Detection of Solidlike Components in Starch Using Cross-Relaxation and Fourier Transform Wide-Line ^{1}H NMR Methods

Junshi Y. Wu, Robert G. Bryant,† and Thomas M. Eads*

Department of Food Science and Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, Indiana 47907-1160

A new proton (^{1}H) NMR method, nuclear magnetic cross-relaxation spectroscopy, permits sensitive detection of immobilized polymer in starch samples. The ^{1}H cross-relaxation spectrum reflects the amount and relative rigidity of immobilized starch chains. Cross-relaxation integrated spectral intensities for freshly gelatinized waxy maize starch were found to depend sensitively on starch concentration above 10 wt %. Retrogradation is observed to introduce a broad component to the cross-relaxation spectrum, interpreted as an increase in fraction of immobilized starch chains. Kinetics of aging may thus be expressed in terms of the changes in amount and relative rigidity of solidlike components. The complementary wide-line Fourier transform ^{1}H NMR spectrum obtained with magic angle spinning verifies the presence of immobilized starch with a very wide ^{1}H resonance and simultaneously shows narrow starch resonances, indicating a highly mobile starch fraction. The combined evidence demonstrates a very wide distribution of starch chain segmental mobilities and that the distribution changes as gelatinized starch ages.

INTRODUCTION

Food mechanical and rheological properties often depend on the flexibility of macromolecular networks. In such networks, the architecture, the rates of making and breaking of network bonds, and the flexibility of intervening molecules or their segments are important (Morris, 1990). Thus, a goal in the molecular science of food is to characterize the structure and dynamics of coexisting structural components. Chain segments near covalent cross-links, in crystallites, or in associated chain forms are expected to be relatively immobile or even rigid. However, these regions may represent a small fraction of the total sample mass and thus may be difficult to detect.

Principal methods for measuring the structure of food biopolymers include X-ray diffraction, optical rotation, vibrational spectroscopy (Raman, infrared), and magnetic resonance spectroscopy. While X-ray and vibrational methods are powerful, they do not conveniently give information about molecular dynamics. Although changes in molecular dynamics are reflected by changes in heat capacity measured calorimetrically and rheological methods can be used to infer structural and sometimes dynamical aspects of network architecture, it is not possible with any of these, except magnetic resonance, to specify the rates and amplitudes of motions of the structural polymer species themselves.

Nuclear magnetic resonance (NMR) has been used in polymer science and biophysics to relate macromolecular structure and conformational dynamics with functional properties. The main issue in the application of NMR to multiphasic materials such as food is that it is often difficult to observe the component of interest selectively, particularly if it is solidlike. In general, polymer chain segments in solidlike domains have restricted mobility that broadens NMR lines severely. Nonetheless, methods exist for detecting solidlike components. For example, ^{1}H spin-echo decay methods have been used to follow starch swelling, gelatinization, and retrogradation (Lechert and Hennig, 1976; Lechert et al., 1980; Blanshard et al., 1990). Alternatively, the magnetization decay after a single excitation pulse or after a solid echo pulse sequence can be analyzed into fast and slow components, corresponding to solidlike and mobile components. The corresponding wide-line NMR spectrum, containing broad and narrow resonances, can be obtained by Fourier transformation. The single pulse wide-line experiment was demonstrated on starch granules by Lechert and Hennig (1976). In this paper we present the solid echo wide-line *and* high-resolution ^{1}H NMR of a starch gel. Continuous wave (CW) wide-line ^{1}H NMR methods can provide similar information. The line width of solid starch was estimated by the CW method to be >16 kHz in early work (Collison and McDonald, 1960), although spectra were not shown. The CW method is rarely used and has significant disadvantages for motionally heterogeneous samples (Andrew, 1953) when compared with pulsed time domain and Fourier transform methods.

Carbon-13 NMR spectra of starch are simple to obtain when starch mobility is high, as in fresh pastes, gels, or dissolved starch (Callaghan et al., 1983). The experiment usually consists of a single short pulse at the carbon frequency and low-power irradiation at the proton frequency to eliminate ^{1}H–^{13}C scalar coupling, i.e., to collapse ^{13}C multiplets. To obtain ^{13}C signals from *solid* domains, however, it is necessary to employ line-narrowing techniques (magic angle spinning, high-power ^{1}H decoupling), and it may be advantageous to employ cross-polarization techniques for sensitivity enhancement (^{1}H–^{13}C cross-polarization) (Hartmann and Hahn, 1962; Pines et al., 1973). The application of high-resolution liquid and solid ^{13}C techniques in starch research has been reported by several groups (Baianu and Förster, 1980; Callaghan et al., 1983; Mora-Gutierrez and Baianu, 1991; Gidley and Bociek, 1985; Dev et al., 1987). In ^{13}C NMR, the liquid methods select highly mobile components, while the conventional solid methods are solid-selective. Dynamics of components that are neither rigid nor highly flexible can also be characterized. This was demonstrated clearly for glycogen (Jackson and Bryant, 1989), a polymer closely related to amylopectin (waxy maize starch). However,

† Departments of Chemistry and Biophysics, University of Rochester, 601 Elmwood Ave, Rochester, NY 14642.

0021-8561/92/1440-0449$03.00/0

such experiments are not routine. Other techniques, perhaps more convenient and applicable for analysis and process situations, would be valuable.

Here we report application of a recently developed magnetization transfer NMR method called cross-relaxation or "Z-spectroscopy" (Wolff and Balaban, 1989; Grad and Bryant, 1990) which selectively detects solidlike domains, is very sensitive to the dynamical characteristics of the solid components over a wide range in mobility, and has the advantage that it is simple to execute on many kinds of pulsed NMR spectrometers. Instead of a direct measurement of the broad resonance spectrum, the liquid-phase ^1H spectrum containing the water resonance is observed. Due to magnetic coupling between liquid and solid, the amplitude of the liquid spectrum can be made to depend on the nuclear magnetic relaxation times T_1 and T_2 of the solid, as well as on the solid–liquid spin concentration ratio, and the magnetization-transfer rate between liquid and solid. The relaxation properties of solid domains depend sensitivity on their internal mobility. Thus, the resulting cross-relaxation spectral line shape is similar to the wide-line ^1H NMR line shape and reflects internal mobility in solidlike components in the sample.

We demonstrate the utility of the cross-relaxation method on the starch–water system, because this system is important in its own right and also affects the texture of many foods. We show that cross-relaxation spectroscopy senses the rigidity of starch in granules, polymer immobilization due to high concentration in gels, and changes in starch chain mobility accompanying the processes of gelatinization and retrogradation. The method thus complements other physical methods by describing states and processes in terms of molecular dynamics of the structural polymer itself.

MATERIALS AND METHODS

Sample Preparation. Waxy maize starch was obtained from Penford Products Co., Cedar Rapids, IA. Xanthan was obtained from Kelco Co., San Diego, CA. Although waxy maize starch contained 12% water as determined by vacuum oven dry, the starch concentrations were referred to the granule as received. For samples to be gelatinized, starch granules were weighed directly into a Wilmad 5-mm NMR tube. Deionized distilled water was then added to the tube to bring the starch concentration to the desired level (between 10 and 45 wt %); the total weight of the sample was about 0.9 g. The starch/water was mixed using a vortex mixer. The suspension was degassed at room temperature using an aspiration pump to reduce the dissolved or trapped air in the mixture. After repeated degassing and vortexing, the NMR tube was transferred into a preheated water bath (95 °C) and kept there for 15 min. Starch gelatinization and pasting occurred quickly, the sample becoming transparent to translucent in appearance, depending on starch concentration. Samples were equilibrated at room temperature for a minimum of 30 min prior to the NMR measurement. Some gelatinized samples were stored at 5 °C and then equilibrated to room temperature before NMR measurement. For NMR measurement of uncooked samples, starch granules were first suspended in 0.25 wt % xanthan solution and then transferred into the NMR tube.

NMR Measurements. All NMR spectra were obtained at room temperature (ca. 23 °C). The ^1H cross-relaxation NMR spectra were obtained on a Nicolet NT200 spectrometer, operating frequency (^1H) of 200.067 MHz, using a single frequency (^1H), single coil, high-resolution probe. The pulse sequence used is summarized in Figure 1. The preparation pulse D1 was applied using the decouple channel (ν_1) in heteronuclear mode. Unless otherwise specified, the preparation pulse was 450 ms at a radio frequency field strength of 500 Hz (proton precession frequency). The frequency offset (Δ) of the preparation pulse from the water resonance was varied from −50 to 50 kHz, usually in 5-kHz increments. The other acquisition parameters were as follows: predelay time (D5) 20 s, 90° pulse (P2) 6 μs, spectral width ±1000 Hz, data size 4K, and a single scan. Free induction decays were Fourier transformed after application of 1-Hz Lorentzian line broadening. The cross-relaxation spectrum is a plot of the normalized intensity of the water peak, M_A^Z (with preparation pulse/M_A^{Z0} (without preparation pulse), vs the frequency offset (Δ).

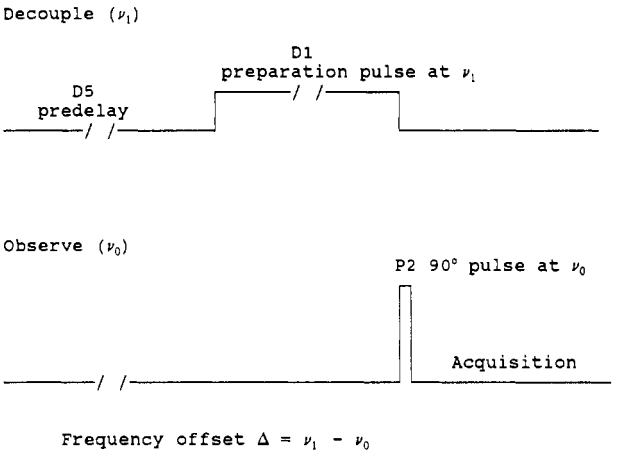

Figure 1. Pulse sequence used in cross-relaxation spectroscopy.

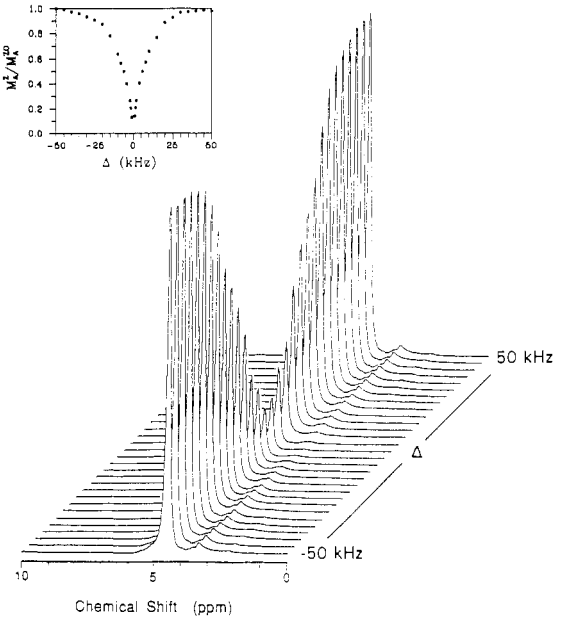

Figure 2. ^1H NMR spectra of an aged 40% waxy maize starch gel sample (room temperature, 5 days) with preparation pulse duration of 200 ms, radio frequency field strength (as precession frequency $\nu_1 = \gamma_H B_1/2\pi$) of 300 Hz applied at offset frequency Δ varying from +50 to −50 kHz. (Inset) Cross-relaxation spectrum constructed from water resonance saturation as a function of Δ.

Figure 2 displays a series of ^1H NMR spectra for 40% waxy maize starch gel aged at room temperature for 5 days with preparation pulse irradiating at offset Δ from −50 to 50 kHz. The cross-relaxation spectrum calculated from these spectra is shown in the inset.

The wide-line *and* high-resolution ^1H NMR spectrum was obtained on a Bruker MSL400 spectrometer, using a solid echo pulse sequence $(90^0_X - \tau_1 - 90^0_Y - \tau_2 - t)_n$ (Mansfield, 1965) with 90° pulse width of 1.8 μs, $\tau_1 = 5$ μs, $\tau_2 = 4$ μs, $t = 10$ s, and $n = 128$. Phase alternation consistent with quadrature detection was employed. Receiver phase was adjusted to place most of the signal in the A channel of the quadrature detector. The time domain signal digitized via a 9-bit ADC with 5-MHz bandwidth was co-added into 64K memory. The signal was Fourier transformed without further data treatment. The spectrum required only very small zero-order phase adjustments. Although the solid signal is obtained with excellent S/N ratio in the time

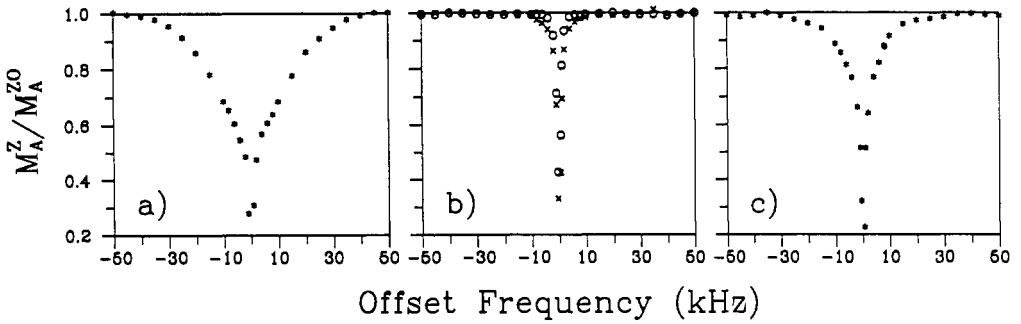

Figure 3. ^{1}H cross-relaxation spectra of 10% waxy maize starch: (a) suspension of granules in 0.25% xanthan aqueous solution; (b) (×) freshly gelatinized and cooled to room temperature, (○) DMSO solution; (c) retrograded gel (5 °C for 30 days).

domain, the corresponding broad resonance in the Fourier transform appears to be noisy. This occurs when the acquisition time is much longer than the solid decay, a requirement when signals with long T_2s, such as liquid water and mobile starch components, are to be detected without truncation of their decays. The sweep width was ±1 000 000 Hz, and the digital resolution in the spectrum is 30.5 Hz/point. The cylindrical sample rotor was spun at the magic angle (54° 44′) at a rate of 2.3 kHz. The rotor volume was 0.44 mL. Excitation and detection of ^{1}H resonance were achieved via the high-frequency channel of a high-power double-resonance probe. A single-frequency MAS probe would suffice.

Theory. The cross-relaxation experiment probes the magnetic and dynamical properties of solid components via observation of the liquid signal. The experiment consists of simply irradiating the sample with a preparation pulse that is off-resonance from the liquid signal to be detected and then reading the effect on the liquid magnetization by sampling with an on-resonance 90° radio frequency pulse. The liquid intensity may be determined directly from a time domain signal or a Fourier transform of the resulting free induction decay, i.e., from the usual spectrum in the frequency domain. Due to the cross-relaxation process, partial saturation of protons in solid components will transfer to water during the preparation period. The degree of saturation of the solid components depends on irradiation frequency and on the rate at which solid magnetization is lost due to nuclear relaxation in the system. The resonance intensity of the liquid, which is magnetically coupled to the solid, is plotted as a function of the frequency of the off-resonance preparation pulse and thus yields a spectrum that reflects the solid NMR spectrum and the relaxation properties of the whole system. The line shape of the spectrum is dependent on the amount and the relative rigidity of the solid component: the more solid component the sample contains and/or the more rigid the solid component is, the broader and more intense will be the resulting cross-relaxation spectrum. An advantage of Fourier transform detection of the liquid signal is that resonances from liquid and dissolved components are also obtained. As shown in Figure 2, the starch resonance peaks at 3.7 ppm corresponding to CH protons (except for the anomeric proton) (Morris and Hall, 1982; McIntyre et al., 1990) indicate the high mobility of a fraction of starch chain segments. The anomeric protons, normally visible as a peak at 5.4 ppm (Morris and Hall, 1982; McIntyre et al., 1990) are often buried in the wings of the water resonance (4.76 ppm).

Cross-relaxation spectroscopy has been discussed in detail by Grad and Bryant (1990). Their result for the steady-state magnetization for a liquid component (A) interacting via intermolecular dipole–dipole interactions (cross-relaxation) with a solid component (B) is

$$\bar{M}_A^Z = \alpha/(\beta + \Delta^2\gamma) \tag{1a}$$

where

$$\alpha = fR_{BA}T_{2B}\omega_1/(2R_AR_B) \tag{1b}$$

$$\beta = R_{BA}/R_B + f(R_{BA}/R_A + 1)(T_{2B}\omega_1^2/R_B + 1) \tag{1c}$$

$$\gamma = T_{2B}(R_{BA}/R_B + f(R_{BA}/R_A + 1)) \tag{1d}$$

The magnetization has been written in terms of a reduced form

$$\bar{M}_A^Z(t) = [M_A^{ZO} - M_A^Z(t)]/2M_A^{ZO} \tag{2}$$

where t is time. The reduced magnetization is a measure of the deviation of the longitudinal magnetization from its equilibrium value in the absence of a radio frequency field. Equation 1a is valid only when $\Delta \gg \omega_1$. In eq 1a f refers to the ratio of the number of B spins (solid) to the number of A spins (liquid); Δ denotes the frequency offset of the preparation radio frequency field from the A resonance frequency (in the cases below, water proton resonance); ω_1 is proportional to the strength B_1 of the radio frequency field of the preparation pulse and is given as the proton precession frequency $\omega_1 = \gamma_H B_1$, where γ_H is the proton gyromagnetic ratio; T_{2B} is the characteristic time of solid transverse relaxation; R_{BA} is the cross-relaxation rate; and R_A and R_B are longitudinal relaxation rates.

In the limit when cross-relaxation between the spin populations is rapid, i.e., when $R_{BA}/R_A \gg 1$

$$\bar{M}_A^Z = (1/2)\omega_1^2T_{1B}T_{2B}/[(1 + 4\pi^2T_{2B}^2\Delta^2)(1 + T_{1B}/fT_{1A}) + \omega_1^2T_{1B}T_{2B}] \tag{3}$$

This equation is similar to the steady-state solution of the Bloch equations for a single population of nuclear spins but contains the relaxation parameters of the solid as well as those of the liquid. The equation shows that liquid signal intensity $\bar{M}_A^Z$ is affected by the magnetic relaxation parameters of the solid spin system and, therefore, the molecular dynamics of the solid components. The line width of the cross-relaxation spectrum increases with decreasing T_{2B}, i.e., increasing rigidity. The intensity of the cross-relaxation spectrum is dependent on the solid to liquid proton ratio f; the area of the cross-relaxation spectrum increases with increasing f.

The spectroscopy is executed on the liquid, obviating the need for special electronics for detecting the rapidly decaying free induction decay signal of the solid, as in the wide-line Fourier transform method.

The theory of cross-relaxation spectroscopy has been recently extended by Wu (1991), in which transverse magnetization of the water spin is considered in the cross-relaxation process.

RESULTS AND DISCUSSION

Cross-Relaxation Spectra of Starch. Cross-relaxation NMR spectra of starch in various states are shown in Figure 3. The low water intensity resulting from the preparation pulse at very small frequency offset (less than 1 kHz) might be caused by the effects of direct saturation and/or finite line width of the water resonance as indicated in Wu's (1991) equation, and those data points would not fit any simple line shape functions for the broad components if included along with the other data points. Thus, only the data points at frequency offsets larger than ±1 kHz were used to estimate the broad component line width of the cross-relaxation spectra. The cross-relaxation spectrum of a 10 wt % suspension of starch granules (Figure 3a) thus shows a width at half-height of about 25 kHz, which reflects the ^{1}H NMR line shape of the

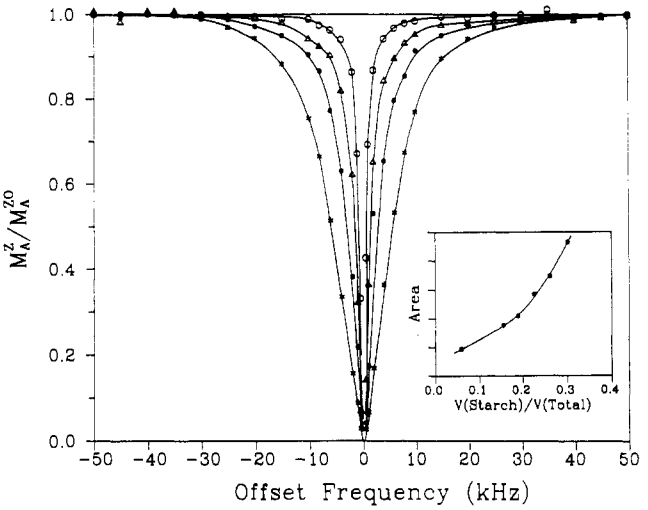

Figure 4. ^1H cross-relaxation spectra of freshly gelatinized waxy maize starch as a function of starch concentration: (○) 10, (△) 25, (●) 35, and (*) 45 wt %.

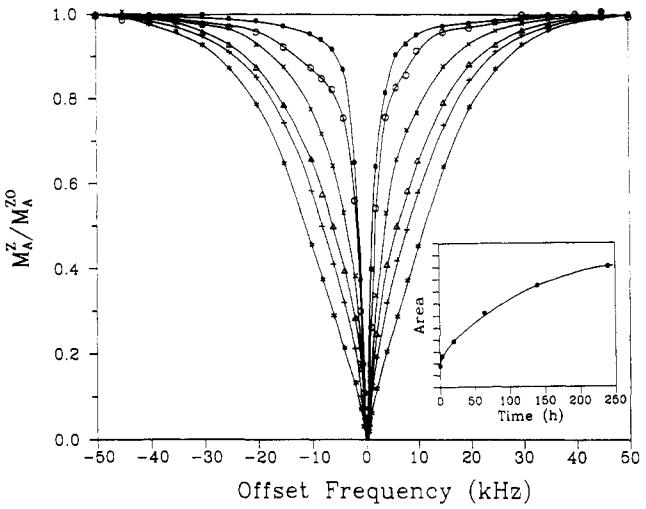

Figure 5. Time dependence of cross-relaxation line shape of gelatinized 25% waxy maize starch during retrogradation at 5 °C: (●) 3 h; (○) 20 h; (×) 64 h; (△) 140 h; (+) 10 days; (*) 67 days.

immobilized components. The width of this component indicates that the starch chains in these wet granules are fairly rigid. Heating such a sample above about 70 °C causes swelling, hydration, loss of crystallinity, and loss of granule integrity in the process known as gelatinization (Zobel, 1984). Consistent with release of immobilizing constraints of high concentration and crystallinity of starch chains in the granule, the cross-relaxation spectrum of the freshly gelatinized sample, cooled to room temperature, lacks detectable evidence of rigid solid; i.e., there is no intensity at offsets larger than about 10 kHz. Figure 3b shows both the relatively narrow cross-relaxation spectra of the fresh gel and of a 10% starch/DMSO solution. The solution spectrum is even narrower than the fresh gel spectrum, as expected from increased motional averaging of the ^1H–^1H dipole–dipole couplings.

However, gelatinized starch may retrograde, which is a process accompanied by development of gellike properties and increasing firmness, crystallinity detectable by X-ray diffraction and/or a melting peak in a differential scanning calorimetry (DSC) thermogram, and increased turbidity and light scattering (Slade and Levine, 1987; Ring et al., 1987; Morris, 1986). These observations are consistent with formation of domains in which polymer is highly concentrated and, at least in the case of crystallites, relatively immobilized. Immobilization of the macromolecules is reflected in the return of the broad features in the cross-relaxation spectrum (Figure 3c). The broad component in the cross-relaxation spectrum permits estimation of the fraction of starch that has returned to the immobilized state.

Concentration Dependence of Starch Chain Rigidity. The absence of detectable intensity at the large frequency offset in the cross-relaxation spectrum of heated 10% starch requires that the starch proton T_2 be quite long. This change is consistent with significantly increased molecular motion that would attend dissolution. Polymer chain segmental mobility may become more restricted at higher polymer concentration because of increased intermolecular contact and excluded volume effects. To test this idea, cross-relaxation spectra were obtained on freshly gelatinized samples ranging in starch concentration from 10 to 45 wt %. The series of room temperature spectra is shown in Figure 4. The spectra were obtained right after equilibrium at room temperature was reached, so that retrogradation of the amylopectin molecules was minimized. The principal features of this series are that there is a detectable cross-relaxation spectrum resulting from starch protons at concentrations greater than 10 wt % and the cross-relaxation spectral width and intensity increase as concentration increases. The inset of Figure 4 is the plot of the spectral area vs the volume fraction of starch, which was calculated from starch granule concentration with the correction of 12% water content and assuming that starch has approximately the same density as crystalline glucose monohydrate. Equation 3 predicts an increased area and line width as the ratio f of "solid" to liquid protons is increased. We observe a greater increase in area and width than predicted for a simple increase in f. This indicates that other parameters are changing. At present we favor the possibility of a decrease in T_{2B} corresponding to partial immobilization of starch chain due to concentration. Crystallinity is not likely to be the source of broadening in any of these spectra, since in a fresh waxy maize gel there is no crystallinity detectable by differential scanning calorimetry (Slade and Levine, 1987) or by X-ray diffraction (Miles et al., 1985). A full explanation of the cross-relaxation line shapes of concentrated gelatinized starch will require further study.

Starch Chain Immobilization during Retrogradation. As illustrated in Figure 3c, the cross-relaxation spectrum is sensitive to the changes in chain motion that accompany retrogradation. For waxy maize starch, this is a relatively slow process that can be monitored by observing the change in cross-relaxation spectrum shape and intensity. The results for a single 25 wt % sample tested at various times are shown in Figure 5. The major change at early times during retrogradation is an increase in intensity of a broad spectral component, which signals the development of rigidity in a starch component. Subsequently, substantial increases in area and width suggest gradual immobilization of a certain fraction of the remaining polymer mass. The concentration dependence of starch molecule immobilization during retrogradation is the subject of a separate study (in progress).

Motional Heterogeneity in Starch. The complex line shape during retrogradation may be interpreted as the superposition of several components corresponding to domains where polymer chains are far more rigid than those in solution but which nonetheless have different internal mobilities.

Independent evidence for motional heterogeneity of hydrated starch derives from a previous study (Lechert et al., 1980), in which a time domain signal due to starch

protons was obtained in a spin echo experiment on 3% starch gels in ^2H_2O. The echo decay could be resolved into fast and slow components, attributed to "solidlike and mobile" starch components, respectively. However, no time axis was provided; thus, the spin echo decay components cannot be compared to directly detected spectral components (see below). Blanshard et al. (1990) also reported spin–echo detection of transverse magnetization decay, noting the existence of at least two components with decay rates of about 10 μs and 1–10 ms, in retrograded amylose gels in ^2H_2O. Spectral component widths can be estimated from these rates using the relation $\Delta\nu_{1/2} \approx 1/\pi T_2$, where $\Delta\nu_{1/2}$ is the line width at half-height and T_2 is the apparent transverse relaxation time, here taken to be equal to each spin echo magnetization decay rate. The predicted component widths would be 30 kHz and 30–300 Hz, respectively. The authors attributed the decay components to rigid, double-helical segments and to mobile interjunction segments, respectively. Motional heterogeneity in starch has also been demonstrated from ^{13}C NMR studies (Gidley, 1989). Integrated intensities in a single pulse high-resolution ^{13}C NMR spectrum of starch gel can be compared with that of standard compounds to obtain an estimate of mobile starch fraction (Callaghan et al., 1983; Hansen et al., 1989).

Other evidence of motional heterogeneity is found in the spectra used to calculate the cross-relaxation spectrum. For example, starch peaks can be detected in the high-resolution ^1H NMR spectrum of starch gelatinized in ^1H_2O and retrograded at room temperature for 5 days (Figure 2, peaks between 3 and 4 ppm). The observation of starch resonances in a high-resolution ^1H NMR spectrum requires the corresponding starch components to be highly mobile. A previous ^1H NMR study alluded to the presence of mobile starch, but neither spectra nor integrals were reported (Jaska, 1971). Since the water (^1H_2O) resonance is large and sometimes broad in such spectra, starch proton resonances are more easily observed in ^1H NMR spectra obtained in a ^2H_2O/starch sample and/or at high temperature (Gidley, 1985). We have observed starch resonance widths in ^2H_2O samples of concentrated starch gels (10–40%) to be in the range 25–80 Hz at room temperature (data not shown), depending on concentration and aging. The range is still orders of magnitude narrower than the width of components detected in cross-relaxation spectra. Thus, neither high-resolution NMR spectra nor cross-relaxation spectra provide a complete picture.

Wide-Line and High-Resolution ^1H NMR of Starch. We demonstrate here a method for observing the total ^1H NMR spectrum. The details of the method, called wide-line and high-resolution, are described under Materials and Methods. Figure 6 shows the wide-line Fourier transform ^1H NMR spectrum of a 40% waxy maize starch/^2H_2O gel aged at 5 °C for 10 days. The spectrum shows a broad component with a width of about 30 kHz, which is comparable to the width of the broadest component in a cross-relaxation spectrum of the similar starch/^1H_2O sample (data not shown). An intermediate component with a width of several kilohertz is evident in the wide-line spectrum. This feature would generally not be observed in a conventional high-resolution ^1H NMR experiment, in which the entire resonance is not excited, and the spectral width is usually smaller than about 4 kHz. An exact value for the widths of intermediate components cannot be given since the line shape is fairly complex, and methods for decomposing the total spectrum into a sum of components of different shapes and widths are not yet perfected. Nonetheless, the correspondence

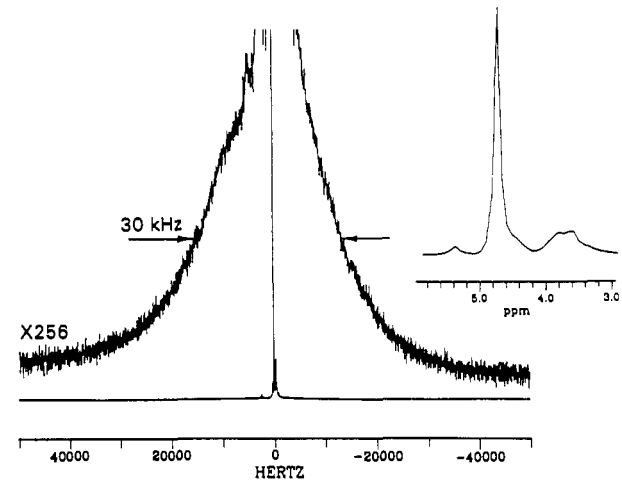

Figure 6. Wide-line and high-resolution Fourier transform ^1H NMR spectrum of 40% waxy maize starch/^2H_2O gel aged at 5 °C for 10 days. Details are given in the text. (Inset) 40× horizontal expansion of the central part of the spectrum, corresponding to a conventional high-resolution ^1H NMR experiment.

between the cross-relaxation spectrum and the total ^1H NMR spectrum of an aged gel is striking, in that each can apparently be decomposed into broad (several tens of kilohertz) and intermediate (several kilohertz) components.

Magic angle sample spinning (MAS) permits resolution which is sometimes lost in a wide-line NMR probe to be recovered (Eads et al., 1991; Badiger et al., 1991; Rutar et al., 1988). Thus, the wide-line spectrum with MAS produces narrow, resolved water and highly mobile starch resonances. The central part of the wide-line spectrum contains the same information as a conventional single pulse ^1H NMR experiment. The fractions of polymer mass producing broad, intermediate, and narrow spectral components in the total NMR spectrum can be estimated by integration of the spectrum in Figure 6 to be about 63:16:21, respectively. While we are not aware of previous reports of the wide-line FT NMR spectrum of starch, the observation of a wide range in line widths, and hence mobility, might have been anticipated from the ^1H spin echo experiments of Lechert et al. (1980) and of Blanshard et al. (1990).

Independent evidence from light scattering, DSC, and X-ray clearly suggest that domains of highly concentrated and sometimes crystalline starch can exist in starch gels. Thus, at least in the case of retrograded gels a relationship may be drawn between spectral components reflecting motional heterogeneity and physical components, i.e., domains in which polymer chains have different degrees of mobility. It should be emphasized that while a multicomponent NMR spectrum may indicate a distribution of motions, it does not necessarily indicate that physical domains exist unless independent experimental evidence, including other NMR experiments (Lacelle and Gerstein, 1987), supports such as interpretation.

High-resolution NMR spectra can also be obtained from rigid components. Resolved chemical shifts in such experiments can provide information about local conformation and packing effects. The relevant ^{13}C experiment involves cross-polarization, magic angle spinning, and high-power proton decoupling (Pines et al., 1973). Applications to starch systems have been reported (Marchessault et al., 1985; Gidley and Bociek, 1985, 1988; Veregin et al., 1987; Blanshard et al., 1990; Mora-Gutierrez and Baianu, 1991). The corresponding ^1H experiment (CRAMPS) involves multiple pulses and magic angle spinning (Taylor et al.,

1979); application of CRAMPS to starch has not yet been reported. However, the distributions of *motional* components are not easily made with either ^{13}C or ^{1}H high-resolution solids methods, unless nuclear relaxation experiments are conducted. These can be time-consuming and may have limited value in process-monitoring schemes or in measurement of processes occurring in times of seconds or less. Finally, it is not straightforward with either ^{13}C or ^{1}H high-resolution methods to obtain resonances from intermediate mobility components.

Conclusion. The potential value of ^{1}H cross-relaxation spectra for monitoring processes involving changes in amount and molecular dynamics of solid and semisolid domains is clearly indicated by the results presented in this paper. Other NMR methods measuring *starch* ^{1}H NMR behavior have been proposed and demonstrated for such purposes (Lechert et al., 1980; Blanshard et al., 1990). There is also substantial literature covering ^{1}H NMR methods observing *water* ^{1}H, ^{2}H, and ^{17}O NMR relaxation behavior; some recent examples include Belton and Colquhoun (1990), Hills et al. (1991), Mora-Gutierrez and Baianu (1989), and Schmidt (1990). While the water studies have practical value, they do not directly detect solid and measure mobility of the macromolecules, as in wide-line NMR, nor do they reflect so clearly the state of immobilized components, as in cross-relaxation NMR.

Most NMR spectrometers can be equipped to execute a ^{1}H cross-relaxation experiment. Although we report the full cross-relaxation spectrum, requiring about 10–20 min to obtain, a simpler, shorter experiment is conceivable, wherein the saturation of the $^{1}H_2O$ signal is monitored after a preparation pulse applied at a single offset frequency. Such an experiment could provide a useful result in less than 2 s. Finally, a method that obtains the entire cross-relaxation spectra in less than 5 s has been reported (Swanson, 1991). The experiment was conducted with application of a magnetic field gradient in a magnetic resonance imaging spectrometer. The cross-relaxation methodology has been applied to systems besides starch, namely animal tissues (Grad et al., 1991; Wolff et al., 1991), and should find wide utility in food science and technology.

ACKNOWLEDGMENT

Paper No. 12945 of the Agricultural Experiment Station, Purdue University, West Lafayette, IN 47907-1160. The research reported in this publication was funded by the Whistler Center for Carbohydrate Research, Purdue University, the National Institutes of Health (GM-34541), and the University of Rochester. Use of the Bruker MSL400 NMR spectrometer at Kraft General Foods Technology Center, Glenview, IL, is gratefully acknowledged. The contributions of P. Pellechia and R. Santini (Purdue) in NT200 NMR spectrometer upgrade design and execution are appreciated.

LITERATURE CITED

Andrew, E. R. Nuclear Magnetic Resonance Modulation Correction. *Phys. Rev.* **1953**, *91*, 425.

Badiger, M. V.; Rajamohanan, P. R.; Kulkarni, M. G.; Ganapathy, S.; Mashelkar, B. A. Proton MASS-NMR: A New Tool to Study Thermoreversible Transition in Hydrogels. *Macromolecules* **1991**, *24*, 106–111.

Baianu, I. C.; Förster, H. Cross-Polarization, High-Field Carbon-13 NMR Techniques for Studying Physicochemical Properties of Wheat Grain, Flour, Starch, Gluten and Wheat Protein Powders. *J. Appl. Biochem.* **1980**, *2*, 347–354.

Belton, P. S.; Colquhoun, I. J. Nuclear Magnetic Resonance Spectroscopy in Food Research. *Spectroscopy* **1990**, *4*, 22–32.

Blanshard, J. M. V.; Jaroszkiewicz, E. M.; Gidley, M. J. The Structure and Behavior of the Starch Granule as Studied by NMR. In *NMR Applications in Biopolymers*; Finney, J. W., Schmidt, S. J., Serianni, A. S., Eds.; Plenum Press: New York, 1990; pp 155–173.

Callaghan, P. T.; Jolley, K. W.; Lelievre, J.; Wang, R. B. K. Nuclear Magnetic Resonance Studies of Wheat Starch Pastes. *J. Colloid Interface Sci.* **1983**, *92*, 332–337.

Chinachoti, P.; White, V. A.; Lo, L.; Stengle, T. R. Application of High-Resolution Carbon-13, Oxygen-17, and Sodium-23 Nuclear Magnetic Resonance to Study the Influences of Water, Sucrose, and Sodium Chloride on Starch Gelatinization. *Cereal Chem.* **1991**, *68*, 238–244.

Collison, R.; McDonald, M. P. Broadening of the Water Proton Line in High-Resolution Nuclear Magnetic Resonance Spectra of Starch Gels. *Nature* **1960**, *186*, 548–549.

Dev, S. B.; Burum, D. P.; Rha, C. K. High Resolution CPMAS and Solution NMR Studies of Polysaccharides—Part 1: Starch. *Spectrosc. Lett.* **1987**, *20*, 853–869.

Eads, T. M.; Weiler, R. K.; Gaonkar, A. G. Detection of Water in the Internal Aqueous Phase of W/O/W Multiple Emulsions Using Magic Angle Spinning Proton NMR. *J. Colloid Interface Sci.* **1991**, *145*, 466–477.

Gidley, M. J. Quantification of the Structural Features of Starch Polysaccharides by N.M.R. Spectroscopy. *Carbohydr. Res.* **1985**, *139*, 85–93.

Gidley, M. J. Molecular Mechanisms Underlying Amylose Aggregation and Gelation. *Macromolecules* **1989**, *22*, 351–358.

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.

Gidley, M. J.; Bociek, S. M. ^{13}C CP/MAS NMR Studies of Amylose Inclusion Complexes, Cyclodextrins, and the Amorphous Phase of Starch Granules: Relationships between Glycosidic Linkage Conformation and Solid-State ^{13}C Chemical Shifts. *J. Am. Chem. Soc.* **1988**, *110*, 3820–3829.

Grad, J.; Bryant, R. G. Nuclear Magnetic Cross-Relaxation Spectroscopy. *J. Magn. Reson.* **1990**, *90*, 1–8.

Grad, J.; Mendelson, D.; Hyder, F.; Bryant, R. G. Application of Nuclear Magnetic Cross-Relaxation Spectroscopy to Tissues. *Magn. Reson. Med.* **1991**, *17*, 452–459.

Hansen, L. M.; Setser, C. S.; Paukstelis, J. V. Investigations of Sugar-Starch Interactions Using Carbon-13 Nuclear Magnetic Resonance. I. Sucrose. *Cereal Chem.* **1989**, *66*, 411–415.

Hartmann, S. R.; Hahn, E. L. Nuclear Double Resonance in the Rotating Frame. *Phys. Rev.* **1962**, *128*, 2042–2053.

Hills, B. P.; Cano, C.; Belton, P. S. Proton NMR Relaxation Studies of Aqueous Polysaccharide System. *Macromolecules* **1991**, *24*, 2944–2950.

Jackson, C. L.; Bryant, R. G. Carbon-13 NMR of Glycogen: Hydration Response Studied by Using Solid Methods. *Biochemistry* **1989**, *28*, 5024–5028.

Jaska, E. Starch Gelatinization as Detected by Proton Magnetic Resonance. *Cereal Chem.* **1971**, *48*, 437–444.

Lacelle, S.; Gerstein, B. C. NMR Studies on the Presence of Domain Structures in Polysaccharides. *Biopolymers* **1987**, *26*, 849–861.

Lechert, H.; Hennig, H. J. NMR Investigations on the Behavior of Water in Starches. In *Magnetic Resonance in Colloid and Interface Science*; Resing, H. A., Wade, C. G., Eds.; ACS Symposium Series 34; Washington, DC, 1976; pp 328–343.

Lechert, H.; Maiwald, W.; Köthe, R.; Basler, W.-D. NMR-Study of Water in Some Starches and Vegetables. *J. Food Process. Preserv.* **1980**, *3*, 275–299.

Mansfield, P. Multiple-Pulse Nuclear Magnetic Resonance Transients in Solid. *Phys. Rev.* **1965**, *137*, A961–A974.

Marchessault, R. H.; Taylor, M. G. Solid-State ^{13}C-C.P.-M.A.S. N.M.R. of Starches. *Carbohydr. Res.* **1985**, *144*, C1–C5.

McIntyre, D. D.; Ho, C.; Vogel, H. J. One-Dimension Nuclear Magnetic Resonance Studies of Starch and Starch Products. *Starch* **1990**, *42*, 260–267.

Miles, M. J.; Morris, V. J.; Orford, P. D.; Ring, S. G. The Roles of Amylose and Amylopectin in the Gelation and Retrogradation of Starch. *Carbohydr. Res.* **1985**, *135*, 271–281.

Mora-Gutierrez, A.; Baianu, I. C. ^{1}H NMR Relaxation and Viscosity Measurements on Solutions and Suspensions of Carbohydrates and Starch from Corn: The Investigation of Carbohydrate Hydration and Stereochemical and Aggregation Effects in Relation to ^{17}O and ^{13}C NMR Data for Carbohydrate Solutions. *J. Agric. Food Chem.* **1989**, *37*, 1459–1465.

Mora-Gutierrez, A.; Baianu, I. C. Carbon-13 Nuclear Magnetic Resonance Studies of Chemically Modified Waxy Maize Starch, Corn Syrups, and Maltodextrins. Comparisons with Potato Starch and Potato Maltodextrins. *J. Agric. Food Chem.* **1991**, *39*, 1057–1062.

Morris, G. A.; Hall, L. D. Experimental Chemical Shift Correlation Maps from Heteronuclear Two-Dimensional Nuclear Magnetic Resonance Spectroscopy. II: Carbon-13 and Proton Chemical Shifts of α-D-glucopyranose Oligomers. *Can. J. Chem.* **1982**, *60*, 2431–2441.

Morris, V. J. Gelation of Polysaccharides. In *Functional Properties of Food Macromolecules*; Mitchell, J. R., Ledward, D. A., Eds.; Elsevier: London, 1986; pp 121–170.

Morris, V. J. Starch gelation and Retrogradation. *Trends Food Sci. Technol.* **1990**, *1*, 2–6.

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

Ring, S. G.; Colonna, P.; I'Anson, K. J.; Kalichevsky, M. T.; Miles, M. J.; Morris, V. J.; Orford, P. D. The Gelation and Crystallization of Amylopectin. *Carbohydr. Res.* **1987**, *162*, 277–293.

Rutar, V.; Kovač, M.; Lahajnar, G. Improved NMR Spectra of Liquid Components in Heterogeneous Samples. *J. Magn. Reson.* **1988**, *80*, 133–138.

Schmidt, S. J. R. Characterization of Water in Food by NMR. In *NMR Applications in Biopolymers*; Finney, J. W., Schmidt, S. J., Serianni, A. S., Eds.; Plenum Press: New York, 1990; pp 415–459.

Slade, L.; Levine, H. Recent Advances in Starch Retrogradation. In *Recent Developments in Industrial Polysaccharides*; Stivala, S. S., Crescenzi, V., Dea, I. C. M., Eds.; Gordon and Breach Science: New York, 1987.

Swanson, S. D. Cross-Relaxation NMR Spectroscopy: Broad-Band Excitation and Detection. *J. Magn. Reson.* **1991**, submitted for publication.

Taylor, R. E.; Pembleton, R. G.; Ryan, L. M.; Gerstein, B. C. Combined Multiple Pulse NMR and Sample Spinning: Recovery of Proton Chemical Shift Tensors. *J. Chem. Phys.* **1979**, *71*, 4541–4545.

Veregin, R. P.; Fyfe, C. A.; Marchessault, R. H.; Taylor, M. G. Correlation of ^{13}C Chemical Shifts with Torsional Angles from High-Resolution, ^{13}C-C.P.-M.A.S. N.M.R. Studies of Crystalline Cyclomalto-Oligosaccharide Complexes, and Their Relation to the Structures of the Starch Polymorphs. *Carbohydr. Res.* **1987**, *160*, 41–56.

Wolff, S. D.; Balaban, R. S. Magnetization Transfer Contrast (MTC) and Tissue Water Proton Relaxation in Vivo. *Magn. Reson. Med.* **1989**, *10*, 135–144.

Wolff, S. D.; Eng, J.; Balaban, R. S. Magnetization Transfer Contrast: Method for Improving Contrast in Gradient-Recalled-Echo Images. *Radiology* **1991**, *179*, 133–137.

Wu, X. Lineshape of Magnetization Transfer *via* Cross Relaxation. *J. Magn. Reson.* **1991**, *94*, 186–190.

Zobel, H. F. Gelatinization of Starch and Mechanical Properties of Starch Pastes. In *Starch Chemistry and Technology*, 2nd ed.; Whistler, R. L., BeMiller, J. N., Paschall, E. F., Eds.; Academic Press: New York, 1984; pp 285–309.

Received for review May 21, 1991. Revised manuscript received November 25, 1991. Accepted December 10, 1991.

Registry No. Amylopectin, 9037-22-3.