell walls treated with CDTA, Na_2CO_3, and 1 M KOH (Residue 2) (**S**), and subspectra associated with relatively long (**A**) or short (**B**) values of $T_{1\rho}$(H).

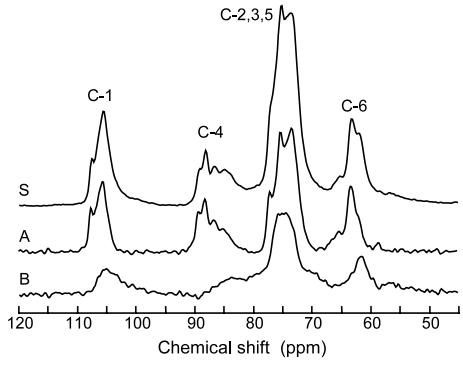

Fig. 7. Solid-state CP/MAS ^{13}C NMR spectrum of cell walls treated with CDTA, Na_2CO_3, and 4 M KOH (Residue 3) (**S**), and subspectra associated with relatively long (**A**) or short (**B**) values of $T_{1\rho}$(H).

Table 1
Combinations of coefficients and corresponding time constants used in PSRE experiments where two subspectra were obtained

Preparation	Combinations of coefficients	Proton spin relaxation time constants [$T_{1\rho}$(H) (ms)]
Cell walls	$\mathbf{A} = -1.5\mathbf{S} + 5.6\mathbf{S}'$	5
	$\mathbf{B} = 2.5\mathbf{S} + 5.6\mathbf{S}'$	3
Residue 1	$\mathbf{A} = -1.9\mathbf{S} + 4.4\mathbf{S}'$	10
	$\mathbf{B} = 2.9\mathbf{S} - 4.4\mathbf{S}'$	5
Residue 2	$\mathbf{A} = -1.8\mathbf{S} + 4.4\mathbf{S}'$	9
	$\mathbf{B} = 2.8\mathbf{S} - 4.4\mathbf{S}'$	5
Residue 3	$\mathbf{A} = -1.6\mathbf{S} + 4.0\mathbf{S}'$	9
	$\mathbf{B} = 2.6\mathbf{S} - 4.0\mathbf{S}'$	4

Table 2
^{13}C NMR chemical shifts for crystal forms of cellulose[12,14,15]

Crystal form	Chemical shift (PPM from TMS)		
	C-1	C-4	C-6
Cellulose I_α (interior)	105.5	90.2, 89.3	65.5
Cellulose I_β (interior)	106.3, 104.6	89.3, 88.4	66.1, 65.5
Cellulose I_α and I_β (surface)	105.5	84.9, 83.9	62.9, 61.7
Cellulose II (interior)	107.7, 105.5	89.3, 88.1	63.6, 63.0
Cellulose II (surface)	Not resolved	88.1, 86.7	61.9

molecules were in the crystallite interior in both the unextracted cell walls and Residue 1. The value for the unextracted cell walls differed from the value of 40% reported by Newman and co-workers,[8] because a different procedure was used for separating signals into subspectra (see Section 4). For Residue 2, it was estimated that 42% of the cellulose molecules were in the crystallite interior. Similar studies on wood[18] lead us to expect a standard error of $< 2\%$ for the percentages of cellulose molecules in the crystallite interiors estimated in the present study. These values are consistent with cellulose crystallites of about 3 nm cross-sectional diameter. A strong correlation (correlation coefficient = 0.99) has been shown between lateral dimensions estimated by this method and those estimated from the widths of X-ray diffraction peaks.[18] Signals assigned to the crystallite interior and crystallite surface of cellulose II were not well resolved in subspectrum **A** obtained for Residue 3 (Fig. 7), so no quantitative interpretation was attempted.

Non-cellulosic material.—In addition to cellulose, the spectrum of the unextracted cell walls showed a broad peak (165–180 ppm) which was probably composed of signals from C-6 of the galacturonic acid residues in homogalacturonans (171–175 ppm)[19] and from protein (165–180 ppm)[20] (Fig. 1). The protein was probably partly from cell-wall protein and partly from cytoplasmic contamination of the cell walls. The size of the broad peak was successively reduced in Residues 1–3, indicating that the material was extracted. A peak at 29 ppm in the spectrum of the unextracted cell walls was not reduced in the spectra of Residues 1–3, which is consistent with it being assigned to polymethylene chains, for example in cutin or waxes associated with the cuticle.

Disordered material.—The total and PSRE subspectra **A** and **B** for the unextracted cell walls, Residues 1, 2 and 3 are shown in Figs. 4–7, respectively. The distribution of total carbon between ordered and disordered material was obtained by integrating PSRE subspectra **A** and **B**, and adjusting the results to correct for exponential proton rotating-frame spin-lattice relaxation [$T_{1\rho}$(H)] during the cross-polarization contact time. The relevant values of $T_{1\rho}$(H) are shown in Table 1. The percentages of carbon contained in disordered material were 64, 49, 57 and 57% for unextracted cell walls and Residues 1, 2 and 3, respectively.

The comparatively low percentage of disordered material in Residue 1 indicated that much of this material, probably mainly pectic polysaccharides, had been extracted by the CDTA and sodium carbonate. The higher percentage of disordered material in Residue 2 indicated partial disruption of the cellulose I crystalline structure by the 1 M KOH. The larger signal at 105 ppm in subspectrum **B** of Residue 2 (Fig. 6) compared with subspectrum **B** of Residue 1 (Fig. 5), was consistent with partial decrystallization, because this signal was assigned to C-1 of the glucosyl residues in disordered cellulose.[14]

There was no difference in the relative proportions of crystalline and disordered material in Residue 3 compared with Residue 2. We interpret this as resulting from a balance between the removal of disordered material and the production of further disordered material by partial decrystallization during the conversion of cellulose I to cellulose II (Fig. 7).

Cellulose–polysaccharide interactions.—Compared with the spectrum of unextracted cell walls, the spectra of the residues obtained after extracting with CDTA and sodium carbonate (Residue 1) and with CDTA, sodium carbonate, and 1 M KOH (Residue 2) showed relatively weak signals at 100.0 and 102.3 ppm (Fig. 1). These signals can be seen more clearly in plot expansions (Fig. 8) and are assigned to C-1 in polysaccharides that are relatively well ordered, for example through interactions with cellulose crystallite surfaces.[15]

The sharp nature of the bandshapes points to well-ordered polysaccharides.[21] The signal at 100.0 ppm was tentatively assigned to C-1 of terminal or 2-linked xylose in xyloglucans.[22] The signal at 102.3 ppm was tentatively assigned to C-1 of mannose in galactoglucomannan.[21]

The sizes of the signals at 100.0 and 102.3 ppm were not lower in the spectrum of Residue 2 compared with the spectrum of Residue 1 (Fig. 8). Thus, there was no evidence for the removal of these well-ordered polysaccharides by 1 M KOH. The spectrum of Residue 3, obtained after successive extraction with CDTA, sodium carbonate and 4 M KOH, had smaller signals at 100.0 and 102.3 ppm. This result is consistent with the 4 M KOH removing the well-ordered polysaccharides from the cellulose crystal surfaces.

3. Discussion

There was no evidence for the conversion of cellulose I to cellulose II after treating the cell walls with 50 mM CDTA at 80 °C for 2 hours and 50 mM sodium carbonate at 4 °C for 20 hours then at 20 °C for 6 hours, or with this treatment followed by exposure to 1 M KOH at 20 °C for 4 hours. However, successive treatment of the cell walls with CDTA, sodium carbonate, and 4 M KOH at 20 °C for 4 hours did cause conversion of cellulose I to cellulose II, leaving only a small fraction in the cellulose I crystal form. Thus, the critical concentration of KOH for effecting the change is between 1 and 4 M. This is consistent with the observations of Chanzy and co-workers[23] concerning mercerization of cellulose in the primary cell walls of rose (*Rosa glauca*) suspension culture cells. After the cell walls had been treated successively with water, ammonium oxalate, 0.5 M NaOH and 1.25 M NaOH, no cellulose II was detected, but a subsequent treatment with 1.5 M NaOH resulted in the total mercerization of the cellulose. Our observations are also consistent with those of Dinand and co-workers[24] who found that NaOH concentrations of 2.5 M or above were necessary to mercerize cellulose microfibrils extracted from sugar beet (*Beta vulgaris* subsp. *vulgaris*) pulp, which contained mostly parenchyma cells with primary walls.

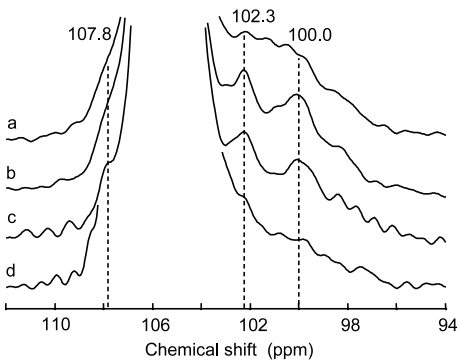

Fig. 8. Expansion of portions of solid-state CP/MAS ^{13}C NMR spectra of (a) untreated cell walls; (b) cell walls treated with CDTA and Na_2CO_3 (Residue 1); (c) cell walls treated with CDTA, Na_2CO_3, and 1 M KOH (Residue 2); and (d) cell walls treated with CDTA, Na_2CO_3, and 4 M KOH (Residue 3). The chemical shift range covers signals assigned to C-1 of cellulose and associated polysaccharides. The plots were normalized so that the height of the tallest peak (offscale in these expansions) was identical in each case.

The cellulose crystallinity was also not affected by treating the cell walls with CDTA and sodium carbonate, but decreased after treatment with CDTA, sodium carbonate, and 1 M KOH. Rumbold[25] reported that detectable amounts of alkali were absorbed by cellulose even when the concentration was too low to cause mercerization. Our observations are consistent with partial decrystallization by the absorption and desorption of such small amounts of alkali. Several authors have reported similar results. Taylor and co-workers[26] extracted the pectic polysaccharides from primary cell walls of bean (*Phaseolus vulgaris*) hypocotyls using ammonium oxalate and oxalic acid, followed by 0.7 M KOH, and used proton NMR to demonstrate partial decrystallization. Mörl and co-workers[27] used ^{13}C NMR spectroscopy to characterize the cellulose in cotton linters after treatment with KOH solutions. Cotton linters have thick, secondary cell walls composed almost exclusively of cellulose. Compared with the cellulose in primary cell walls, the cellulose in cotton linters was mercerized at higher KOH concentrations: cellulose II was not detected among the products until the KOH concentration was 2.8 M. However, partial decrystallization was found after treatment with 2.2 M KOH. Fengel and co-workers[28] treated cotton linters with various concentrations of NaOH and used FTIR to detect partial decrystallization, which occurred at 2.5 M NaOH, and X-ray diffraction to detect cellulose II, which formed only at concentrations > 3.25 M. Kim and co-workers[29] treated ramie cellulose with NaOH and found that the decrease of cellulose I was slightly faster than the increase of cellulose II, implying that some might be converted into an amorphous state.

The process of chemical extraction in the present study may have caused the interaction of non-cellulosic polysaccharides with cellulose molecules at the crystallite surfaces. The solid-state CP/MAS ^{13}C NMR spectra of the unfractionated cell walls showed no evidence for well-ordered non-cellulosic polysaccharides. However, the differences in the spectra after treatment with CDTA and sodium carbonate indicated that non-cellulosic polysaccharides, possibly xyloglucans and galactoglucomannans, had become well-ordered through this extraction procedure. Leaf cell walls of *A. thaliana* are known to contain xyloglucans[13] as well as galactoglucomannans (L. M. Davies and P. J. Harris, unpublished

results). It is proposed these non-cellulosic polysaccharides may have become well-ordered because of the loss of acetyl groups. These changes would have improved molecular interactions with cellulose crystallites.

Such a chemically induced interaction between cellulose and non-cellulosic polysaccharides has important implications for these commonly used polysaccharide extraction procedures. The interaction can be reversed by subsequent treatment with 4 M KOH, and this is consistent with reports that high concentrations of alkali are required to disrupt hydrogen bonds between xyloglucan and cellulose,[30] but the residue is mostly cellulose II and therefore not suitable as a model substance for studies of molecular interactions at the cellulose–water interface. Hayashi and Maclachlan[31] and Hayashi and co-workers[32] used cellulose extracted from pea epicotyls using 4.3 M KOH to examine the binding of xyloglucan molecules to cellulose. Our results suggest that those authors studied binding to cellulose II, not to cellulose I. The binding data are therefore of little relevance to models for plant cell walls.

4. Experimental

Plant material.—Plants of *A. thaliana* (L.) Heynh. ecotype Columbia were grown in a controlled environment and harvested as described by Newman and co-workers.[8] Cell walls were isolated from *A. thaliana* rosette leaves, as described by Newman and co-workers.[8]

Model substances.—A portion of Avicel microcrystalline cellulose (E. Merck) was moistened to 40% moisture content. A second portion was mercerized by soaking in 4 M NaOH for 16 h, followed by thorough washing and part-drying to approximately 40% moisture content.

Fractionation of the cell walls.—Three leaf cell-wall residues, which were obtained by extracting cell walls that had not been dried or treated with organic solvents, were examined by NMR spectroscopy. Residue 1 (CDTA and Na_2CO_3 extracted residue) was obtained by extracting cell walls (equivalent to 265 mg dry weight) successively with 25 mL 50 mM CDTA (adjusted to pH 6.5 with KOH) at 80 °C for 1 h (twice) and 25 mL 50 mM Na_2CO_3 (containing 25 mM $NaBH_4$) at 1 °C for 20 h, then at 20 °C for 3 h (twice at 20 °C). All incubations were done under Ar with frequent mixing, and after each incubation, the suspension was centrifuged (450 g, 5 min) and the supernatant was discarded. Residue 2 (CDTA, Na_2CO_3 and 1 M KOH extracted residue) was obtained by extracting cell walls (equivalent to 330 mg dry weight) with CDTA and Na_2CO_3 as described above, but in addition, the cell-wall material was further extracted with 1 M KOH containing 25 mM $NaBH_4$ (25 mL) at 20 °C for 2 h (twice) with frequent mixing. Residue 3 (CDTA, Na_2CO_3 and 4 M KOH extracted residue) was obtained by extracting cell walls (equivalent to 429 mg dry weight) as for Residue 2, except 4 M KOH was used instead of 1 M KOH. Each final residue was suspended in water (50 mL), and the pH was adjusted to 5.0 with 18 M AcOH, and 4 °C). The suspension was centrifuged (450 g, 5 min), the pellet was washed five times in 80% EtOH, and an aliquot (0.5 g) was taken for dry weight determination. The residues were stored in 80% EtOH until NMR spectroscopy was carried out.

CP/MAS ^{13}C NMR spectroscopy.—This was done on the cell-wall fractions as described by Newman and co-workers[8] for rosette leaf cell walls.

'Normal' spectra (**S**) were obtained with a 6 μs proton preparation pulse, a 1 ms cross-polarization contact time, 30 ms of data acquisition, and a delay of 0.6 s before the sequence was repeated. The proton transmitter power level was increased to provide radiofrequency field strengths in the range $\gamma B_h/(2\pi) = 56$ to 63 kHz during data acquisition for all samples. Transient signals from 39,000 contacts were averaged.

Proton rotating-frame relaxation time constants $T_{1\rho}(H)$ (Table 1) were estimated by a pulse sequence in which a proton spin-locking pulse, set at 4 ms, was inserted between each 90° proton preparation pulse and the cross polarization contact time to give a partly relaxed spectrum **S′**. Standard errors of $< 10\%$ are typically encountered in such measurements.

Proton spin relaxation edited (PSRE) NMR.—This is a method designed to exploit differences in $T_{1\rho}(H)$ associated with different spatial domains, i.e., crystalline and non-crystalline domains.[14,33,34] The method was used here to examine NMR signals from cellulose in the cell-wall residues. The theoretical basis for PSRE NMR has been described previously[14,33] and applied in studies of cellulose in primary cell walls.[5,8]

PSRE NMR involves mixing the spectra (**S** and **S′**) described above to generate subspectra **A** and **B**. In the present study, the coefficients for the mixtures (Table 1) were calcd from estimates of the relevant values of $T_{1\rho}(H)$ for signals in subspectra **A** and **B**. A peak at 89 ppm (assigned to C-4 in crystalline cellulose) was taken as representative of subspectrum **A**, and the signal strength in a dip at 80 ppm (assigned to disordered polysaccharides) was taken as representative of subspectrum **B**. Relative proportions of crystalline interior and crystalline surface chains were estimated by comparing the areas of peaks at 89 and 84 ppm. An arbitrary boundary was drawn at 87 ppm, as illustrated in Smith and co-workers.[7]

Acknowledgements

This research was supported by the Marsden Fund of the Royal Society of New Zealand (contract number UOA 406).

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