A New NMR Method for the Study of Local Mobility in Solids and Application to Hydration of Biopolymers in Plant Cell Walls

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ABSTRACT: A new solid-state NMR experiment, J-WISE, is presented for studying local mobility in solids with atomic resolution. The experiment correlates the wide-line proton spectrum with the isotropic chemical shift of carbon-13 via the *J* coupling between both nuclei. The wide-line proton dimension contains information about the relative mobility of the *directly bonded* protons. The *through-bond* correlation to the isotropic chemical shift via *J*-coupling ensures a very selective detection of molecular motion. This experiment is notably particularly useful to distinguish between bonded and nonbonded interactions such as those involved in hydration or hydrogen bonding. This experiment is first demonstrated experimentally on the model system L-alanine. Finally, it is applied to onion cell wall material in order to localize water in the cell wall architecture. By comparison with complementary dipolar WISE spectra, we were able to attribute unambiguously some of the signal intensity in the latter experiments to bound water molecules, which must be intimately mixed with polysaccharide chains of the primary plant cell wall. A semiquantitative estimation of the hydrated cellulose part allowed us to discount oversimplified models of the structure of cellulose microfibrils in primary plant cell walls, which locate the hydrated cellulose only at the surface of the microfibril. The possibility to use faster sample spinning speeds for WISE-type experiments is demonstrated.

Introduction

Solid-state NMR has established itself as a very powerful probe of the details of molecular motion in solids. Indeed, since its discovery, various methods have been developed and applied to study molecular motion in different regimes and in a very diverse range of materials.[1]

The proton spectrum in the solid state constitutes one of the most widely used indicators of rapid motion,[2] since the width of the proton spectrum is dominated by (orientation dependent) dipolar couplings. Any molecular motion which is rapid compared to the inverse of the dipolar couplings will tend to reduce the effective dipolar couplings and therefore lead to a characteristic change in proton line width.

In 1992, the utility of proton line widths was substantially increased with the introduction of the WISE (WIde-line SEparation) experiment by Schmidt-Rohr et al.[3] The 2D-WISE experiment (see Figure 1a) correlates the wide-line proton spectrum with the isotropic chemical shift of the neighboring carbons. The great advantage of the WISE experiment is that in principle differences in local mobility across the molecule can be detected since the dynamic information contained in the proton wide-line is separated according to the isotropic chemical shift of the individual carbon atoms. Thus, differences in proton line widths from one carbon to another can be interpreted in terms of differences in mobility of the neighboring hydrogen nuclei within a molecule.

In WISE, the transfer of polarization from protons to carbons is performed with a cross-polarization (CP) step[4−6] driven by the through-space dipolar coupling between both nuclei. Using this method, magnetization

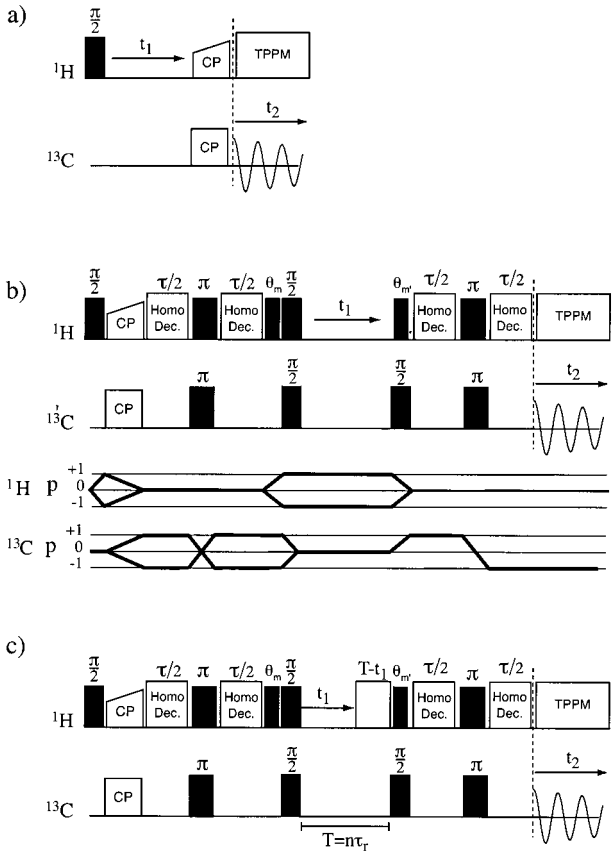

Figure 1. Pulse sequences for (a) dipolar 2D WISE, (b) J-WISE, and (c) constant time J-WISE. The phase cycle of sequences (b) and (c) is the same as for the MAS-J-HSQC sequence.[8] The pulse angle θ_m represents the angle between the static magnetic field and the effective field direction of the homonuclear sequence used. $\theta_{m'}$ is the complementary angle, $\theta_{m'} = \pi/2 - \theta_m$.

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can be transferred to a particular carbon from protons more remote than just the directly bound ones. Therefore, for a particular carbon resonance, the observed proton wide-line spectrum contains dynamic information averaged over all the protons which were able to transfer some polarization to that carbon during the CP step. As a result, the localization of the dynamic information depends on the length of the CP step. This feature can be exploited for the measurement of domain sizes in heterogeneous mixtures of compounds showing different molecular mobility.[2,3]

In certain cases, however, the lack of spatial selectivity during the CP transfer in dipolar WISE experiments may be a handicap. Notably, the CP dynamics depends sensitively on the local molecular mobility, which is the parameter the experiment is supposed to measure. In complex systems the results can become ambiguous, as reported for example in hydrated plant cell walls.[7] In that system, previously reported dipolar WISE experiments clearly showed two types of mobility. This result could be interpreted with two different hypotheses: either an inhomogeneity of mobility across the sample or the presence of bound water molecules intimately mixed in the biopolymer structure. The first explanation was however in contradiction with relaxation experiments which showed essentially monoexponential curves. The second hypothesis seemed in contradiction with accepted models of the structure of cellulose microfibrils.

To remedy this problem, in this paper we present a new complementary wide-line experiment called J-WISE, which restricts the motional information detected by a carbon nuclei to only its directly bonded protons. This is achieved by using a polarization transfer step driven by the proton−carbon heteronuclear J coupling instead of the dipolar driven CP step. The J-WISE sequence is demonstrated on the model system L-alanine and then successfully applied to hydrated onion cell wall material (CWM). By comparison of through-bond and through-space correlations, we are able to demonstrate that the amorphous regions of cellulose are not limited to the surface of the microfibrils but that the interior of the microfibril contains a mixture of amorphous and crystalline regions on a length scale of <3 nm. In passing, we also demonstrate that both the dipolar and the J-WISE experiment can be performed under higher magic angle spinning (MAS) speeds than usually used.

Pulse Scheme

The J-WISE sequence is shown in Figure 1b. It is based upon the MAS-J-HSQC sequence[8] without decoupling during the proton evolution period t_1. The carbon magnetization obtained through CP is subsequently transferred to protons during the first τ period via the heteronuclear J coupling. After the two 90° pulses, the resulting antiphase single-quantum proton magnetization then evolves in the indirect dimension t_1 in the absence of decoupling, leading after Fourier transform to the wide-line proton spectrum in the F_1 dimension. After the t_1 evolution, the proton coherence is transferred back via J coupling during the second τ period to in-phase carbon magnetization, which is detected under heteronuclear TPPM[9] decoupling during t_2. All pulse sequences and phase cycles are available from our Web site[10] or on request to the authors.

Traditionally, the standard dipolar 2D-WISE experiment (see Figure 1a) is performed under relatively slow rotation of the sample at the magic angle (MAS). This yields a proton line shape dominated by the dipolar coupling, without significant disturbance from MAS. In such cases, the proton resonance can normally be fitted well by a Gaussian line shape function:

$$S(\omega) = I \exp\left\{-4 \ln 2\left(\frac{\omega - \omega_0}{\Delta}\right)^2\right\} \qquad (1)$$

with I the overall intensity, ω_0 the center frequency of the resonance, and Δ the line width (full width at half-height, fwhh) of the Gaussian function which reflects the degree of motional averaging. However, sensitivity and resolution can be substantially improved in the WISE experiment, especially in the carbon dimension, by using higher MAS rates. This induces splitting of the wide proton lines into spinning sideband patterns. As the extended proton network is typical of a homogeneous spin system, the resolution of the spinning sidebands will increase with the spinning speed.[11] The information about the degree of molecular mobility is no longer simply contained in the width of the individual proton resonances but in the intensity distribution of the spinning sideband pattern. As has been discussed extensively in the literature, to a good approximation one can consider that for moderate spinning speeds the width of the spinning sideband pattern envelope reflects the dipolar interaction[11,12] and is therefore sensitive to molecular motion. The intensity of the spinning sidebands can therefore be modeled by a Gaussian distribution:

$$S(\omega) = \sum_{i=-n}^{n} I \exp\left\{-4 \ln 2\left[\left(\frac{n\omega_r}{\Delta}\right)^2 + \left(\frac{\omega - \omega_0 - n\omega_r}{\lambda_1}\right)^2\right]\right\} \qquad (2)$$

with I the overall intensity, ω_0 the center frequency, λ the line width (fwhh) of each spinning sideband separated by the spinning frequency ω_r, and Δ the width (fwhh) of the Gaussian distribution, which as before reflects the degree of local mobility. This simple analysis is expected to be valid at moderate MAS rates (typically up to about 20 kHz); at higher MAS frequencies, "spin-pair" type models[13,14] may become more relevant in the interpretation of the sideband envelope. In any case eq 2 is expected to be valid in all the cases treated in this article.

For a proper in-phase detection of the spinning sidebands in the indirect dimension, the rotor position at the beginning and the end of the t_1 evolution should be synchronized for each t_1 delay.[15] This is achieved by replacing the t_1 evolution step by a constant time T, which is an integer multiple of the rotor period. The constant time T is composed of the incremented time t_1 and a decremented spin-lock field which stores one component of the magnetization until the end of T. This applies to both the dipolar and J-WISE versions of the experiment. The constant time version of the J-WISE pulse sequence is shown in Figure 1c. Because T has to be at least as long as the longest t_1 increment, the constant time version of the J-WISE experiment is less sensitive. However, experimentally we find that the constant time version is only necessary in cases where the sideband pattern of the proton spectrum is very well resolved.

Experimental Section

The natural abundance sample of L-alanine was purchased from Sigma and used without further recrystallization. The preparation of onion CWM is described in Hediger et al.[7] The hydration level of the CWM sample was about 35% (obtained by rehydration of dried CWM with H_2O). It was shown previously that this hydration level was adequate to induce noticeable changes in the mobility of the cell wall components when compared to dry CWM.[7] The spectral assignment of the carbon resonances in CWM was based on published data.[16,17] All NMR experiments were performed on a Bruker DSX 500 spectrometer (proton frequency 500 MHz) using a 4 mm CP/MAS probe. For the J-WISE experiments, the sample volume was restricted to the center $^1/_3$ of the rotor using flat spacers to ensure a better rf homogeneity over the sample. The proton rf field was set during both the τ delays (FSLG homonuclear decoupling[18−20]) and the acquisition (TPPM heteronuclear decoupling[9]) to 75 kHz in the case of L-alanine and to 100 kHz for the onion CWM sample. The recycle delay was 2 s for L-alanine and 1 s for onion CWM. Quadrature detection in t_1 was achieved using TPPI[21−23] and States[24] methods for the dipolar WISE and J-WISE experiments, respectively.

Results

J-WISE on L-Alanine. The J-WISE experiment was first tested on powdered L-alanine. At the MAS frequency used (12.5 kHz), the sideband pattern of the proton spectrum was well resolved. The constant-time J-WISE sequence of Figure 1c was necessary to give a distortion-free spectrum. The 2D spectrum and traces through both carbon resonances are shown in Figure 2. Comparing the proton sideband pattern for the CH and CH_3 sites in L-alanine, it is evident that the much narrower envelope for the CH_3 group reflects the higher mobility compared to that of the CH group. An analysis of the sideband pattern was obtained by fitting the experimental data with the model of eq 2. We found a sideband pattern width Δ of 20.4 kHz for the CH group and 13.7 kHz for the CH_3, which shows the clear difference in relative mobility between the two groups. (The CH_3 group is known to be undergoing rapid rotation about its C_3 axis at room temperature.[25]) Although less visible, this fact can also be seen in the difference between the individual line widths of the spinning sidebands for both groups (2.2 kHz for CH and 1.7 kHz for CH_3), the smaller effective dipolar coupling of the CH_3 group being better averaged than the CH at the same spinning speed. The qualitative information about dynamics obtained here at higher spinning speed from the width of the spinning sideband pattern is essentially the same as that which could be obtained at lower MAS rotation frequency from the width of the wide-line spectrum**.** However, the possibility of performing the experiment at higher spinning speeds yields more flexibility with regards to resolution of the carbon-13 spectrum and to sensitivity. Indeed, in principle the reliability of the determination of the width of the proton spectrum is expected to be significantly greater for the spinning spectrum than for the static spectrum.[26]

Hydration of Onion Cell Wall Material. Plant cell walls are a natural material composed of several polysaccharides showing different degrees of crystallinity and hydration.[27] The main component, cellulose, is arranged in crystalline fibers and forms a dense network. In most species the cellulose microfibrils are coated with xyloglucans, which span the space between the microfibrils. In primary cell walls, as in onions for example, this network is embedded in a highly hydrated matrix of polysaccharides called pectin. Using solid-state

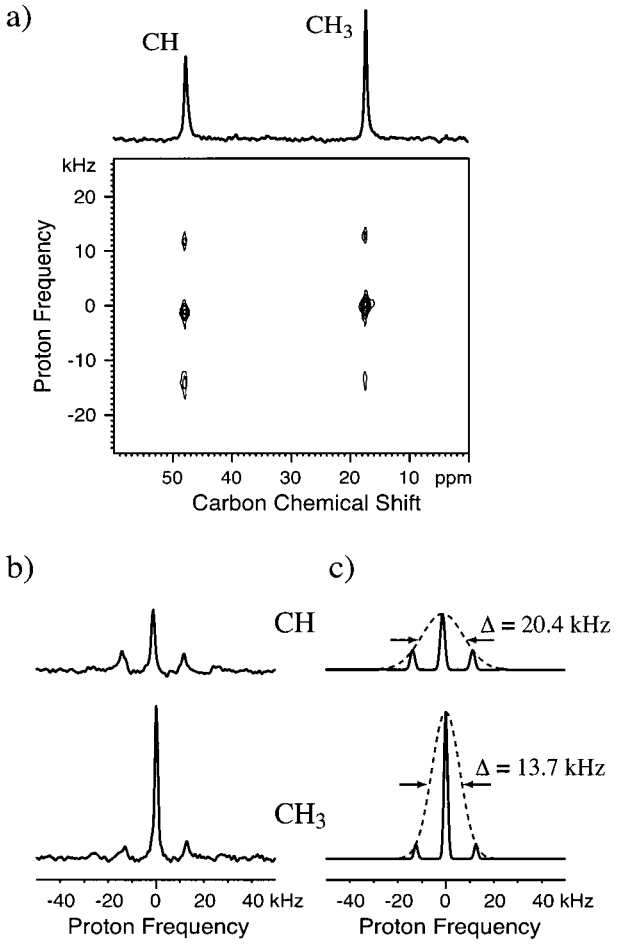

Figure 2. Constant-time J-WISE experiment on L-alanine (a) and traces along the proton dimension through the CH and CH_3 resonances (b). The spinning speed was set to 12.5 kHz. A total of 128 t_1 increments with 208 scans each were collected. The contact time for cross-polarization was set to 700 μs and the τ delay to 2.8 ms. A constant time T of 640 μs was used. The best least-squares fit according to eq 2 of the traces through the CH and CH_3 resonances is given in (c). The dashed line represents the Gaussian spinning sideband envelope.

NMR data, several authors have shown that water molecules contained in hydrated cell wall material (CWM) mostly affect the pectin molecules by increasing the mobility of this polymer. The cellulose on the contrary seems almost unaffected by the degree of hydration.[7,17,28−30] This is in agreement with the crystal structure of cellulose microfibrils which does not contain any water molecules[31] and with molecular dynamics simulations, which have shown that only the topmost surface of the cellulose fibril is structurally affected by the water present near the surface.[32] In this respect, the results of recent dipolar 2D-WISE experiments on onion cell walls are puzzling. For pectin as well as for cellulose resonances, the proton line showed two components.[7] A broad component of about $\Delta = 40$ kHz was attributed to the polysaccharide. The origin of the second narrower component (less than 10 kHz) is not clear. As discussed by Hediger et al.,[7] it could be explained in two ways: either (i) a spatial inhomogeneity of the mobility of the cellulose inside the cell wall or (ii) by bound water molecules located in close proximity to the polysaccharides. Considering that the narrow component appears at short CP contact times (300 μs), the second hypothesis would imply that water molecules are entangled *inside* the cellulose microfibrils, which

Table 1. Fitted Values with Estimated Errors for Carbon Traces of the Dipolar WISE Experiment at 5 KHz MAS, According to the Two Gaussian Model of Eq 3

ppm	assignment	I_1	Δ_1/kHz	I_2	Δ_2/kHz	S_2/S_1[a] (%)
			Pectin Resonances			
101	C-1	0.36 ± 0.03	34.8 ± 0.2	0.61 ± 0.03	10.7 ± 0.2	52
80	C-4	0.50 ± 0.01	27.6 ± 0.1	0.51 ± 0.01	7.1 ± 0.1	26
69	C-2,3	0.62 ± 0.01	26.2 ± 0.1	0.39 ± 0.01	6.2 ± 0.1	15
54	methoxyl			1.01 ± 0.01	8.2 ± 0.1	
21	CH_3			1.06 ± 0.03	5.2 ± 0.2	
			Cellulose Resonances			
105	C-1	0.60 ± 0.01	41.7 ± 0.1	0.40 ± 0.01	8.0 ± 0.1	13
84	surf. C-4 [b]	0.73 ± 0.03	40.8 ± 0.3	0.27 ± 0.03	6.6 ± 0.3	6
74	gen	0.44 ± 0.01	39.6 ± 0.1	0.55 ± 0.01	8.1 ± 0.1	26
72	gen	0.54 ± 0.01	40.1 ± 0.1	0.46 ± 0.01	7.9 ± 0.1	17
65	cryst C-6	0.78 ± 0.03	47.9 ± 0.3	0.21 ± 0.03	10.1 ± 0.3	6
62	surf. C-6	0.27 ± 0.03	35.9 ± 0.5	0.72 ± 0.03	8.4 ± 0.3	62

[a] Ratio of the areas of the narrow (S_2) and the broad (S_1) Gaussian components. [b] The signal-to-noise ratio of the trace at 89 ppm assigned to crystalline (interior) C-4 was not good enough to allow a significant fitting of the curve.

would be a surprising result according to Heiner et al.[32] At this point it is worth noting that other authors have reported this kind of line shape in 2D-WISE experiments on the same kind of sample and attributed the narrow line to bound water.[33,34] However, these experiments were performed either on amorphous cellulose or with longer CP times as well as with an additional spin-diffusion mixing time, which clearly allows for correlation of cellulose carbons in the interior of the fibril with water molecules bound *at the surface* of the microfibrils. The question of the origin of the narrow component in the dipolar WISE experiments could be answered by rehydrating the CWM with D_2O. Indeed, if the narrow component arises from water protons, it will disappear if all water molecules are deuterated. However, this procedure is delicate as hydroxyl protons can exchange with water deuterons, and care has to be taken that all the water molecules are replaced by deuterated analogues and that they remain deuterated with time.

To solve the problem of water localization in cellulose, we compared the results obtained with the dipolar and the J-WISE sequences on the H_2O-rehydrated CWM sample. Traces from the slow-spinning 2D-dipolar WISE spectrum through various carbon resonances of onion CWM (the carbon spectrum is given in Figure 3a) are shown in Figure 3b. The MAS frequency set to 5 kHz was not fast enough to generate sideband patterns. The CP contact time was 300 μs. The proton traces are fitted very well with the model of eq 1, considering *two* Gaussian components:

$$S(\omega) = I_1 \exp\left\{-4 \ln 2\left(\frac{\omega - \omega_{01}}{\Delta_1}\right)^2\right\} + I_2 \exp\left\{-4 \ln 2\left(\frac{\omega - \omega_{02}}{\Delta_2}\right)^2\right\} \quad (3)$$

Fitted values with estimated errors for the relative intensity and line width of both components are given in Table 1 (maximal signal intensity was set to 1 for all traces prior to fit), together with the relative signal area of both Gaussian components. Except for the methoxyl (at 54 ppm) and methyl (21 ppm) resonances in the pectin, which could be fitted with one Gaussian component, *all carbon resonances originating from both the pectin and the cellulose* show, as already reported,[7,33,34] a broad (rigid) and a narrow (mobile) component.

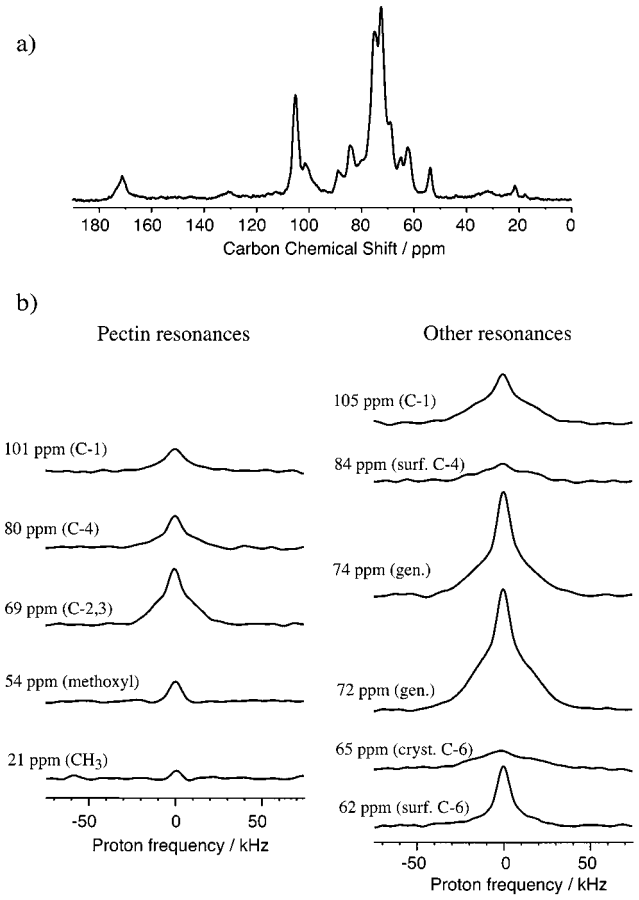

Figure 3. (a) CP/MAS spectrum of onion CWM. The spinning speed was set to 5 kHz, and the contact time for CP was 1 ms. (b) Traces along the proton dimension of a slow spinning dipolar WISE experiment on onion CWM. The corresponding carbon resonances are indicated. The spinning speed was set to 5 kHz. A total of 48 t_1 increments with 2400 scans each were collected. The contact time for cross-polarization was set to 300 μs. A proton spectral width of 150 kHz was used. Results from the two-component fit of the experimental line shapes according to eq 3 are given in Table 1. Fitted curves are not shown, as they match perfectly the experimental ones.

The dipolar 2D-WISE experiment was repeated at a faster MAS frequency (10 kHz) to provide a direct comparison with the J-WISE experiment. We note that the CP/MAS spectrum of onion CWM at this higher spinning speed gave no indication of a centrifugation of the bound water toward the sides of the rotor. Indeed,

Table 2. Fitted Values with Estimated Errors for Some Carbon Traces of the Dipolar WISE Experiment at 10 kHz MAS, According to the Two Gaussian Model of Eq 4

ppm	assignment	I_1	Δ_1/kHz	λ_1/kHz	I_2	Δ_2/kHz	S_2/S_1[a] (%)
			Pectin Resonances				
69	C-2,3	0.35 ± 0.01	26.4 ± 0.1	5.55 ± 0.03	0.64 ± 0.01	4.5 ± 0.1	31
54	methoxyl				0.98 ± 0.01	4.9 ± 0.1	
21	CH_3				1.00 ± 0.01	3.9 ± 0.1	
			Cellulose Resonances				
105	C-1	0.41 ± 0.01	39.7 ± 0.5	8.17 ± 0.03	0.58 ± 0.01	5.1 ± 0.1	18
74	gen	0.35 ± 0.01	32.2 ± 0.1	7.13 ± 0.01	0.65 ± 0.01	4.8 ± 0.1	28
72	gen	0.38 ± 0.01	34.2 ± 0.2	7.26 ± 0.01	0.62 ± 0.01	4.7 ± 0.1	22
62	surf. C-6	0.22 ± 0.01	33.4 ± 0.5	7.66 ± 0.01	0.77 ± 0.01	5.3 ± 0.1	56

[a] Ratio of the areas of the narrow (S_2) and the broad (S_1) Gaussian components.

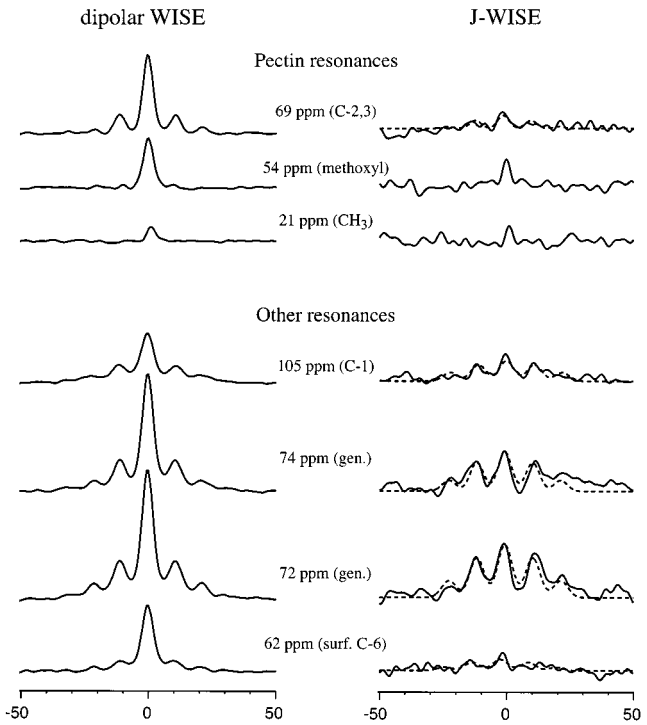

Figure 4. Traces along the proton dimension of a dipolar WISE (left column) and a J-WISE (right column) experiment on onion CWM. Only traces with sufficient signal-to-noise ratio to allow a significant fitting are given. The corresponding carbon resonances are indicated. The spinning speed was set to 10 kHz in both experiments. A total of 96 t_1 increments with 2400 scans each were collected for the dipolar WISE and 50 t_1 increments with 8192 scans each for the J-WISE. Both data sets were obtained using the "standard" version of the pulse sequences, since the constant-time version was not found to be necessary. The contact time for cross-polarization was set to 300 μs for the dipolar WISE and 1 ms for the J-WISE. Proton spectral widths of 150 and 100 kHz were used for the dipolar and J-WISE experiments, respectively. Fitted curves for the dipolar WISE experiment are not shown but match very closely the experimental line shapes. The results of the fit using the model of eq 4 are given in Table 2. The broad component obtained by least-squares fit of the dipolar WISE experiment (given in Table 2) was adjusted only in intensity to match the corresponding J-WISE curve. It is shown as dash lines on top of the different J-WISE traces.

this would have implied a decrease in the observed CP/MAS hydration level with time and notably a degradation of the resolution, which was not observed. Traces along the proton dimension of the dipolar 2D-WISE experiment are given in the left column of Figure 4. The splitting of the proton line into spinning sidebands is obvious. The traces could once again be fitted very well with a two component model according to

$$S(\omega) = S_1(\omega) + S_2(\omega), \quad \text{with}$$

$$S_1(\omega) = \sum_{i=-n}^{n} I_1 \exp\left\{-4 \ln 2\left[\left(\frac{n\omega_r}{\Delta_1}\right)^2 + \left(\frac{\omega - \omega_{01} - n\omega_r}{\lambda_1}\right)^2\right]\right\}$$

$$S_2(\omega) = I_2 \exp\left\{-4 \ln 2\left(\frac{\omega - \omega_{02}}{\Delta_2}\right)^2\right\} \qquad (4)$$

The first component $S_1(\omega)$ was considered to give a sideband pattern according to eq 2. For the narrow component $S_2(\omega)$, which is estimated to be at most 10 kHz in width (see Table 1) from the 5 kHz MAS spectrum, it can be considered that only the centerband will have a noticeable intensity at 10 kHz MAS. The result of the numerical fit according to eq 4 is given in Table 2 for the different traces. (As before, maximal intensity was set to 1 for all traces prior to fit.) The best values for the fit were found for a number of spinning sidebands n for the first component fixed to $n = 4$.

These traces can be compared in Figure 4 to the ones obtained with the J-WISE experiment (right column). Especially for the cellulose resonances, it is obvious that the sideband pattern has changed. The narrow component which was concentrated in the centerband intensity has disappeared. As shown in Figure 4, the envelope of the sideband pattern in the J-WISE experiment corresponds very well to only the broad Gaussian component found in the dipolar WISE traces (dash lines on top of the J-WISE traces). If the narrow component was due to a pool of more mobile polysaccharides, the protons of these polysaccharides would still have been able to transfer their polarization to their attached carbons via the J-coupling in the J-WISE experiments, and the narrow component would have been conserved. We can therefore conclude that the narrow component in the dipolar WISE spectrum arises indeed from water molecules.

Discussion

The comparison of the dipolar and J-WISE experiments has important consequences for the structural model for cellulose in cell walls. The generally accepted model for the cellulose microfibrils in plant cell walls is that the cellulose units in the interior of the microfibrils are crystalline whereas the ones at the surface are noncrystalline (amorphous). The amorphous parts could

contain bound water molecules, but the crystalline parts cannot. This model was formulated from the observation that both the C-4 and C-6 sites each have two distinct chemical shifts, one broader than the other. The relative intensity of the two resonances was found to correlate with the number of cellulose units considered as inside or at the surface of the microfibrils based on their diameter.[35−37] Thus, the resonances at 89 and 65 ppm are generally attributed to C-4 and C-6 sites, respectively, of crystalline cellulose units located inside the microfibrils (crystalline) and the peaks at 84 and 62 ppm to C-4 and C-6 sites of surface amorphous cellulose. On the basis of resolution enhanced CP/MAS spectra and the similar proton $T_{1\rho}$ relaxation rates of the two C-4 sites, Newman and co-workers maintained the assignment of surface and interior cellulose units but considered that both are mainly crystalline, with the noncrystalline part being reduced to a very broad background signal in that region.[38−40] The experiments described in this paper yield complementary information which forces us to review to some extent this rather rough model of cellulose fibrils.

Prediction of the "Surface" Model. The J-WISE experiment shows that the narrow component in the dipolar WISE traces of the pectin and cellulose resonances arises only from sites that are close enough to a water molecule to get polarization from its protons, whereas the broad component arises from all the sites of the particular ^{13}C resonance. The presence of an intense narrow component is understandable for the pectin sites, as it is accepted that these polysaccharides are hydrated. It is however improbable that the water molecules located in the pectin are responsible for the narrow component observed at the cellulose sites, considering the short CP contact time (300 μs) used. Indeed, the length scale $\langle x \rangle$ covered by spin diffusion during the contact time τ can be estimated by the following equation:[2]

$$\langle x \rangle = \sqrt{4D\tau/\pi} \qquad (5)$$

Taking for the spin diffusion coefficient D the value of 0.15 nm^2 $(ms)^{-1}$ found by Radloff et al.[33] as determined from the line width of the mobile component of the proton spectra in cellulose, the water−cellulose distance can be estimated in our case to be less than 0.3 nm. This is at least 1 order of magnitude less that the diameter of the microfibrils expected for primary cell walls, which ranges from 3 to 15 nm.[27] Considering a microfibril of 3 nm diameter, the volume which is 0.3 nm or less from the surface corresponds to 36% of the total volume of the microfibril. This is illustrated graphically in Figure 5. This 0.3 nm deep "surface part" lowers to 23% for a 5 nm diameter microfibril, to 12% for a diameter of 10 nm, and to 8% for 15 nm.

Comparison with the Experiments. Although carbon signal intensity obtained from CP experiments cannot easily be interpreted quantitatively, an analysis of the relative intensity of the broad and narrow components in the dipolar WISE experiments is very informative. In Tables 1 and 2, the area of the narrow Gaussian component S_2 is given as a percentage of the broad Gaussian component S_1. The ratios are relatively consistent for both MAS spinning speeds (5 and 10 kHz) that were measured here. Notably, for the resonance assigned specifically to the crystalline cellulose inside the microfibrils (at 65 ppm), the S_2/S_1 ratio is very low

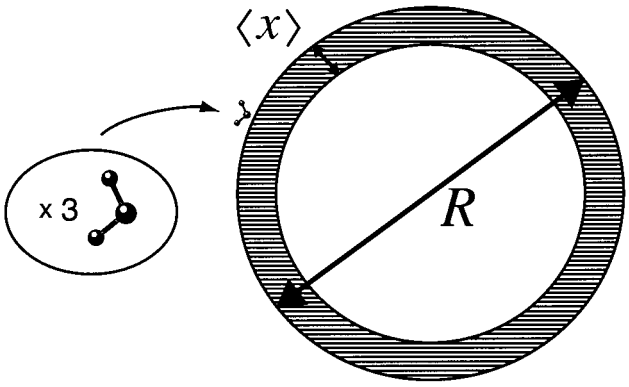

Figure 5. Spin diffusion originating from water protons using the "surface" model. We consider a cellulose microfibril diameter $R = 3$ nm. The length scale $\langle x \rangle$ covered by spin diffusion during the CP contact time is estimated at 0.3 nm, according to eq 5. The shaded surface corresponds to 36% of the microfibril.

(6%) as expected. However, for the other cellulose resonances, the "hydrated" cellulose amounts to 20−30% on average, *only slightly less than in pectin (about 30%)*. Even at this level of interpretation, this ratio is in accord with the prediction of the surface model of Figure 5 only for the smallest microfibril size (<5 nm). However, we have to consider in addition that the cross-polarization transfer from a water proton to a cellulose carbon can only be slower than the transfer from the directly bound proton (due to the significantly longer distance and the much greater mobility of the water molecule). The ratio S_2/S_1 is therefore underestimated from these data. The values of S_2/S_1 thus represent the lowest limit on the fraction of the cellulose that contains water molecules and which must therefore be considered as noncrystalline or amorphous. The underestimation of the ratio S_2/S_1 can be roughly estimated from the comparison of the ratios for the cellulose sites with the ratios for the pectin sites. Indeed, these ratios are about the same. Considering that experimentally the CP behavior is found not to be very different between the pectin and the cellulose for very short contact times (300 μs),[7] we can assume that about the same amount of cellulose units can be considered as hydrated as of pectin units. This is certainly not what is expected from the surface model. We therefore deduce that our experimental data are in contradiction with Figure 5.

In conclusion, the data are not consistent with only the topmost surface layer of the cellulose microfibrils being hydrated. These results imply that a significant amount of cellulose is in contact with water molecules, rendering the postulate of a purely crystalline interior improbable. We suppose therefore that the interior of the fibril is composed of crystalline and noncrystalline regions which are mixed on a length scale of <3 nm. Molecular mobility in both domains is probably very similar due to the very dense structure of cellulose. Indeed, even if molecular bound water is present in amorphous cellulose, the glucan chains are still tightly packed via hydrogen bonding, which limits the increase in mobility with the degree of hydration. This would explain why the proton $T_{1\rho}$ and carbon T_1 relaxation rates of the cellulose are less sensitive to the hydration level of the sample than those of pectin,[7] even if the overall level of hydration appears to be about the same.

Conclusions

In this article we have proposed a new solid-state NMR experiment, J-WISE, which allows a very selective detection of relative mobility inside the sample. As for the dipolar WISE experiment, the J-WISE experiment correlates the proton line which is sensitive to motion with the isotropic chemical shift of carbons. However, because of the J-transfer of polarization, the dynamic information for a carbon site is limited in the J-WISE experiment to only directly bonded protons, giving access to very local information about dynamics. Indeed, in our test model alanine, the increased mobility of the CH_3 protons compared to that of the CH proton is perfectly clear in the proton traces of the 2D J-WISE experiment. We have also demonstrated that dynamic information can be gained from WISE experiments (dipolar or J-) performed at higher spinning speeds, which can be useful to optimize the sensitivity and resolution of these experiments.

J-WISE and the dipolar WISE should be considered as complementary methods, leading to different types of dynamic information in solids. On one hand, the assignment of motion to specific sites can only be obtained unambiguously with J-WISE. The dipolar WISE experiment, on the other hand, offers the possibility to detect and measure the size of spatial heterogeneities, when they are distinguished by different relative molecular mobilities.

The complementary nature of dipolar and scalar correlations in solids is highlighted by the comparison of dipolar WISE experiments and J-WISE experiments on an onion CWM sample. These experiments allowed the unambiguous assignment of the narrow component in the proton traces of dipolar WISE experiments to bound water, since this component completely disappeared in the J-WISE spectrum and therefore must originate from nonbonded protons. The contribution of water protons to the proton wide-line of the cellulose signals in the dipolar WISE experiment allowed us to conclude that water molecules are present *inside* the cellulose microfibrils, in contradiction with the common model of cellulose. As a result, we are able to put forward a model of cellulose fibril structure containing both crystalline and amorphous (noncrystalline) domains in the interior of the fibril.

Finally, we remark that the technique is applicable in principle to all types of organic solids, ranging from the biopolymers used as an example here to synthetic polymers or crystalline solids (notably, for example, to identify dynamic mechanisms in supramolecular host−guest systems).

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