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Solid-state NMR characterization of hydration effects on polymer mobility in onion cell-wall material

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Abstract

The effect of hydration on the mobility of polysaccharides in onion cell-wall material (CWM) was studied by solid-state NMR. The application of proton $T_{1\rho}$, carbon T_1 relaxation measurements, and 2D-WISE (wideline separation) experiments led to a coherent overall picture of the mobility inside the cell wall, combining domain-averaged as well as site-specific motional information. Whereas the mobility of all individual chemical sites increased upon hydration, only the average chain mobility of the pectin was strongly affected, indicating the predominant interaction of water molecules with the pectin. The 2D-WISE experiment revealed a spatial heterogeneity of the polysaccharide dynamics across the sample, showing at least two different motional regimes for pectin and cellulose domains.

Keywords: Solid-state NMR; Cell walls; Polysaccharides; Mobility; Relaxation; Cross polarization; WISE

1. Introduction

Plant cell walls are a natural material with remarkable physical properties, having both good load resistance and flexibility. The molecular basis for these mechanical and dynamic properties is not yet well understood, but is certainly related to the complex structure of the cell wall. Increased knowledge of the organization and interactions between components in the cell wall should lead to better understanding of changes in physical properties related to chemical transformations during growth or ripening. Cell walls are composed of several polysaccharides showing different degrees of crystallinity and hydration. Cellulose, the main component, is arranged in crystalline fibers and builds a dense network responsible for the mechanical strength properties. In most species, the cellulose microfibrils are coated with xyloglucans that span the space between the microfibrils. In primary cell walls of type I, this framework is embedded in a highly hydrated jelly-like matrix composed of complex pectic polysaccharides [1]. Structural proteins form a third domain.

For a structural study of such a complex matrix, solid-state NMR offers several advantages. As a non-invasive technique, it is able to track information in the native cell wall without disturbing its arrangement. Chemical shift differences in the carbon spectrum can be used to distinguish different types of structures and to localize domains showing various levels of crystallinity [2–10]. Information about the spatial distribution of the different

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components in the cell wall can be obtained using proton spin–diffusion experiments [11].

In recent years, an increasing effort has been made to monitor mobility inside plant cell walls using different solid-state NMR techniques [12–21]. They can be divided into two types of experiments leading to complementary information about molecular dynamics: measurement of either relaxation parameters or dipolar interactions. Relaxation of proton or carbon spin magnetization is directly related to molecular motion. However, the strong dipolar interactions present in solid polymers can lead to an accelerated decay of magnetization. As it is impossible to separate these two effects completely, NMR relaxation data have to be interpreted with care, especially in the case of proton relaxation. Because of the poor resolution of the proton spectrum, individual proton relaxation time constants have to be indirectly detected through the neighboring carbons in order to obtain differentiated results for the various components of the cell wall. Spin diffusion during the magnetization-transfer step leads to an averaging of the observed relaxation time constants for molecular sites that are in close proximity. Thus, indirectly detected proton relaxation techniques will only deliver information about average mobility of well-defined domains in the cell wall. This concerns mainly slow motion (kHz range) of the polymer chain, which can be detected through the relaxation parameter T_2 (motion-induced transverse relaxation) or $T_{1\rho}$ (tilted rotating-frame longitudinal relaxation time). Proper measurement of these relaxation parameters requires the complete averaging to zero of homonuclear interactions. This is impossible in both experiments, either by using magic-angle spinning (MAS) [22] or CPMG sequences [23] as averaging techniques. However, as the homonuclear dipolar interaction is scaled down by a factor of two in the rotating frame with respect to the laboratory frame, spin diffusion is expected to be less efficient in $T_{1\rho}$ than in T_2 experiments. Therefore, the measurement of proton $T_{1\rho}$ relaxation time constants is probably the most appropriate method to probe the average mobility in different parts of the cell wall, e.g. the pectin or the cellulose.

The mobility of specific chemical sites in the polymer can best be probed with ^{13}C relaxation measurements, as long as the different chemical sites have resolved chemical shifts. Since spin diffusion among carbons is negligible in natural-abundance compounds, the measured ^{13}C-relaxation time constants reflect site-specific motions. Interesting dynamics information in the MHz range is available from the carbon longitudinal relaxation parameter T_1.

A second source of dynamic information comes from unaveraged proton dipolar interactions, since their strength is modified by molecular motion. The homonuclear proton–proton interaction dominates the proton linewidth. Its strength is therefore directly available from the proton spectrum. More detailed and site-specific information is obtained from the wideline separation (WISE) experiment, where the different ^1H wideline resonances are separated in a two-dimensional experiment by the corresponding ^{13}C isotropic chemical shift [24]. Heteronuclear dipolar couplings can also be used to probe mobility. Since this interaction is used in cross-polarization (CP) techniques, molecular mobility can be detected through the dynamics of polarization transfer observed in a variable-contact-time experiment. In model compounds with well-isolated $^1H-^{13}C$ two-spin systems, the heteronuclear dipolar coupling can easily be determined from the CP build-up curves in both static [25] and fast MAS experiments [26]. However, in solids with strong multiple spin interactions, the polarization transfer process becomes complex, especially under MAS. As a wide range of mobilities is present in cell walls, data interpretation from CP experiments can be difficult and misleading [17,18].

In this study, we evaluated systematically the different NMR techniques mentioned above for their ability to deliver information about mobility in natural polymers. Onion cell-wall material (CWM), which is known to have a large content of mobile pectic material [27], was chosen as a test material. Changes in mobility were induced through hydration of the sample. The combination of proton $T_{1\rho}$, carbon T_1 relaxation, and WISE experiments

Table 1
Monosaccharide composition and yield of CWM prepared from fresh onions

Yield (g/kg FW)	Sugar composition (mol%)							Total (μg/mg)
	Rha	Ara	Xyl	Man	Gal	Glc	Uronic acid	
12.4	1.3	2.6	3.4	1.2	27.2	30.7	33.1	764

gave a consistent picture of the mobility inside the cell wall, combining site-specific as well as domain-averaged information. The interaction of water molecules with the different polysaccharides and the effect on their mobility could be derived from these experiments.

2. Results and discussion

The pectic polysaccharide content of the CWM was over 50% (Table 1), which is consistent with published values [27].

The ^{13}C carbon spectra obtained for onion CWM at hydration levels of about 10 and 35% are shown in Fig. 1. The spectral frequency assignment, given in Table 2, was based on published data [16,18]. An increase in spectral resolution due to hydration of the sample is observed. However, in both spectra some signal overlap is present. For these resonances, several sites can contribute to the signal intensity. The experiments performed are discussed for six different pectin signals and seven resonances attributed to cellulose or general carbohydrates. Their chemical shifts are listed in Table 2. The lack of uniquely assigned resonances does not allow a distinction between the different polysaccharides of the pectin family, e.g., galactan and polygalacturonan. The discussion will therefore be focused on pectin in general. Peaks at 105 and 62 ppm were assigned to both cellulose and pectin signal (galactan) [16]. However, for the CP contact times used in the different experiments presented here, the intensity of both peaks is dominated by the cellulose contribution [16].

Proton $T_{1\rho}$ relaxation experiments.—The proton $T_{1\rho}$ relaxation time constants observed for the different sites are presented in Fig. 2. Pectin and cellulose resonances are separated to simplify the comparison. The $T_{1\rho}$ values were uniform among the pectin and cellulose sites, which confirmed that relaxation time constants are averaged over a certain distance by spin diffusion. The spatial range of this averaging effect was however smaller than the dimension of the pectin or cellulose domains, which behave differently. The property of the $^1H-T_{1\rho}$ experiment to homogenize the relaxation times of the different chemical sites to a few averaged values makes this technique ideal for editing ^{13}C subspectra according to mobility [28,20]. For both samples, the pectin relaxation time constants were lower than the cellulose ones. This can be interpreted as a difference in average mobility.

To evaluate the contribution of transverse relaxation (T_2^*) in the '$T_{1\rho}$' behavior of the

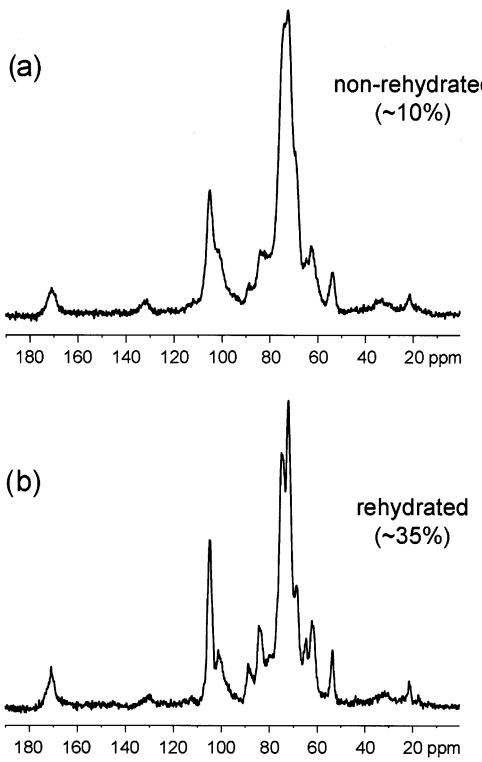

Fig. 1. Carbon spectra of (a) 10% and (b) 35% hydrated CWM of onion. The spectra were acquired at 5 kHz MAS with a CP contact time of 1 ms. 10,000 scans were accumulated for spectrum (a) and 13,200 for spectrum (b).

Table 2
Assignment of the ^{13}C spectrum of onion CWM [a]

Pectin resonances	ppm	Cellulose and general carbohydrate resonances	ppm
Pectic carboxyl, Me ester	171	Cellulose + galactan C-1	105
Pectic and other C-1	101	Crystalline cellulose C-4	89
Pectic C-4	80	Surface cellulose C-4	84
Pectic C-2, C-3	69	General carbohydrates	74
Pectic methoxyl	54	General carbohydrates	72
Acetyl, CH_3	21	Crystalline cellulose C-6	65
		Surface cellulose + galactan C-6	62

[a] Chemical shift differences between the 10 and 35% hydrated samples were less than 1 ppm.

different polymers [29], the relaxation experiment was repeated at a lower spin-lock field of 40 kHz for the 10% hydrated sample (data not shown). As expected, all time constants decreased as a result of greater contribution of transverse relaxation dephasing. The pectin resonances with $T_{1\rho} = 3.3 \pm 0.6$ ms remained faster than the cellulose ones ($T_{1\rho} = 5.5 \pm 0.8$ ms). The decrease of the pectin time constants from 4.6 ± 0.4 ms at the higher spin-lock field (60 kHz) to 3.3 ± 0.6 ms was slightly more marked than for cellulose (from 6.5 ± 0.6 to 5.5 ± 0.8 ms). This effect seems to indicate a higher proton density in the pectin compared to cellulose, which is in agreement with the fact that water penetrates more easily in the pectin network. Recently, molecular dynamics simulations have shown that in all crystalline forms of cellulose, water molecules are not able to penetrate into the microfibrils but only bind at their topmost surface [30]. Indeed, this is observed in the behavior of the C-4 and C-6 resonances in cellulose (Table 3). These chemical sites show different chemical shifts for molecules inside or on the surface of the microfibrils [3–5]. By lowering the spin-lock field in the $T_{1\rho}$ experiment, only time constants corresponding to surface sites decreased significantly because of larger linewidths in a denser proton environment (see Table 3). We neglect here the influence of an eventual galactan contribution to the surface cellulose peak at 62 ppm. Indeed, from the carbon T_1 experiments presented below, this contribution can only be very small, if present at all for the experimental conditions used. This is in agreement with Ha et al. [16].

The effect of hydration on the cell wall is obtained by comparing the $T_{1\rho}$ time constants in both samples (Fig. 2). At a hydration level of 10%, the time constants of the pectin varied around 4.6 ± 0.4 ms. The cellulose resonances relaxed somewhat more slowly, with an averaged time constant of 6.5 ± 0.6 ms. At a hydration level of 35%, the pectin relaxation time constants systematically decreased to a mean value of 3.2 ± 0.2 ms. On the contrary, the average $T_{1\rho}$ value for the cellulose sites ($T_{1\rho} = 6.1 \pm 1.1$ ms) did not change signifi-

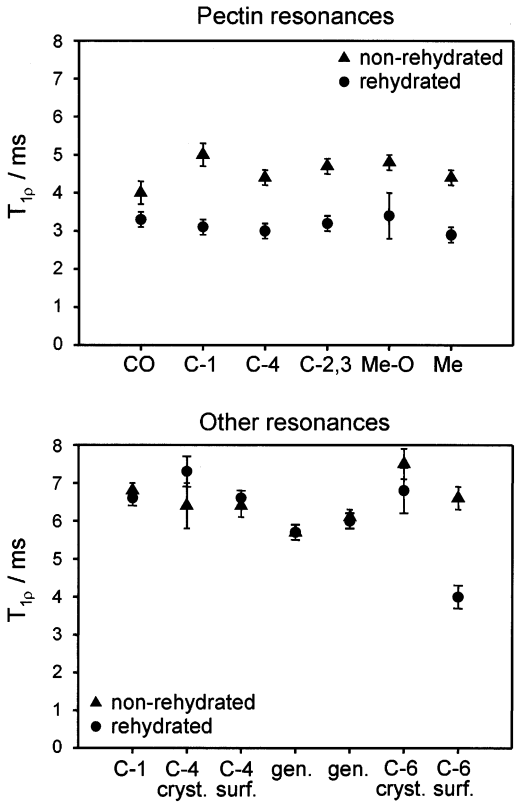

Fig. 2. Fitted proton $T_{1\rho}$ relaxation time constants for the various assigned resonances in 10% hydrated (triangles) and 35% hydrated (circles) onion CWM. To simplify the comparison, the values resulting from pectin resonances (upper graph) were separated from the other resonances (lower graph). The isotropic chemical shift for the different sites can be found in Table 2.

Table 3
^{1}H $T_{1\rho}$ relaxation time constants for the C-4 and C-6 resonances of cellulose in 10% hydrated CWM of onion [a]

Assignment	ppm	$T_{1\rho}$ (ms) 60 kHz	$T_{1\rho}$ (ms) 40 kHz
Crystalline cellulose C-4	89	6.4 ± 0.6	6.3 ± 0.5
Surface cellulose C-4	84	6.4 ± 0.3	5.1 ± 0.3
Crystalline cellulose C-6	65	7.5 ± 0.4	6.9 ± 0.5
Surface cellulose + galactan C-6	62	6.6 ± 0.3	5.1 ± 0.4

[a] The experiment was performed with proton spin-lock fields of 60 and 40 kHz.

cantly. Indeed, the only cellulose resonance sensitive to hydration was the surface C-6, which is consistent with a higher mobility of the hydroxyl group pointing outside the cellulose microfibrils. These results clearly show that hydration does not greatly affect the cellulose. The significant decrease of the average $T_{1\rho}$ value for the pectin reflects the rise in proton density and mobility of the polymer, which is due to the presence of water molecules.

Carbon (^{13}C) T_1 relaxation experiments.— The results of ^{13}C-T_1 relaxation experiments in 10 and 35% hydrated CWM are shown in Fig. 3. Except for six curves (peaks at 74, 72, 69, 65, and 62 ppm for 10% hydrated sample, and 74 ppm for 35% hydration level), the experimental points could be fitted with a mono-exponential function within the intensity variation estimated from the noise. The less intense second component is denoted in Fig. 3 by open symbols for the remaining bi-exponential curves. Bi-exponential behavior was principally observed in the sample with the lower hydration level. In most cases, the T_1 value of the second exponential component approached the value found in the more hydrated sample. We conclude tentatively therefore that the second component arose from more hydrated molecules, the molecular sites of which already tend towards the mobility range found in the more hydrated sample. Bi-exponential relaxation could also come from homogeneous movement across the sample, which has two distinct components.

The T_1 relaxation time constants were spread over a broad range (from 1 to 55 s) in both the pectin and the cellulose, pointing to different mobilities for the various nuclear sites in the polymers. In the 10% hydrated sample, the T_1 values were well correlated with the chemical structure of the sugars. The non-protonated carbonyl group had the longest time constant with T_1 of 55 s. Among the pectin resonances, the lowest time constants (about 7–8 s) were found for the most mobile sites involving rotating CH_3 groups. In cellulose, all sites involved in the sugar ring had long T_1 time constants (28–41 s). A large difference in T_1 was found between the two C-6 resonances of cellulose, in agreement with Ref. [15]. As this carbon atom is not part of the sugar ring, it was expected to have higher mobility than the sites located within the ring. In fact, higher mobility was only found to be the case for cellulose molecules at the surface of the microfibrils, which indeed had a lower T_1 (6 s) resulting from interactions with more mobile pectin polymers or rapidly exchanging

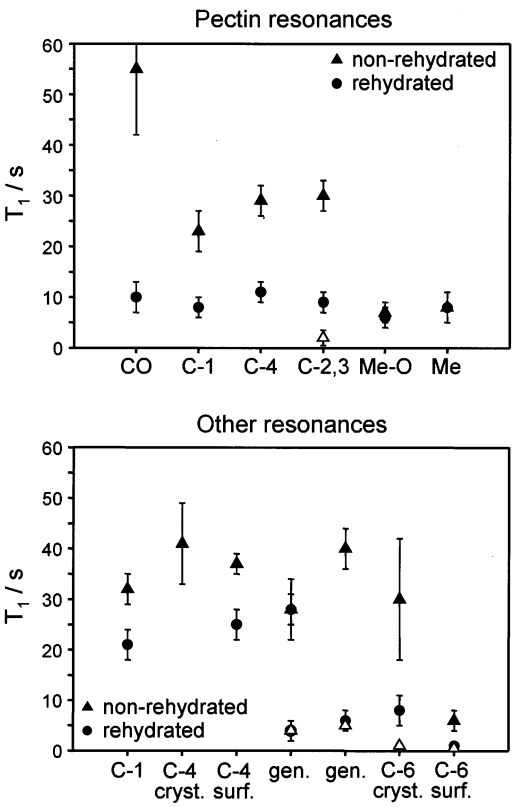

Fig. 3. Fitted carbon T_1 relaxation time constants for the different assigned resonances in 10% (triangles) and 35% (circles) hydrated onion CWM. For resonances showing a marked bi-exponential behavior, the second component (the less intense one) is given with open symbols. In the 35% hydrated sample, experimental data for the C-4 crystalline cellulose site at 89 ppm were too noisy to be properly fitted.

water molecules. Inside the cellulose fibril, the time constant for C-6 (30 s) was close to the values found for the other carbon atoms in the sugar rings. This reflects the compact packing of the glucan chains, as well as potential hydrogen bonds involving the C-6 of the glucose units.

The addition of water induced an overall decrease of the T_1 time constants, pointing to an overall increase of the carbon site mobility. The T_1 values of the pectin resonances became homogeneously distributed around 9 ± 2 s, indicating a common relaxation mechanism for the different sites. In cellulose, the mobility of the chemical sites generally also increased, but the relaxation times remained heterogeneous. The longer T_1 time constants were now found for C-1 and C-4 positions, both involved in the glycosidic linkage. The mobility of the C-6 position inside the microfibrils increased markedly (T_1 decreased from 30 to 8 s). However, it remained slightly slower than that of the corresponding site at the surface of the fibril ($T_1 = 0.5$ s).

Polarization transfer experiments.—A problem in analyzing the CP build-up curves is the choice of the appropriate fit model. In static samples, the magnetization evolution during CP can be described phenomenologically by a single-exponential build-up in the case of strong proton–proton interactions [31]:

$$I(t) = I_0\left[1 - \exp\left\{-\frac{t}{T_{dip}}\right\}\right]\exp\{-t/T_{1\rho}(CP)\} \quad (1)$$

by a two-step transfer in the case of a partially resolved heteronuclear coupling [32]:

$$I(t) = I_0\left[1 - s\exp\left\{-\frac{t}{T_{dip}}\right\} - (1-s) \times \exp\left\{-\frac{3t}{2T_{dip}}\right\}\right]\exp\{-t/T_{1\rho}(CP)\} \quad (2)$$

or by transient oscillations added to a slow exponential build-up in the extreme and rare case of a well-resolved $^1H-^{13}C$ coupling of strength b (dependent on crystallite orientation) [25]:

$$I(t) = I_0\left[1 - s\exp\left\{-\frac{t}{T_{dip}}\right\} - (1-s) \times \exp\left\{-\frac{3t}{2T_{dip}}\right\}\cos(bt)\right]\exp\{-t/T_{1\rho}(CP)\} \quad (3)$$

In each case T_{dip} is the time constant associated with the CP build-up process and $T_{1\rho}(CP)$ the time constant for the decay of the magnetization. The type of evolution depends on the relative sizes of homonuclear and heteronuclear spin interactions, i.e., on the nuclear spin network in the sample. With the introduction of MAS, CP dynamics will be modified as the dipolar interactions become modulated with time. The signal evolution will depend on the MAS frequency used, on the effective strength of dipolar interactions at the different chemical sites, and on the setting of the radio-frequency (rf) field strengths during CP [26,32]. Depending on these parameters, all three theoretical models are possible. In our experiments, the rf field strength was set as close as possible to the original Hartmann–Hahn condition used for static samples (same nutation frequency on both channels). For this rf field match, the sample rotation leads to an averaging of dipolar interactions (which was indeed observed for several resonances in the form of a slight modulation of the build-up curve during the first two rotor periods), and the transfer will phenomenologically approach the single-exponential function of Eq. (1). It is noteworthy that the CP matching profile (signal intensity as a function of the rf field difference) was very broad for the dominant cellulose signals, and the structural features of matching profiles under high-speed MAS [33] were not visible at the spinning speed used. The precise determination of the experimental matching condition was therefore difficult.

The results according to Eq. (1) for both fitted time constants, T_{dip} for the CP transfer and $T_{1\rho}(CP)$ for the exponential decay, are represented in Fig. 4 for 10 and 35% hydrated CWM of onion. For all resonances, the build-up parameter T_{dip} was close to 100 μs, except for the carbonyl signal that exhibited a much slower transfer because of the lack of a directly bonded proton. Surprisingly, the in-

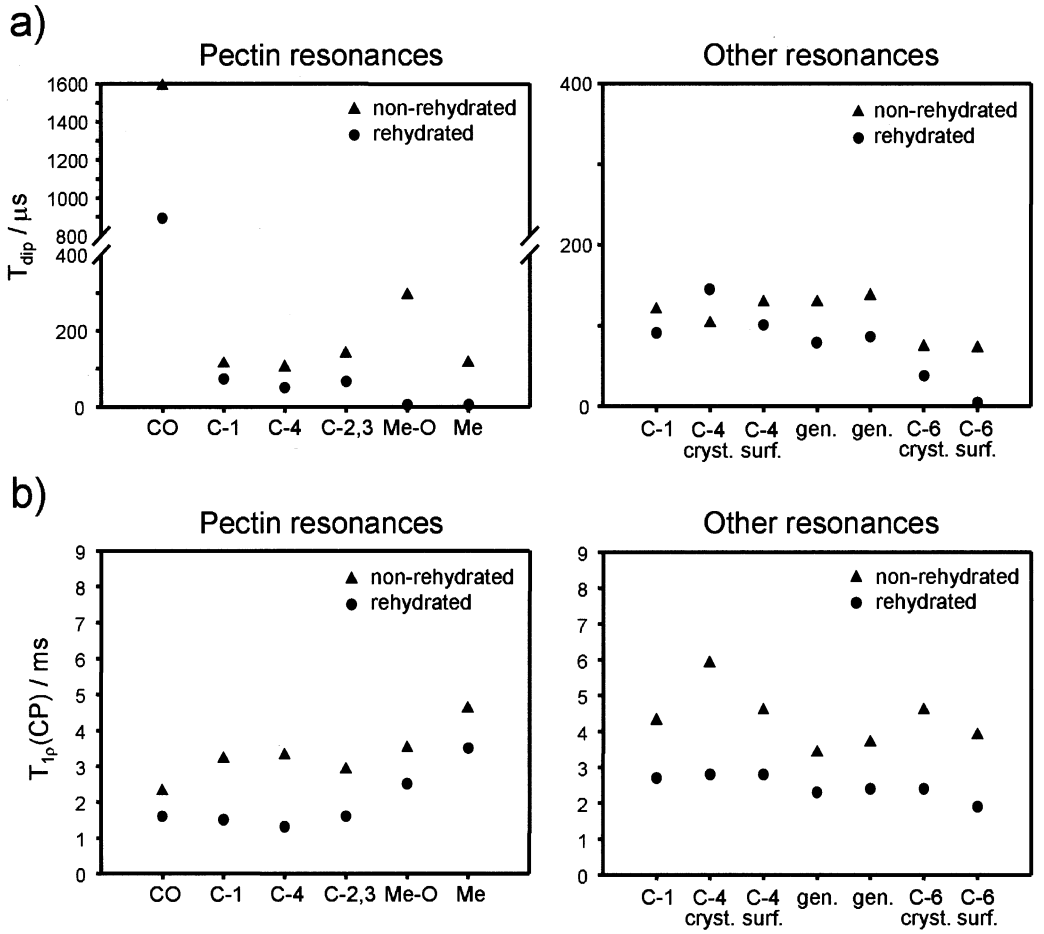

Fig. 4. Fit results of CP build-up experiments performed on 10% (triangles) and 35% (circles) hydrated onion CWM. The time constants for the CP transfer (T_{dip}) and the decay ($T_{1\rho}$(CP)) are presented in (a) and (b), respectively, for all assigned resonances. To simplify the comparison, the values resulting from pectin resonances (left graphs) were separated from the other resonances (right graphs).

crease in the water content generated shorter build-up time constants for most of the resonances (see Fig. 4(a)). Although increased CP efficiency for wet samples was also observed by other authors [34], it would be expected that the increased mobility in the 35% hydrated sample would average the dipolar interactions, and lead to slower polarization transfer (longer T_{dip}). The reason for the contradictory behavior is not clear. One explanation could be a difference in the rf field matching conditions between the experiments. Deviations from the Hartmann–Hahn condition between the two experiments could explain the differences in CP transfer rates [35]. It is not easy to verify this hypothesis because of the difficulty in setting the rf field strength at a precise matching condition. Another hypothesis could be a self-decoupling effect in the 10% hydrated sample. Large proton–proton interactions would lead to heteronuclear self-decoupling and thus to a slower CP transfer. In the 35% hydrated sample, the increased mobility may be sufficient to eliminate this self-decoupling effect by reducing the homonuclear interaction, resulting in a larger effective heteronuclear coupling. In conclusion, it is difficult to interpret the build-up time constants in terms of polymer mobility because of the complexity of the CP transfer. Other experiments are therefore more advantageous.

All decay time constants $T_{1\rho}$(CP) extracted from the build-up experiments (Fig. 4(b)) are lower than the proton $T_{1\rho}$ values (Fig. 2) but follow almost the same relative behavior. This is explained by the facts that a lower proton spin-lock field was used in the CP (40 kHz)

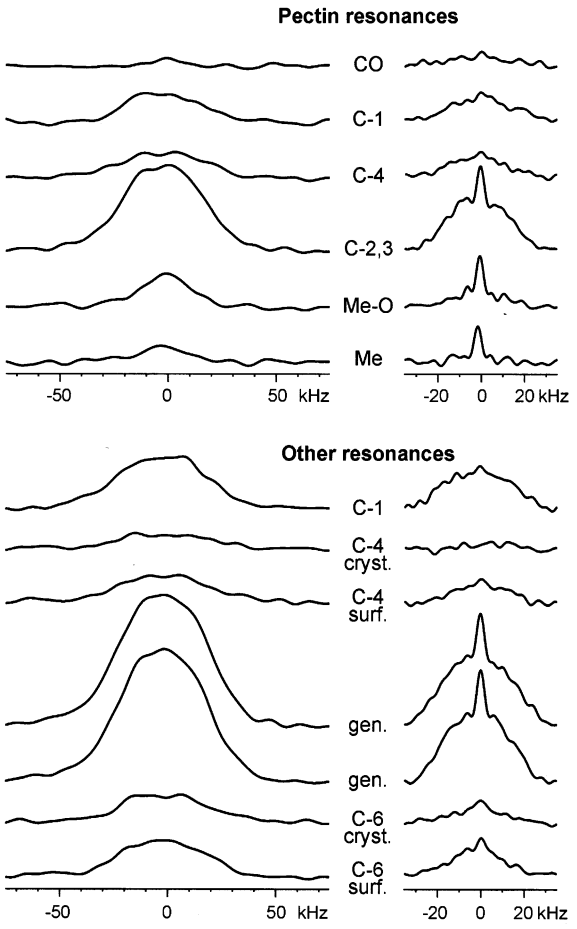

Fig. 5. Traces along the proton dimension of 2D-WISE experiments performed on 10% hydrated (left) and 35% hydrated (right) onion CWM. The corresponding carbon resonances are given. Proton spectral widths of 150 and 70 kHz were used for the 10 and 35% hydrated samples, respectively.

than in the proton $T_{1\rho}$ measurements (60 kHz), and that the signal decay in build-up experiments is due to both proton and carbon $T_{1\rho}$ relaxation. This result, however, is in contradiction with that of Jarvis and co-workers [18], who found that the signal decay in CP experiments was slower than the effective proton $T_{1\rho}$. These authors explained this behavior by the presence of slow build-up components, which minimize the effective decay. Such an effect was not visible under the experimental conditions used here.

2D-WISE experiments.—Traces in the proton dimension are shown for different pectin and cellulose carbon resonances in Fig. 5. Because of spin diffusion during the CP contact time used in the experiment, the information obtained in a 2D-WISE [24] experiment is averaged over a certain distance, as was the case for the proton $T_{1\rho}$ experiments. However, the contact time was kept sufficiently short (300 μs) to avoid a spreading of the information across different polysaccharide domains. In the 10% hydrated sample, very large proton lineshapes (about 40 kHz full-width at half-height) were found for both pectin and cellulose sites. Only CH_3 groups seemed to have a slightly sharper proton linewidth (20 kHz FWHH). Upon hydration, most of the ^{13}C resolved proton wideline spectra developed a sharp component of less than 10 kHz FWHH in addition to the broad component, whose width is only slightly decreased. This feature seems to indicate a spatial inhomogeneity of the mobility inside the cell wall induced by hydration. In pectin and cellulose polymers, parts of the sample appeared to be more mobile than others. The proportion of the narrow component with respect to the larger one differed depending on the carbon site. In the C-1 and C-4 positions of both pectin and cellulose this sharp component was not observed. On the contrary, for acetyl and methoxyl groups, the broad component was almost not visible. The narrow component of the wideline proton spectra might also be assigned to mobile water protons located in proximity to the polysaccharide chains. However, for the cellulose resonances, this explanation is unlikely. The presence of the narrow component would also be consistent with hydroxyl exchange with water, although it is unlikely that so much intensity from the hydroxyl proton can be transferred to the carbon in the short contact time used. The 2D-WISE experiment thus offers the advantage of being very sensitive to mobility differences across the sample. This kind of spatial inhomogeneity could not be detected from the relaxation data, which were generally mono-exponential (exceptions were mainly found for some T_1 relaxation curves in the 10% hydrated sample, which do not correspond to the observations in WISE).

3. Conclusions

The combination of different solid-state NMR experiments was able to deliver a coher-

ent picture of the mobility inside onion cell-wall polysaccharides. The experiments were selected for the complementarity of their motional information. The results obtained for two different hydration level of the CWM enabled information on the interactions of water molecules with the various polysaccharides to be derived.

The average chain mobility was probed by indirect detected proton $T_{1\rho}$ relaxation parameters. Because of spin diffusion during the experiment, the relaxation time constants were uniform through domains of pectin or cellulose, yielding the average mobility at low frequencies in these different domains. The selective decrease of the $T_{1\rho}$ time constants of the pectin sites only, under hydration of the sample, reflected the increase of both the proton density due to water molecules and the mobility of the polysaccharide chains. On the contrary, the cellulose resonances remained unaffected. This experiment clearly shows that the water molecules preferentially penetrate the pectin network, thus increasing its mobility.

Information about the higher-frequency mobility of specific chemical sites was obtained using carbon T_1 relaxation data. The time constants reflected the chemical structure of the polymers. Thus, under hydration the longer relaxation times in cellulose were maintained for the C-1 and C-4 sites, revealing the restricted dynamics of the atoms involved in the glycosidic linkage. The differences between relaxation times for atoms inside or at the surface of the cellulose microfibrils were indicative of the various interactions between the polymers. Upon hydration, all chemical sites of both pectin and cellulose developed a shorter T_1 relaxation time constant. Thus, the carbon sites in the cellulose became more mobile in the MHz frequency range without changing the slow motions of the microfibril as was found in $T_{1\rho}$ experiments. For the pectin polymers, the relaxation time constants of the different sites became uniform at the hydration level of 35%, indicating a common relaxation mechanism for all sites. The discussion on $T_{1\rho}$ and T_1 parameters was limited to a qualitative interpretation using the simplest mathematical models. The molecular system was too complicated to apply reasonable existing quantitative models appropriate for restricted motion.

For most resonances, the relaxation decay curves could be fitted within experimental error with a mono-exponential function, giving no clear indication of possible variation of motional regime within the same polymer type. An overall picture of the mobility range across the sample was best obtained with 2D-WISE experiments. The proton wideline spectra corresponding to most of the pectin and cellulose resonances revealed both a broad (rigid) and a narrow (mobile) component. This experiment seemed to indicate that the mobility is not uniform across the sample, but that some heterogeneity in the dynamics is present, showing at least two different motional regimes.

The combination of proton $T_{1\rho}$, carbon T_1 relaxation data, and 2D-WISE experiments was shown to give a coherent picture of the mobility inside the plant cell wall. These results are also consistent with results from other authors on similar compounds [13,15,21]. An attempt to get further motional information form CP build-up curves was carried out. The results, however, were inconsistent with the other experiments and seem to be in contradiction to published data [18] (a serious comparison is however difficult considering that experimental parameters like spinning speed and rf field strength are different from those in Ref. [18]). The complexity of the polarization transfer kinetics as well as the dependence of the transfer on the experimental conditions seems to be a serious drawback of variable-contact experiments in terms of motional information.

4. Experimental

Preparation and characterization of CWM.—The dried, as well as the first layer of fresh peel, were removed from 1 kg of fresh onions, which were then cryo-milled and homogenized in phenol–acetic acid–water (2w:1v:1v) solution. The tissue was then centrifuged and the residue extensively washed

with cool water. After dialysis, the residue was freeze-dried, yielding 10 g of dried CWM [36]. With time, the CWM naturally rehydrates to a level of about 10%. The neutral sugar composition and uronic acid content were determined by established methods [27,36].

NMR spectroscopy.—All NMR experiments were performed on a Varian Unity + spectrometer operating at a proton resonance frequency of 500 MHz and equipped with a Jakobsen 5 mm CP/MAS probehead. For all experiments, the MAS frequency was set to 5 kHz. A proton rf field strength of 60 kHz was used for decoupling and spin-locking in the $T_{1\rho}$ experiments (exceptions are mentioned in the text). For the CP contact time, the rf field strengths on both channels were matched in the center of the matching profile at about 40 kHz each. Precise matching was first determined for a sample of ^{13}C-labeled alanine. After retuning the probe, the same matching condition (based on the most intense cellulose signal) was found for the different cell-wall samples. The improved heteronuclear decoupling sequence TPPM was used during acquisition [37]. The repetition delay was set at 1 s for all experiments. The NMR experiments were performed on the CWM as described above (10% hydrated) as well as on a more hydrated sample (hydration level of about 35%). Possible loss of water during the experiments was monitored by regularly weighing the rotor. The maximum loss was 3% of the total sample weight.

Proton $T_{1\rho}$ measurements.—The proton $T_{1\rho}$ experiments were performed by inserting a variable delay of 0, 200 μs, 400 μs, 600 μs, 1 ms, 1.4 ms, 2 ms, 3 ms, 5 ms, 8 ms, 12 ms, and 18 ms prior to CP during which proton spins were irradiated with a constant rf field strength. For resolution purposes, the proton magnetization was then transferred to the neighboring carbons using CP. A short contact time of 400 μs was used to avoid a spread off of the information due to proton spin diffusion during CP. For each experiment, 10,000 scans were accumulated. For all carbon peaks, the relaxation curve obtained using maximal peak height could be fitted within experimental error by a mono-exponential function. Error bars for the relaxation parameter $T_{1\rho}$ were estimated from the fit result for five different positions distributed around the maximum of each resonance.

Carbon T_1 measurements.—The carbon T_1 relaxation rates were monitored after the ^{13}C magnetization (obtained with a CP contact time of 500 μs) had been flipped back along the static magnetic field. A proper phase cycling of the flip-back 90° pulse led to an exponential decay of the signal intensity [38]. No heteronuclear decoupling was applied during the relaxation delay set successively to 20 μs, 10 ms, 100 ms, 1 s, 2.5 s, 5 s, 10 s, and 20 s. A total of 4800 scans were accumulated for each delay. The majority of T_1 curves obtained from maximal peak heights could be fitted with a mono-exponential function (deviations were within the noise level). However, the signal decay at 74, 72, 69, 65, and 62 ppm for the 10% hydrated sample, and 74 ppm for the 35% hydrated sample showed a significant bi-exponential behavior. Error bars for the relaxation parameter T_1 were estimated from the fit result of three to five different positions distributed around the maximum of each resonance.

CP build-up experiments.—The build-up of carbon signal intensity as a function of contact time was monitored using 11 different CP contact times ranging from 0 to 8 ms. During the first few rotor periods, points were only taken at full cycles of the rotor to avoid a possible modulation of the curve due to MAS. A total of 10,000 scans were accumulated for each experiment. The evolution of the maximal peak heights was fitted with the single-exponential model of Eq. (1). However, it is noteworthy that the corresponding Gaussian model for the build-up gave about the same quality of fit.

2D-WISE experiments.—For the two-dimensional WISE experiments, 16 complex points were acquired in the proton evolution period. The States method for phase-sensitive detection in the indirect dimension was used [39]. The signal was then transferred to carbon spins using a short CP contact time of 300 μs. Longer contact times would lead to an equilibration of the proton magnetization due to spin diffusion, and consequently to the same proton lineshape for all carbon sites. For each

t_1 increment, a number of 4800 for the 10% hydrated sample, and 2480 scans for the more hydrated one, were accumulated.

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