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Evolution of polymer mobility during ageing of gelatinized waxy maize starch: a magnetization transfer ^{1}H NMR study

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The immobilization of polymer during ageing of gelatinized 25 wt% and 40 wt% waxy maize starch at room temperature and 5°C was studied using proton Nuclear Magnetic Resonance (NMR) spectroscopy. Cross-relaxation NMR was used to monitor the increase of relatively immobile starch components, while high-resolution ^{1}H NMR was used to monitor the decrease of components with liquid-like mobility. The cross-relaxation spectra of aged starch gel were analyzed into two spectral components. The broader component intensity was found to correlate with the degree of crystallinity as measured by differential scanning calorimetry; and the narrower component was attributed to starch chains having mobility intermediate between those in crystalline and liquid-like states. The kinetics of crystallization-induced immobilization were analyzed by fitting the intensity of the broader component to an Avrami equation. The results were compared with those obtained from other physical techniques. The events of ageing are discussed in terms of changes in fractions of starch components having different molecular dynamics.

INTRODUCTION

Starch is a main source of carbohydrate in the human diet. It also contributes to the texture of food by forming a gel network. Natural starch is partially crystalline in its native granule form. During food processing starch granules gelatinize as a result of heating in the presence of water, i.e. the granules swell, crystalline regions melt and starch chains become hydrated. After being cooled, ageing of the gelatinized starch is accompanied by increasing turbidity, firmness, and degree of crystallinity (Morris, 1986). 'Retrogradation' was first used to refer to the return of crystallinity in the starch of staling bread (Katz, 1934), and is also used to refer to the analogous process in starch. The several processes accompanying ageing are important for texture and stability of starch-containing foods. In order to understand the processes of ageing, the starch/water system has been extensively investigated using physical methods such as turbidity (Miles *et al.*, 1984; Ring *et al.*, 1987), dilatometry (Miles *et al.*, 1985*a*; Ring *et al.*, 1987), thermal analysis (McIver *et al.*, 1968; Longton & LeGrys, 1981; Russell, 1987), rheology (Miles *et al.*, 1985*a,b*; l'Anson *et al.*, 1988; Biliaderis & Zawistowski, 1990), X-ray diffraction (Hellman *et al.*, 1954; Zobel & Senti, 1959; Miles *et al.*, 1985*a,b*; l'Anson *et al.*, 1988), vibrational spectroscopy (Bulkin *et al.*, 1987), microscopy (Fannon & BeMiller, 1992), and NMR spectroscopy (Lechert & Hennig, 1976; Blanshard *et al.*, 1990). These methods follow rather different properties. For example, turbidity measures distribution of refractive index (hence density); dilatometry measures volume change; thermal analysis measures heat capacity change and the latent heat of melting of crystalline regions; X-ray diffraction measures long-range three-dimensional order in crystalline starch domains; vibrational spectroscopy monitors conformation- and crystallinity-dependent vibrational frequencies of chemical bonds; microscopy measures the spatial distribution of refractive mass (over the range in dimensions from molecular to subvisible); and NMR monitors chain segmental motions, conformation-dependent chemical shifts (resonance frequencies) and degree of crystallinity. The physical studies show that the time course of change depends on the property being measured, and therefore, that some changes,

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such as increase in turbidity, can precede others. It is possible to propose mechanisms based on the sequence of events and their individual time course. The molecular picture emerging from this analysis is that during ageing of gelatinized starch, partial phase separation of polymer-rich and solvent-rich domains occurs, followed by slower crystallization of some starch chains (Miles *et al.*, 1984, 1985*a,b*). The relationship between microscopic events and development of texture is not clear. Fundamentally speaking, development of elasticity requires formation of a network that resists deformation, and viscosity requires internal dissipation of mechanical work done on the material. Thus, for example, the modulus of starch gels (i.e. stiffness) has been closely linked to the association of amylopectin chains, as monitored by dilatometry and DSC (Ring *et al.*, 1987). Crystallites might thus be regarded as nodes in an elastic network, and they contribute particle-like resistance to shear-induced flow. However, elasticity can develop due to chain entanglements or non-crystalline association of chains, as well as by formation of crystallites. In all cases, network formation implies that at any instant, some fraction of polymer is conformationally restricted. The distribution of segmental motions in such a system should show a fraction that is relatively immobile. Regardless of detailed mechanism, development of gel-like rheological properties in the starch system should therefore be accompanied by the appearance of immobilized starch fractions. While several NMR studies of starch have supported this idea (Lechert & Hennig, 1976; Blanshard *et al.*, 1990), most of the NMR methods used are inherently selective of either highly mobile or rigid components.

In a previous paper (Wu *et al.*, 1992) we showed that a magnetization-transfer ^{1}H NMR method called cross-relaxation spectroscopy (Wolff & Balaban, 1989; Grad & Bryant, 1990) can be used to detect the immobilization of chains in gelatinized waxy maize starch during ageing. ^{1}H cross-relaxation spectroscopy was shown to be a convenient alternative to direct detection of the total NMR spectrum (very broad resonances from immobilized molecules, intermediate resonances, and very narrow resonances from liquid-like molecules) by wide line Fourier transform, magic angle spinning ^{1}H NMR spectroscopy. The latter method was demonstrated on a starch gel in that paper. Waxy maize starch contains mainly amylopectin, which is a highly branched α-D-glucan macromolecule, with $(1 \rightarrow 4)$ linked linear chains and $\alpha(1 \rightarrow 6)$ linkage at branch points (Young, 1984). Amylopectin has been shown to participate in the slow rate crystallization of starch, which in turn is associated with textural change (Miles *et al.*, 1985*a*; Ring *et al.*, 1987; Russel, 1987). Here we present the application of ^{1}H cross-relaxation NMR spectroscopy to study waxy maize starch ageing in terms of starch chain immobilization. We have also measured the change in highly mobile starch by conventional high resolution ^{1}H NMR, and the degree of crystallinity by DSC.

MATERIALS AND METHODS

Materials

Waxy maize starch was obtained from Penford Products Co., Cedar Rapids, Iowa. Deuterium oxide (99·9%) was purchased from Cambridge Isotope Laboratories.

NMR measurements

Cross-relaxation spectra of starch samples were obtained from the offset frequency dependence of the water signal intensity in a saturation transfer NMR experiment (Grad & Bryant, 1990) as described earlier (Wu *et al.*, 1992). The details of sample preparation were also given in that paper. The high resolution ^{1}H NMR spectra which were used to construct the cross-relaxation spectra were obtained on a spectrometer (Nicolet NT-200) which had been upgraded with a computer-controllable spectrometer interface (TecMag 'Libra') and fast data system (Apple Macintosh IIfx) using 'MacNMR' software (TecMag) for data acquisition and processing. The spectrometer operating frequency (^{1}H) was 200·067 MHz. A single frequency (^{1}H), single coil, high resolution probe was used. The pulse sequence is D5 (relaxation delay)-D1(preparation pulse)-P2(90° pulse)-acquisition. In all cross-relaxation spectral measurements, the preparation pulse was applied for 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 kHz to $+50$ kHz, usually in 5 kHz increments. The other acquisition parameters were: relaxation delay time (D5) 20 s, 90° pulse (P2) 6 μs, spectral width ± 1000 Hz, data size 4K, and a single scan. High-resolution ^{1}H NMR spectra of starch gelatinized in D_2O were measured on a General Electric QE-300 NMR spectrometer. All NMR measurements were carried out at room temperature. For samples aged at 5°C, samples were equilibrated at room temperature for 15 min before the measurement, then restored at 5°C after the NMR measurement. The typical time for measurement of a cross-relaxation spectrum was 15 min. In order to reduce the experimental error of the ageing temperature, three individual samples were used in the case of 40 wt% aged at 5°C for 3, 6 and 9 h.

Differential scanning calorimetry (DSC) measurements

DSC measurements were performed on a Perkin-Elmer DSC-4 instrument, equipped with a System 4

microprocessor controller and a thermal analysis data station Model 3600. Suspensions of waxy maize starch granules of various concentrations, having a total weight of 10–15 mg, were transferred to preweighed, coated aluminum, hermetic DSC sample pans (TA instruments, Wilmington, DE), which were then sealed and reweighed. The sample pans were heated in an oven at 100°C for 20 min and then stored either at room temperature or 5°C for certain times before DSC analysis. The DSC traces from 25°C to 100°C at a heating rate of 10°C/min were recorded using an empty sample pan as a reference.

Theory of cross-relaxation NMR spectroscopy

The cross-relaxation experiment probes the solid components via their interaction with the liquid. The experiment consists of simply irradiating the sample with a radiofrequency pulse that is off-resonance from the liquid signal to be detected, then reading the effect on the liquid magnetization by sampling it with an on-resonance 90° radiofrequency pulse. The amplitude of the liquid signal plotted as a function of the frequency of the off-resonance preparation pulse yields a spectrum whose shape reflects the properties of the solid-like components.

Cross-relaxation spectroscopy has been discussed in detail by Grad & 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:

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

where

$$\alpha = f R_{BA} T_{2B} \omega_1/(2R_A R_B) \tag{1a}$$

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

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

The magnetization is written in terms of a reduced form:

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

which is a measure of the deviation of the longitudinal magnetization from the equilibrium in the absence of a r.f. field. Equation (1) is only valid when $\Delta \gg \omega_1$; in eqn (1) 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 r.f. field from the A resonance frequency (in the cases below, water proton resonance), ω_1 refers to the amplitude of the r.f. field of the preparation pulse, 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 $R_{BA}/R_A \gg 1$,

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

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 decrease in T_{2B}, i.e. increasing rigidity. The intensity of cross-relaxation depends on the solid to liquid proton ratio f; thus the area of the cross-relaxation spectrum increases with increasing f.

Lineshape analysis

The complex lineshapes of the cross-relaxation spectra of retrograded starch samples were analyzed using simple lineshape functions. Most lineshapes in NMR spectra can be approximated either by Lorentzian or Gaussian functions, whose mathematical expressions are:

$$I_L = \frac{C_L}{1 + (2(\nu - \nu_0)/\Delta\nu_L)^2} \tag{4}$$

$$I_G = C_G \exp\left(\frac{\ln 2(2(\nu - \nu_0))^2}{(\Delta\nu_G)^2}\right) \tag{5}$$

where I is the intensity, C is the coefficient, ν is the frequency in Hz, $\Delta\nu$ is the line width at half height in Hz, ν_0 is the central position, and the subscripts L and G denote Lorentzian and Gaussian functions, respectively. The cross-relaxation spectrum is a plot of the normalized intensity of water peak, M_A^Z (with preparation pulse)/M_A^{ZO} (without preparation pulse) versus the frequency offset (Δ). This plot is 'up-side down' compared with most NMR spectra: thus for convenience of analysis and inspections, the function $1 - M_A^Z(\Delta)/M_A^{ZO}$ was used. The resulting lineshape was fitted by computer using a least squares procedure (SigmaPlot curve fitter, Jandel Scientific, Corte Madera, CA) to a sum of Lorentzian and Gaussian functions:

$$1 - M_A^Z(\Delta)/M_A^{ZO} = \frac{C_L}{1 + (2000\Delta/\Delta\nu_L)^2} + C_G \exp\left(-\frac{\ln 2(2000\Delta)^2}{(\Delta\nu_G)^2}\right) \tag{6}$$

where Δ is the offset frequency in kHz, and the other parameters are the same as in eqns (4) and (5).

The areas of the cross-relaxation spectrum and its components are the integrals of the fitting functions, which equal the sum of the values of the functions over the total frequency range. As an approximation, the areas of the cross-relaxation spectrum and its components were calculated as the sum of the values at intervals of 1 kHz over the frequency range of −100–100 kHz and expressed in arbitrary units:

$$\text{Area} = \sum_{\Delta_i = -100}^{100} \frac{C_L}{1 + (2000*\Delta_i/\Delta\nu_L)^2} \qquad (7)$$

$$+ C_G \exp\left(-\frac{\ln 2*(2000*\Delta_i)^2}{(\Delta\nu_G)^2}\right)$$

where the values of C_L, C_G, $\Delta\nu_L$ and $\Delta\nu_G$ were given from the curve fitting.

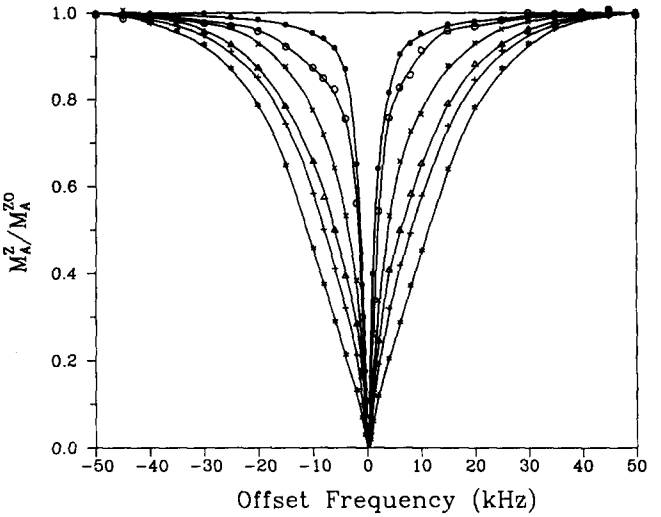

Fig. 1. Time dependence of cross-relaxation line shape of 25 wt% waxy maize starch gel during retrogradation at 5°C. (●) 3 h; (○) 20 h; (X) 64 h; (△) 140 h; (+) 10 days; (*) 67 days.

RESULTS AND DISCUSSION

Starch gel ageing monitored by cross-relaxation spectra

The cross-relaxation method detects the solid-like components in a water-containing system, producing a spectrum whose shape and width are closely related to the shape and width of the ^{1}H NMR resonance detected in a wide line NMR experiment (Wu *et al.*, 1992; Grad & Bryant, 1990). The cross-relaxation spectrum is thus sensitive to the changes in starch chain motion that accompany ageing. For waxy maize starch, starch chain immobilization is a relatively slow process that can be monitored by observing the change in cross-relaxation spectrum shape and intensity. We measured the cross-relaxation spectra of gelatinized waxy maize starch at concentrations of 25 wt% and 40 wt% during ageing at 5°C and room temperature. The results for a single 25 wt% sample tested at various times are shown in Fig. 1. There is a substantial increase in overall width and area, and the line shape changes dramatically during ageing.

The area of a cross-relaxation spectrum may be used as an index of the degree of starch chain immobilization. According to eqn (3) cross-relaxation spectral intensity is expected to increase as the solid to liquid proton ratio (f) increases; and the line width is expected to increase as the rigidity (T_{2B}^{-1}) of the solid-like component increases. Figure 2((a) and (b)) displays the increase of cross-relaxation spectral area of starch gel during ageing at 5°C and room temperature, respectively. At 5°C the area of the 40 wt% sample increases rapidly and approaches its limiting value in 3 days, while the area of the 25 wt% sample approaches its limiting value at a time longer than 10 days. At room temperature the process is much slower than at low temperature: the 40 wt% sample approaches its limiting value at 50 days, while the 25 wt% sample only reaches one half of its limiting value in the same time. We note that the area of a cross-relaxation spectrum can become less sensitive to further solidification when the proton ratio f is large; this was determined on suspensions of starch granules in water (unpublished data). Nevertheless, the areas of spectra reported in this work are in the sensitive region of change.

Clearly, immobilization kinetics is strongly concentration and temperature dependent (Fig. 2). The temperature dependence is consistent with results from other physical property measurements (Ring *et al.*, 1987; Biliaderis & Zawistowski, 1990), while the concentration dependence is not clear from those previous measurements. Therefore it is of interest to examine the evolution of cross-relaxation spectra in detail.

Multicomponent nature of cross-relaxation spectra of retrograded starch gel

Equation (3) implies that the cross-relaxation spectral lineshape should be a single Lorentzian-like function of Δ for a single motional component. Indeed it is possible to fit the room temperature cross-relaxation spectrum of freshly gelatinized waxy maize starch to a single Lorentzian function, which suggests that motion in the solid-like material detectable by cross-relaxation spectroscopy is effectively characterized by a single value of T_{2B}. In contrast, it is not possible to fit the cross-relaxation spectra of an aged gel to a single simple function; this is due to the appearance of an additional broad component.

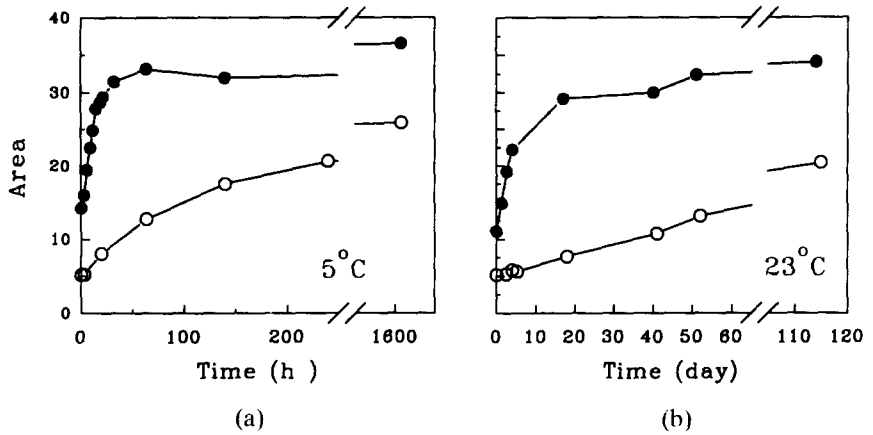

Fig. 2. The area of cross-relaxation spectra for (○) 25 and (●) 40 wt% waxy maize starch gel as a function of retrogradation time, (a) in h and (b) in days, at 5°C and room temperature.

The complex lineshape for an aged gel may be interpreted as the superposition of spectral components corresponding to solid-like polymer fractions with different mobilities. In order to explore this further, cross-relaxation spectra were fitted to sums of simple Lorentzian and/or Gaussian functions of Δ (see Material and Methods). Figure 3 demonstrates that the cross-relaxation spectrum of 40 wt% gel aged at 5°C for 9 h fits to a sum of Lorentzian (dashed line) and Gaussian (dotted line) functions. The experimental data can be accurately fitted in this way (standard deviation less than 7%); and among the various possible combinations, the best fits are found to be a broader Gaussian plus a narrower Lorentzian. This is true for all the spectra of ageing gels. The changes of line width and area of spectral components during ageing are illustrated in Fig. 4.

Following these data we find that the width of the broader component increases at an early stage and soon achieves a stable value (25–30 kHz for the 25 wt% sample and 30–35 kHz for 40 wt%), and that the area of this component increases significantly. These observations suggest that the mass fraction of the corresponding starch component increases steadily while its rigidity first increases then remains fairly constant.

In the 25 wt% gel the width of the narrower component appeared to increase while its area remained stable. This suggests that during the ageing process in the 25 wt% gel, progressive immobilization occurs in this less rigid solid-like phase, while its net mass does not change. For the 40 wt% gel, the narrower component width first increases and then decreases, while the area decreases first and then becomes stable. The interpretation offered for the early stage is essentially the same as that for the 25 wt% gel, while the slight decrease in area over long periods may be associated with further changes in distribution of mass not detected in the cross-relaxation experiment. An alternative possibility, which has not been fully tested, is that changes in nuclear relaxation parameters are occurring.

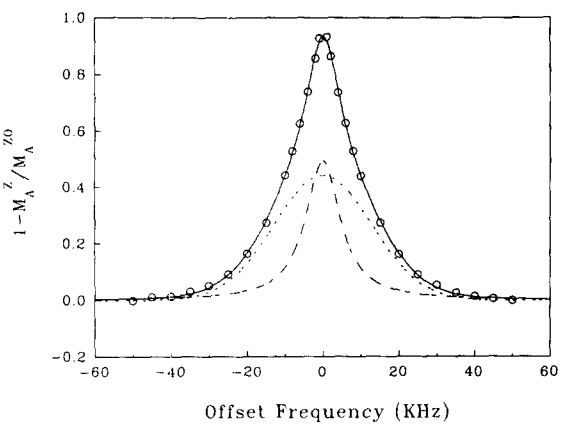

Fig. 3. The deconvolution of cross-relaxation spectrum as sum of a Lorentzian line and a Gaussian line for 40 wt% waxy maize starch retrogradation at 5°C for 9 h. The width of the Lorentzian line (dashed line) is 11 kHz and the width of the Gaussian line (dotted line) is 30 kHz; the ratio of the area under Lorentzian to that under Gaussian is about 1:1·7.

Origin of the broader component

The increase in area of the broader (Gaussian) component suggests an increase in the fraction of starch chain in highly restricted conformation, as might occur in domains of aggregated chains or in crystallites. To test this idea, we measured the enthalpy of melting of crystalline domains using the differential scanning calorimetry (DSC) method. The value of melting enthalpy ΔH is generally taken to be proportional to the degree of starch crystallinity in the sample (Russell, 1987). The values for the enthalpy of melting observed here are in agreement with those reported previously (Biliaderis & Zawistowski, 1990; Russell, 1987). For example, Biliaderis and Zawistowski reported that ΔH for 40 wt% waxy maize stored at 6°C for 24 h was 5·2–6·4 J/g while in this work ΔH for 40 wt% waxy maize gel aged at 5°C for 19 h was 7·3–7·7 J/g of starch. For long ageing times, our ΔH values

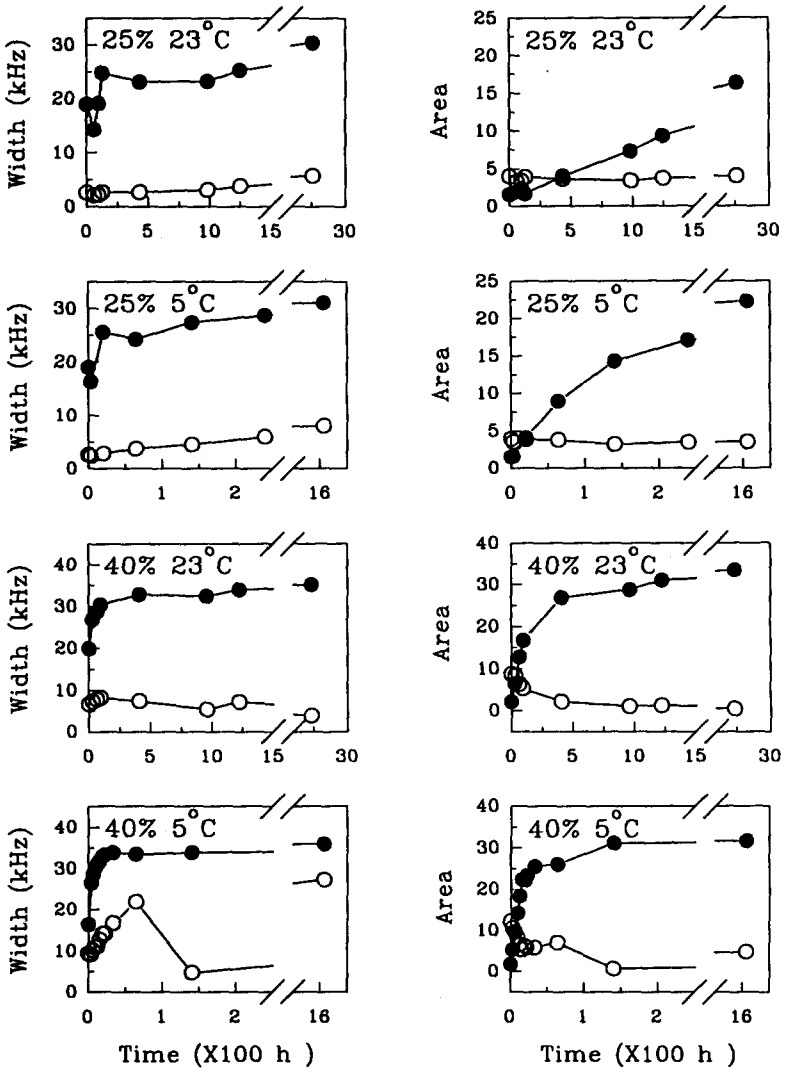

Fig. 4. Summary of deconvolution of the cross-relaxation spectra of 25 and 40 wt% waxy maize starch gel aged at 5°C and room temperature. (○) Lorentzian function; (●) Gaussian function.

approach the enthalpy of gelatinization of waxy maize starch (16·7 J/g), which is also in agreement with previously reported values of 12·5–20·0 J/g (Russell, 1987; Zobel, 1988).

The relationship between melting enthalpy and the area of the broad (Gaussian) spectral component is given in Fig. 5, which contains all the data collected. The good correlation of ΔH with the area of broad component for most of the data indicates that the area of the broad component of cross-relaxation spectra is proportional to the crystalline content when the crystallinity is well-developed in the sample. However, the data for the 40 wt% sample aged at room temperature and short times ($\leqslant$ 4 days) do not fall on the line. Thus under these conditions, some starch becomes nearly as rigid as crystalline, but does not present the latent heat associated with the melting of crystalline domains. In time ($\geqslant$ 17 days), crystallinity develops in this sample, and the corresponding data points return to the line (Fig. 5).

It was established previously that during ageing at room temperature, the crystallinity of amylopectin develops very slowly (Slade & Levine, 1987; Ring *et al.*, 1987). However, direct evidence for chain aggregation preceding crystallization has been difficult to obtain. Ring *et al.* (1987) reported that the gelation of 20% amylopectin solution at 1°C developed in 30–40 days, as indicated by the approach to the limiting values of shear modulus, volume change, melting enthalpy, and crystallinity from X-ray diffraction, all with the same time course. Recently Biliaderis & Zawistowski (1990) reported that for a 40 wt% waxy maize starch gel the modulus reached a limiting value after 24 h storage at 6°C, while melting enthalpy continued to increase up to 48 h of storage. Our results plus Biliaderis & Zawistowski's (1990) may provide evidence that chain aggregation precedes crystallization. We previously offered a general interpretation that the broad component observed in cross-relaxation spectra might arise from microscopic phase separation, during which

starch chains become immobilized due to their associations in a polymer-rich phase (Wu *et al.*, 1992). Thus the ability to fit the data to a sum of broader and narrower components should not in general be taken to identify a crystalline phase separate from other solid-like but less rigid components, except when the degree of crystallinity is established by independent measurement.

Analysis of kinetics of starch chain immobilization during ageing

The kinetics of starch ageing is usually analyzed using the theory of Avrami (Avrami, 1941; McIver *et al.*, 1968; Brennan & Sodah-Ayernor, 1973; Kim & D'Appolonia, 1977; Ciacco & Fernandes, 1979; Longton & LeGrys, 1981; Germani *et al.*, 1983; Rosario & Pontiveros, 1983; Russell, 1983, 1987; Bulkin *et al.*, 1987). The theory accounts for the time course of crystallization of matter in terms of the processes of nucleation and growth of crystallites. The theory of Avrami can be used to describe isothermal crystallization of supercooled polymer melts in such terms with the following relation (Mandelkern, 1964):

$$\ln(\theta) = -kt^n \qquad (8)$$

where θ is the fraction of material uncrystallized at time t, k is a rate constant containing both the nucleation and growth parameters, and n is the Avrami exponent, whose value is determined by the mode of nucleation and growth of the crystalline phase. For a semi-crystalline material, such as starch gel $(1 - \theta)$ is the degree of crystallinity determined from the measurement of different physical properties.

Although the application of the Avrami equation in a concentrated polymer-solvent system has been criticized (Mandelkern, 1964), using this approach to quantify the kinetics results in order to make comparisons with similar systems is still valuable (Russell, 1987). We applied the Avrami analysis to the time change of the area (A) of the broader component of the cross-relaxation spectra, with $\theta = (A_\infty - A_t)/A_\infty - A_0)$. Here the areas obtained from the longest retrogradation time were taken as A_∞. Avrami plots are presented in Fig. 6, and the resulting parameters (from a least squares fit) are summarized in Table 1. From Table 1, it can be seen that the values of n are close to 1. Values in this range have been reported for a variety of starch gels, and this is taken to suggest that crystallization follows rod-like growth from instantaneous nuclei. In order to compare the rate constants k, we also fit the data with the value of n fixed at 1·0. From the resulting k values it may be concluded that both temperature and concentration determine the immobilization rate in waxy maize starch gel, and that at least over the ranges measured, low temperature and high concentration

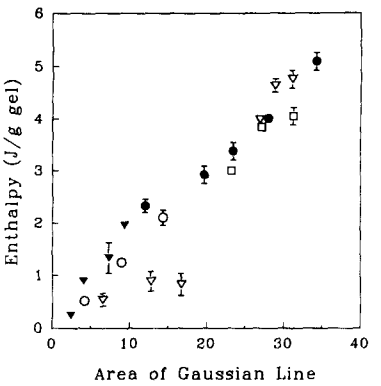

Fig. 5. The correlation of the melting enthalpy of crystalline starch with the area of the broad component of the cross-relaxation spectra. (●) starch gel concentration of 20, 25, 35, 40 and 45 wt% aged at 2°C for 14 days; (○) 25 wt% gel aged at 5°C for 20, 64 and 140 h; (▼) 25 wt% gel aged at room temperature for 97 h and 18, 41 and 52 days; (□) 40 wt% gel aged at 5°C for 18, 63, 144 h; (▽) 40 wt% gel aged at room temperature for 31, 65, 97 h and 17, 40 and 51 days.

Table 1. Avrami parameters

Sample (%)	Temp. (°C)	$k \times 10^3$	n
40	5	43	1·11
40	23	9·1	0·88
25	5	1·4	1·33
25	23	0·29[a]	1·04[a]
Sample (%)	**Temp. (°C)**	**$k \times 10^3$ (h^{-1})**	**n**
40	5	56	1
40	23	5·2	1
25	5	4·8	1
25	23	0·36[a]	1

[a]The area for 25 wt% retrogradation at 5°C for 67 days was taken as the infinite value.

speed up the process. A value of 46×10^{-3} h^{-1} of k for waxy maize starch-water 43:57 (w/w dry basis) ageing at 21°C has been reported by Russell (1987) using the DSC method. Our waxy maize starch contains about 12% moisture as determined by vacuum oven drying; thus the dry starch content for a 40 wt% sample is actually about 35 wt%. Taking into account the higher temperature and lower starch concentration of our sample, the k values determined from cross-relaxation spectroscopy (Table 1) are roughly comparable to those from the DSC method.

The decrease in the mobile fraction of starch during ageing

The observation of an increase in mass fraction of immobilized starch during ageing (Figs 2 and 4) raises the question of its origin. Since total starch concentration in the sample is constant, there must be a conversion of mass from higher mobility fractions.

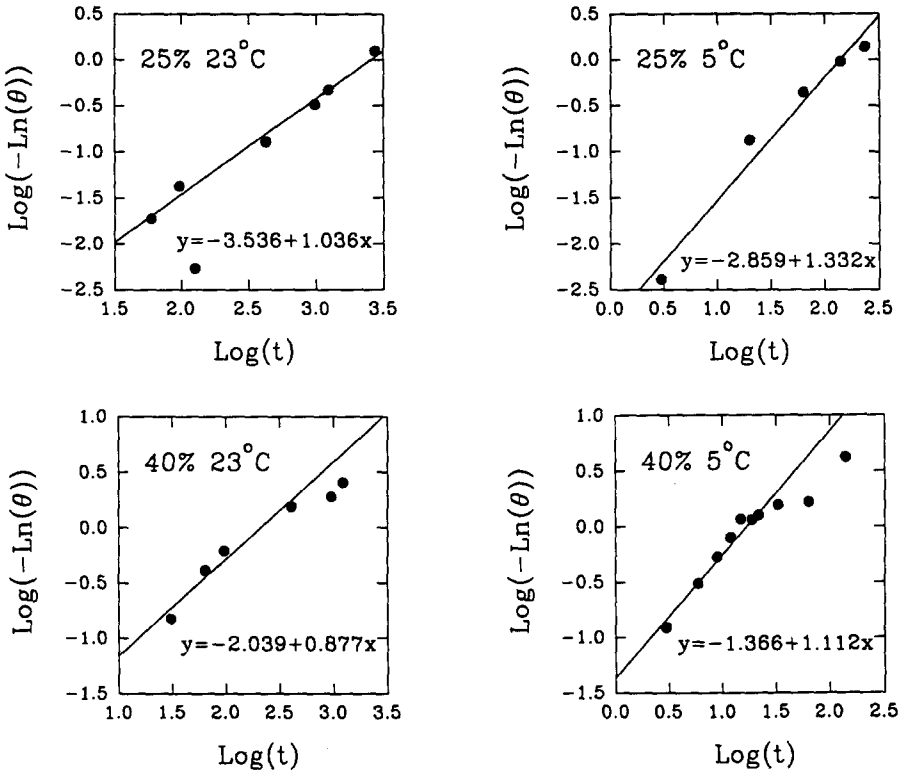

Fig. 6. Avrami plots of ageing as monitored by the broad component of the cross-relaxation spectra.

However a net conversion from the less rigid starch fraction contributing to the Lorentzian spectral component does not seem likely, since the area of this component apparently does not change much during ageing (Fig. 4). Components with mobility producing line widths of about 2 kHz or greater are detectable by cross-relaxation spectroscopy. Therefore, highly mobile fractions must be detected directly using high resolution ^1H NMR. This method selectively detects small molecules in liquids, such as water molecules and solutes, as well as highly mobile segments of macromolecules. In order to appear in the high resolution spectrum, starch chain segments would have to possess values of correlation times for an effectively isotropic motion of less than 10^{-9} s. We measured ^1H NMR spectra of starch/^2H_2O gels during ageing at 5°C. Deuterated water was chosen to reduce the intensity of the ^1H_2O peak, and thus obtain better detection of starch peaks. The NMR spectra of a single 25 wt% starch/^2H_2O gel during ageing are presented in Fig. 7. The starch resonance peaks at 3·7 ppm correspond to CH protons (except for the anomeric proton) and the peak at 5·4 ppm corresponds to anomeric protons (Morris & Hall, 1982; McIntyre *et al.*, 1990). The integrated intensity of the starch peaks apparently decreases, and their widths increase during ageing. The intensity of the residual ^1H peak at the water ^1H frequency (4·8 ppm) arises as a result of proton-deuteron exchange of ^2H_2O with $-O^1H$ of starch (Taylor *et al.*, 1961) and moisture (^1H_2O) in the starch

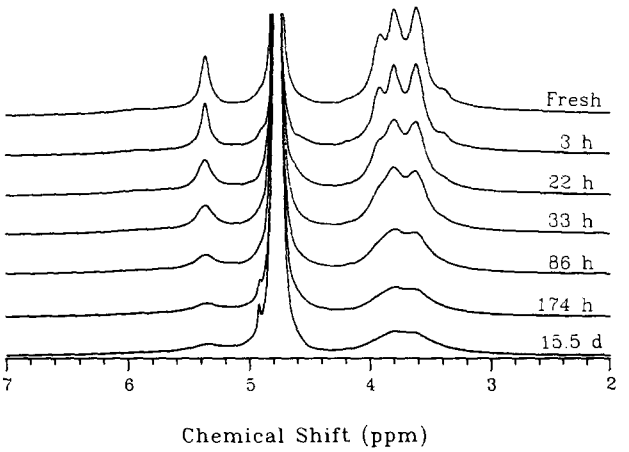

Fig. 7. Proton high resolution NMR spectra of 25 wt% waxy maize starch/D_2O gel. Spectral parameters: 1 pulse experiment, observe frequency 300 MHz, sweep width +/− 1500 Hz, data size = 4K, pulse width = 9 μs (flip angle 80°), relaxation delay = 5 s, number of acquisition = 8. The numbers marked on the spectra are the time (h) of the ageing at 5°C.

granules (≈ 12%, as determined by vacuum oven drying). If we make the reasonable assumptions that this peak contains all the protons from the granule moisture and all starch hydroxyl protons, that all water molecules are highly mobile, and that the fraction of immobile exchangeable protons is negligible, then this peak can serve as an internal standard. The fraction of starch which is highly mobile can be calculated as follows:

$$f_{\text{mobile}} = \frac{I_{\text{starch}}}{I_{\text{water}}^{*}\,(X_{\text{CH}}/X_{\text{OH}})} \qquad (9)$$

where I_{starch} is the sum of NMR integrals of the peaks at 3·7 and 4·5 ppm, I_{water} is the integral of the water peak, X_{CH} is the mole fraction of non-exchangeable protons of starch, and X_{OH} is the mole fraction of exchangeable protons from the starch hydroxyl group and moisture. The mole fractions are readily obtained from the stoichiometry of the starch-water mixture. The decrease of the function f_{mobile} during ageing is illustrated in Fig. 8. From Fig. 8 it is clear that the highly mobile fraction in the 40 wt% sample decreases much faster than in the 25 wt% sample. This is consistent with the observation that the immobile component of the 40 wt% sample increases faster than the 25 wt% sample (Fig. 2). The initial value of the highly mobile fraction is higher for the more dilute sample. This is expected on the basis of previous results (Wu *et al.*, 1992, and Figs 2 and 4, this paper). We note that the values of f_{mobile} are somewhat greater than the previous reported values from ^{13}C NMR measurements (Callaghan *et al.*, 1983; Hansen *et al.*, 1989), which were 0·6 for a fresh 10% wheat starch paste (Callaghan *et al.*, 1983) and 0·8 for a fresh 30% wheat starch gel in 0·5 M sucrose solution (Hansen *et al.*, 1989). Great caution is required when comparing results from experiments involving different nuclei, spectrometers, sample types and NMR methods. Nonetheless, our weakest assumption may be that all water molecules are highly mobile in concentrated starch gel. While further experiments may be necessary, f_{mobile} still serves as an indicator of the highly mobile fraction of starch.

The curves in Figs 8 and 2 (5°C data) taken together imply that some starch mass converts from a highly mobile liquid-like state to an immobile solid-like state during ageing. At the same time, certain fractions of starch retain high mobility and intermediate mobility since all three fractions are present in the fully aged sample.

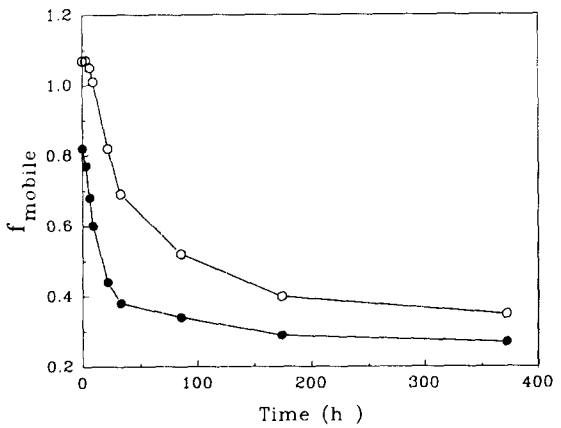

Fig. 8. The decrease of starch mobile component as a function of ageing time at 5°C for 25 wt% starch/D_2O gel (○) and 40 wt% starch/D_2O gel (●), respectively.

CONCLUSION

The ageing of gelatinized waxy maize starch has been studied using ^{1}H cross-relaxation NMR and high resolution NMR spectroscopy. During the ageing process, the immobile fractions of starch increase and the mobile fractions of starch decrease. Polymer chains in starch gels appear to be in three different motional states: (1) The most flexible: the correlation time of nearly isotropic segmental motion is short enough ($<10^{-9}$ s) to produce fairly narrow resonances in high resolution ^{1}H NMR spectra. (2) The most rigid: the motion of the segment is geometrically highly restricted and slow enough to give rise to a very broad component in the cross-relaxation spectrum. At least in the case of starch aged at lower temperatures, this component corresponds to the crystalline fraction of the polymer. In the case of starch aged at room temperature this component probably corresponds to associated starch chains in highly concentrated polymer domains. (3) The less rigid: the motions are intermediate between those in crystalline and dissolved states, and produce the narrower component of the cross-relaxation spectra. This component may correspond to unassociated or partially associated chains in polymer rich regions. Direct observations of polymer flexibility may be valuable in developing and testing models for the molecular origin of rheological (texture) response in carbohydrate polymers. Further studies based on such methods as simultaneous wide line and high resolution NMR (Wu *et al.*, 1992) are required to measure the absolute values of all motional classes in the multiphasic system.

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