Molecular Characterization around a Glassy Transition of Starch Using ^{1}H Cross-Relaxation Nuclear Magnetic Resonance

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The aim of this work was to characterize the glassy–rubbery transition in starch gels using molecular (NMR) techniques. Proton cross-relaxation (^{1}H CR) NMR spectra of gelatinized starch (~50% mc) were obtained by cooling stepwise from 20 to −30 °C. A significant line broadening was observed in the CR spectra between 0 and −10 °C. Deconvolution of the spectra into its component curves (broad and narrow) yielded a peak amplitude, width at half-height, and peak area for each curve. Between 0 and −10 °C (temperatures around T_g), a significant line width change in the broad component (rigid solid) was apparent. These observed qualitative changes may be evidence of a glassy–rubbery transition at a molecular (short-range) level which are strengthened by a similar transition temperature range found previously with ^{13}C CP-MAS and DMA tan $\delta(T)$ measurements. However, the increase in the relative quantity of rigid protons observed by ^{1}H CR NMR spectra could also be attributed to ice. The ^{1}H CR NMR method showed its potential application for probing solid components in gels using a simple and economical NMR spectrometer, without the need for a solid-state instrument.

Keywords: *NMR; proton cross-relaxation; starch gels; glass transition*

INTRODUCTION

Characterization of thermal transitions occurring in starch gels containing phase separated "freezable" water has posed a serious challenge to food scientists. For example, typical differential scanning calorimetry (DSC) thermograms of such gels show a main transition occurring at ~0 °C due to ice melting accompanied by a small baseline shift. The baseline shift has been suggested by many to be due to the glass transition of the maximally freeze concentrated matrix or T_g' (Slade and Levine, 1988; Roos and Karel, 1993; Simatos and Blond, 1993; Ablett et al., 1992, 1993). This has been debated, and the interpretation remains inconclusive (Hatley et al., 1991; Reid et al., 1993). Various errors could affect the conclusion, such as endotherm area measurement errors or failure to account for the temperature dependence of the enthalpy and kinetics parameters. Data have been published to show T_g' in simple (small molecules) model systems, but more data are needed for complex polymers such as starch.

Nuclear magnetic resonance (NMR) provides alternative approaches for studying molecular motions. ^{13}C CP-MAS NMR can be used to analyze the starch carbon-chain motion in the crystalline structures of starch as affected by hydration (Blanshard et al., 1990). It can also be used to study motion in the amorphous component of starch (Morgan et al., 1992; Kalichevsky et al., 1992).

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Cross-relaxation (CR) NMR spectroscopy is a high-resolution ^{1}H method for determining the information on the solid component relaxation via the observable liquid spin system (Grad and Bryant, 1990). A sample is irradiated with a radio frequency pulse which is off-resonance from the liquid signal. Due to the dipolar coupling between the liquid and solid protons, the amplitude of the liquid spectrum will change with the offset frequency and a *Z*-spectra is obtained (Grad and Bryant, 1990; Wu et al., 1992). Wu and Eads (1993) found an increase in the *Z*-spectral line shape, area, and width for waxy starch gels during aging that was dependent on concentration and storage (Wu et al., 1992).

These NMR methods are important tools in describing molecular motions of starch. ^{1}H CR NMR offers a means to investigate solid protons based on the information one obtains from a relatively inexpensive and more widely available NMR spectrometer used for liquid samples. However, preliminary investigation must be done to understand how to characterize the contributions of various solid protons. This important first step is critical to an assignment of phase transitions. The objective of this study was to characterize the molecular transition of starch gels by ^{1}H CR NMR.

MATERIALS AND METHODS

Cross-Relaxation ^{1}H Nuclear Magnetic Resonance. Wheat starch (Gemstar 100, Manildra Milling Corp., Minneapolis, MN) was weighed and placed inside a 1 mm i.d. capillary, and appropriate amounts of water were added to make a 40% starch suspension (60% moisture). The samples were mixed with a thin wire inside the capillary and gelatinized at 90 °C for 5 min. The capillary was then inserted into a Teflon spacer and placed inside a 5 mm i.d. NMR tube. NMR spectra were obtained as described in Wu et al. (1992). A 200 MHz spectrometer (Bruker Instruments, Billerica, MA)

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A

Pulse sequence

preparation pulse (v₁)

predelay
15 sec

decouple

90°/1 sec (v₀)

observe

↓

vary v₁

↓

obtain peak intensity

B

decouple
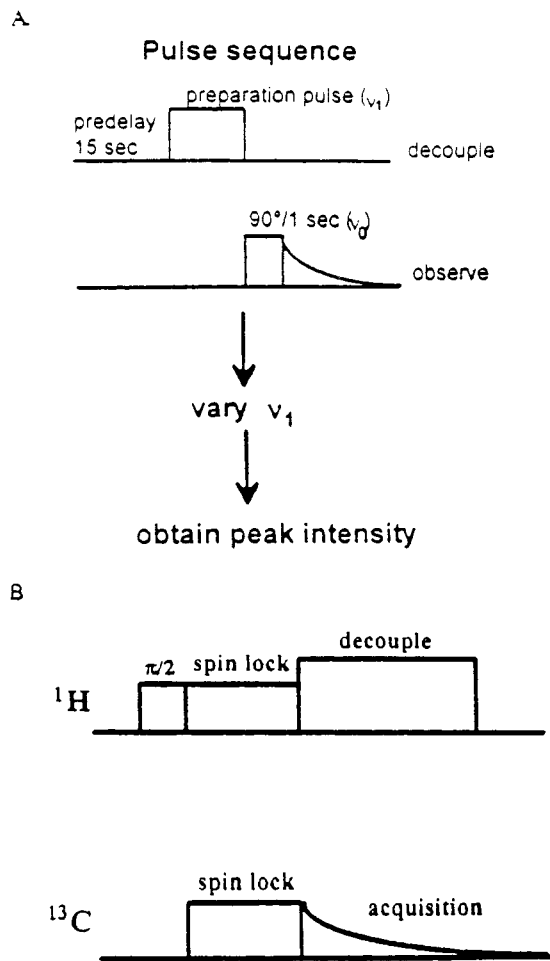
π/2 spin lock

¹H

spin lock

acquisition

¹³C

Figure 1. (A) Pulse sequence for the cross-relaxation ¹H NMR experiment followed by procedure to obtain solid spectra. (B) Pulse sequence for ¹³C CP-MAS experiment.

was utilized to acquire the data. The following parameters were used: decoupling power was adjusted between samples to ensure full saturation of the liquid peak; preparation pulse ranging between 0.3 and 3 s; recycle delay, 15 s; ¹H 90° pulse width, 4.3 μs; number of scans, 4; and acquisition time, 0.3 s. The pulse sequence is schematically represented in Figure 1A. The frequency offset from the water resonance was varied from −50 to 50 kHz. The offset frequencies were calculated from the difference between the frequency of the off-resonance preparation pulses used to irradiate the solid component and the frequency of the on-resonance 90° pulse of the liquid component.

For the variable-temperature experiments, a Bruker variable-temperature controller fitted with liquid nitrogen and nitrogen as the carrier gas was utilized. The power necessary for saturation at the various temperatures was calibrated prior to data acquisition. The desired temperature (20 °C to −30 °C) was reached within 5 min and held there for at least 15 min prior to analysis. The experiment time was ~30 min.

All cross-relaxation solid spectra were decomposed into either Lorentzian and Gaussian (at ≥0 °C) or Lorentzian and double-cumulative (at <0° C) curves with Peakfit (Jandel Scientific, San Rafael, CA). Although the cumulative curve has no physical meaning (as compared to the Gaussian and Lorentzian curves), the overall fit was excellent, and the parameters obtained from the fit helped discern qualitative trends in the overall *Z*-spectrum. The equations describing these curves are presented in Table 1. The parameters obtained from these curves (peak temperature, peak amplitude, width at half-height, and area) were monitored at different temperatures.

¹³C Cross Polarization Magic Angle Spinning (CP-MAS) Measurements. A gelatinized wheat starch (Gemstar

Table 1. Equations of Curves Used for Decomposition of ¹H Cross-Relaxation NMR Spectra[a]

curve	equation
Lorentzian	$y = \dfrac{a}{1 + \left(\dfrac{x-b}{c}\right)^2}$
Gaussian	$y = a \exp\left[-0.5\left(\dfrac{x-b}{c}\right)^2\right]$
symmetric double cumulative	$y = 0.5a\left[1 + \mathrm{erf}\left(\dfrac{x-b+\frac{c}{2}}{\sqrt{2}d}\right)\right]\left[1 - \mathrm{erf}\left(\dfrac{x-b-\frac{c}{2}}{\sqrt{2}d}\right)\right]$

[a] *a*, *b*, and *c* are constants.

100, Manildra Milling Corp.) sample was prepared by mixing equal parts of starch and water and heating the solution to 90 °C between two sheets of aluminum in a hot press (F. S. Carver, Inc., Summit, NJ). This method yields a 47% moisture sample. The starch gel was packed inside a NMR rotor with Macor end caps for analysis.

A 200 MHz spectrometer (Bruker Instruments) equipped with an IBM solids unit was used to acquire the data. Cross-polarization (CP) and magic angle spinning (MAS) were applied. Two hundred scans were obtained using the following parameters: spinning rate of 4 kHz; 90° proton pulse width of 5 ms; carbon pulse width of 2 ms; a recycle delay of 3 s; contact time of 2 ms; spin lock time ranging from 0.5 to 30 ms; and a decoupling field of 50 MHz. The FID was Fourier transformed using a line broadening of 50. A Bruker variable-temperature controller was used to adjust the sample temperature. The desired temperature (20 to −50 °C) was reached within 5 min and held there for at least 15 min prior to analysis.

RESULTS AND DISCUSSION

Samples Containing "Freezable" Water. ¹H CR NMR. Figure 2A shows typical results of a cross-relaxation ¹H NMR experiment in which the liquid signal (single resonance) was observed at various offset frequencies. At sufficiently large offset frequencies, solid protons were not saturated and no cross-relaxation occurs between the solid and liquid protons after a 90° pulse. As the frequency offset difference decreased (small offset frequencies, Figure 2A), the excited solid protons transfer magnetization to liquid protons, resulting in an NMR liquid signal decrease due to the cross-relaxation between the spins of restricted mobility and those of the mobile bulk-water pool (Tessier et al., 1995). The amplitude of the liquid spectrum (water resonance saturation) as a function of offset frequency (Figure 2B) thus contains information of the internal mobility of the solid components in the sample (Wu et al., 1992). An inverted plot of the same data (the *y*-axis being $1 - M^z/M^{z0}$) would closely resemble a solids ¹H spectrum (data not shown).

Figure 3 shows the effect of temperature on the ¹H cross-relaxation (CR) spectrum of a freshly gelatinized starch sample (60% moisture). Spectra at various temperatures were obtained in an effort to identify the phase transition region observed in earlier dynamic mechanical analysis (DMA) experiments (Vodovotz and Chinachoti, 1996). As the sample was cooled (stepwise) from 20 to 0 °C, little variation in the shape was noted in the CR spectra, except for a slight broadening. A dramatic change in the curve shape was observed, between 0 and −10 °C, as a result of the smaller amplitude of the liquid spectrum at smaller frequency offsets (Figure 3). Below −10 °C, the cross-relaxation spectrum broadened further.
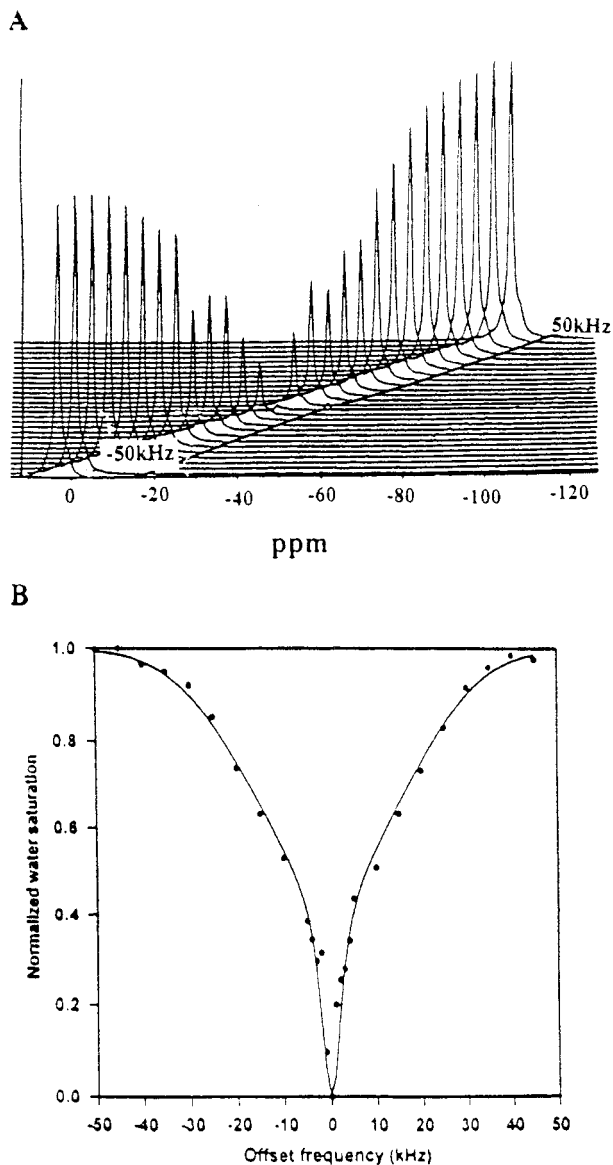
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A

B

Figure 2. (A) Typical ¹H NMR spectra of freshly gelatinized starch at different offset frequencies ranging from −50 to 50 kHz. (B) Cross-relaxation spectra constructed from water resonance saturation as a function of offset frequency for a 60% moisture starch gel.

As described by Grad and Bryant (1990), using a two spin−pool model, the Bloch equations [see eqs 1a−1f in Grad and Bryant (1990)] are formulated with the following parameters: longitudinal relaxation rates of the solid and liquid components, the cross-relaxation rate, the spin−spin relaxation time of the solid component, the ratio of the number of solid to liquid spins (f), and the amplitude of the radio frequency field of the preparation pulse. However, as Henkelman et al. (1993) pointed out, the Bloch equations have no solutions if a line shape other than Lorentzian is used because a single T_2 rate cannot give rise to anything other than a Lorentzian line shape. In view of this limitation, Henkelman et al. (1993) proposed a least-squares solution to the line shape in which they include the contribution of Gaussian and Lorentzian functions. In their derivation of the equation, they normalized the total number of proton spins in the liquid pool, whereas Grad and Bryant (1990) utilized the f value. The results from the fitted model yielded five parameters: R_b (rate of the

solid proton pool longitudinal magnetization recovery), T_{2b} (transverse relaxation time of the solid proton pool), R (fundamental rate constant that characterizes the exchange rate between the liquid and solid proton pools), RM_0^b/R_a (ratio of pseudo-first-order rate constants of the solid proton pool to the rate of liquid proton pool recovery from the longitudinal magnetization), and $1/R_a T_{2a}$ (the inverse of the rate of liquid proton pool recovery from the longitudinal magnetization times the transverse relaxation time of the liquid proton pool) (Henkelman et al., 1993).

Various researchers have found that the two spin−pool model did not adequately represent their data (Adler et al., 1996; Tessier et al., 1995; Tzou et al., 1997). Three spin−pool models (water, mobile macromolecules, and fixed macromolecules) have been used successfully by Tessier et al. (1995) to describe the cross-relaxation spectra of biological tissues at room temperature. Tzou et al. (1997) worked on samples containing ice and used a three spin−pool model to describe the system. In this case the three spin-pool model consisted of immobile protons, "freezable" water protons, and "unfreezable" water protons. Cross-relaxation between liquid water and ice has been reported previously (Turner et al., 1991).

Wu and Eads (1993) used the equation derived by Grad and Bryant (1990) to fit the cross-relaxation spectra of starch gels with a combination of Gaussian and Lorentzian curves. Changes in the parameters of these decomposed curves (such as area and line width) were attributed to certain factors in the original cross-relaxation equation. A similar approach was used in this work. The cross-relaxation spectra fitted with a single Lorentzian curve were of unacceptable quality (data not shown) so they were decomposed into Gaussian (broad) and Lorentzian (narrow) curves for temperatures ranging from 0 to 20 °C (Figure 4A) and into double-cumulative (broad) and Lorentzian (narrow) curves for temperatures between −10 and −30 °C (Figure 4B). A cumulative curve was used below 0 °C instead of a Gaussian curve because a much better fit was obtained with the former rather than the latter. The Lorentzian curves (L) represent the more mobile solid protons and are narrower, whereas the Gaussian and double-cumulative curves (G/C) are due to the more rigid solid protons and are broader (Wu and Eads, 1993).

Each of the decomposed curves was further analyzed to obtain peak height or amplitude, width at half-height, and peak area (Wu and Eads, 1993). Figure 5 shows these different parameters for the decomposed curves as a function of temperature. The normalized peak amplitude refers to the relative *amount* of narrower and broader components. The width at half-height is interpreted as an index of *rigidity*. Thus, at ambient temperature (Figure 5A,B), the starch gel consisted of 68% mobile solid protons (with a line width of ∼6 kHz) and ∼30% rigid solid protons (with a line width of ∼27 kHz). At 0 °C, the fraction of narrow component decreased to 50−55% and that of the broad component increased to ∼40% (Figure 5A) with little change in line width in either fraction (Figure 5B). The most significant change occurred between 0 and −10 °C, where the broad component increased in relative intensity to ∼70% at the expense of the narrower component intensity, which decreased to ∼22% (Figure 5A). Broadening of the G/C component was observed (from 30 to ∼50 kHz), whereas no significant change in line width of the narrow

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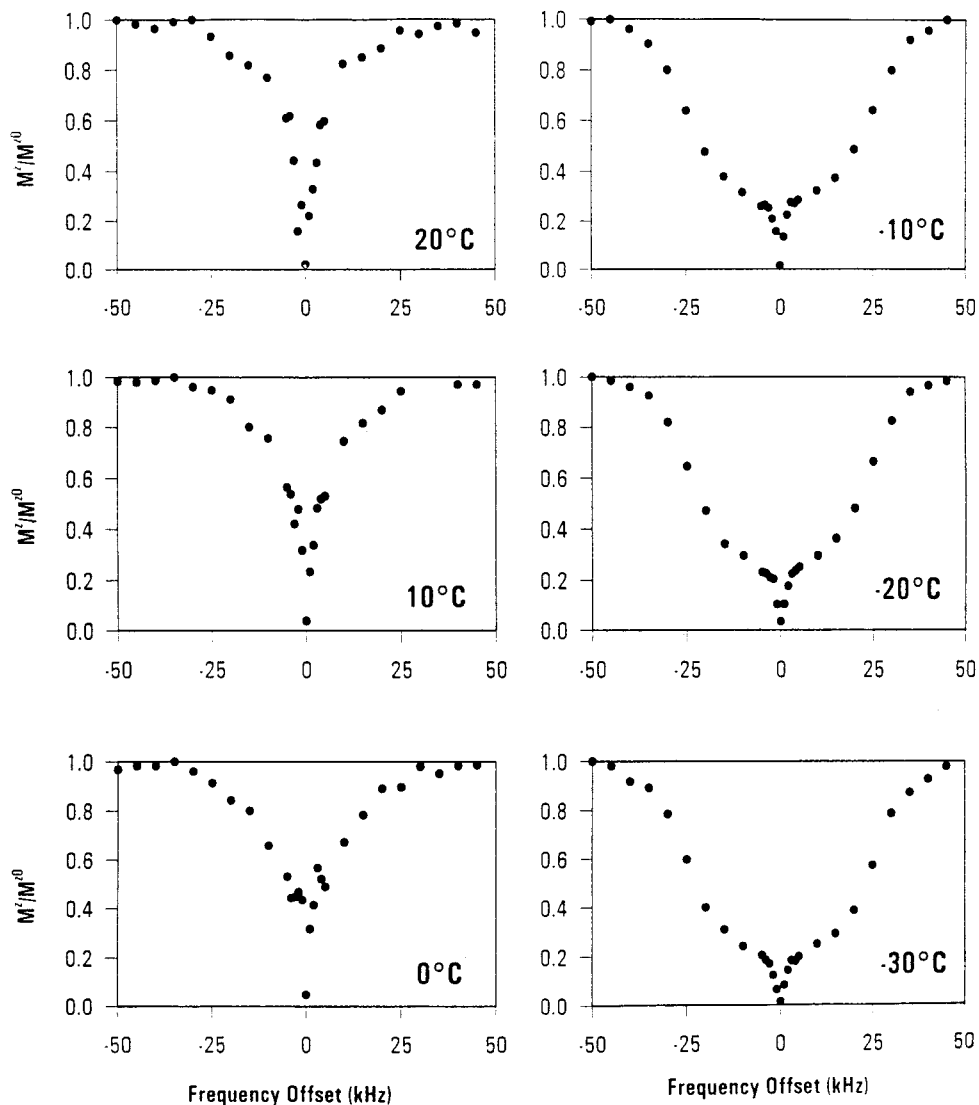*J. Agric. Food Chem.,* Vol. 48, No. 10, 2000 **4951**

Figure 3. Cross-relaxation ^{1}H NMR spectra of a 60% moisture starch gel at various temperatures.

component was evident (Figure 5B). Decreasing the temperature further (< -10 °C) resulted in little change in the intensity or line width of either of the components.

These results indicate that the main molecular transition of the starch sample occurred between 0 and -10 °C, leading to broadening and rigidification of the solid spectra. In addition, the broad component shape was also transformed from a Gaussian to one which could be fit by a double-cumulative function in this temperature range, suggesting some potential change in the ice and/or glassy starch fraction (Vodovotz and Chinachoti, 1996), namely ice melting and/or a glassy–rubbery transition of starch.

The relative intensity of the water ^{1}H NMR signal not influenced by the solid (at a sufficiently large offset frequency) in the starch gel was monitored at decreasing temperatures (Figure 6). Ice formation was expected between 0 and -10 °C and is depicted as a drop in liquid ^{1}H NMR intensity (Figure 6). The drop of almost two-thirds of the signal area indicated a decrease in the amount of liquid ^{1}H detected due to the formation of ice (solid proton signals became too broad to be detected by solution NMR). Similar results were found by Tzou et al. (1997) and indicated a drop in the amount of "freezable" water in the sample.

The resulting protons of the ice may have played a significant role in the relaxation process with the liquid water protons (Tzou et al., 1997). These authors used a formulation proposed by Yeung et al. (1994), based on modified Bloch equations (to account for the saturation of a macromolecular component with a non-Lorentzian line shape) and utilizing the concept of spin temperature to fit their data. The equation used was

$$W_A(\infty) = (1/2\alpha_{1sat})\{[\alpha_{AB}\pi g^G_B(\delta)]/$$
$$[\alpha_B + \alpha_{AB}/f_{AB} + \pi g^G_B(\delta)] + \pi g^L_A(\delta)\} \quad (1)$$

where A and B denote the spin baths of the mobile water protons and immobile protons, respectively, and $W_A \equiv (M^o_{ZA} - M_{ZA})/2M^o_{ZA}$, M^o_{ZA} = equilibrium longitudinal magnetization, M_{ZA} = longitudinal magnetization,

$$\alpha_{1sat} = \alpha_A + \alpha_B - \{(\alpha^2_{AB}/f_{AB})/$$
$$[\alpha_B + \alpha_{AB}/f_{AB} + \pi g^G_B(\delta)]\} + \pi g^L_A(\delta) \quad (2)$$

$$\alpha_x = r_x/\omega_2 \quad \text{with } x = \text{A, B, and AB} \quad (3)$$

r_B and r_A = intrinsic relaxation rates of A and B, r_{AB} = cross-relaxation rate, ω_2 = amplitude of the saturating

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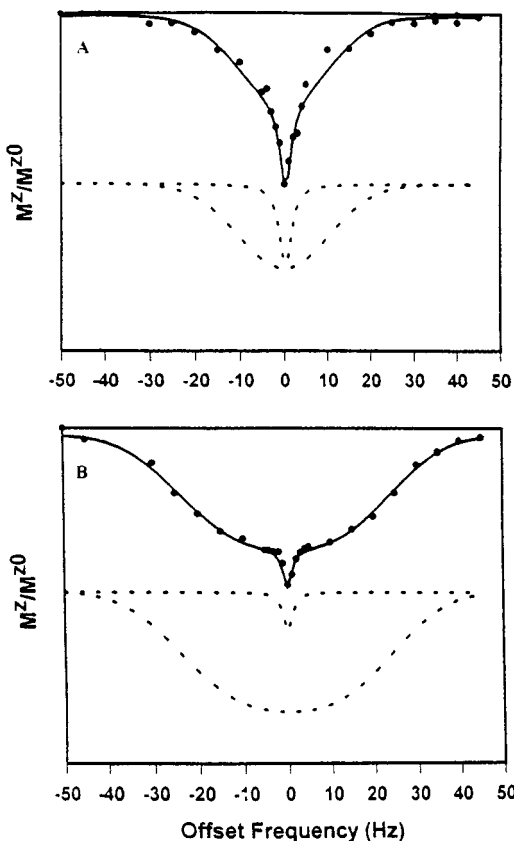
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Figure 4. Cross-relaxation ^{1}H NMR decomposition results for a 60% moisture starch gel. At temperatures >0 °C (A), the measured values (symbols) were best fitted (solid line) with Lorentzian and Gaussian functions (dotted lines). In contrast, at temperatures <0 °C (B), the measured values (symbols) were best fitted (solid line) with Lorentzian and cumulative functions (dotted lines).

radio frequency field expressed in angular frequency units, f = molar ratio of the B-spin moiety to the A-spins, and $g^G_B(\delta)$ and $g^L_A(\delta)$ = line shape functions of the corresponding spin components with the superscript L denoting a Lorentzian and G a Gaussian form.

It is important to note that Tzou et al. (1997) fit the above model to their data and obtained a quantitative data for a set of parameters (r_{1A}, r_{1B}, r_{AB}, T_{2A}, T_{2B}, f), whereas Wu and Eads (1993) fit their data with a combination of Lorentzian and Gaussian curves (through a least-squares fitting) and qualitatively described the changes in T_{1A}, T_{2B}, T_{1B}, and f.

Upon freezing, Tzou et al. (1997) found that the cross-relaxation rate increased, the f value increased (due to a greater number of less mobile spins), and the T_2 of the liquid decreased (reflected in the line shape functions), which led to an increased line width and area of the cross-relaxation spectra. Similar increases in cross-relaxation rate (Henkelman et al., 1993) and f value (Wu et al., 1992) were found for samples with increased solids concentrations at room temperature.

An increase in the solid component's area and line width resulted in a change from a Gaussian to a shape that was fit to a double-cumulative curve below 0 °C. Such increased area and line width in the order of 50–60 kHz would be due to an increase in the number of solid proton spins, which may be attributed to the formation of ice and/or changes in the starch molecules. As liquid water turns to solid ice below 0 °C, a decrease in the liquid water signal is noted (as described above),
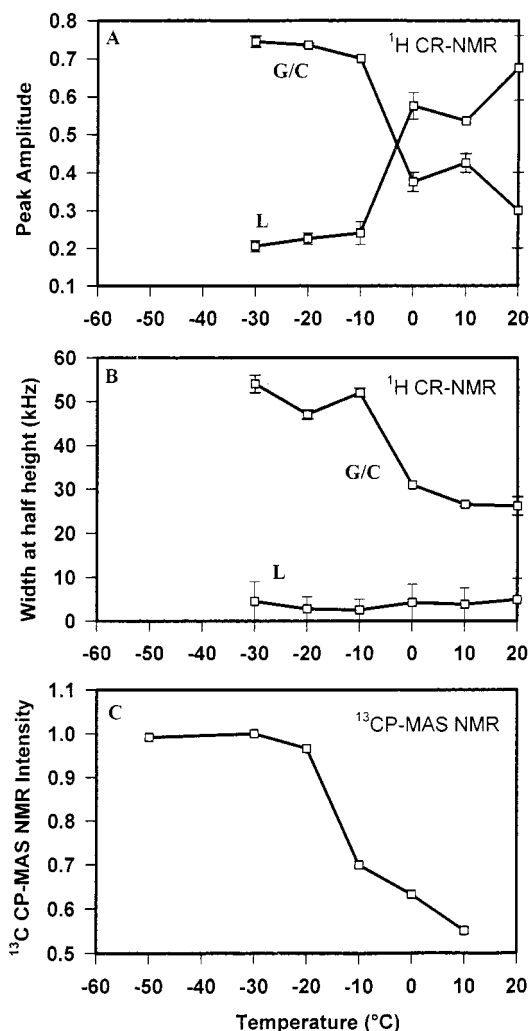

Figure 5. Liquid and solid NMR results. Peak parameters were obtained from the decomposed curves of the cross-relaxation ^{1}H NMR spectra of a 60% moisture starch gel: (A) peak amplitude; (B) peak width at half-height [L = Lorentzian curves (narrow); G/C = Gaussian or double cumulative (broad)]; (C) ^{13}C CP-MAS NMR intensity of a 47% moisture starch gel.
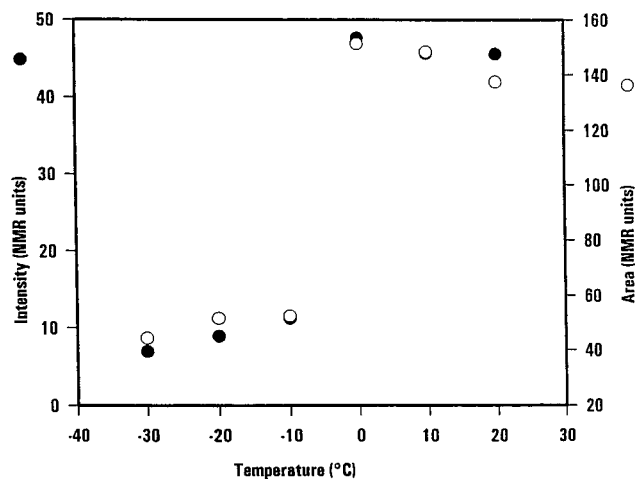

Figure 6. Intensity and area of the liquid water peak obtained from ^{1}H NMR solution measurements at large offset frequencies (−50 kHz/50 kHz) in the cross-relaxation experiment versus temperature of a 60% moisture starch gel,

and it is assumed that the f value also increased due to more solidlike protons (Tzou et al., 1997). However, in a protein system (hydrated collagen), the relaxation

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times (both T_{1B} and T_{2B}) associated with the immobile protons did not change upon freezing and therefore did reflect the contribution from ice formation. Similarly, Bryant (1978) reviewed solute−solvent interactions in solid protein systems noting that the ^{1}H NMR relaxations for water protons for both frozen lysozyme solutions (Hsi and Bryant, 1975) and powders (Hilton et al., 1977) were very similar, implying little contribution from ice. In our data, ice likely contributed to the increase in the total *f* (immobile to mobile protons), which would result in a broader line width and increased area. However, changes in the macromolecule from a rubbery to a glassy state (increased immobile component) upon cooling below 0 °C may have contributed to a dramatic change in the *shape* of the broad component observed (G/C curves).

The cross-relaxation broad component (G/C) below 0 °C resulted in a line width between 45 and 50 kHz (Figure 5B), which was in good agreement with that obtained from dry waxy corn starch (<2% moisture) using solid state ^{1}H NMR at 20 °C [∼42 kHz (Li et al., 1996)]. It can thus be interpreted that such agreement was due to the similar physical states of the starch; that is, starch from this work at <0 °C and the dry starch were both in a glassy state and, therefore, the broad component (G/C) represented the protons in the glassy (rigid) solid.

Additionally, results from carbon-13 CP-MAS NMR experiments of 47% moisture starch gels showed a sharp increase in the intensity of carbon-1 between −10 and −20 °C (Figure 5C). This increase in carbon intensity (decreased segmental mobility) with decreasing temperature was similar to that found in rubbery-to-glassy transition in synthetic polymers (Dickinson et al., 1988). Additionally, similar changes were detected in low-moisture systems analyzed between 10 and 60 °C. Carbon-1 intensity (^{13}C CP-MAS NMR) of a 17% moisture starch gel showed a gradual decrease in intensity at temperatures >20 °C, which paralleled DMA tan $\delta(T)$ data (Vodovotz, 1996). Such consistent results suggest ^{1}H CR spectral change was related to the change in carbon chain mobility, possibly due to glass transition.

As mentioned previously, aging of waxy starch at ambient temperature has been shown by ^{1}H CR NMR to result in an increased spectral area and line width likely due to recrystallized starch (Wu and Eads, 1993). Work in progress in our laboratory has confirmed these findings for wheat starch gels (manuscript in preparation). In addition, accompanying ^{13}C CP-MAS NMR analysis showed a significant increase in ^{13}C intensity with aging, implying greater rigidity in the starch chains. This indicates that ^{1}H CR NMR is sensitive to and can detect rigid starch components.

Future applications of this technique can be further enhanced if the contributions from various solids can be deconvoluted and identified. In this work, a ^{1}H CR NMR method has shown its potential to serve as an economical means to investigate solid protons in aqueous environments without the need to access an expensive solid state NMR.

Conclusions. ^{1}H CR NMR spectra of a 60% moisture starch gel showed an increase in the area of the broad component and an increase in its line width upon cooling below 0 °C. Decomposition of the CR spectra yielded a sum of Gaussian and Lorentzian curves at temperatures ≥0 °C and cumulative and Lorentzian curves at temperatures <0 °C. Increases in the amount and line width

of the broad component are attributed to ice and possible stiffness of the amorphous starch chain, which may be related to the starch fraction that underwent a rubber-to-glass transition. This ^{1}H CR NMR method showed its potential application for probing solid components in gels using a simple and economical NMR spectrometer, without the need for a solid state instrument.

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