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Solid-state NMR studies on the structure of starch granules

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Abstract

Resonances expected for carbon atoms in the amorphous regions of moistened starch granules are not visible in ^{13}C CP/MAS NMR spectra. These resonances could be observed in spectra obtained by using a single-pulse, Bloch-decay sequence, with magic-angle spinning (SP/MAS). This implies that the amorphous regions in moistened starch granules are mobile and have properties similar to a rubberlike polymer. For moistened wheat starch granules, proton rotating frame relaxation times for resonances assigned to the amylose–lysophospholipid inclusion complexes were shorter than those of the crystalline starch. Relaxation differences were used to generate subspectra of the crystalline starch and the amylose–lysophospholipid inclusion complex. Our results suggest that the inclusion complexes occur in distinct regions of the starch granule. There are thus three distinct components making up the wheat starch granule: (i) highly crystalline regions formed from double-helical starch chains, (ii) solid-like regions formed from lipid inclusion complexes of starch, and (iii) completely amorphous regions, associated with the branching regions of the amylopectin component of starch and possibly the lipid-free amylose. All these components can be observed separately using different solid-state NMR techniques and would be expected to contribute to the physical properties of a wheat starch granules.

Keywords: Starch granules; ^{13}C CP/MAS NMR spectroscopy; Structure, solid-state

1. Introduction

Cereal starches are important constituents of a wide variety of baked goods. We are interested in examining the structure and properties of cereal starches since they

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determine the structure and properties of baked goods. Cereal starches are in general composed of two polysaccharides, amylose and amylopectin. Both contain 4-linked α-D-glucopyranosyl units. Amylose is a linear polymer, whereas amylopectin is highly branched, since 5–6% of the 4-linked units are also 6-linked. The branching is not random but bunched into distinct portions of the amylopectin polymer. It is generally accepted that this arrangement results in a structure that is organised on the nanometer scale. The widely accepted cluster model proposes that amylopectin is composed of alternating regions of amorphous starch, containing the branch points, and highly crystalline starch, composed of double helices of starch chains [1]. Both regions are about 5 nm across [2]. The other major components of cereal starches are lipids. Lysophospholipids are the dominant lipid components of wheat starch, but not in other cereal starches [3]. Certain cereal hybrids, the so-called waxy starches, have low lipid content and correspondingly low amylose content.

Three distinct crystalline forms of starch have been characterised. The A and B forms, found in cereal and tuber starches, respectively, consist of double-helical arrangements of starch chains with difference in water association and crystal symmetry. The V form, which consists of starch chains in a single-helical arrangement, has a cylindrical hydrophobic cavity that can include molecules such as iodine and the alkyl chains of surfactants. Each form can be unambiguously identified from characteristic X-ray diffraction patterns and by ^{13}C CP/MAS NMR resonances.

A number of previous papers [4–7] have examined the structure of starch using ^{13}C CP/MAS NMR spectroscopy. Most of the resonances cannot be distinguished or have not been assigned, but the C-1 carbon atoms have chemical shifts characteristic for each of the three types of conformation. For the A conformation, which has three non-identical sugar residues, the C-1 peak is a cluster of three peaks (~ 102, 101 and 100 ppm), while for the B conformation, which has two non-identical sugar residues, the C-1 peak is a cluster of two peaks (~ 101 and 100 ppm) [4]. Although the C-1 carbon of the V conformation has a peak at 103.7 ppm, in non-waxy cereal starches it appears only as a shoulder on the downfield C-1 resonance of the A conformation. More clearly differentiated is the peak at ca. 82.0 ppm due to the C-4 carbon of the V conformation.

Morrison et al. [8,9] have been able to show using ^{13}C CP/MAS NMR spectroscopy that the lipids in non-waxy starch granules form inclusion complexes with the amylose component of starch. This result was based on finding that a small peak at 31.2 ppm had a chemical shift similar to that observed for alkyl carbons in lipid inclusion complexes of V-amylose, and noting that the shoulder, expected for starch in the V conformation, is more intense in non-waxy than waxy starches. Similarities between the lines in the X-ray diffraction spectra of wheat starch, and amylose complexed with fatty acids, prompted Katz and van Italie [10] and Hellman et al. [11] to suggest a similar result more than 40 years ago. The results of Morrison et al. are, however, more convincing. We present new results that not only support their interpretation, but have also allowed us to obtain new insights into the structure of starch granules. We also correct some misconceptions in the literature on the interpretation of ^{13}C CP/MAS NMR spectra of starch.

2. Experimental

^{13}C solid-state NMR spectra were acquired on a Varian Unity 500 MHz spectrometer. Samples were packed into 5-mm rotors and spun at speeds of 5 kHz in a probe from Doty Scientific. Spectra of native starch samples were acquired moistened (33% water) and dry. To retain moisture in wet samples over the time period of the experiment, end caps of the rotor were smeared with a thin layer of halocarbon grease. Proton and ^{13}C rf field strengths were about 60 kHz, corresponding to a 90° pulse width of 4 μs. Cross-polarisation times were about 1000 μs.

Subspectra of moistened wheat starch were generated by the method of Newman and Hemmingson [12] as follows. Two spectra were acquired with a similar number of transients. The first was a normal ^{13}C CP/MAS NMR spectrum. The second was acquired in a similar manner but with 5000 μs of proton spin-locking preceding the cross-polarisation. This spin-locking time was near optimal for obtaining the greatest S/N ratio in the sub-spectra. Sub-spectra were generated by a weighted subtraction of these two spectra. The weighting was adjusted so that in each sub-spectrum no peaks fell below the base line.

Liquid-like components were observed using a single-pulse Bloch-decay sequence and magic-angle spinning, ^{13}C SP/MAS [13]. For the variable temperature studies, the temperature of the rotor was calibrated by acquiring ^{1}H spectra of ethylene glycol under identical spinning speeds and heating conditions. The chemical shift differences between the two proton resonances of the ethylene glycol was used to calculate the temperature [14].

Proton rotating frame relaxation times, $T_{1\rho}$, were determined on a modified Varian XL200 spectrometer using a 7-mm rotor and probe from Doty Scientific. For the proton $T_{1\rho}$ determinations the time of proton spin-lock before cross-polarisation was systematically varied. Peak heights of the carbons associated with different relaxing protons were then measured and results fitted to Eq. (1) to obtain proton $T_{1\rho}$.

$$I = I_0(1 - e^{-t/T_{1\rho}}) \tag{1}$$

In Eq. (1) I is the peak height at time t and I_0 is the peak height at time $t = 0$.

3. Results and discussion

Branching regions of the amylopectin component of starch granules.—Comparison of ^{13}C CP/MAS NMR spectra of moistened samples of wheat starch [Fig. 1(a)] and waxy maize starch [Fig. 1(b)] shows some marked differences. A shoulder at 103.5 ppm, which occurs as a distinct peak in the resolution-enhanced spectrum of Fig. 1(a), and the peak at 82.4 ppm for the wheat starch are absent from the spectrum of the moistened waxy maize starch. However, in the dry waxy maize starch, [Fig. 1(c)] both of these spectral features are present. The moistened waxy maize starch appears to be more crystalline than dry waxy maize starch, as the line-widths in Fig. 1(b) are narrower than those in Fig. 1(c).

Horii et al. [6] have attributed similar observations of apparent line-width narrowing

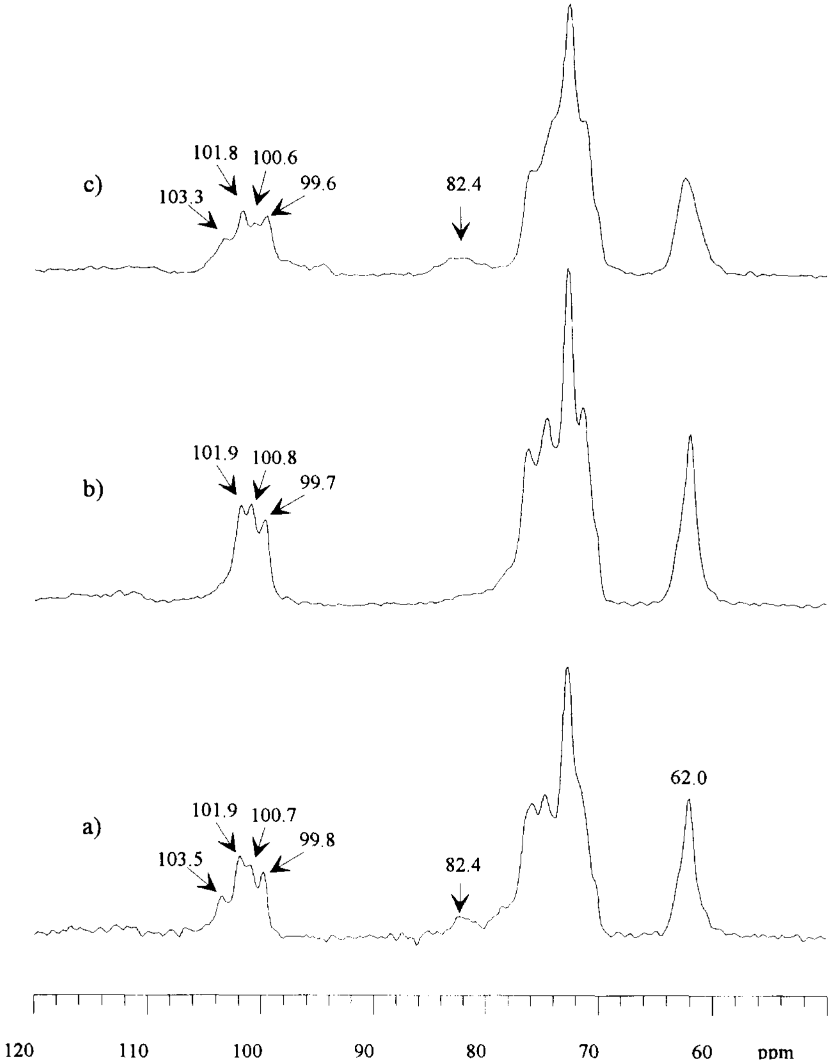

Fig. 1. ^{13}C CP/MAS NMR spectra of (a) wheat starch with 33% added water, (b) waxy maize starch with 33% added water, and (c) dry waxy maize starch. All spectra have been resolution enhanced by applying a mild Lorentzian to Gaussian transformation to the FID.

on addition of moisture to an increase in the fraction of starch that is in the double-helical arrangement. They assumed that dehydration of starch causes some of the double helices to unwind. In contrast, Veregin et al. [4,7] postulated that the effect may originate from increases in the mobility of polymer chains in the crystalline regions of starch, due to the plasticising effect of water. As they noted, this explanation is inconsistent with ^{13}C T_1 relaxation data for signals associated with these crystalline components, which are similar for the dry and moistened starches [6], and also it did not seem reasonable to them that the more mobile component should be the more crystalline. Both of these interpretations, however, are based on misconceptions about the physical changes that occur to the components in starch granules on moistening.

Only in the last decade has it been perceived that the amorphous regions of amylopectin, that is the branching regions, undergoes a glassy transition at a temperature, T_g, that depends on moisture content [15]. For gelatinised starch, T_g can decrease by as much as 10°C/wt% of water [16]. The water appears to act as a plasticiser, increasing the mobility of the polymer chains in the amorphous regions. This has important effects on the physical properties of starch and starch gels. Previously we have shown [17] that a large proportion of the components of freshly gelatinised starch are mobile or liquid-like, and as a result these components are invisible in spectra obtained using ^{13}C CP/MAS NMR spectroscopy. Cross-polarisation depends on heteronuclear dipolar couplings between ^{1}H and ^{13}C nuclei. If these couplings are averaged because of rapid molecular motion, then cross-polarisation does not occur. Resonances of mobile components will not be seen in a ^{13}C CP/MAS NMR spectrum, but can be observed by a ^{13}C SP/MAS sequence, a type of sequence commonly used in 1D solution NMR spectroscopy.

The above results for starch gels have led us to consider that the resonances of the amorphous regions of moistened native starch may similarly not appear in ^{13}C CP/MAS NMR spectra.

We have obtained spectra using ^{13}C SP/MAS NMR of moistened wheat starch and moistened waxy maize starch at 25 and 35°C (Fig. 2). The resonance intensity in these spectra is similar to that in the corresponding ^{13}C CP/MAS spectrum, if allowance is made for the fourfold sensitivity enhancement that occurs during cross-polarisation [18]. This indicates that a major component (about half) of the moistened starches has liquid-like behaviour. The peaks are surprisingly sharp, and the spectra are similar to, albeit broader than, solution-state spectra of amylopectin dissolved in deuterated dimethyl sulfoxide, acquired at 80°C [19]. Measured line-widths are 700 and 400 Hz for the C-1 carbons of wheat and waxy maize starch at 25°C [Figs 2 (a) and (b)], but these values are approximately halved for only a 10°C increase in temperature [Figs 2 (c) and (d)]. Since waxy maize starch contains only small amounts of amylose and lipids, for this starch the mobile components must be associated with the amylopectin component. In wheat starch, which contains significant amounts of lipid-free amylose, some of the signal intensity in the SP/MAS spectra may be due to mobile amorphous lipid-free amylose.

That amylopectin is organised, according to the cluster model, as a series of alternating regions containing crystalline starch, formed by a double-helical association of two linear starch chains, and amorphous starch, associated with the 1 → 6 branch points of amylopectin, has been widely accepted. This is based in part on the results of extensive enzymatic analysis of amylopectins [2], and electron diffraction studies of acid degraded starch granules [20]. The melting point of the crystalline regions of wheat starch starts at about 58°C [21], a temperature too high to account for the room temperature mobility observed in starch granules. The mobility must therefore be associated with the amorphous regions of amylopectin and possibly, when present, with lipid-free amylose.

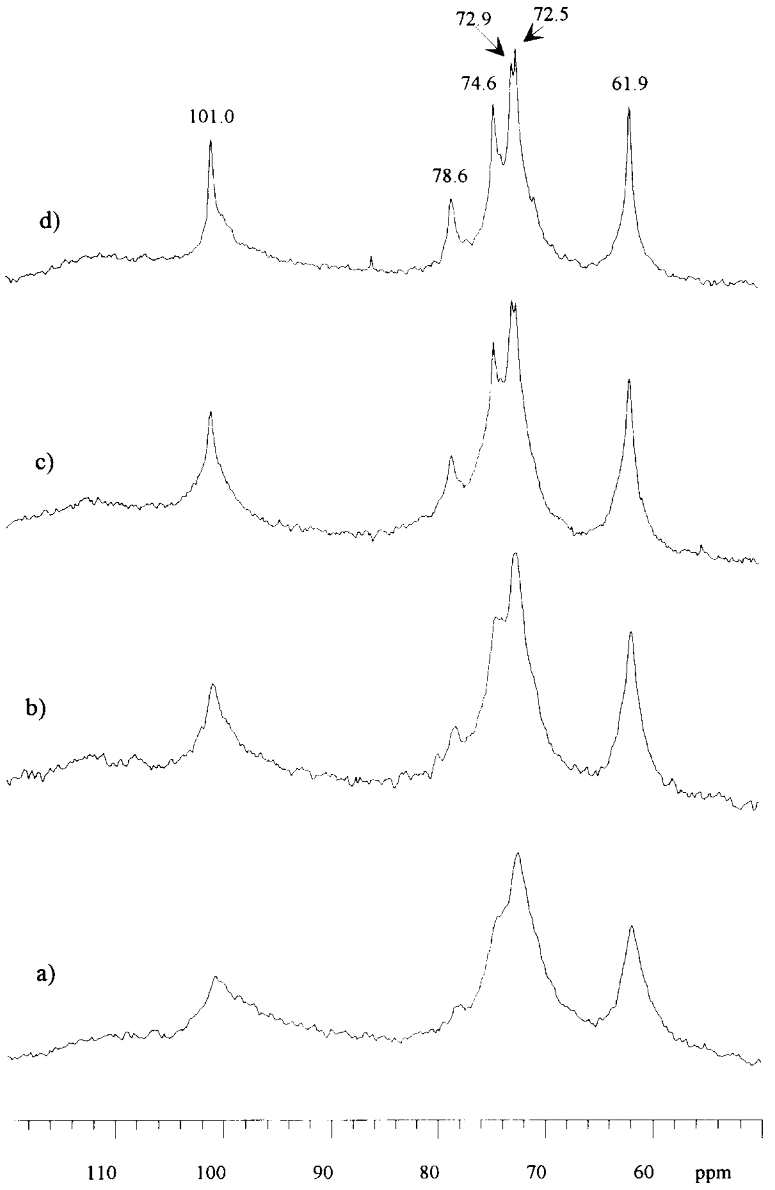

Fig. 2. ^{13}C SP/MAS NMR spectra of starches with 33% added water: (a) wheat starch at 25°C, (b) waxy maize starch at 25°C, (c) wheat starch at 35°C and (d) waxy maize starch at 35°C. The broad background signals are due to Kel-F, which forms part of the stator in the MAS probe.

The ^{13}C relaxation times (T_1) for the amorphous component of the moistened wheat starch are short, about 300 ms for the C-6 carbon atoms and 600 ms for the other carbon atoms. For comparison, values of about 5 s for the C-6 carbon atoms and 17 s for the others have been obtained for the crystalline A and B conformation [6]. Examination of the same samples in a solution probe using normal proton decoupling power levels failed to reveal any signals. This suggests that MAS is still required to average chemical shift anisotropy, and high-power decoupling is needed to remove heteronuclear dipolar couplings.

Slade and Levine [22] found for freshly gelatinised wheat starch a reversible glassy transition temperature, T_g, at $-5°C$. They concluded that at $-5°C$ the amorphous regions of starch become mobile due to the plasticising effect of water. They did not observed a T_g for native, i.e., non-gelatinised, wheat starch at this temperature, but instead assigned a DSC endotherm occurring at temperatures of ~ 50–60°C to the glass transition for the amorphous region. In contrast, Noel and Ring [23] observed no glass transition near the melting point of crystalline starch in amylopectin prepared from potato starch.

Our NMR results show significant mobility of moistened native wheat and waxy maize starch in the amorphous regions and significant increases in mobility occurring over a small temperature range of 10°C. This is consistent with a partial plasticising, since the line widths are very broad compared with those of starch gels acquired at 90°C.

These NMR results explain the apparent increase in crystallinity that occurs when starch is moistened, as observed by Horii et al. [6] and Vergrin et al. [7]. When starch is moistened the signals of the amorphous region become invisible in the CP/MAS NMR spectra. Therefore, the masking effects of the broad resonances due to the amorphous region are lost and only the sharp resonances of the crystalline components are left.

Amylose–lipid inclusion complex.—Gidley and Bociek [24] have shown that the C-1 and C-4 chemical shifts of dry amylose–lipid inclusion complexes observed by CP/MAS NMR spectroscopy are coincident with shifts of starch from the amorphous regions of dry starch. They concluded that a portion of the starch in the amorphous regions must be in a single-helical conformation, known to occur in the inclusion complex. The similarity between the NMR resonances of two starch components led us, in a previous paper [17], to incorrectly assign signals in the spectra of moistened starches to the amorphous regions of starch [14] when they are in fact due to the amylose–lipid inclusion complex, as we show below. We also concluded that the amorphous parts of a starch granule form distinct regions within a starch granule and that these are in intimate contact with the crystalline regions. Our previous results now need to be reinterpreted in terms of amylose–lipid inclusion complexes.

The proportion of lysophospholipids in a starch granule is quite small, about 1 g/100 g of dry wheat starch [3]. This proportion would appear too small to account for all of the intensity of the shoulder at 103.5 ppm in moistened wheat starch, but this is not so. The average molecular weight of the lysophospholipids in wheat starch is about 500. Therefore, the molar ratio of lipids to starch glucose residues is about 1:300. Morrison et al. [9] prepared an inclusion complex of extracted starch lipids with amylose in which the molar ratio of lipids to glucose residues was about 1:3. This is quite high and probably represents the maximum amount of lysophospholipids that can be included into the amylose. Data published by Godet et al. for fatty acid complexes with a series of malto-oligosaccharides [25] suggest that the most stable complexes were formed with oligomers of dp 40 containing two fatty acid groups. Thus, stable inclusion complexes could still form with molar ratios of lysophospholipid to starch glucose residues as low as 1:20 or 13.4 wt%. This is consistent with values of about 13 wt% for the amount of lysophospholipids in amylose complexes determined using a colorimetric assay devised by Morrison et al. [9]. In moistened starches about half of the starch is visible in the CP/MAS NMR spectra because the other half, associated with the amorphous regions, is mobile. The proportion of the C-1 peak manifold due to the amylose–lysophospholipid inclusion complexes could thus be calculated to be about 13%. This is close to the proportion contributed by the 103.5 ppm shoulder as calculated by curve-fitting the C-1 peak manifold [26].

Additional evidence that the 103.5 ppm shoulder is due to amylose–lysophospholipid inclusion complexes can be obtained by heating moistened wheat starch. Spectra acquired immediately after heating the samples to 80°C show little decrease in the signal intensity of the shoulder at 103.5. Even with prolonged heating at 100°C for 0.5 h, the shoulder decreases in intensity only by about 40%. In contrast, there is a large decrease in the signal due to crystalline starch at both these temperatures. This indicates that the crystalline starch is melting, and that there is little melting of the amylose inclusion complexes, as expected [27]. These results are consistent with the ^{13}C CP/MAS NMR spectra of dry and moistened waxy maize starch [Figs 1(c) and 1(b)]. In spectra of dry waxy maize starch, resonances are observed at 103.5 and 82.4 ppm, but these resonances are not observed in moistened waxy maize starch. This is because there are negligible lipids in waxy maize starch and consequently no starch in the V conformation.

Protons in solids are coupled to one another by strong homonuclear dipolar couplings. Generally for a homogeneous solid, all protons, even though they may be chemically distinct, have identical relaxation times due to proton spin diffusion, mediated by these dipolar couplings. However, for spatially distinct regions greater than about 1 nm across, spin diffusion is not fast enough to average the proton relaxation times, and each region can have a different proton relaxation time [28]. In this case proton relaxation times could be distinct because different molecular motions occur within each region. For starch, the rotating frame proton relaxation time, $T_{1\rho}$, is the most sensitive proton relaxation time to this regional difference.

Proton $T_{1\rho}$ values were determined for different regions in the moistened starch as explained in the Experimental. Values associated with the crystalline regions were determined from peak heights of the C-1 carbon resonance at 100.7 ppm and were found to differ from those determined from peak heights of both the C-1 carbon signal at 103.5 ppm and the C-4 carbon resonance at 82.4 ppm. To show that these latter two resonances are due to the amylose–lysophospholipids inclusion complex, it should be possible to show that proton $T_{1\rho}$ values determined from these resonances and the resonances of the alkyl chain of the lysophospholipids are the same. Although a resonance for the lysophospholipids observed at 31.5 ppm is distinct from those of starch, it was not possible to measure proton $T_{1\rho}$ values from this resonance because of poor S/N in the ^{13}C CP/MAS spectra. However, the ^{31}P CP/MAS spectra have good S/N because of the larger γ and 100% natural abundance of the ^{31}P nuclide, and proton $T_{1\rho}$ values were determined by measuring the peak height of the ^{31}P resonance in these spectra, as shown in Fig. 3. The lysophospholipid protons have $T_{1\rho}$ values the same as those determined from the resonances at 103.5 and 82.4 ppm ($T_{1\rho} = 3500 \pm 300$ μs),

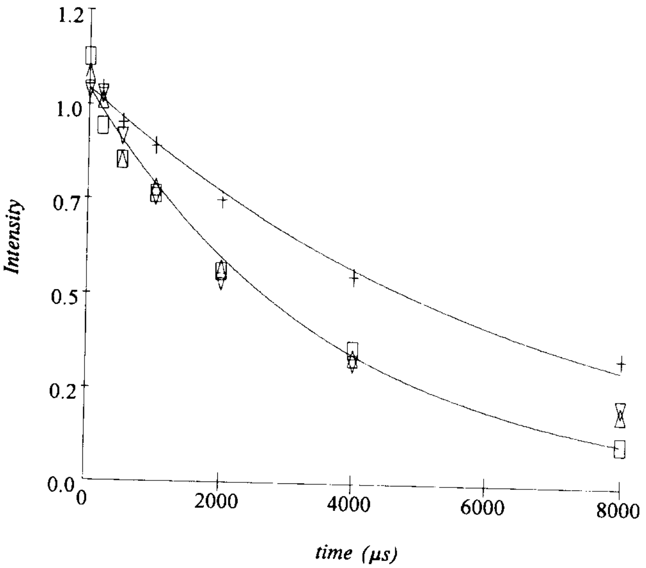

Fig. 3. Intensity of proton magnetisation at differing proton spin-lock times, measured indirectly by peak heights in the ^{13}C CP/MAS NMR and ^{31}P CP/MAS NMR spectra of wheat starch moistened with 33 wt% water. Peak positions, plot symbols and values obtained for proton $T_{1\rho}$ are: at 103.5 ppm, △, $T_{1\rho} = 3600 \pm 400$ μs; at 101 ppm, +, $T_{1\rho} = 6600 \pm 300$ μs; at 82.4 ppm, □, $T_{1\rho} = 3400 \pm 300$ μs; and for the ^{31}P resonance, ▽, $T_{1\rho} = 3500 \pm 400$ μs. Lines of best fit are shown for data obtained from peak heights at 101 and 82 ppm.

but different to those of the protons associated with starch in the crystalline regions ($T_{1\rho} = 6700 \pm 300$ μs).

From these results, we propose that the wheat starch CP/MAS NMR resonances at 103.5 and 82.4 ppm are due to the amylose–lipid inclusion complex. Additionally, it can be deduced that the crystalline starch and the amylose complex with lysophospholipids form distinct regions within a starch granule. Previous spin-diffusion measurements suggested that these regions are no more than 6 nm across [17].

Previously we had used differences in NMR relaxation times between these regions to generate separate subspectra for both of these regions [17]. Sub-spectra were generated by subtraction of 50 MHz ^{13}C CP/MAS NMR spectra acquired at different spin-locking times (see Experimental). New results obtained at 125 MHz, (Fig. 4) show improved resolution for the subspectrum of the amylose–lipid inclusion complex; Fig. 4(c). The spectral features are similar to those reported for amylose in the V conformation [6]. The region of the spectrum assigned to C-1 carbon atoms is interesting because it shows, besides the most intense peak at 103.1 ppm, other smaller peaks at 100.9, 99.0 ppm and, possibly, a broad band underlying all these peaks from 105 to 95 ppm. This indicates considerable disorder in the structure of the amylose complex, probably as a result of disruption of the single-helical conformation of the amylose complex by the polar head group of the lysophospholipids. Molecular modelling studies have shown that the head group, because it is hydrophilic and of a large size, will not be comfortably

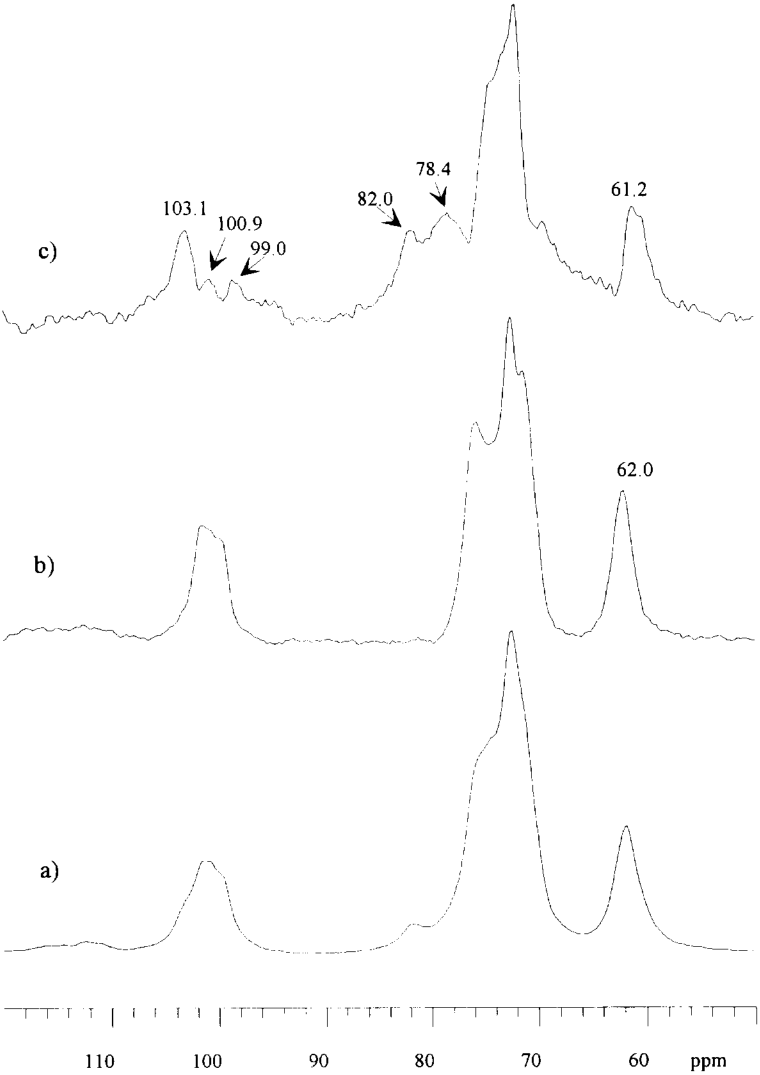

Fig. 4. Spectra of wheat starch with 33 wt% added water: (a) ^{13}C CP/MAS NMR spectrum, (b) sub-spectrum of the crystalline component of amylopectin, and (c) sub-spectrum of the amylose–lysophospholipid inclusion complex.

accommodated within the hydrophobic centres of the single helix formed from the amylose [25].

X-ray diffraction studies of cereal starch granules show no pattern due to starch in the V conformation as expected for crystalline arrays of amylose–lipid inclusion complexes, although V patterns are observed when wheat starch is heat and moisture treated and persist through starch retrogradation [11]. If iodine vapours are adsorbed onto maize starch granules that are subsequently moistened, a blue colour develops, and the V pattern appears when the starch is X-rayed [29]. These results seem to indicate that in the native starch cereal granules the amylose–lipid inclusion complexes are not crystalline, but that they do form, as Zobel notes [29], a 'separate entity' within the starch granule. Zobel [29] considered amylose to be a separate entity within the starch granule since, among other reasons, it is easily leached from the granule with hot water, and (as noted above) the interaction of iodine with starch gives rise to the V pattern when maize starch is X-rayed. These results are consistent with the NMR data showing that the amylose–lipid inclusion complexes occur in separate regions within the starch granule. The smaller $T_{1\rho}$(H) relaxation times for the amylose–lipid inclusion complex indicate that in these regions the amylose–lipid inclusion complexes are less rigid than the starch double helices in the crystalline regions.

Although the X-ray evidence shows that the amylose–lipid inclusion complexes are not ordered in the starch granule and therefore, are amorphous, most of the 4-linked α-D-glucopyranosyl units of the amylose–lipid inclusion complex are in a similar environment because the amylose is in a single-helical conformation. Therefore, the peak at 103.1 ppm is relatively narrow. Since the amylose–lipid inclusion complex appears to be more ordered than the branching regions of the amylopectin component of starch, it could lead to confusion to call these regions amorphous as well. Also, in the current literature the amorphous component of starch has usually been used for the branching regions of the amylopectin component, as we ourselves have done here. We have therefore decided to refer to the regions formed by the amylose–lipid inclusion complexes of starch as being solid-like.

Organisation of the starch granule.—Recently Oostergetel and van Bruggen proposed a fascinating structure for the amylopectin in potato starch [30]. Based on X-ray diffraction and transmission electron microscopy studies, they concluded that the crystalline regions of potato starch, though composed of double helices, were organised on a larger scale into interpenetrating 'super' helices. They report this to be a unique structure that no other polymer is known to adopt. The voids inside these 'super' helices seem to be empty and are roughly 8 nm wide. The molecular weight of α- and β-amylase is about 45 000 [31] and 64 200, [32] respectively. From comparison with the known diameter of proteins of similar molecular weight [33] we estimate the size of these enzymes to be 5–6 nm. This is small enough for the starch-degrading enzyme to fit inside the voids, and perhaps the voids inside the 'super' helices allow access of starch degrading enzymes into the starch granule.

The size and type of crystalline regions and the gelatinisation temperature of starch granules may vary, but the variation is not sufficient to suppose that cereal and tuber starch granules have a different physical structure. Nature is inherently conservative in design, and the structural arrangement for amylopectin found in potato starch by Oostergetel and van Bruggen will probably be similar in other granular starches. It is only the voids within the super helices formed from the crystalline regions of amylopectin that appear to be a suitable site for the amylose complex. Since starch in the V conformation is resistant to attack from amylases, the function of the amylose complexes may be to plug these voids and thus restrict enzyme access to the inside of the starch granule.

Other models for the structural arrangements of starch granules have been proposed. Morrison et al. suggest that the starch granule contains alternating crystalline and amorphous regions arranged in concentric shells, and that the amorphous regions are composed of both lipid-free and lipid-complexed amylose [34]. However, this model explains neither the structure of waxy maize starch, which is essentially amylose-free, nor why starch reversibly swells with addition of water. If starch is composed of a complete concentric crystalline shell, it should fracture irreversibly upon water addition. Cameron and Donald [35] found that a model involving alternate amorphous and crystalline regions embedded in an amorphous matrix is consistent with small-angle X-ray scattering data, but they did not try to fit those data to any other type of model. Electron microscopy studies conducted by Yamaguchi et al. [20] have shown lamellar structures for the crystalline regions in acid-treated waxy maize starch granules, but Oostergetel and van Bruggen [30] do not mention whether this is consistent with their proposed structure. The model of Oostergetel and van Bruggen, and also that of Morrison et al., fail to explain the presence of growth rings in a starch granule [20]. We feel the model of Oostergetel and Van Bruggen is the most satisfactory at the moment, based on the available experimental evidence.

Morrison et al. have concluded that non-waxy starch granules should be regarded as consisting of three types of polysaccharide: amylopectin, lipid-free amylose and lipid-complexed amylose [34]. Such a categorisation was useful in explaining the chemical constituents of starch. Of possibly more importance to cereal chemists are the physical characteristics of starch, since these affect properties such as gelation temperature, glassy transition temperature and softness of starch gels. As a structural material, non-waxy cereal starch granules contain three important components situated in distinct regions: (i) highly crystalline starch, consisting of a double-helix of two starch chains, possibly arranged as a super helix, (ii) amorphous starch, which separates the crystalline regions, and (iii) solid-like amylose–lipid inclusion complexes.

Our NMR results have shown that the amorphous component of moistened starch granules is mobile and is therefore similar to a rubbery polymer. Synthetic and natural polymers that contain both crystalline and amorphous components are strong without being brittle. The rubbery regions in a starch granule appear to function similarly and would act as shock absorbers if the starch granule was subject to compressive forces. Perhaps the intricate structure of starch evolved to help maintain the integrity of the starch granule during swelling caused by moistening of the starch granule.

4. Conclusions

New results reported in this paper have shown that the resonances expected for carbon atoms in the branching region of the amylopectin component of wheat starch granules are invisible in the CP/MAS NMR spectrum of moistened starches, but appear using the SP/MAS sequence. The amorphous regions are mobile at room temperature due to the plasticising effect of water. The direct observation of the amorphous regions should be a powerful method for examining starch gelation occurring in processed foods.

The amylose complex with lysophospholipids was shown to occur in distinct regions of the starch granule since proton rotating frame relaxation times associated with this complex are shorter than those of the crystalline components.

In non-waxy starches it is important when discussing physical properties to consider the three structural components in the non-waxy cereal starch granule: (i) amorphous starch formed from the branching regions of the amylopectin, (ii) crystalline starch formed from double helices in the linear region of the amylopectin, and (iii) solid-like starch formed from amylose–lipid inclusion complexes.

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