Water Organization and Molecular Mobility in Maize Starch Investigated by Two-Dimensional Solid-State NMR

Andrzej S. Kulik,* J. R. Chris de Costa, and Johan Haverkamp

Unilever Research Laboratorium, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen, The Netherlands

The molecular mobility of polysaccharide chains and the water organization in maize starch forms A and B were investigated with the recently developed 2D ^{1}H$-^{13}$C heteronuclear wideline separation (WISE) method of solid-state NMR spectroscopy. Proton lines of starch yield information about the molecular motion. No evidence for molecular motion of the polysaccharide chains on a time scale faster than microseconds was found. From the WISE spectra measured with a short mixing time it was estimated that 28% of the water present was bound to the polysaccharide chains in forms A and B. It was found that there is no preference for water to bind to particular sites of the glucose rings. Comparison of WISE spectra obtained with different mixing times t_m revealed differences in water organization between the starch forms. From the rate of the spin diffusion process active during t_m it was concluded that the molecular mobility of water in form A is higher than in form B. This explains the lower gelation temperature of A-form starch.

INTRODUCTION

Knowledge of the water distribution in starch granules is important for understanding their properties. On the basis of X-ray diffraction three distinct crystalline forms (A, B, C) of starch have been determined (Guillot and Mercier, 1985). Forms A and B are well-defined, while form C is considered to be a mixture of the A and B forms. In form A the structure is based on parallel-strained double helices in the unit cell, which produce a monoclinic crystal. It was proposed that four water molecules per unit cell are located between these helices (Imberty et al., 1988). In form B chains are also organized in double helices; however, crystal packing and water content are different. It is well-accepted that form B has a hexagonal unit cell (Guillot and Mercier, 1985). Imberty and Perez (1988) proposed a model in which the double helices in form B are arranged around a channel with well-localized water molecules. Half of the water molecules are connected to the carbohydrate chains by hydrogen bond, whereas the remainder are connected to other water molecules. All of the water molecules are connected by at least one hydrogen bond. The water organization as suggested by the X-ray investigations differs between the forms.

Solid-state NMR has proved to be a particularly successful technique in characterizing the local molecular structure, the conformational order, and the dynamics in solids (Mehring, 1983; Schmidt-Rohr and Spiess, 1994). Carbon-13 NMR spectra obtained with cross-polarization (CP) and magic angle spinning (MAS) yield high resolution through well-separated chemical shifts which contain information about molecular moieties (Mehring, 1983; Pines et al., 1973). In ^{13}C spectra of starch reported earlier clear distinction between forms A and B could be made only by measuring samples soaked in water (Gidley and Bociek, 1985). Fine splittings were observed at the C-1 position, namely, a triplet for A-form starch and a doublet for B-form starch. However, spectra for both forms taken in the dry state

are almost identical. Compared to ^{13}C CP/MAS spectra, proton NMR lines measured in the solid state are wide and structureless (Abragam, 1961). The width of the ^{1}H line characterizing the strength of the dipolar coupling between protons gives information about the molecular mobility in the tens of kilohertz regime. Motions fast on this time scale will result in the narrowing of the line shape to a few kilohertz or even less, while motions slow on this time scale will remain multispin dipolar interaction unaffected, yielding line shapes with the width in the tens of kilohertz. Wu et al. (1992) reported ^{1}H NMR spectra of native starch containing between 10 and 45% water. The proton spectrum exhibited a relatively narrow water line, whereas signals from starch are absent. This reveals the high mobility of water molecules. Thus, ^{13}C CP/MAS spectra characterize molecular structure, while ^{1}H lines give information about the molecular mobility. Those concepts of correlating structural information from ^{13}C CP/MAS and mobility from ^{1}H were combined recently by Schmidt-Rohr et al. (1992) in a two-dimensional (2D) ^{1}H$-^{13}$C heteronuclear wideline separation experiment (WISE). Since 2D NMR is far superior to 1D techniques for studying the local structure, it has been used for the present study (Ernst et al., 1987; Schmidt-Rohr and Spiess, 1994).

The purpose of the present investigation is to probe various water environments in maize starch by means of 2D WISE-NMR. Further, this experimental technique will be used to obtain information about mobility of the polysaccharide chains in the dry state.

EXPERIMENTAL PROCEDURES

WISE-NMR. To facilitate the understanding of the spectra presented below, the relevant features of the experimental technique employed in this paper are briefly summarized. The WISE sequence (Schmidt-Rohr et al., 1992; Tekely et al., 1993), shown in Figure 1, starts with a 90° pulse on the protons. After the pulse, proton magnetization evolves under the influence of dipolar coupling during the time t_1. In the simplest version of this experiment with the mixing time $t_m = 0$, the proton magnetization is transferred to the carbons by means of Hartmann–Hahn cross-polarization (Hartmann and Hahn, 1962; Pines et al., 1973). The signal is detected under MAS

* Author to whom correspondence should be addressed (e-mail andrzej.kulik@urlnl.sprint.com at Internet; fax int+31104605671).

2804 *J. Agric. Food Chem.*, Vol. 42, No. 12, 1994

Kulik et al.
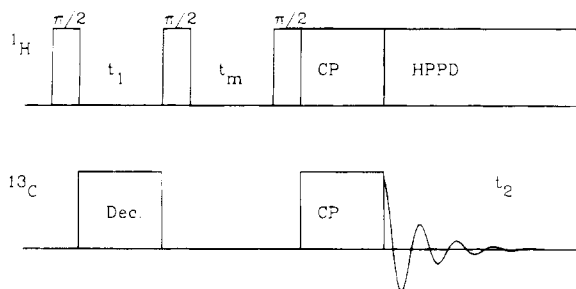
Figure 1. Pulse sequence of the heteronuclear $^1H-^{13}C$ 2D WISE-NMR experiment.

conditions during the time t_2. Incrementation of the time for proton evolution t_1 modulates the ^{13}C signal recorded during t_2. Subsequent 2D Fourier transformation yields a 2D spectrum in which the chemical structure is reflected by the signals along the ^{13}C chemical shift axis. For each of these resolved ^{13}C signals, a corresponding ^1H line is obtained indicating local mobility.

The WISE experiment can be utilized to probe the contact between the water and the polymer matrix (Schmidt-Rohr and Spiess, 1994; Zumbulyadis, 1986). For this purpose, the ^1H magnetization remaining after the evolution period is flipped to the z-axis for a subsequent mixing period with a duration t_m. During this time the proton magnetization of the water can exchange with the magnetization of the protons in the polymer chain by means of spin diffusion. This process is mediated by homonuclear dipolar coupling (Bloembergen, 1948). During the mixing time, transfer of magnetization between protons occurs only when they are sufficiently close in space.

The NMR experiments were carried out on a Bruker MSL-300 spectrometer operating at resonance frequencies of 75.47 and 300.13 MHz for ^{13}C and ^1H, respectively. Carbon-13 spectra were recorded with CP, applying a contact time of 500 μs in a variable-temperature double-resonance probe. The samples were spun at 4 kHz. The ^1H 90° pulse was 4.5 μs. For the ^1H spectra 128 scans were averaged. The 2D spectra were taken with up to 128 increments of 8 μs. Typically about 1000 scans were averaged. Fourier transformation of the data and the data analysis were performed on a Silicon Graphics computer using a modified PV-Wave software package (Visual Numerics Inc.). All experiments were performed at ambient temperature.

Starch. Samples were obtained from National Starch (Zutphen, The Netherlands) and were used without any chemical treatment. The A-starch used was a waxy maize starch (Amioca 85), whereas the B-starch was an amylomaize VII starch (Hylon VII). The moisture level in the samples was approximately 12%, as determined by vacuum oven drying.

RESULTS AND DISCUSSION

One-Dimensional Spectra. Figure 2 shows ^{13}C CP/MAS and ^1H spectra of starch in forms A and B. The line shapes in the ^{13}C spectra are similar to those reported earlier (Gidley and Bociek, 1985). The only difference between the spectra of A- and B-starch is the shape of the shoulder peak at 82 ppm. This peak represents C-4 carbons. As mentioned in the Introduction, it is difficult to distinguish between various forms of starch on the basis of these spectra. The proton lines which are broadened by $^1H-^1H$ dipolar couplings also shown in Figure 2 are narrow and have Lorentzian shape. Lorentzians are characterized by their half-width at half-maximum (hwhm). For Hylon VII the hwhm is 490 Hz, while for Amioca 85 the hwhm is 850 Hz. Such relatively narrow lines point to mobile water present in the polymer matrix (Wu et al., 1992). The marked difference in line width indicates that the water environments in both forms are different. As can be
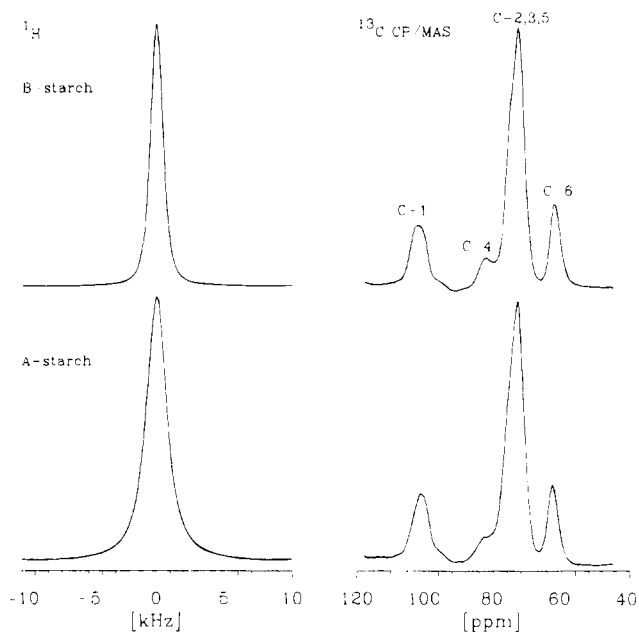

Figure 2. NMR spectra of starch in forms A and B: (lower) Form A (amylomaize VII, Hylon VII); (upper) form B (waxy maize, Amioca 85); (left) ^1H; (right) ^{13}C CP/MAS.
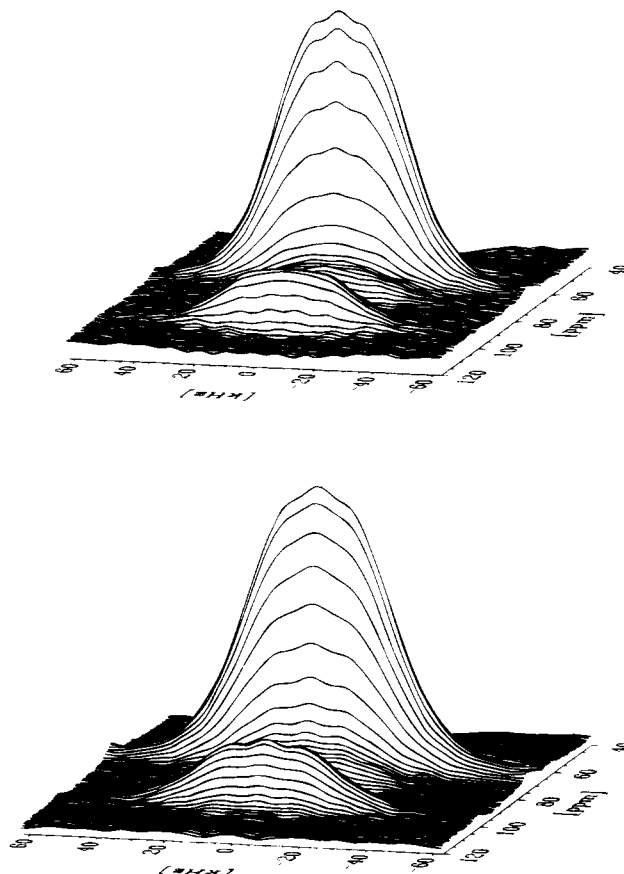
Figure 3. WISE-NMR spectra of starch measured without mixing time ($t_m = 0$). The resolution along the ^{13}C frequency is clearly visible. For every carbon position the corresponding proton line is seen. (Upper plot) A-starch; (lower plot) B-starch. Scale is in kilohertz in the proton dimension and in parts per million in the carbon-13 dimension.

seen, the structural information obtained with one-dimensional (1D) NMR spectroscopy is limited.

Molecular Motion in Starch. Figure 3 shows 2D WISE spectra of starch in forms A and B measured without the mixing time. For every distinct carbon
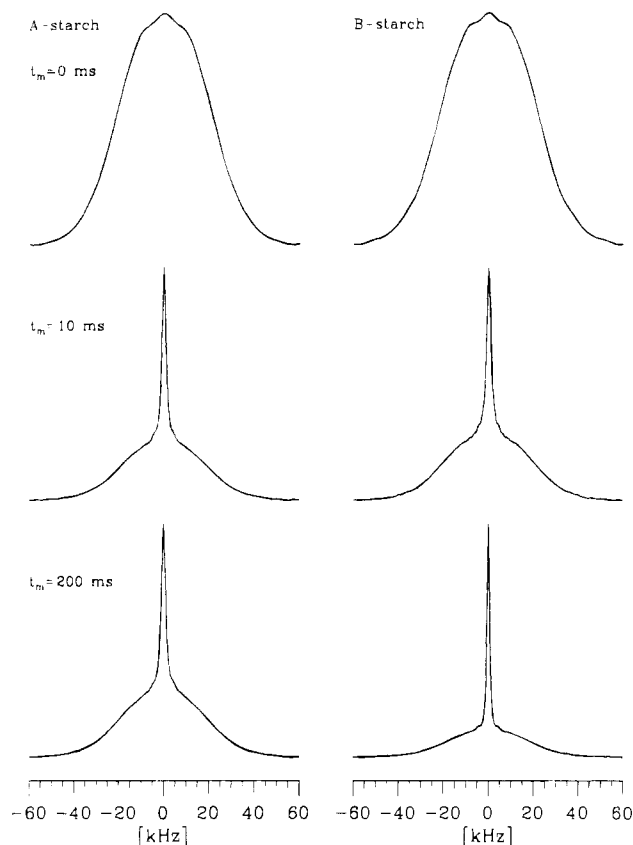
Figure 4. Projections on the ^{1}H dimension of the spectra for various mixing times: (left) A-starch; (right) B-starch.

position a proton line is obtained. All proton lines have the same shape; they are wide and structureless. For convenience, projections on the ^{1}H axis of the spectra have been collected in the top line of Figure 4. The hwhm of the lines is 25 kHz, which is larger by about 2 orders of magnitude than for lines measured in a ^{1}H one-pulse experiment (see Figure 2). Such widelines are typical for a rigid solid in the WISE experiment. The proton spectra are obtained indirectly by monitoring the ^{13}C signal (Schmidt-Rohr et al., 1992; Tekely et al., 1989; Zumbulyadis, 1986). The proton magnetization is transferred to the carbons only during the CP. In the short CP time of 500 μs carbons are polarized by protons in the polysaccharide chain and only partially by protons in water bound via hydrogen bonds. It is clear that ^{1}H lines in the 2D spectra are much broader than in 1D proton spectra corresponding to the mobile water (Figure 2). This indicates that the protons in mobile water are not in close proximity (subnanometer scale) to the ^{13}C nuclei detected in the ^{13}C spectrum. More precisely, the WISE experiment clearly demonstrates that there exist proton reservoirs of mobile water which are not coupled with ^{13}C in the polysaccharide chains through dipolar interactions.

The shape of the ^{1}H line reflects the nature of the multispin dipolar interaction between the protons. It should be noted that it is virtually impossible to calculate the proton line shape, as this results from a specific motional process for the multispin dipolar interaction. [1]H wideline spectra contain only limited qualitative information about the motional processes involved. Roughly, molecular motion on the time scale slower than $\tau_c \approx \Delta\omega^{-1} = (2\pi\Delta\nu)^{-1} = 3$ μs has no effect on proton lines. Molecular motion is then too slow to average the homonuclear dipolar coupling. From the

WISE spectra it can be concluded that molecular motion of polysaccharide chains on a time scale faster than microseconds is absent. It will be interesting to characterize in detail the slow dynamics of the polysaccharide chains in starch. Work along these lines is in progress.

WISE spectra reveal the absence of an effect of motional averaging on the C-6 proton line (primary alcoholic side chain) (Figure 3). This is in contradiction with the results reported by Froneman and Reynhardt (1991). Their proton spin—lattice relaxation $(T_{1\rho})$ studies of lyophilized amorphous starches revealed that C-6 performs fast anisotropic reorientation. This process of relaxation should be ascribed to the residual water and impurities and not to molecular motion (vide supra).

We note that starch samples measured at a moisture ratio of 12% and at ambient temperature are well below the glass transition temperature (T_g) and the melting point (Zeleznak and Hoseney, 1987). Thus, an amorphous part is in the glassy state, in which the chains are essentially fixed in the structure. In this state the diffusional motion and structural changes are highly restricted and slow in contrast to the relatively fast relaxation of the vibrational and librational degrees of freedom (Jäckle, 1986). At T_g structural relaxation times are on the order of minutes (Götze and Sjögren, 1992). In contrast to the main-chain dynamics, localized motions like those of the side groups do not stop at T_g but persist in the glassy state (McCrum et al., 1967). In starch the side group is formed by a CH_2OH group, and the position of this carbon has a characteristic position in the ^{13}C spectrum (peak C-6 at 62 ppm, see Figure 2). However, the proton line measured in the WISE experiment at 62 ppm (^{13}C dimension) exhibits a broad line typical for a rigid solid, indicating the absence of molecular motions in the tens of kilohertz regime (vide infra). This result shows that structural constraints of the polysaccharide chains in the amorphous part are high. Presumably this leads to a coalescence of the glass transition and the melting temperature in the DSC curves (Biliaderis et al., 1986).

Water Organization in Form A. Information about the proximity of water in native starch can be obtained by introduction of a mixing time into the WISE experiment. Figure 5 displays spectra of starch in form A for two mixing times, $t_m = 10$ and 200 ms, respectively. Pronounced changes are observed with respect to the spectra in Figure 3. The proton lines for each carbon site exhibit the same shape, and the spectra at both mixing times do not differ significantly. Every ^{1}H line clearly consists of two components with 1:14 intensity ratio and line widths of 0.86 and 25 kHz, respectively. Even after 200 ms, the proton lines measured in the WISE experiment differ significantly from [1]H [1]D spectra.

The fact that the proton lines at all carbon positions are the same proves contact between the water and the polysaccharide chains on a nanometer scale. As mentioned previously, it is possible to estimate the distance between water and the polymer matrix from the rate of spin diffusion. An attempt was made to estimate the distance over which the magnetization can be transported during 10 ms. It was assumed that molecular motion of bound protons is too slow to average the dipolar interaction. A mean absolute displacement $\langle |x| \rangle$ during time t_m is given by (Crank, 1975; Schmidt-Rohr et al., 1992)

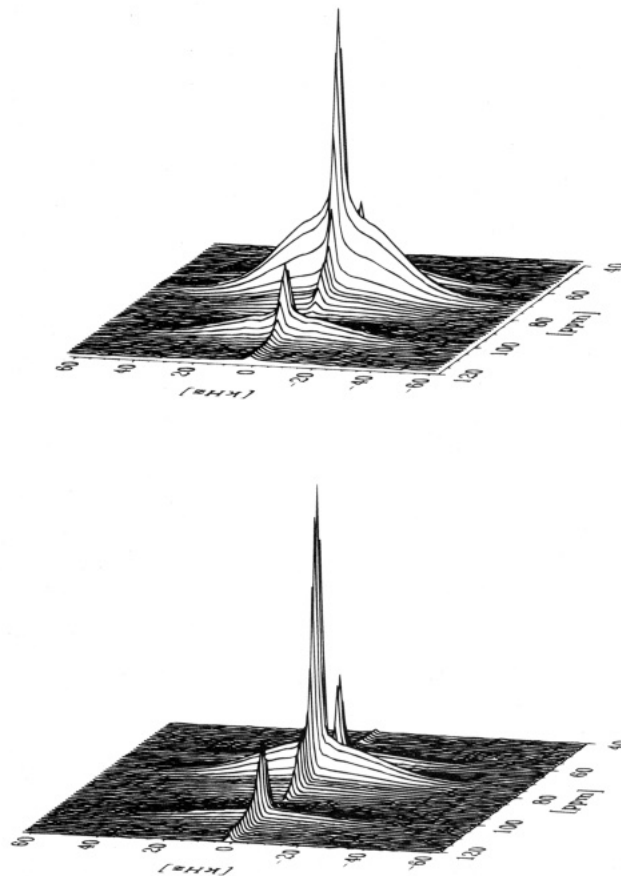**2806** *J. Agric. Food Chem.*, Vol. 42, No. 12, 1994

Kulik et al.
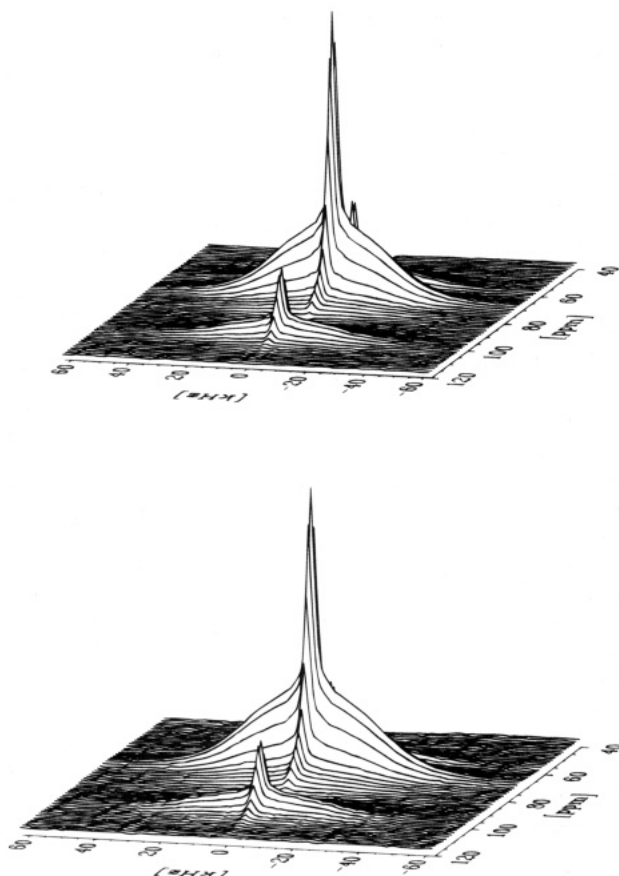
Figure 5. WISE-NMR spectra of A-starch for mixing times of 10 and 200 ms: (upper plot) t_m = 10 ms; (lower plot) t_m = 200 ms.

$$\langle |x| \rangle = \sqrt{D t_m \pi/4} \qquad (1)$$

Taking a proton spin diffusion coefficient D = 0.5 nm²/ms, in line with values found for synthetic polymers (Clauss et al., 1993), the estimated distance is 2 nm. This result is in accordance with data obtained by X-ray crystallography (Imberty and Perez, 1988; Imberty et al., 1988). It roughly corresponds to the dimensions of a unit cell (~1 nm). Thus, within 10 ms water magnetization can reach all carbons in the unit cell. Another important observation is that all proton lines change in the same way. The detection of narrow proton lines of mobile water connected with all ¹³C resonances in the ¹³C spectrum indicates that there is no preference for mobile water to bind to particular sites of the glucose ring. In other words, mobile water is in close proximity to all sites of the polysaccharide chains. The spectra obtained for t_m = 10 and 200 ms are highly similar. The fact that the proton line shapes do not change as a function of the mixing time suggests that the spin diffusion rate in A-starch is restricted. The spin diffusion rate depends on the strength of the dipolar coupling. The dipolar coupling is averaged by molecular motion. Fast translational motion in water would average dipolar interaction and reduce the spin diffusion rate. Thus, it can be inferred from the spectra that polysaccharide chains are surrounded by highly mobile water which is in close proximity to the chains. This reduces the rate of spin diffusion, making the process of averaging proton lines extremely slow.

Another important observation is that proton lines exhibit hwhm of 0.86 and 25 kHz, in full agreement with the hwhm of the lines for water and starch, respectively.

Figure 6. WISE-NMR spectra of B-starch for mixing times of 10 and 200 ms: (upper plot) t_m = 10 ms; (lower plot) t_m = 200 ms.

The experiment with short t_m shows contact only between the starch and the water bound via hydrogen bridges. Thus, the WISE experiment is a convenient way to measure the ratio of bound/free water. At moisture level of 12% the ratio of protons water/starch is about 1:4. The WISE experiment at short t_m = 10 ms reveals the ratio of the two components is 1:14. Consequently, about 28% of the water appears to be bound to the polysaccharide chains via hydrogen bonds in form A.

Water Organization in Form B. Figure 6 displays spectra of B-starch for the same mixing times as measured for form A, namely, 10 and 200 ms. Proton lines for each carbon exhibit the same shape in the spectra for each mixing time. However, in contrast to the spectra for A-starch, the ¹H line shapes at 10 and 200 ms are different. From visual inspection of the spectrum recorded at t_m = 10 ms, it appears that proton widelines have a similar shape as those for form A. They can be described as being composed of two components with 1:14 intensity ratio and line widths of 0.86 and 25 kHz, respectively. This is in excellent agreement with the line shapes observed for form A. Thus, similar to A-starch, it can be concluded that in B-starch about 28% of water is bound to the polysaccharide chain. Proton spectra measured at t_m = 200 ms differ significantly, the narrower component being much more pronounced. Lines can be described as consisting of two components with line widths of 0.5 and 25 kHz. The intensity ratio has been changed to 1:8.

From the similarity of the proton lines measured for t_m = 10 ms it is concluded that the phase separation on the nanometer scale is the same in both forms of starch.

Water Organization and Molecular Mobility in Maize Starch

J. Agric. Food Chem., Vol. 42, No. 12, 1994 **2807**

Also in both forms all carbons are coupled with water via hydrogen bonds in the same way, indicating homogeneous distribution of bound water along the polysaccharide chains. The spectra measured at $t_m = 200$ ms (Figure 6) show that the transfer of the proton magnetization from water is faster. This faster spin diffusion rate is due to lower water mobility. Thus, in the proximity of polysaccharide chains the molecular motion of water molecules in B-starch is slower than in A-starch. This results in a marked change of the proton lines in the spectrum obtained for $t_m = 200$ ms.

The presented spectra indicate that the molecular mobility of water in A-starch is higher than in B-starch. This conclusion seems to be in contrast with that derived from 1D ^{1}H spectra (vide infra). Clearly, drawing conclusions about water mobility on the basis of 1D NMR proton lines can give rise to erroneous results due to observation of all (bound and mobile) water molecules. The conclusions derived from 2D WISE are consistent with the macroscopic properties of the starch. In form A gelation and breaking down of the granular structure occur at lower temperature (347 K) than in form B (359 K) (Zobel, 1988). This process is connected with the presence of water. Apparently, the higher mobility of water molecules facilitates the gelation process and thus reduces the gelation temperature. We note that the same experiments have been performed on another starch, Ultra-Set LT (National Starch), which is also an A-starch and has low gelation temperature. The obtained spectra were the same as for form A, revealing high water mobility.

Summary and Outlook. 2D heteronuclear ^{1}H$-^{13}$C WISE experiments were employed to investigate the molecular motion of the polysaccharide chains in A- and B-starch. Introduction of the mixing time in the WISE experiment yielded information about the water mobility in both forms. The proton lines of starch exhibited the absence of molecular motion on the time scale of microseconds or faster. From the WISE spectra measured with short mixing time the amount of water bound to the polysaccharide chains was estimated to be about 28%. There is no preference for water to bind to particular sites of the glucose rings. The WISE spectra for long mixing times revealed that water organization in form A differs from that in form B. In the former, the molecular mobility of water is higher. This explains the lower gelation temperature of A-starch. WISE-NMR appears to be a convenient way to probe the water environment and molecular mobility in biopolymer matrices. The WISE experiment including data processing and data analysis is easy to implement and yields much more information than conventional 1D experiments.

LITERATURE CITED

Abragam, A. *The Principles of Nuclear Magnetism*; Clarendon: Oxford, U.K., 1961.

Biliaderis, C. G.; Page, C. M.; Maurice, T. J.; Juliano, B. O. Thermal Characterization of Rice Starches: A Polymeric Approach to Phase Transitions of Granular Starch. *J. Agric. Food Chem.* **1986**, *34*, 6−14.

Bloembergen, N. On the Interaction of Nuclear Spins. *Physica* **1949**, *15*, 386−426.

Clauss, J.; Schmidt-Rohr, K.; Spiess, H. W. Determination of Domain Sizes in Polymers. *Acta Polym.* **1993**, *44*, 1−17.

Crank, J. *The Mathematics of Diffusion*; Clarendon: Oxford, U.K., 1975.

Ernst, R. R.; Bodenhausen, G.; Wokaun, A. *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*; Clarendon: Oxford, U.K., 1987.

Froneman, S.; Reynhardt, E. C. An NMR Investigation of Some Amorphous Polysaccharides. *J. Phys. D: Appl. Phys.* **1991**, *24*, 387−391.

Gidley, M. J.; Bociek, S. M. Molecular Organization in Starches: A ^{13}C CP/MAS NMR Study. *J. Am. Chem. Soc.* **1985**, *107*, 7040−7044.

Götze, W.; Sjögren, L. Relaxation Processes in Supercooled Liquids. *Rep. Prog. Phys.* **1992**, *55*, 241−376.

Guillot, A.; Mercier, C. Starch. In *Polysaccharides*; Aspinall, G. O., Ed.; Academic Press: Orlando, FL, 1985; Vol. 3, pp 209−282.

Hartmann, S. R.; Hahn, E. L. Nuclear Double Resonance in the Rotating Frame. *Phys. Rev.* **1962**, *128*, 2024−2053.

Imberty, A.; Perez, S. A Revisit to the Three-Dimensional Structure of B-Type Starch. *Biopolymers* **1988**, *27*, 1205−1221.

Imberty, A.; Chanzy, H.; Perez, S.; Buleon, A.; Tran, V. The Double-Helical Nature of the Crystalline Part of A-Starch. *J. Mol. Biol.* **1988**, *201*, 365−378.

Jäckle, J. Models of the Glass Transition. *Rep. Prog. Phys.* **1986**, *49*, 171−231.

McCrum, N. G.; Read, B. E.; Williams, G. *Anelastic and Dielectric Effects In Polymeric Solids*; Wiley: London, 1967.

Mehring, M. *High Resolution NMR in Solids*; Springer: Berlin, 1983.

Pines, A.; Gibby, M. G.; Waugh, J. S. Proton-Enchanced NMR of Dilute Spins in Solids. *J. Chem. Phys.* **1973**, *59*, 569−590.

Schmidt-Rohr, K.; Clauss, J.; Spiess, H. W. Correlation of Structure, Mobility and Morphological Information in Heterogeneous Polymer Materials by Two-Dimensional Wideline-Separation NMR Spectroscopy. *Macromolecules* **1992**, *25*, 3272−3277.

Schmidt-Rohr, K.; Spiess, H. W. *Multidimensional NMR and Polymers*; Academic Press: New York, 1994.

Tekely, P.; Canet, D.; Delpuech, J. J. Observation of ^{1}H Nuclei in Heterogeneous Solids via Cross-Polarization ^{13}C NMR. *Mol. Phys.* **1989**, *67*, 81−96.

Tekely, P.; Palmas, P.; Mutzenhardt, P. Elimination of Heteronuclear Dipolar Interaction Effects from ^{13}C-Detected Proton Spectra in Wideline-Separation Nuclear Magnetic Resonance Spectroscopy. *Macromolecules* **1993**, *26*, 7363−7365.

Wu, J. Y.; Bryant, R. G.; Eads, T. M. Detection of Solidlike Components in Starch Using Cross-Relaxation and Fourier Transform Wideline ^{1}H NMR Methods. *J. Agric. Food Chem.* **1992**, *40*, 449−455.

Zeleznak, K. J.; Hoseney, R. C. The Glass Transition in Starch. *Cereal Chem.* **1987**, *64*, 121−124.

Zobel, H. F. Molecules to Granules: A Comprehensive Starch Review. *Starch* **1988**, *40*, 44−50.

Zumbulyadis, N. ^{1}H$-^{29}$Si Nuclear Spin Correlations in α-Si: H: A Two-Dimensional NMR Study. *Phys. Rev. B* **1986**, *33*, 6495−6496.