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RESEARCH PAPER

Solid-state ^{13}C-NMR spectroscopy shows that the xyloglucans in the primary cell walls of mung bean (*Vigna radiata* L.) occur in different domains: a new model for xyloglucan–cellulose interactions in the cell wall

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Abstract

Xyloglucans (XG) with different mobilities were identified in the primary cell walls of mung beans (*Vigna radiata* L.) by solid-state ^{13}C-NMR spectroscopy. To improve the signal:noise ratios compared with unlabelled controls, Glc labelled at either C-1 or C-4 with ^{13}C-isotope was incorporated into the cell-wall polysaccharides of mung bean hypocotyls. Using cell walls from seedlings labelled with D-[1-^{13}C]glucose and, by exploiting the differences in rotating-frame and spin-spin proton relaxation, a small signal was detected which was assigned to Xyl of XGs with rigid glucan backbones. After labelling seedlings with D-[4-^{13}C]glucose and using a novel combination of spin-echo spectroscopy with proton spin relaxation-editing, signals were detected that had ^{13}C-spin relaxations and chemical shifts which were assigned to partly-rigid XGs surrounded by mobile non-cellulosic polysaccharides. Although quantification of these two mobility types of XG was difficult, the results indicated that the partly-rigid XGs were predominant in the cell walls. The results lend support to the postulated new cell-wall models in which only a small proportion of the total surface area of the cellulose microfibrils has XG adsorbed on to it. In these new models, the partly-rigid XGs form cross-links between adjacent cellulose microfibrils and/or between cellulose microfibrils and other non-cellulosic polysaccharides, such as pectic polysaccharides.

Key words: Cellulose microfibrils, cell-wall models, primary cell wall, solid-state ^{13}C NMR, *Vigna radiata*, xyloglucan.

Introduction

Primary cell walls of dicotyledons contain cellulose microfibrils embedded in a matrix of non-cellulosic polysaccharides (Bacic *et al*., 1988). Models of the architecture of dicotyledon primary cell walls commonly assume that xyloglucans (XGs) are adsorbed on to and coat the cellulose microfibrils. In these models, reviewed by Cosgrove (2001), the XGs cross-link adjacent microfibrils (Fig. 1A), or cross-link microfibrils and other matrix molecules, for example, pectic polysaccharides (Fig. 1B). Such cross-links will constrain the movements of the microfibrils and must be disrupted to allow cell-wall expansion. This expansion is thought to occur either by expansin detaching XG adsorbed on to cellulose (Cosgrove, 2001) and/or by xyloglucan endotransglycosylase (XET) breaking and rejoining cross-linking XG molecules (Thompson and Fry, 2001).

Several lines of evidence indicate that XGs are associated with cellulose microfibrils in primary cell walls. First, cross-links, possibly XG, between adjacent cellulose microfibrils have been observed in primary cell walls by

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Abbreviations: CP/MAS, cross-polarization/magic-angle spinning; GalUA, galacturonic acid; NMR, nuclear magnetic resonance; PSRE, proton spin relaxation editing; PDMS, polydimethylsiloxane; SE-PSRE, spin echo-proton spin relaxation editing; SPE, single pulse excitation; TEM, transmission electron microscopy; XG, xyloglucans; XET, xyloglucan endotransglucosylase.

TEM after preparing them using fast-freeze, deep-etch, and rotary shadowing (McCann *et al*., 1990; Fujino *et al*., 2000). Second, immunogold labelling using antibodies against XG showed that XG molecules were closely associated with cellulose microfibrils in the primary walls of suspension-cultured cells of *Rubus* sp. (Joseleau *et al*., 1992) and in suspension-cultured cells of tobacco (*Nicotiana tabacum*) (Sabba *et al*., 1999). In the latter cells, no labelling was found in the middle lamella where no cellulose could be detected using cellulase conjugated to colloidal gold. Third, the cell walls of suspension-cultured tobacco grown in the presence of 2,6-dichlorobenzonitrile, an inhibitor of cellulose synthesis, contained no cellulose and their XGs could be extracted easily without the use of alkali (Sabba *et al*., 1999). *In vitro* systems have also been used to show that XG can associate with cellulose. For example, if a solution of XG is passed down a column of cellulose, some of the XG associates with the cellulose (Mishima *et al*., 1998). Moreover, if the cellulose-producing bacterium *Acetobacter xylinus* is grown in the presence of XG, the latter associates with the cellulose ribbons produced (Whitney *et al*., 1995).

However, none of the above studies provide evidence for the cellulose microfibrils in primary cell walls being coated with XG. Indeed, it is possible that only a relatively short length of each XG molecule is actually adsorbed on to a cellulose microfibril, and only a small proportion of the total surface area of the cellulose microfibrils may have XG adsorbed on to it.

Solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy is a technique that can be used to identify polymers with different mobilities. These polymers may be cell-wall polysaccharides, such as xyloglucans, which are structurally similar, but have different mobilities because of their locations and interactions with other molecules within the cell wall. For example, the glucan backbones of the XG molecules, or parts of molecules, adsorbed on to the surface of cellulose microfibrils *in muro* would be predicted to adopt a rigid, flattened conformation, rather than the twisted-backbone conformation of free XG (Levy *et al*., 1997). These two different conformations can potentially be detected by solid-state ^{13}C NMR spectroscopy (Horii *et al*., 1984; Jarvis, 1994). This technique has the added advantage that cell walls that have never been dried can be examined. By studying proton or ^{13}C spin relaxation properties, it should be possible to detect differences in spin relaxation and in chemical shift between xyloglucans of different mobilities. However, solid-state ^{13}C NMR studies of primary cell walls of six dicotyledon species have provided no conclusive evidence for rigid XG molecules (Newman *et al*., 1994, 1996; Koh *et al*., 1997; Smith *et al*., 1998*a*; Thimm *et al*., 2002). This may be because there are no rigid XG molecules in these cell walls, or because the amount is insufficient to be detected by solid-state ^{13}C NMR spectroscopy using the natural abundance of ^{13}C-isotope (1.1% of all carbon atoms).

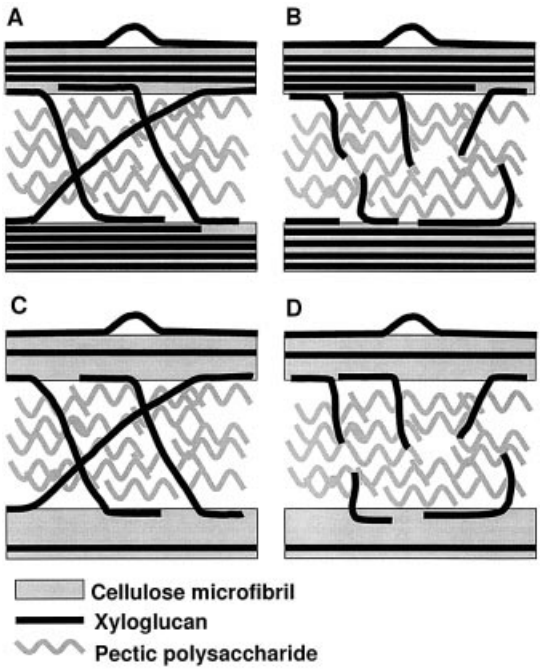

Fig. 1. (A, B) Current models of primary cell walls of dicotyledons showing the postulated locations of XG molecules. In both of these models, the XGs coat the entire surface areas of the cellulose microfibrils, and a small proportion of the XGs are within the cellulose microfibrils (not shown). (A) The XGs crosslink adjacent cellulose microfibrils. (B) The XGs crosslink cellulose microfibrils and pectic polysaccharides. (C, D) New models for the primary cell walls of mung bean seedlings. These are as (A) and (B), but XGs occupy only a small proportion of the surface area of the cellulose microfibrils. No XGs are within the cellulose microfibrils.

In the present study, the proportion of ^{13}C-isotope in the cell-wall polysaccharides was increased to improve the signal-to-noise ratio and thereby improve the sensitivity of the solid-state ^{13}C-NMR spectroscopy. Etiolated hypocotyls of mung beans (*Vigna radiata* L.) were used as a source of ^{13}C-enriched primary cell walls for these experiments as these seedlings are easy to incubate with ^{13}C-labelled Glc, the hypocotyls consist mostly of cells with primary walls, and the XG in these cell walls has been characterized (Kato and Matsuda, 1976, 1980*a*, *b*). Plant tissues take up Glc and convert it to nucleoside diphosphate sugars, the precursors of the cell-wall polysaccharides (Fry, 1985). By using Glc labelled with ^{13}C either at C-1 or at C-4, the monosaccharides in the cell-wall polysaccharides of mung bean seedlings can be labelled specifically at C-1 or C-4.

The mobility of XGs in the cell wall was investigated using solid-state cross-polarization/magic-angle spinning (CP/MAS) ^{13}C-NMR spectroscopy in combination with proton spin relaxation editing (PSRE). This combination of techniques is useful in studying the mobilities of

Table 1. *Water content of cell-wall preparations from seedlings incubated with ^{13}C-labelled Glc; suppression factors and resulting linear combinations used to obtain PSRE subspectra from CP/MAS NMR spectra are also shown*

		D-[1-^{13}C]glucose[a]	D-[1-^{13}C]glucose[b]	A-[4-^{13}C]glucose[a]
Water content (% w/w)		35	40	47
Supression factors				
$T_{1\rho}$(H) (ms)	f_a	0.61	0.68	0.73
	f_b	0.22	0.28	0.29
T_2(H) (μs)	f_a	0.30	0.30	–[c]
	f_b	0.54	0.56	–[c]
Linear combinations				
$T_{1\rho}$(H) (ms)	Subspectrum *A*	–0.47S+2.33S′	–0.70S+2.50S′	–0.66S+2.27S′
	Subspectrum *B*	1.47S–2.33S′	1.70S–2.5S′	1.66S–2.27S′
T_2(H) (μs)	Subspectrum *A*	2.25S–4.17S″	2.15S–3.85S″	–[c]
	Subspectrum *B*	1.25+4.17S″	–1.15S+3.85S″	–[c]

[a] Walls treated with ethanol.
[b] Walls not treated with ethanol.
[c] Not determined.

components in multi-component systems, such as the plant cell wall (Newman, 1999*a*; Tang and Hills, 2003). Two series of experiments were conducted. In the first series, cell walls from seedlings incubated with D-[1-^{13}C]glucose were used to detect XGs in the cell-wall domain containing rigid polysaccharides. In the second series of experiments, the seedlings were incubated with D-[4-^{13}C]glucose, and then a novel NMR technique was used on the cell walls that combined ^{13}C spin-echo experiments with proton spin relaxation editing (SE-PSRE) to measure ^{13}C spin-spin relaxation time constants (T_2(C)). This technique was used to detect XGs in the cell-wall domain containing semi-rigid and mobile polysaccharides.

Materials and methods

Chemicals

D-[1-^{13}C]glucose (99%) and D-[4-^{13}C]glucose (99%) were from Novachem Pty Ltd, South Yarra, Victoria, Australia.

Plant material

Seeds of mung bean (*Vigna radiata* L. cv. Berkin) were surface-sterilized in 30 g l^{-1} NaOCl for 20 min, soaked in sterile water in the dark for 7 h at 25 °C, and germinated on sterile, damp vermiculite in the dark at 25 °C.

Microscopy

Bright-field and UV-fluorescence microscopy was carried out on fresh transverse sections cut from midway along the hypocotyls as described by Carnachan and Harris (2000). Lignin was detected by the red colour given with phloroglucinol–HCl, by the blue autofluorescence in UV radiation when mounted in 0.1 M NH_4OH, and by the green or blue staining produced with the polychromatic stain toluidine blue O (Carnachan and Harris, 2000). Starch was detected using I_2 in KI.

Lignin was detected only in the annular and spiral thickenings of the few protoxylem vessels present in the hypocotyls. Small starch granules, detected in the layer of cortical parenchyma cells nearest the endodermis, were removed by filtration during cell-wall isolation.

Incubation of seedlings with ^{13}C-labelled Glc

Batches of five seedlings with hypocotyls 3.5–4.5 cm long were selected, the primary roots were cut off, and the cut-ends placed in a solution of unlabelled Glc, D-[1-^{13}C]glucose or D-[4-^{13}C]glucose (7.5 mg Glc ml^{-1}, 2 cm^3). The seedlings were incubated (18 h at 25 °C) in the dark in a sealed glass container with the air saturated with water vapour. For each treatment, 15 batches of seedlings were used, making a total of 75 seedlings.

Isolation of cell walls

After incubation with ^{13}C-labelled Glc or unlabelled Glc, the cotyledons were removed from the seedlings by cutting 0.5 cm below the cotyledons. All the steps in the isolation procedure were carried out at 4 °C. The hypocotyls were cut into pieces (0.5 cm long) and homogenized in 100 cm^3 of 20 mM HEPES–KOH buffer (pH 6.7) containing 10 mM dithiothreitol, using a Polytron blender (Model PT10-35, Kinematica GmbH, Luzern, Switzerland) on full power (three times for 20 s). Further homogenization was done using a Tenbroeck ground-glass homogenizer (15 cm^3; Kontes Glass Company, Vineland, NJ, USA). Breakage of the cells was monitored using bright-field microscopy after staining with Ponceau 2R, which stains cytoplasmic protein red ((Harris, 1983). The homogenate was centrifuged (250 *g* for 10 min), and the pellet washed three times by centrifugation with buffer (with dithiothreitol omitted), followed by six times with water. The pellet was resuspended, washed on to nylon mesh (pore size 11 μm), and the residue on the mesh washed with water (500 cm^3). The residue (cell-wall preparation) was washed three times by centrifugation with 80% (v/v) ethanol and the final suspension was kept at 4 °C until NMR spectroscopy could be carried out.

An additional cell-wall preparation was obtained from the hypocotyls of seedlings incubated with D-[1-^{13}C]glucose, but was not washed with 80% (v/v) ethanol. Instead, this preparation was frozen and dried under vacuum to a water content of approximately 40% (w/w) and NMR spectroscopy was carried out immediately. The exact water content of each sample was determined after completing the NMR spectroscopy by heating at 105 °C until dry (Table 1).

NMR spectroscopy

Cell-wall preparations in 80% (v/v) ethanol were prepared for NMR by filtering portions of the suspensions onto a glass-fibre filter (GF/C, Whatman Scientific Ltd, Kent, UK) and part-drying in air to a water content of approximately 40% by weight. A sample was then

packed in a 7 mm diameter, cylindrical silicon nitride rotor, and retained with Vespel end caps. An internal standard of PDMS (polydimethylsiloxane) was added to all samples. The rotor was spun at 4 kHz in a Doty Scientific magic-angle spinning probe for ^{13}C NMR spectroscopy at 50.3 MHz on a Varian Inova-200 spectrometer.

In the cross-polarization (CP) NMR experiments, each 6 μs 90° proton preparation pulse was followed by a 1 ms CP contact time, 51 ms of data acquisition and a recovery delay of 1.0 s before the sequence was repeated. Newman (1987) showed that a 6 μs proton preparation pulse is sufficient to excite protons in the rigid domain. Preliminary T_1(H) experiments indicated that a 1.0 s delay was adequate for the recovery of proton magnetization, as was also shown in the experiments on other cell walls (Newman *et al*., 1994, 1996). The proton transmitter output was increased to its maximum during data acquisition, corresponding to a precession frequency of 59 kHz.

Proton relaxation experiments

Proton rotating-frame spin relaxation with time constant $T_{1\rho}$(H), and proton spin-spin relaxation with time constant T_2(H) were characterized by inserting relaxation intervals of duration t_1 or t_2, respectively, between the proton preparation pulse and the CP contact time. The values for t_1 and t_2 were chosen to be within the ranges of values for $T_{1\rho}$(H) and T_2(H), respectively, in order to optimize signal:noise ratios in separate subspectra (Newman and Hemmingson, 1990). Protons were spin-locked during t_1, but the proton transmitter was switched off during t_2.

PSRE NMR subspectra were generated by combining spectra labelled *S*, *S*′ and *S*", where *S* was obtained by the normal cross-polarization pulse sequence, *S*′ with t_1=4 ms and *S*" with t_2=15 μs. The principles behind PSRE NMR have been described elsewhere (Newman, 1999*a*; Tang *et al*., 2000). In the simplest case, a spectrum *S* is the sum of subspectra *A* and *B* from two distinct types of domains. A partly-relaxed spectrum *S*′ is then

$$S'=f_aA+f_bB \quad (1)$$

where f_a and f_b are signal suppression factors. The subspectra can then be separated by computing:

$$A=kS+k'S' \quad (2a)$$

$$B=(1-k)S-k'S' \quad (2b)$$

where

$$k=f_b/(f_b-f_a) \quad (3a)$$

$$k'=-1/(f_b-f_a) \quad (3b)$$

In the present context, *A* and *B* contain signals from the cellulose microfibrils and the non-cellulosic polysaccharides, respectively.

Two NMR signals were selected for proton rotating-frame relaxation experiments. The signal at 89 ppm (assigned to C-4 of cellulose) was selected as representative of cellulose crystallites, appearing at a chemical shift for which there is little risk of overlap with signals from other polysaccharides. A signal at 69 ppm (assigned to C-2, C-3, and C-5 of GalUA residues in pectic homogalacturonan (Jarvis and Apperley, 1995; Sinitsya *et al*., 1998)) was selected as representative of mobile polysaccharides. Because the $T_{1\rho}$(H) relaxation for the 69 ppm signal was non-exponential, it was not possible to achieve total elimination of signals from non-cellulosic polysaccharides without also suppressing signals from cellulose, so linear combinations of *S* and *S*′ were generated to enhance signal suppression. The suppression factors and the resulting linear combinations used to generate the PSRE subspectra from the experimental PSRE spectra are shown in Table 1. The slight differences in these values may reflect differences in the water content of the cell-wall preparations (Hediger *et al*., 1999; Tang *et al*., 2000). The separation was successful in that the signals assigned to cellulose and non-cellulosic polysaccharides appeared in subspectra *A* and *B*, respectively.

The editing process was repeated for T_2(H) relaxation for the cell walls from seedlings labelled with D-[1-^{13}C]glucose. This spin relaxation process suppressed cellulose signals more than the other signals, but did not eliminate them entirely. Linear combinations were again generated to enhance the amount of suppression (Table 1). The separation was successful in that signals assigned to cellulose and non-cellulosic polysaccharides appeared in subspectra *A* and *B*, respectively.

The combinations of spectra were generated off-line using software written to run under MS-DOS. Spectra were then processed using MS Excel.

SE-PSRE experiments

T_2(C) relaxation was characterized by a spin-echo sequence in which a delay of duration t_2 was inserted between the CP contact time and commencement of data acquisition (Newman *et al*., 1996). A 180° refocusing pulse was applied halfway through t_2, and values of t_2 were chosen so that $0.5t_2$ was always a multiple of the rotor rotation period, and protons were decoupled with an attenuated power output corresponding to a precession frequency of 43 kHz throughout t_2. PSRE NMR subspectra were generated for the T_2(C) spectrum using the *S* and *S*′ values calculated as described above.

Single pulse excitation/magic-angle spinning (SPE/MAS) experiments

The single pulse excitation/magic-angle spinning (SPE/MAS) spectrum was obtained with a pulse sequence in which each 6 μs ^{13}C excitation pulse was followed by 51 ms of data acquisition time and a 1.0 s recovery delay (Smith *et al*., 1998*b*). The 1.0 s delay was used to maximize the response from mobile components and minimize the response from rigid components of the cell walls (Smith *et al*., 1998*b*). The proton decoupler transmitter power was increased to provide radiofrequency field strengths of 59 kHz during data acquisition.

Cellulose content of the an unlabelled cell-wall preparation

The cellulose content of the cell-wall preparation was determined by the method of Brendel *et al*. (2000).

Neutral monosaccharide composition of an unlabelled cell-wall preparation

An unlabelled cell-wall preparation was hydrolysed with sulphuric acid, and the released neutral monosaccharides reduced and acetylated to produce alditol acetates, which were quantified by gas chromatography (Smith and Harris, 1995).

Results

CP/MAS ^{13}C NMR spectroscopy of cell-wall preparations from hypocotyls of seedlings incubated with ^{13}C-labelled Glc

CP/MAS solid-state ^{13}C NMR spectra of cell-wall preparations from seedlings incubated with D-[1-^{13}C]glucose or D-[4-^{13}C]glucose showed increases in signal strengths at C-1 or C-4, respectively, compared with the spectrum of a

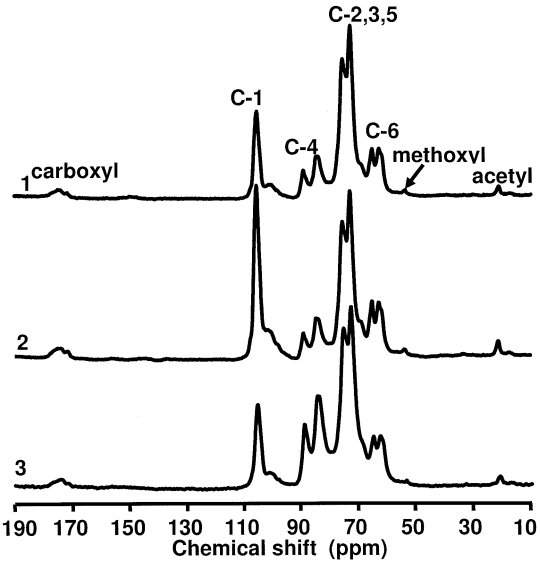

Fig. 2. CP/MAS NMR spectra of cell-wall preparations from hypocotyls of mung bean seedlings. (1) Seedlings incubated with unlabelled Glc; (2) seedlings incubated with D-[1-^{13}C]glucose; (3) seedlings incubated with D-[4-^{13}C]glucose. Carbon numbers refer to the Glc residues of cellulose.

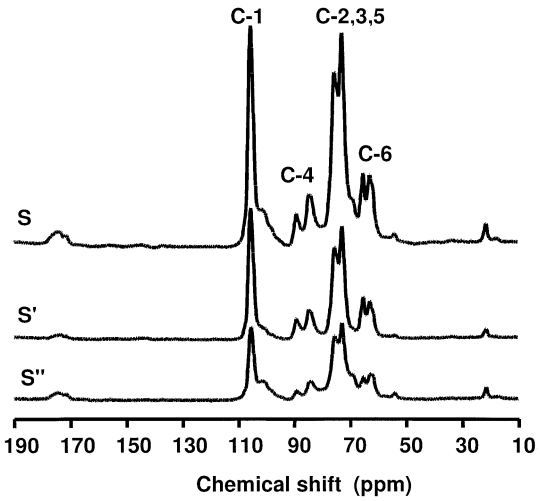

Fig. 3. Solid-state ^{13}C NMR spectra of a cell-wall preparation from hypocotyls of mung bean seedlings incubated with D-[1-^{13}C]glucose. (*S*) obtained by the normal CP/MAS NMR pulse sequence; (*S*′) obtained with 4 ms of proton rotating-frame spin relaxation; (*S*″) obtained with 15 μs of proton spin-spin relaxation. Carbon numbers refer to the Glc residues of cellulose.

cell-wall preparation from seedlings incubated with unlabelled Glc (Fig. 2). Measurements of peak areas over the chemical-shift range assigned to C-1 or C-4 (93–108 ppm and 80–93 ppm, respectively) showed that isotopic enrichment for cell walls obtained from seedlings incubated with D-[1-^{13}C]glucose was approximately 2.5 and that for cell walls obtained from seedlings incubated with D-[4-^{13}C]glucose was approximately 2.3. These enrichment factors are averages for all polysaccharides responding to the cross-polarization pulse sequences. Moreover, they are only approximations as they do not take into account the efficiency of the magnetization transfer in the CP process, where molecules will have different mobility and strength of H-C dipolar interactions (Hediger *et al*., 1999). The signals include the dominant signals assigned to the rigid cellulose crystallites and the broader underlying signals assigned to non-cellulosic polysaccharides.

PSRE of CP/MAS NMR spectra obtained for a cell-wall preparation from hypocotyls of seedlings incubated with D-[1-^{13}C]glucose

Proton spin relaxation experiments on a cell-wall preparation from seedlings incubated with D-[1-^{13}C]glucose were used to search for NMR signals with proton relaxation properties appropriate for rigid XG. PSRE involved exploiting the differences between the normal CP/MAS spectrum and experimental spectra obtained with proton spin relaxation (Fig. 3). The CP/MAS spectrum obtained for this cell-wall preparation was then edited using the $T_{1\rho}$(H) time constants into two subspectra (Fig. 4A): subspectrum *A* contained signals with long values of $T_{1\rho}$(H), assigned to the rigid cell-wall polysaccharides, mostly cellulose, and subspectrum *B* contained signals with short values of $T_{1\rho}$(H), assigned to polysaccharides more mobile than cellulose. When the CP/MAS spectrum was edited using the T_2(H) time constants (Fig. 4B), subspectrum *A* contained signals with short values of T_2(H), assigned to rigid polysaccharides, mostly cellulose, and subspectrum *B* contained signals with long values of T_2(H), assigned to more mobile polysaccharides. Amplification of the signals in the C-1 portion of subspectrum *A* separated using $T_{1\rho}$(H) and using T_2(H) both showed a small signal at 100.4 ppm (Fig. 5A). This signal can be assigned to Xyl in XG, based on chemical shifts reported for XG (Table 2). Full spin relaxation curves could not be analysed for this signal, since the initial portions of these curves were dominated by an overlapping signal at 101 ppm from GalA of pectic homogalacturonans. It is also possible that the signal at 100.4 ppm may contain a contribution from pectic homogalacturonans. Proton spin diffusion can account for the transfer of spin information over distances of nanometers during the 1 ms cross-polarization contact time of a typical solid-state ^{13}C-NMR experiment (Zumbulyadis, 1983), so the values of $T_{1\rho}$(H) and T_2(H) are averaged within a monosaccharide residue and between adjacent residues (Hediger *et al*., 1999). Therefore, the signal strength observed through cross-polarization will reflect the molecular ordering over dimensions several times larger than a Glc residue (Newman, 1992). The presence of the 100.4 ppm signal in subspectrum *A* indicates that the polysaccharide

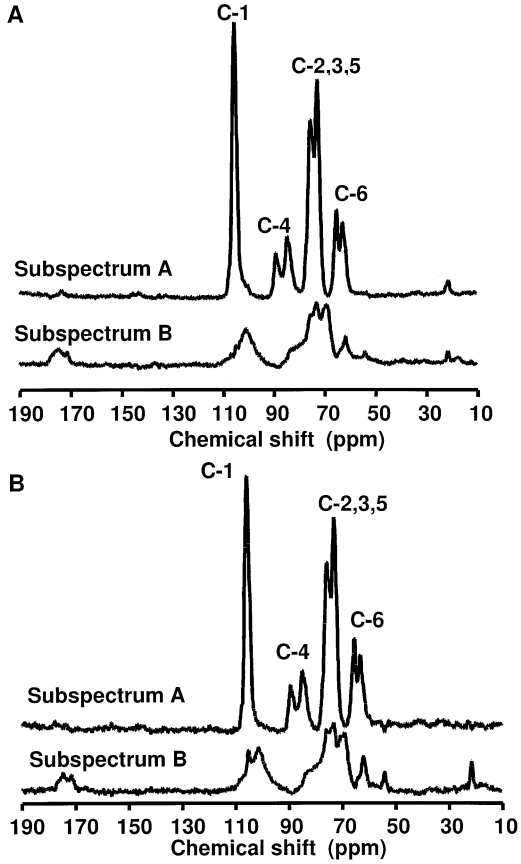

Fig. 4. PSRE subspectra separated from the normal CP/MAS spectrum of a cell-wall preparation from hypocotyls of mung bean seedlings incubated with D-[1-^{13}C]glucose. (A) Subspectra obtained by exploiting differences in proton rotating-frame spin relaxation. Subspectra *A* and *B* display signals assigned primarily to cellulose and the non-cellulosic matrix, respectively. (B) Subspectra obtained by exploiting differences in proton spin-spin relaxation. Subspectra *A* and *B* display signals assigned primarily to cellulose and the non-cellulosic matrix, respectively. Carbon numbers refer to Glc residues of cellulose.

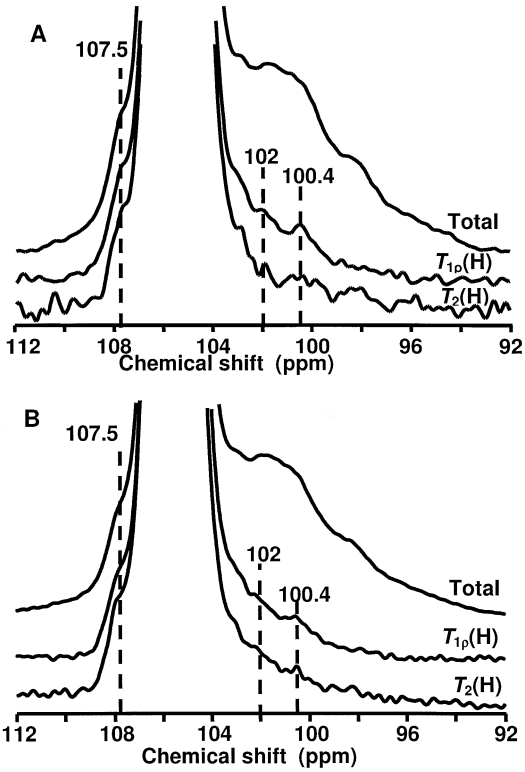

Fig. 5. Expanded portions of (Total) normal CP/MAS NMR spectra of cell-wall preparations from hypocotyls of mung bean seedlings incubated with D-[1-^{13}C]glucose and corresponding portions of rigid subspectra separated by exploiting differences in ($T_{1\rho}$(H)) proton rotating-frame spin relaxation and (T_2(H)) proton spin-spin relaxation. (A) and (B) are with and without treatment with 80% (v/v) ethanol, respectively. Signals in this region are assigned primarily to C-1 of Glc residues in cellulose I. Three signals assigned to polysaccharides other than cellulose I have been marked with broken lines.

producing this signal, which was assigned to XG, is in the vicinity of another rigid polysaccharide, namely cellulose. This signal at 100.4 ppm was small and only just above the background and it could not be detected in the total CP/MAS spectrum or subspectrum *A* of cell walls from unlabelled seedlings (results not shown). However, it was reproducibly detected in two different cell-wall preparations (Fig. 5A, B). The small Xyl signal (at 100.4 ppm) detected in subspectrum *A* accounted for less than 1% of the total C-1 signals. Therefore, it must be stressed that it would be difficult to detect this signal without using isotopic labelling.

A quantitative estimate of the proportion of the cell-wall XG that is rigid is difficult because of the low strength of the signal at 100.4 ppm, and because it is not known whether the ^{13}C-label on either C-1 or C-4 divides equally among the cell-wall polysaccharides, including XGs and cellulose. In addition, the quantification does not take into account the efficiency of the magnetization transfer in the CP process, where molecules will have differing mobility and strength of H-C dipolar interactions (Hediger *et al*., 1999). However, it was considered important to place an upper limit on the signal strength and then use this upper limit to obtain an estimate of the proportion of rigid XG. First, the contribution of this signal at 100.4 ppm to the total signal in the C-1 region of the rigid domain of the cell wall was obtained by integrating over an arbitrary 2 ppm range centred on 100.4 ppm. This was then compared with the area of the cellulose C-1 signal at 105.5 ppm which showed that for every C-1 atom in Xyl there were 30–40 cellulose C-1 atoms; the lower value was obtained using the $T_{1\rho}$(H) subspectrum (Fig. 4A) and the higher using the T_2(H) subspectrum (Fig. 4B), with a mean ratio of 1:35. As the cellulose content of the cell-wall preparations was 41% (w/w) (cell-wall dry weight), the amount of Xyl in the rigid domain was 1.2% (w/w). Moreover, these cell-wall preparations contained 4.4% (w/w) Xyl, which originated principally from XG, although the cell-wall preparations probably also contained small proportions of heteroxylans (Bacic *et al*., 1988). If it is assumed that all the Xyl was

Table 2. *Cited NMR assignments for C-1 and C-4 of Xyl and Glc residues of XG*

Assignment	Chemical shift ppm	Plant material	Reference
C-1 Xyl	99.8	Cyclamen seed XG	Braccini *et al*. (1995)
	99.8	Tamarind XG	Gidley *et al*. (1991)
	100.0	*Arabidopsis* cell walls[b]	Davies *et al*. (2002)
	100.3	Tamarind XG/cellulose	Whitney *et al*. (1995)
	100.4[a]	*Rubus* suspension cells	Joseleau *et al*. (1992)
	99.1–100[a]	Tamarind XG	York *et al*. (1993)
	100.2[a]	Cyclamen seed XG	Braccini *et al*. (1995)
C-4 Xyl	70.1–70.4[a]	Tamarind XG	York *et al*. (1993)
	70.8[a]	Cyclamen seed XG	Braccini *et al*. (1995)
C-1 Glc	103.6	Tamarind XG	Gidley *et al*. (1991)
	103.9[a]	*Rubus* suspension cells	Joseleau *et al*. (1992)
	103.1–103.7[a]	Tamarind XG	York *et al*. (1993)
	103.5[a]	Cyclamen seed XG	Braccini *et al*. (1995)
C-4 Glc	82–85	Cyclamen seed XG	Braccini *et al*. (1995)
	70.3–80.5[a]	Tamarind XG	York *et al*. (1993)
	79.5–80.8[a]	Cyclamen seed XG	Braccini *et al*. (1995)

[a] Solution NMR spectroscopy; all other values were obtained with CP/MAS of solid material.
[b] Chemically extracted to remove pectic polysaccharides.

from XG that had the monosaccharide composition reported by Kato and Matsuda (1980*a*) for mung bean hypocotyl XG (Glc:Xyl:Gal:Fuc=10:5.7:2.5:2.9 anhydro w/w), then the maximum XG content of the cell-wall preparations was 13.5% (w/w). Approximately 27% of the total wall XG will be in the rigid domain, accounting for 3.9% (w/w) of the cell-wall preparation.

Because the xylose residues of XGs are attached to the glucan backbone by (1→6)-linkages, it is possible that the xylose is able to rotate around this linkage and so be under-represented in the rigid domain. However, this was considered unlikely because models of rigid XGs show straight backbones with the xylose residues protruding on either side; such Xyl residues cannot rotate freely around the (1→6)-linkage (Levy *et al*., 1991). Moreover, the Xyl residue may appear more rigid than it actually is because of the averaging effects of proton spin diffusion from dominant, inherently rigid polysaccharides.

Rigid XG does not account for all the signals between 92 ppm and the main C-1 peak of cellulose at 105.5 ppm (Fig. 5). Small amounts of Man (2.8% w/w) were found in these cell-wall preparations and the small signal at 102 ppm may be from the C-1 of Man in a gluco- or a galactoglucomannan. A signal at 102 ppm has previously been assigned to C-1 of Man in rigid galactomannans (Newman and Hemmingson, 1998) and in galactoglucomannans in the cell walls of *Arabidopsis thaliana* (Davies *et al*., 2002).

Proton spin diffusion averages the spin information over dimensions of nanometres (Zumbulyadis, 1983; Hediger *et al*., 1999). Therefore, if the Xyl in the side-chains of XG is present in the NMR subspectra of the rigid domain of the cell walls (Fig. 4A, B), the Glc signals from the glucan backbone of this XG should also be present in these subspectra. A shoulder at 107.5 ppm was found on the side of the main cellulose peak in both the total spectrum and in the subspectra of the rigid domain (Fig. 5), which may be from the Glc of XG with a rigid, flattened, glucan backbone. The flattened backbone of rigid XG is predicted to have two Glc residues per helical turn and is, therefore, similar in conformation to β-glucan chains in crystalline cellulose (Levy *et al*., 1997). The flattening of a β-glucan backbone is predicted to cause increased torsion in the linkage carbons, C-1 and C-4, compared with that in a β-glucan backbone in a twisted conformation, resulting in different ^{13}C NMR chemical shifts (Horii *et al*., 1984; Jarvis, 1994). Data for cellulose provide an estimate of the likely chemical-shift displacement for the Glc residues of rigid XG. For the twisted conformation of the backbone, the C-1 chemical shift is 102.7 ppm, obtained from a solution of cellulose oligosaccharides generated by partial acid hydrolysis (Gast *et al*., 1980). However, for the flat-backbone conformation of cellulose I and II, the C-1 chemical shifts are in the range 105 to 108 ppm (Hemmingson and Newman, 1995), so the chemical-shift displacements are in the range 2 to 5 ppm. Similar chemical-shift displacements for the Glc residues in XG would move the C-1 signal from 103 ppm (Table 2) to the range 105 to 108 ppm, which includes the shoulder found in these subspectra at 107.5 ppm. Because the shoulder at 107.5 ppm was poorly resolved, it was not possible to estimate its area, but it is clearly no more than a minor component of the cell wall. The assignment of the shoulder at 107.5 ppm to the Glc in the flattened backbone of rigid XG is reasonable as it is consistent with the small signal at 100.4 ppm, assigned to Xyl in the sidechain of XG.

However, the signal at 107.5 ppm could also be assigned to C-1 of cellulose II. Hemmingson and Newman (1995) assigned a signal at 106.7 ppm to C-1 of cellulose II. They also assigned signals at 88.2 ppm and 89.4 ppm to C-4 of cellulose II crystallite-interior molecules, coinciding with the area assigned to the interior chains of cellulose I, and a signal at 87 ppm to C-4 in cellulose II crystallite-surface

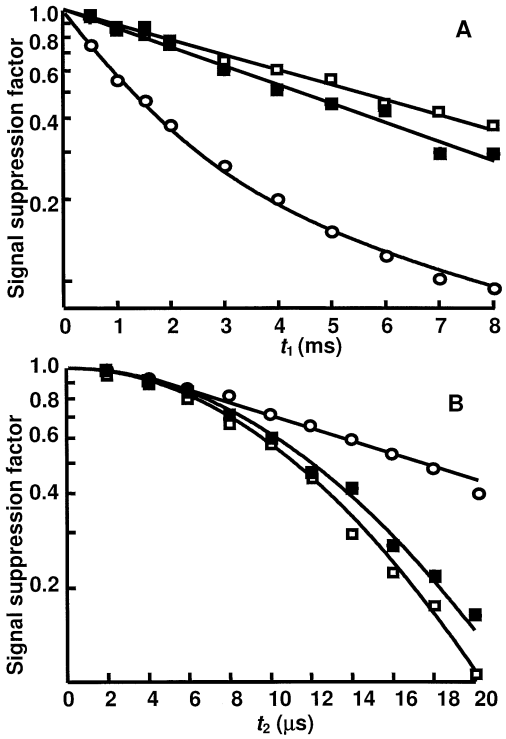

Fig. 6. NMR proton spin relaxation curves for a cell-wall preparation from hypocotyls of mung bean seedlings incubated with D-[1-^{13}C]glucose. Suppression factors for signals at 69 ppm (circles), assigned to GalUA of pectic homogalacturonan, 89 ppm, assigned to crystallite interior of cellulose (open squares) and 107.5 ppm (closed squares), assigned to the Glc in the flattened backbone of rigid XG, were determined from CP/MAS NMR experiments in which the relaxation intervals t_1 and t_2 for (A) proton rotating-frame relaxation, and (B) proton spin-spin relaxation pulse sequences were varied, respectively.

chains. This signal at 87 ppm would fall on the boundary between signal areas assigned to interior and surface chains of cellulose I, but no signal was detected at 87 ppm, suggesting that cellulose II is absent.

To confirm that the polysaccharide associated with the signal at 107.5 ppm was indeed rigid, complete relaxation curves were constructed by varying the relaxation intervals t_1 and t_2 in the $T_{1\rho}$(H) and T_2(H) NMR pulse sequences ((Tekely and Vignon, 1987*a*, *b*). These curves (Fig. 6) show the relaxation characteristics for the signal at 107.5 ppm compared with those for the signal at 89 ppm (assigned to C-4 of Glc in cellulose crystallite interior) and for the signal at 69 ppm (assigned to C-4 of GalUA in pectic homogalacturonans). The $T_{1\rho}$(H) relaxation curve for the signal at 107.5 ppm was similar to that obtained for crystalline cellulose (Fig. 6A). The $T_{1\rho}$(H)) time constants for the signal at 89 ppm and 107.5 ppm were also similar, with $T_{1\rho}$(H)=8.0 and 6.4 ms, respectively. A curve for the C-4 signal at 85 ppm was also exponential with $T_{1\rho}$(H)= 7.4 ms (data not shown). This signal at 85 ppm was assigned to cellulose on the microfibril surfaces. The relaxation curve for pectic homogalacturonan (Fig. 6A) was distinctly different, with rapid initial decay ($T_{1\rho}$(H)=1.3 ms) followed by a slower phase attributed to replenishment of magnetization through spin diffusion from cellulose to pectic homogalacturonan. Furthermore, the Gaussian shape of the T_2(H) relaxation curve (Fig. 6B) for the signal at 107.5 ppm was typical of the response of rigid, solid matter (Cheung and Gerstein, 1981) and was, therefore, consistent with the assignment of this signal to a rigid component of the cell wall. The T_2(H) time constants for the signal at 89 ppm and 107.5 ppm were similar, with T_2(H)=10.2 and 9.5 μs, respectively. A curve for the C-4 signal at 85 ppm was also Gaussian with T_2(H)=9.7 μs (data not shown). By contrast, the exponential shape of the T_2(H) curve for the signal at 69 ppm (Fig. 6B) is typical of mobile material, with T_2(H)=24 μs.

To prevent microbial contamination, the cell-wall preparations were kept in 80% (v/v) ethanol until NMR experiments could be carried out. To confirm that the signal at 100.4 ppm was not a result of this exposure to 80% (v/v) ethanol, a cell-wall preparation that had not been exposed to 80% (v/v) ethanol was also examined. This cell-wall preparation was also obtained from seedlings incubated with D-[1-^{13}C]glucose. No differences were found in the PSRE NMR spectra for these two cell-wall preparations. The same signals at 100.4, 107.5 and 102 ppm were found in the rigid domain of the PSRE subspectra (Fig. 5B).

SE-PSRE NMR of a cell-wall preparation from hypocotyls of seedlings incubated with D-[4-^{13}C]glucose

A novel combination of spin-echo relaxation and rotating-frame experiments were used to detect partly-rigid XGs. Unlike $T_{1\rho}$(H) and T_2(H), T_2(C) is sensitive to the segmental motion of the polysaccharide at the site of the ^{13}C nucleus and is insensitive to the motion of more distant polysaccharides (Rothwell and Waugh, 1981). Therefore, in these SE-PSRE NMR experiments, the T_2(C) values for a polysaccharide will indicate the mobility of that particular polysaccharide, whereas the $T_{1\rho}$(H) and T_2(H) values will reflect the mean molecular motion of the surrounding polysaccharides. Because the spin relaxation of the ^{13}C is dominated by the motion of the relevant carbon atom, the T_2(C) time constants of these XGs should be different from those of polysaccharides with different mobilities in the matrix of the cell wall. The same suppression factors, obtained from rotating-frame experiments (Fig. 7A) and used to separate the subspectra of rigid from partly rigid and mobile domains (Fig. 7B) in PSRE, were also used to edit the SE spectrum into subspectra *A* and *B*. Figure 8 shows the SE spectrum and PSRE subspectra for a pulse sequence with a relaxation interval of t_2=5 ms. The T_2(C) time constants shown in Table 3 were determined by repeating the SE-PSRE experiment for other values of t_2. Signals at 101, 80, and 69 ppm, assigned

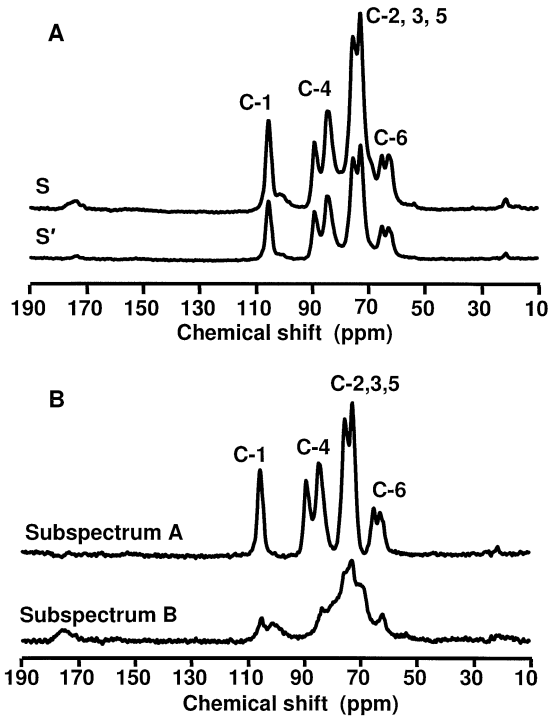

Fig. 7. Solid-state ^{13}C NMR spectra of a cell-wall preparation from hypocotyls of mung bean seedlings incubated with D-[4-^{13}C]glucose. In (A), *S* is the CP/MAS NMR obtained by the normal CP/MAS NMR pulse sequence and *S′* is the proton relaxation spectrum with 4 ms of proton rotating-frame spin relaxation. In (B), PSRE subspectra are separated from the normal CP/MAS NMR spectrum by exploiting differences in proton rotating-frame spin relaxation. Subspectra *A* and *B* display signals assigned primarily to cellulose and non-cellulosic matrix, respectively. Carbon numbers refer to Glc residues of cellulose.

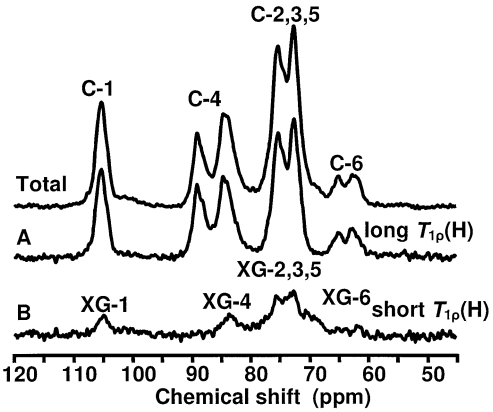

Fig. 8. Spin echo (SE) NMR spectrum (T_2(C) relaxation) with PSRE (proton spin relaxation editing) subspectra of a cell-wall preparation from hypocotyls of mung bean seedlings incubated with D-[4-^{13}C]glucose. The total SE spectrum was obtained with a t_2 delay of 5 ms and no proton spin locking. The PSRE subspectra were separated from the SE spectrum by exploiting the differences in proton rotating-frame relaxation. (A) Signals assigned to cellulose ($T_{1\rho}$(H)=10 ms); (B) signals assigned to non-cellulosic polysaccharides ($T_{1\rho}$(H)=4 ms). Carbon numbers refer to those of Glc residues of cellulose (e.g. C-1) or XG (e.g. XG-1).

to pectic homogalacturonan (Table 3), relaxed with T_2(C) values of 3–4 ms. The ^{13}C spins of these mobile polysaccharides had almost completely relaxed within 5 ms and, therefore, the pectic homogalacturonan signals were not detected in subspectrum *B* of Fig. 8.

XGs with partly-rigid glucan backbones should have a long T_2(C) time constant compared with mobile cell-wall polysaccharides. However, if the XGs are surrounded by mobile polysaccharides, they should have a relatively short $T_{1\rho}$(H) time constant as the proton spin is influenced by the other polysaccharides in the surrounding matrix. Therefore, when the T_2(C) spectrum is edited using the $T_{1\rho}$(H) time constants, it would be expected that signals from the backbone of such partly-rigid XGs surrounded by mobile polysaccharides would separate into subspectrum *B*, whereas signals assigned to cellulose would separate into subspectrum *A*.

Using this SE-PSRE technique, two signals at 105 and 84 ppm were detected in subspectrum *B* and assigned to C-1 and C-4, respectively, of the Glc residues in partly-rigid XG (Fig. 8) (Braccini *et al*., 1995). Both of these signals relaxed with the same time constant (9 ms), which was shorter than those of the cellulose signals, but longer than the time constants of the pectic homogalacturonans signals (Table 3). Hence, the 105 and the 84 ppm signals in subspectrum *B* (Fig. 8) are assigned to partly-rigid XGs surrounded by more mobile polysaccharides. Compared with the C-4 Glc signal of XG in the solution state, the C-4 signal assigned to Glc in XG is displaced from 80 to 84 ppm. This displacement is consistent with the XG being partly rigid and can be attributed to a degree of order in the XG backbone compared with the twisted conformation of the backbone of XG in solution (Table 2).

Other NMR chemical shifts in subspectrum *B* (Fig. 8) can also be assigned to partly-rigid XG. The shifts at 62 and 74 ppm can be assigned primarily to C-6 and C-2, 3, 5 of the Glc residues of the XG backbone, respectively (Braccini *et al*., 1995). The weakness of the 62 ppm signal may be due to T_2(C) relaxation promoted by rotation of the –CH_2OH group (Rothwell and Waugh, 1981). Shifts at 72 and 75 ppm superimposed in this C-2, 3, 5 region of the spectrum may result from minor contributions from other polysaccharides.

Unlike the Xyl residues in rigid XGs which, as indicated above, are unable to rotate freely around the (1→6)-linkage (Levy *et al*., 1991), the Xyl residues in the partly-rigid XGs would be expected to rotate around the (1→6)-linkage as occurs in XG in solution. Thus, the absence of a signal from these Xyl residues at 100.4 ppm is consistent with the XG being in this partly-rigid state.

It is also possible that the signals at 105 and 84 ppm originate from disordered cellulose. However, previous studies have not detected such cellulose in subspectrum *B* of PSRE-NMR spectra obtained from angiosperm primary cell walls, unless these cell walls had first been treated with

Table 3. *T_2(C) time constants for NMR signals detected after combining spin-echo detection (to measure T_2(C)) and rotating-frame relaxation (to separate subspectra* A *and* B*)*

Abbreviations for carbon numbers: C, cellulose; GU, pectic homogalacturonan; XG, xyloglucan; i, crystallite interior; s, crystallite surface.

	Chemical shift (ppm)									
	105	101	89	84	80	75	72	69	65	62
Subspectrum *A*										
Assignment	C-1		C-4i	C-4s		C-2,3,5	C-2,3,5		C-6i	C-6s
T_2(C) (ms)	12		13	11		10	10		7	8
Subspectrum *B*										
Assignment	XG-1	GU-1		XG-4	XG/GU-4	XG-2,3,5	XG-2,3,5	GU-2,3,5		XG-6
T_2(C) (ms)	9	4		9	3	6	6	4		3

strong alkali (Davies *et al*., 2002). Nevertheless, other researchers have published evidence indicating the presence of disordered cellulose in such cell walls (Wickholm *et al*., 1998; Hediger *et al*., 2002).

As indicated above, quantification of XGs with different mobilities is difficult. However, to obtain an indication of the proportion of the total XG in the cell wall which is partly rigid, the areas of the C-4 signals for Glc residues of both cellulose and XG were extrapolated back to t_2=0. The ratio of Glc in partly-rigid XG (Fig. 8) to Glc in cellulose was 1:5. Based on a maximum XG content of the mung bean cell-wall preparation of 13.5% and a cellulose content of 41% (w/w), all of the cell wall XG could be accounted for by the 84 ppm signal.

Single pulse excitation/magic-angle spinning (SPE/MAS) NMR of cell-wall preparations from hypocotyls incubated with D*-[1-^{13}C]glucose*

The CP/MAS NMR spectra of the cell-wall preparations were dominated by signals assigned to cellulose and the broader, underlying signals were assigned to non-cellulosic polysaccharides, along with signals at 173, 54, and 22 ppm assigned to carboxylic acid, methoxyl, and acetyl carbons, respectively (Fig. 2). As cellulose makes up less than half the weight of the cell wall, a portion of the non-cellulosic material must have been too mobile to respond to the cross-polarization pulse sequence. Although SPE/MAS NMR can potentially detect all components in the cell-wall preparations (Tang *et al*., 1999), the highly mobile cell-wall components, which are less responsive to CP/MAS NMR (Foster *et al*., 1996; Smith *et al*., 1998*b*) are particularly responsive to SPE/MAS NMR. By using a short recovery delay in the SPE/MAS pulse sequence (1 s), signals which have long T_1(C) relaxations, such as the rigid cellulose molecules, are not detected (Smith *et al*., 1998*b*; Tang *et al*., 1999). SPE/MAS is thus an excellent complementary technique to CP/MAS NMR (Tang and Hills, 2003) for investigating mobile polysaccharides in cell-wall preparations. The SPE/MAS NMR spectra of the cell-wall preparations were dominated by signals assigned to galactans of the pectic polysaccharides (results not shown), so it was difficult to distinguish signals that could be assigned to highly mobile XG. Hence, although the two CP/MAS techniques accounted independently for all the estimated XG in the cell-wall preparations, the possibility that some XG also exists in a highly mobile domain cannot be excluded.

Discussion

Using solid-state ^{13}C-NMR, XGs of two different mobilities in the primary cell walls of mung beans have been identified: XGs with a rigid glucan backbone, which account for the smaller proportion of the total cell-wall XG, and XGs with a partly-rigid glucan backbone. The latter XGs were surrounded by mobile polysaccharides. It is also possible that there were small quantities of highly mobile XGs; however, interference from signals from mobile pectic polysaccharides precluded their detection. These results are consistent with the findings of Mackay *et al*. (1988) and Taylor *et al*. (1990), who, using solid-state ^{1}H-NMR, found that the XGs in primary cell walls of common bean (*Phaseolus vulgaris*) had constrained molecular mobility. Pauly *et al*. (1999) also identified XGs in different cell-wall domains based on their solubility in XG-specific endoglucanase, concentrated alkali and cellulase, applied sequentially to the cell walls. From the results reported in the present paper, it is not possible to identify the locations of the two different mobility types of XGs relative to other components in the cell wall, it is possible to make predictions based on current models of the primary cell walls of dicotyledons.

The XG molecules with the rigid glucan backbone may have been adsorbed on to the surfaces of the cellulose microfibrils. XG molecules adsorbed in this way would be expected to have a rigid, flattened backbone and not the twisted backbone conformation of free XG (Levy *et al*., 1997). Despite the difficulties involved in quantifying the amounts of the different mobility types of XGs, approximations can be made about the proportion of total cellulose microfibril surface area that has rigid XGs adsorbed to it. This was done by first expanding the NMR signals

assigned to C-4 of cellulose in PSRE subspectra separated using $T_{1\rho}$(H) and T_2(H) to estimate the total surface area of the cellulose microfibrils available for adsorption. Evidence has recently been obtained that the cellulose microfibrils in angiosperm primary cell walls consist of only one cellulose crystallite (Davies and Harris, 2003). Thus, in the present study, it may be assumed that the cellulose crystallite accounts for the entire width of the cellulose microfibril. The integrated signal areas assigned to interior and surface cellulose in the cellulose crystallites (Newman, 1999*b*) for all three cell-wall preparations showed that 64±1.4% (using $T_{1\rho}$(H)) or 62.7±1.3% (using T_2(H)) of the cellulose was on the surface of the crystallite. As the cell walls contained 41% (w/w) (cell-wall dry weight) cellulose, and 63% of this cellulose was on the surface of the microfibrils, then 26% (w/w) of the cell walls was surface cellulose. As it was estimated that the cell walls contained an upper limit of 3.9% (w/w) rigid XG with a Glc:Xyl ratio of 10:5.7 (Kato and Matsuda, 1980*a*), then there would be one XG Glc residue for every 13 surface cellulose Glc molecules. Thus, a maximum of only 8% of the surface of the cellulose microfibrils has adsorbed XG.

This conclusion is inconsistent with present models of cell walls that show XGs coating the entire surface of the cellulose microfibrils (Fig. 1A, B). It is, therefore, suggested that, at least for mung bean cell walls, these models need to be modified to indicate this small proportion of the cellulose microfibril surfaces having XG adsorbed on to it (Fig. 1C, D). It is possible that these adsorbed XGs are equivalent to the small proportion of XGs that is difficult to remove from the primary cell walls by treatment with aqueous solutions of alkali (Edelmann and Fry, 1992). In addition to XGs, the small amounts of rigid gluco- or galactoglucomannans, tentatively identified in the mung bean cell walls, may also be adsorbed on to the microfibril surfaces.

Another possible location for at least some of the rigid XG in the mung bean cell walls is within the cellulose microfibrils. Cell-wall models show a small proportion of XG in this location (Cosgrove, 2001). However, it was found that the cellulose molecules at the surface of the cellulose crystallite were similar in rigidity to those in the interior of the crystallite. This was particularly evident in the T_2(C) values for the interior and surface cellulose, where proton spin diffusion is not a confounding factor. Thus, there was no disruption to the integrity of the cellulose and no evidence to support a model in which some of the XG is within the microfibrils.

The XGs with partly-rigid glucan backbones surrounded by mobile polysaccharides that have been identified may be constrained within the mung bean cell walls. This property is consistent with them forming cross-links between adjacent cellulose microfibrils or loops on the same cellulose microfibril (Fig. 1A, C). It is also consistent with them forming cross-links between cellulose microfibrils and other non-cellulosic polysaccharides, such as pectic polysaccharides (Fig. 1B, D). Evidence has recently been obtained for covalent linkages between XG and pectic polysaccharides in the primary cell walls of rose suspension-cultured cells, where up to 30% (w/w) of the XG was estimated to be involved in these interactions (Thompson and Fry, 2000). It is possible that these are not the only locations for the partly-rigid XG molecules within the cell wall. In addition, although there is evidence that XG in cell walls is associated with the cellulose microfibrils, it is also possible that some of the partly-rigid XG molecules are constrained by being cross-linked between two non-cellulosic polysaccharides.

In conclusion, the results lead to the proposal that, at least for the primary cell walls of mung bean, a modification of current models is required. In these modified models (Fig. 1C, D), no XG is located within the cellulose microfibrils and only a small proportion of the total surface area of the cellulose microfibrils has XGs adsorbed on to it. Adsorbed XG occupying only a small proportion of the total surface area of the cellulose microfibrils in primary cell walls is consistent with recent models of α-expansin action (Cosgrove, 2001). Expansins are thought to mediate wall expansion by breaking XG-cellulose interactions. These models of expansin action propose that slippage between XG and cellulose is induced at only a restricted number of sites in the cell wall; they are therefore inconsistent with abundant XG-cellulose interactions. However, they fit well with the finding that these interactions are infrequent.

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