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Observation by solid-state ^{3}C CP MAS NMR spectroscopy of the transformations of wheat starch associated with the making and staling of bread

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ABSTRACT

Wheat starch and wheat starch gels were characterised by ^{13}C CP MAS NMR spectroscopy. It was found that rotating-frame relaxation rates were significantly different for NMR resonances attributable to crystalline and amorphous regions in the wheat starch. Thus ^{13}C CP MAS NMR sub-spectra of crystalline and amorphous regions before and after gelation could be constructed from spectra obtained at different spin-locking times. Our results indicated that the amorphous regions in the native starch were unaltered by gelation and retrogradation, and we conclude, therefore, that they correspond to the branching regions of the amylopectin component. Upon gelation the amount of crystallinity decreased and a large proportion of the starch became mobile. Since the mobile components of the starch gel were not observed by CP MAS NMR, the kinetics of starch retrogradation could be determined by observation of the increase in crystallinity over a period of time. Starch gels were found to contain three distinct components: amorphous regions, crystalline regions, and liquid-like regions.

INTRODUCTION

Wheat and other cereal grains have as a major component small granules of starch along with proteins and oils. Starch is a mixture of two polysaccharides, amylopectin and amylose, which in wheat are present to the extent of 73–83% and 17–27%, respectively[1]. Both of these polysaccharides are composed exclusively of α-D-glucopyranosyl residues which are largely (1 → 4)-linked. In amylopectin 5–6% of the (1 → 4)-linked residues are also (1 → 6)-linked. This creates a highly branched structure. In contrast amylose is a lightly branched linear polymer. Amylopectin has a very high degree of polymerisation with molecular weights ranging from 10^{6} to 10^{9} daltons. A number of models based on X-ray analysis, enzymatic cleavage studies, and viscosity measurements have been proposed for the arrangement of the amylopectins within the starch granule[2]. The most recent of these are based on

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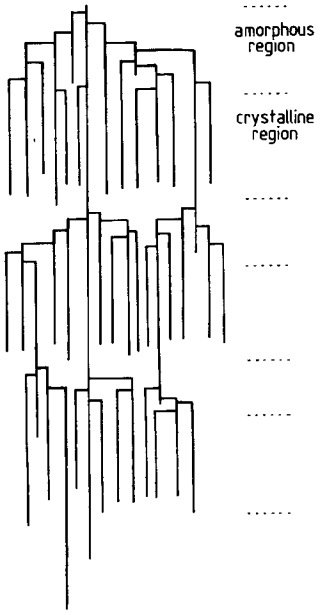

Fig. 1. Hypothetical cluster model of amylopectin in starch granules based on models discussed in ref. 2. Formation of double helices is not shown.

cluster models, such as the one illustrated in Fig. 1 (ref. 3). The function of amylose and its location within a starch granule is not well understood.

Two major crystalline types of starch are found in native starch granules. The so-called A form is found in cereal starches, while the B form occurs in tuber starches. The different forms are readily and unambiguously identifiable by X-ray diffraction[4] or by ^{13}C CP MAS NMR[5,6]. In both forms the polysaccharide chains are thought to associate in left-handed double helix conformations[7], but to differ in their unit cell dimensions and in water association.

The most notable difference between the ^{13}C CP MAS NMR spectra of the A and B forms is the occurrence of the C-1 peak as a triplet ($\approx$ 102.5, 101.5 and 100.6 ppm) in the A form but as a doublet ($\approx$ 101.5 and 100.5 ppm) in the B form[5,6]. Both forms contain a distinct C-6 peak at $\sim$ 62.7 ppm.

Another form, the V form, can be obtained by precipitation of amylose with a variety of organic solvents and compounds. The polysaccharide of the V form is thought to exist in a single helix conformation. Solid-state NMR spectra of V forms have been reported by Horii et al.[8], Veregin et al.[9], and Gidley and Bociek[10].

On heating above $\sim$ 70° starch readily absorbs water and gelatinizes. The starch gel so formed is not stable, and, on cooling, starch crystallization or retrogradation occurs, usually over a period of a few days. This action is the principal cause for the staling of bread[11]. The retrogradation has been followed by a number of

techniques, including differential thermal analysis, elasticity measurements, and X-ray diffraction. X-ray diffraction studies have shown that native wheat starch gels give a weak diffraction pattern typical of starches in the V form[12], and that the recrystallisation occurs to either an A or B form depending on the amount of water present[13].

We have re-examined starch and starch gels using ^{13}C CP MAS NMR and found that it is possible to obtain separate sub-spectra for crystalline and amorphous regions in both moistened wheat starch and wheat starch gels. Our results indicate that the amorphous regions in the native starch are unaltered by gelation and retrogradation, and we conclude therefore that they correspond to the branching regions of the amylopectin component. Upon gelation the amount of crystallinity decreases and a large proportion of the starch becomes mobile and cannot be observed by cross-polarisation. The kinetics of starch retrogradation can be determined by observing the increase in intensity of resonances for crystalline starch components.

EXPERIMENTAL

^{13}C CP MAS NMR spectra were acquired using a Varian XL200 NMR spectrometer. Wheat starch samples were packed into 7-mm sapphire rotors and spun at 2–3 kHz in a probe from Doty Scientific. Moisture in wet samples was retained by a thin film of halocarbon grease between the rotor and tight fitting Vespel end caps. Gelled samples were prepared directly in the rotor by heating the assembly in boiling water until the sample became translucent ($\sim$ 15 min). Proton rotating frame relaxation times, $T_{1\rho}(H)$, were determined by spin-locking the proton magnetization before cross-polarizing to measure the residual proton magnetization.

Subspectra were obtained following the method of Newman and Hemmingson[14]. Pairs of spectra were acquired at $T_{1\rho}(H)$ of 0 and 4000 μs. Subspectra were then generated by a weighted substraction of the pairs of spectra. To generate the sub-spectrum of the crystalline component, the weighting was adjusted to remove the peak at 82 ppm, whereas the peak at 101.5 ppm was eliminated to generate the sub-spectrum of the amorphous component.

RESULTS AND DISCUSSION

The spectra observed for the solid-like components of native wheat starch are similar to those reported for A starches. However, additional peaks occur at 103.3 and 82.0 ppm, which are thought to arise from the amorphous regions within the starch. Protons associated with these peaks were found to relax at a significantly faster rate in the rotating frame [$T_{1\rho}(H) = 4000$ μs] than those associated with the peaks due to the A form [$T_{1\rho}(H) = 8000$ μs].

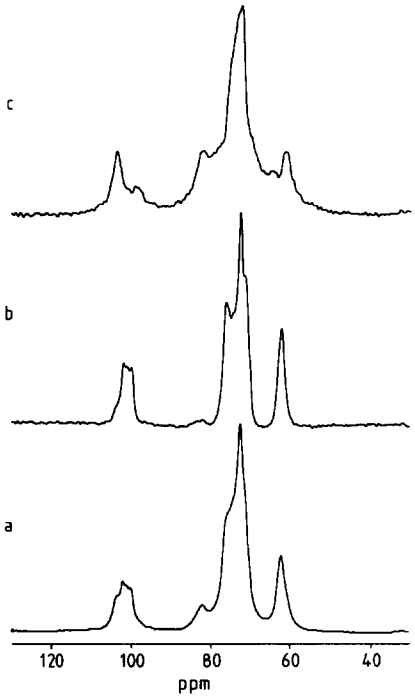

Fig. 2. ^{13}C CP MAS NMR spectra of moistened wheat starch: (a) normal spectrum; (b) A form sub-spectrum; and (c) amorphous form sub-spectrum.

Proton spins in solids are in contact with each other via strong dipolar couplings and generally have identical relaxation times due to mixing by spin diffusion. However, spin diffusion is not sufficiently fast to mix proton spins in large enough, spatially distinct, regions. That the peaks at 103.3 and 82.0 ppm have different rotating frame relaxation rates, suggests that in the starch granules there are distinct regions of two types corresponding to crystalline and less crystalline regions. In order to observe significant differences in rotating frame relaxation, the regions must be greater than 1 nm in dimension[15]. By conducting spin diffusion experiments similar to those of Zumbulyadis[16], however, it could be shown that the separation between regions is probably not more than 6 nm. This size is similar to that expected for the average separation of the crystalline and amorphous regions in the cluster model[2] (Fig. 1). It is possible to obtain separate sub-spectra of the two regions by taking combinations of spectra at different rotating frame relaxation times, as shown in Fig. 2. This method has recently been used to determine cellulose crystallinities in wood. The sub-spectrum in Fig. 2b contains resonances characteristic of the A form. The resonances of the other sub-spectrum are broader and are similar to published spectra of amorphous amylose. As Gidley and Bociek have noted[10], spectra of amorphous amyloses are in many aspects similar to spectra of single helical V forms of amylose. The similarity of this latter sub-spec-

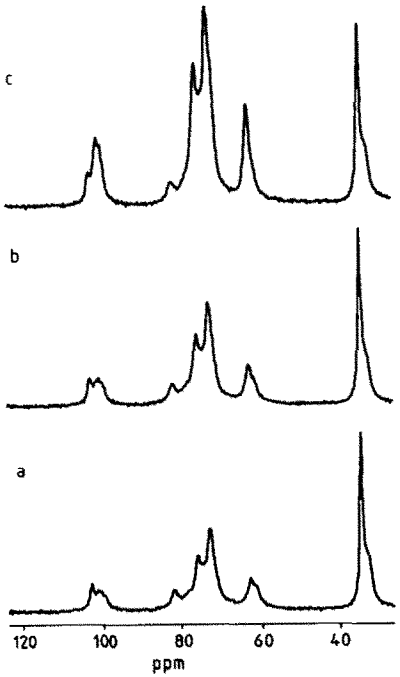

Fig. 3. ^{13}C CP MAS NMR spectra of moistened wheat starch (500 μL of water per g of starch) that has been gelatinised by heating. Spectra were recorded after (a) 0, (b) 6, and (c) 140 h. The peak at 33.0 ppm is due to polyethylene which is used as an internal intensity reference.

trum to that of amorphous amylose, and the faster $T_{1\rho}(H)$ for this component, suggest that this sub-spectrum is associated with random single helices, present in the (1 → 6)-linked branching regions of the native wheat starch. Chains in the branching region are expected to be unable to associate to form double helices.

Although our sub-spectra of the amorphous component of wheat starch is similar to that of amorphous amyloses, two additional weaker peaks are also observed at 98 and 64 ppm. These peaks may be due to the C-1 and C-6 carbons, respectively, of the (1 → 6) linkages, or different conformation of the chains within the branching regions.

Heating starch in the presence of water results in gelatinization. The degree to which this occurs depends on the water content. When moistened wheat starch (500 μL of water per g of starch) was heated in a sealed container at 95° for 15 min, the starch became translucent as gelatinization occurred. The NMR spectrum of the cooled product is shown in Fig. 3a. The most noticeable changes between this and the spectrum of the moistened unheated starch in Fig. 2a were a 65% loss in signal intensity, an increase in the relative intensity of the peaks at 82.0 and 103.3 ppm peak and the splitting of the C-6 peak into two peaks at 60.6 and 62.0 ppm. That is, upon gel formation the dominant component observable by CP MAS NMR is the amorphous form. That the amorphous form can be observed by

cross-polarization is probably due to its association with the branching regions of the amylopectins. Large amounts of branching should anchor chains close to the branching points, within the gel matrix and restrict their mobility. By using polyethylene as an internal standard, it was shown that the amount of the amorphous form remained constant before and after gelation. The spectrum (Fig. 3a) also shows that the gel still contains a small amount of another more crystalline form. Sub-spectra of the starch immediately after gelation could not be obtained because of a low signal-to-noise ratio. The minor crystalline form may be due to double helices formed from small regions of entanglement between adjacent chains, crystallisation occurring during the course of the experiment and a rapid crystallising amylose component[17]. The loss in signal intensity upon gelation appears to be caused by the formation of mobile liquid-like components of the starch polymers. These are unable to cross-polarise due to long T_{CH} and short $T_{1\rho}(H)$. Gidley[18] reported similar behaviour for gels of amylose. He showed that these gels have two ranges for the proton T_2 values of $\sim 10\ \mu s$ and 1–10 ms which correspond to the solid and mobile components, respectively. As Gidley has shown and we have also found, it is possible to obtain spectra for these mobile components, using normal Bloch decay experiments.

Over a period of days the starch recrystallised and the spectra showed increasing contributions from the crystalline components (Fig. 3b and 3c). After ~ 3 days the intensity was 71% of that observed for the original moistened unheated sample. A spectrum of retrograded starch obtained after 2 days is shown in Fig 4a. A sub-spectrum of the crystalline component (Fig. 4b) showed that retrogradation had occurred to the B form since the C-1 peak is a doublet. From X-ray diffraction studies, Hellman et al.[13] obtained similar results for retrogradation at these water contents. The other sub-spectrum (Fig. 4c) was similar, as expected, to the amorphous form. When the experiment was repeated but with less water, ^{13}C CP MAS NMR demonstrated that the retrogradated starch had crystallised in the A form.

The kinetics of starch retrogradation were investigated by measuring the total intensity for all peaks in the NMR spectrum relative to an internal standard of polyethylene, as the starch crystallised. Crystallisation of starch can be analyzed in terms of the Avrami equation[19] (eq *1*) which was developed to explain rates of growth of crystalline regions in polymers,

$$\theta = \exp(-kt^n) \qquad (1)$$

where θ is the fraction of the mobile non-crystalline components, k is the rate constant and n is the Avrami exponent. From our results we obtained $n = 1.04 \pm 0.03$, a value similar to that obtained by McIver et al.[19] using differential thermal analysis, and $1/k = 40 \pm 6$ h. McIver et al. noted that an Avrami exponent of one implies rod-like growth and instantaneous nucleation. The nucleation sites probably correspond to the regions of chain entanglement formed during gelation. Crystallisation then proceeds between two adjacent chains to form double helices.

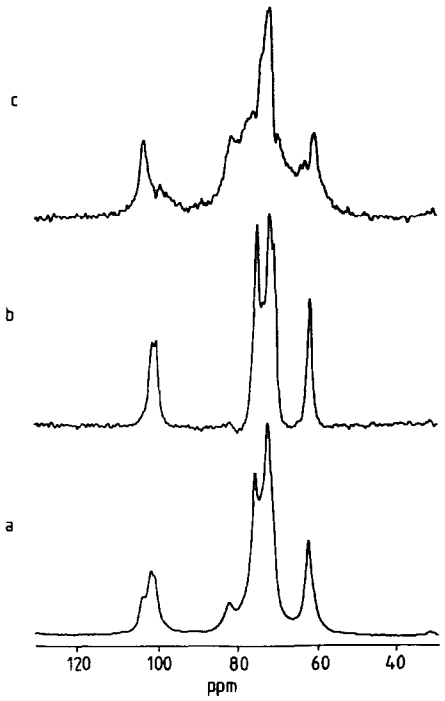

Fig. 4. ^{13}C CP MAS NMR spectra of moistened wheat starch (500 μL of water per g of starch) that has been gelatinised by heating, cooled and left for 48 h. (a) Normal spectrum, (b) B form sub-spectrum, and (c) amorphous form sub-spectrum.

However, not all the starch recrystallizes even after several days and some 30% of the starch still remained unobserved by cross-polarization. As in the case of amylose[18] it appears that the retrograded starch consists of crystalline regions composed of double helices connected by more mobile single helix segments, with the branching regions of the starch remaining essentially unaltered by gelation and retrogradation.

Recent work by Russel[20] has re-examined starch retrogradation using differential scanning calorimetry after very long periods of retrogradation. Although their results seem to indicate that the short-term behaviour (< 250 h) was satisfactorily explained by the Avrami equation with exponent $n = 1$, for time periods of several weeks an Avrami exponent of $n = 0.62$ gave a better fit to the experimental data. However no error analysis was reported, and visual inspection of their results appeared to indicate that they are less accurate than the CP MAS NMR results reported herein. Regardless of this, kinetic data extending to several weeks seems unlikely to be relevant to the bread staling problem. Ring and coworkers[17,21] have also re-examined starch retrogradation using a variety of techniques. Their results indicated that a starch gel consists of swollen starch granules embedded in an amylose matrix. The gels examined by these workers contained more water than in

this study, and no analysis of the kinetic data was attempted, so that their results are not directly comparable to those reported herein. They implied, however, that the amylopectin and amylose components of starch crystallised at different rates. The excellent fit to the Avrami equation for the NMR data of this study would seem to indicate that the retrogradation behaviour of the amylose and the linear chains of the amylopectins was identical at the water contents used in this study.

^{13}C CP MAS NMR provides an intuitive picture of the starch retrogradation process as peaks in the NMR spectrum can be assigned directly to crystalline or non-crystalline components. It has been shown in this study that a starch gel consists of a mixture of at least three components, crystalline regions, amorphous regions, and liquid-like regions.

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