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Solid state NMR studies on the structural and conformational properties of natural maize starches

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Abstract

Natural maize starches having a range of amylose contents have been characterised by ^{13}C CP/MAS NMR spectroscopy. Chemical shifts, relative resonance intensities, line-widths and spectral shapes were compared at different moisture contents. At 10% moisture content, these parameters showed few significant differences across a range of apparent amylose levels from 0 to 84%. After hydration of the granules to $\approx$ 30% moisture, it was found that the amylose content significantly affected the relative signal intensities and line-widths especially of C-1 and C-4 resonances. Narrower line-widths after hydration were attributed to (i) an increased degree of crystallinity, and (ii) disappearance of the signals of amorphous material which, on becoming more mobile, became invisible to the CP/MAS experiment. The enhanced resolution at higher moisture levels revealed signals which were assigned to the amylose–lipid complex, i.e. V-type amylose. The amount of V-amylose detected by NMR increased with both amylose content and lipid content of the granule. Prolonged treatment of the granules with iodine vapour significantly increased the amount of V-type amylose in the high amylose samples, but caused a decrease in their degree of crystallinity. Waxy-maize starch was barely affected by iodination. The results provide evidence that amylose tends to disrupt the structural order within amylopectin crystallities. This effect is enhanced by the formation of the amylose–iodine complex, indicating that V-amylose could be a major crystallite-disrupting agent in native starch granules.

Keywords: Solid state NMR; Structural properties; Conformational properties; Natural maize starches

1. Introduction

Native maize granules consist of starch, water, lipid and protein. They occur in several crystalline types designated A, B, C (Zobel, 1988). In starches, Crystal types vary from A to B via C with an increase in amylose content and average chain length in amylopectin. Crystallinity has a good linear correlation with the amount of short chain fraction of DP about 10–13 glucose units (Cheetham and Tao, 1997). These short chains have been widely considered to form the crystalline regions by intertwining into double helices and packing into extended, regular clusters (Eliasson et al., 1987; Morrison et al., 1994).

It is still a matter of controversy whether or not amylose plays a role in the formation of crystalline lamellae. Naegeli amylodextrin granules, obtained by dilute sulfuric acid treatment on starch, retain or increase in crystallinity but no longer stain blue with iodine (Kainuma and French, 1971), which suggests that amylose is associated with the amorphous regions. On the other hand, Jenkins and Donald (1995) considered that amylose might take part in formation of crystalline lamellae by affecting the size of the crystalline portion of the cluster because (i) the difference between crystalline A-chain lengths and the crystalline lamellar size for the maize starch series increases with increase in amylose content, and (ii) the electron-density difference between crystalline and amorphous lamellae ($\Delta\rho$), which depends on a tightly or loosely packed array of amylopectin chains, decreases when the amylose content is increased.

In recent years, ^{13}C solid-state NMR has been widely used to study native products because it can observe structural changes of starch samples in the solid, and is essentially non-destructive. Although X-ray powder diffraction can monitor crystal structure and relative amounts of crystalline and amorphous phases in starch, it is only sensitive to long range order, while NMR is sensitive to short range order (Saitô et al., 1991), being especially suitable for less crystalline samples. Combination of both techniques has provided much important information about secondary structures and molecular order of a number of molecular systems (Gidley and Bociek, 1985; Cooke and Gidley, 1992). Several studies on the structural and conformational

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changes of different amylose crystal types and hydration levels have appeared (Veregin et al., 1987; Gidley and Bociek, 1988; Saitô et al., 1991; Morgan et al., 1995). Relatively little work has been reported on structural changes of native maize starches with different amylose contents and hydration levels.

Our previous X-ray powder diffraction investigation of maize starches with amylose contents ranging from 0 to 84% showed that there is a transition of crystalline types from A through C to B in these starches, corresponding crystallinity decreases, and an increase in average chain length in amylopectin (Cheetham and Tao, 1997). Because crystallinity changes resulted from certain treatments (Saitô et al., 1989), we decided to use ^{13}C CP/MAS NMR to monitor these changes in terms of local molecular structure and conformational order. The purpose of the present work was to carry out a comparative study of the properties of starches containing different ratios of amylose to amylopectin in the dry and hydrated states, and in the native and iodinated states.

2. Materials and methods

2.1. Sources and pretreatment of starch samples

Native maize starch (MS) samples with different amylose contents (WMS, MSA, MSB, MSC, MSD and MSE) were provided by Starch Australasia Ltd., Australia. Their apparent amylose contents were 0%, 28%, 40%, 56%, 65% and 84% as determined by an amperometric iodine binding method. The preparation of hydrated samples and iodinated samples was the same as described previously (Cheetham and Tao, 1998).

2.2. Solid state ^{13}C CP/MAS spectra of starches

Solid-state ^{13}C CP/MAS spectra were collected at a ^{13}C frequency of 75.46 MHz on a Bruker MSL-300 spectrometer operating at 297 K using a double bearing (DB/MAS) probe head. Samples were spun at the magic angle (54.5° or 54.7°). A magic-angle spinning rate of 2.5 KHz and decoupling field of 61 KHz were used. The 90° pulse width was 4 μs with a recycle time of 5 s. A contact time of 1 ms was used for all samples; spectral width was 20 KHz; acquisition time, 27 ms. Each sample was packed into a 7 mm magic-angle spinning (m.a.s.) sample rotor with tight push-fitting caps. The rotor held 200–240 mg of starch sample. Spectra are referenced to external Me_4Si via the low field resonance of adamantane (38.6 ppm). 1000–2500 scans were accumulated for each spectrum. A polynomial baseline was manually corrected where necessary after Fourier transformation and phasing. Resonance intensities were evaluated as either peak heights or by peak integration. Line shapes were evaluated by deconvolution in term of fit of least-squares of the peaks to a Lorentzian equation with the spectral deconvolution program (Bruker) of 1D NMR 5.1 software. Line-widths were half-height values for each peak. Two measurements were done for each spectrum. The parameter values (intensity of peak, width at half height) were reproducible to at least three significant figures.

3. Results and discussion

3.1. The effect of amylose content on ^{13}C CP/MAS NMR spectra of native maize starches at low moisture levels.

Fig. 1 summarises the ^{13}C CP/MAS NMR spectra of native maize starches with amylose contents ranging from 0 to 84%. Some properties of these starches and ^{13}C chemical shift values for all resolved signals are given in Table 1. All samples show similar spectra, though MSA–MSE show distinct resonances at $\approx$ 103 ppm. Assignments of resonances are consistent with literature data (Veregin et al., 1986; Gidley and Bociek, 1985; Dais and Perlin, 1982; Saitô and Tabeta, 1981). Signals at 99–104, 81–84 ppm are attributed to C-1 and C-4 respectively. The signal at 59–62 ppm is assigned to hydroxymethyl carbon-6 in hexopyranoses. The large signal around 70–73 ppm, is associated with C-2, 3, 5 in hexopyranoses. The signal at ~81.6 ppm for C-4 is about 4 ppm downfield from the solution value. This downfield shift and the

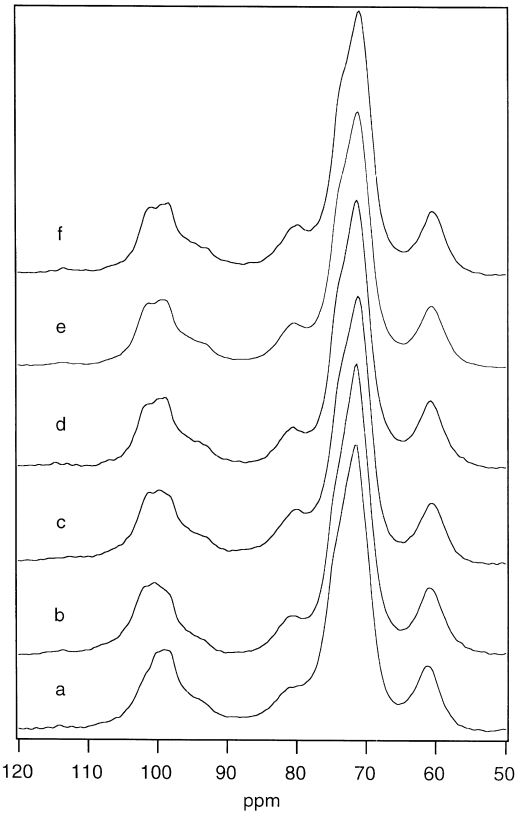

Fig. 1. ^{13}C CP/MAS NMR spectra of maize starches with different amylose contents. a, WMS; b, MSA; c, MSB; d, MSC; e, MSD; f, MSE.

Table 1
^{13}C CP/MAS NMR chemical shifts of maize starches

Sample	Amylose	Chemical shifts			
	(%)	C1	C4	C2,3,5	C6
WMS	0	100.97 99.68 98.90	81.82	71–76	61.62
MSA	28	102.52 100.97 99.16	81.56	71–76	61.37
MSB	40	102.27 100.45 99.16	82.36	71–76	61.37
MSC	56	102.27 100.71 99.42	81.30	71–76	61.37
MSD	65	102.27 100.45 99.42	81.04	71–76	61.37
MSE	84	102.07 100.71 99.41	80.78	71–76	61.37
Amylose	100	102.26 100.58 99.09	81.68	71.85	61.11
Amylopectin	0	102.26 96.63	81.69	72.17	60.72
Amylose in DMSO [a]		99.86	78.77	73.07(C3) 71.93(C2) 71.48(C5)	62.46
Amylose in water [b]		100.30	77.90	74.20(C3) 72.40(C2) 72.00(C4)	61.40

[a] Dais and Perlin (1982).
[b] Saitô and Tabeta (1981).

shoulder at ~95 ppm are believed to arise from non-crystalline material (Gidley and Bociek, 1988; Veregin et al., 1986). The chemical shift differences between solid and liquid spectra occur because ^{13}C chemical shifts in the solid state are determined by a range of fixed values of the dihedral angles, while those in aqueous solution are time-averaged as a result of rapid conformational isomerisation (Saitô et al., 1982). It is reasonable to expect the solution and the solid state conformations to be different.

In the solution state, the amylose content has a significant effect on ^{13}C NMR spectra of starches. The ^{13}C NMR spectra of starch vary greatly with solvent type (Jane and Robyt, 1985) and the degree of branching (Dais and Perlin, 1982; Gidley, 1985; Peng and Perlin, 1987). In aqueous solution, the non-reducing end-unit of branches shows distinctive minor resonances in ^{13}C NMR, and the differences in average branched chain lengths are reflected in the differences in the relative signal intensities (Dais and Perlin, 1982). These properties are likely to be closely related to the secondary or tertiary structure of these polysaccharides in the solution state. Quantitative analysis can provide an estimate of the proportion of branched molecules present, i.e the ratio of (1–4) and (1–6) linkages (Gidley, 1985), as well as a measure of the degrees of branching in the amylopectin components (Peng and Perlin, 1987).

However, in the solid state, maize starches with different amylose contents have similar ^{13}C CP/MAS spectra (Fig. 1), indicating that their overall conformation is almost independent of the amylose/amylopectin ratio, although amylose and amylopectin differ in average chain length, linkage mode, physical and chemical properties. In addition, non-crystalline amylose and amylopectin yield spectra in which the resonances have similar chemical shift ranges to those of the semi-crystalline starches (Fig. 2 and Table 1). A similar result (Saitô et al., 1986) was also

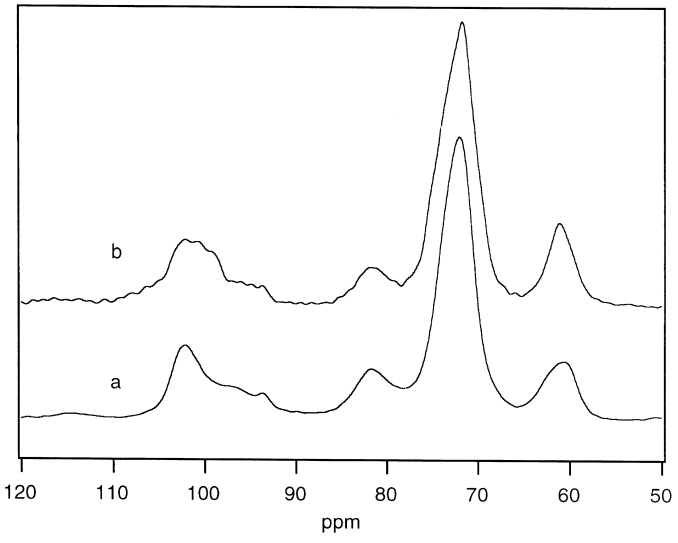

Fig. 2. ^{13}C CP/MAS NMR Spectra of amorphous amylose and amylopectin. a, Amylopectin; b, amylose.

found for curdlan, pachyman and lentinan. Although these polysaccharides have different degrees of branching in the main chain, their spectra have similar chemical shifts in the solid state. These data were considered as evidence that the presence of branching at the C-6 position does not greatly affect secondary structures of these branched glucans in the solid state (Saitô et al., 1986). The results indicate that α-(1-4) linked glucans probably exist in a partially-ordered extended conformation. Orientation manner and proportion of α-(1-6) linked side-chains do not significantly affect the resulting main chain conformation.

3.2. ^{13}C CP/MAS NMR spectra of hydrated maize starches

In Fig. 3, CP/MAS ^{13}C NMR spectra of several maize starches with different amylose contents are presented at three hydration levels. Table 2 lists the ^{13}C chemical shifts and line-widths thus obtained. To ensure that the results for different moisture contents were directly comparable and valid, uniform experimental conditions were employed for all the samples. After 18 days hydration, they had similar moisture contents. No increase in moisture content was found after further exposure to moisture.

At the lower moisture content (~10%), line-widths and

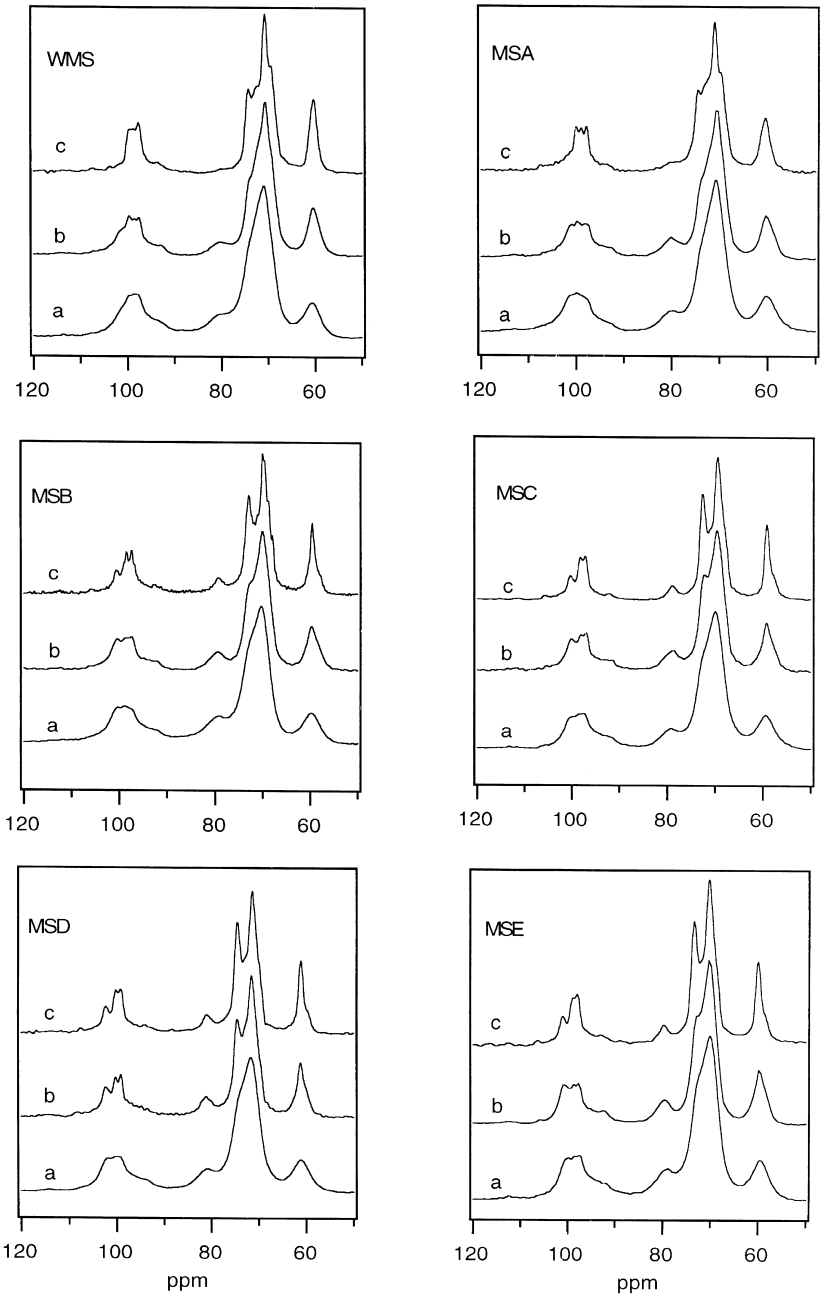

Fig. 3. ^{13}C CP/MAS NMR spectra of WMS, MSA, MSB, MSC, MSD and MSE at different hydration levels. a, ~10%; b, ~18%; c, ~30%.

Table 2
^{13}C chemical shifts and line-widths of maize starches at various hydration levels

Samples	Moisture (%)	C1	C-4 [a]	C-2, C-3, C-5	C-6
WMS	8.8	100.97(313.44) [b]	81.82	71.98(232.70)	61.62
		99.68(231.25)	(508.29)	75.08(358.47)	(211.53)
		98.90(610.69)			
	16.8	100.91(102.55)	81.56	75.15(201.51);71.72(196.38)	61.49
		99.81(100.74)	(154.43)	70.36(181.43)	(158.83)
		98.71(98.82)			
	29.3	100.59(121.57)	81.17	75.22(137.51); 73.54(107.12)	61.37
		99.55(176.58)	(13.91)	71.60(109.13); 70.17(174.15)	(126.52)
		98.65(56.65)			
MSA	9.4	102.52(205.53)	81.30	71.72(248.21)	61.37
		100.97(251.65)	(137.51)	75.02(331.16)	(226.92)
		99.16(337.95)			
	18.6	102.27(224.66)	80.79	74.96(215.74)	61.24
		100.59(217.92)	(133.67)	71.60(234.95)	(187.96)
		98.78(305.63)			
	29.5	100.58(156.61)	80.71	75.02(180.46);73.34(233.09)	61.11
		99.57(154.66)	(134.45)	71.59(52.92); 70.10(156.01)	(156.35)
		98.45(132.81)			
MSB	10.7	102.27(208.58)	80.78	71.72(198.94)	61.37
		100.45(280.39)	(388.59)	74.56(346.05)	(221.56)
		99.16(560.68)			
	17.4	102.14(209.31)	80.97	74.31(171.09)	61.37
		100.32(200.41)	(162.29)	71.72(233.65)	(178.38)
		99.09(278.70)			
	28.6	102.27(99.86)	80.91	74.57(135.02); 72.37	61.24
		100.20(94.67)	(134.86)	71.46(1882.5); 70.17	(111.66)
		99.17(172.61)			
MSC	9.5	102.27(155.53)	81.30	74.31(198.92)	61.37
		100.71(423.30)	(541.47)	71.98(274.08)	(213.54)
		99.42(441.88)			
	18.8	102.14(124.06)	80.59	74.05	61.11
		100.06(149.96)	(127.67)	71.59	(144.63)
		98.83(140.08)			
	29.8	102.40(97.77)	81.04	74.70(97.65)	61.24
		100.33(71.62)	(199.22)	71.60(180.65)	(85.89)
		99.17(89.62)			
MSD	9.70	102.27(213.71)	81.04	74.57(336.21)	61.37
		100.45(121.70)	(451.88)	71.98(203.04)	(228.99)
		99.42(436.83)			
	18.1	102.40(117.78)	80.91	74.95(280.15)	61.11
		100.59(159.86)	(191.80)	71.85(192.35)	(156.35)
		98.65(179.73))			
	30.1	102.40(126.89)	81.04	74.57(122.25)	61.24
		100.20(81.80)	(170.66)	71.47(182.68)	(105.05)
		99.30(136.57)			
MSE	11.5	102.27(146.47)	80.78	74.05(325.27)	61.37
		100.71(219.51)	(361.45)	71.98(213.52)	(208.52)
		99.41(403.93)			
	18.2	102.39(243.46)	81.10	74.18(141.51)	61.24
		100.19(205.35)	(270.62)	71.59(228.11)	(162.25)
		99.22(180.97)			
	30.2	102.27(122.99)	81.04	74.57(119.67)	61.24
		100.20(98.11)	(145.60)	71.47(186.44)	(107.94)
		99.17(118.26)			

[a] After hydration, especially in low amylose samples, much of the C-4 signal at ~81 ppm disappears.
[b] The data in parentheses represent line-widths (Hz).

intensities for different maize starches are almost the same even though their crystal types and crystallinities have obvious differences (Cheetham and Tao, 1998). Differences in the spectra of these samples became apparent with increasing water content.

(1) Changes in resolution and line-width. The reduction in line-width caused by hydration leads to the appearance of some new resonances in the 66–77 ppm region. The number of peaks and line-width are dependent on the extent of hydration (Table 2). The largest change occurred in WMS. When the moisture content is about 10%, the C-2, 3, 5 spectral region is dominated by an intense and broad signal centred at 71.98 ppm, while at 95 and 82 ppm, very broad ^{13}C resonances reflect the amorphous nature of the sample. When this sample was hydrated to 15.4%, the C-2, C-3, C-5 peaks became slightly better resolved, and a shoulder appeared at ~75 ppm. At 30% moisture content, it exhibits two peaks and two shoulder peaks. By comparison with the spectra of amylose in aqueous solution, the peaks are assigned as follows: 75.22 ppm, C-4; 73.54 ppm, C-3; 71.62 ppm, C-2; 70.17 ppm, C-5. MSA also exhibits similar hydration-induced spectral changes as summarized in Table 2. On the other hand, high amylose starches (MSB–MSE) show only two obvious peaks in the C-2, C-3, C-5 region at similar hydration levels. We attribute the increased spectral resolution induced by hydration to two effects.

(i) Increased degree of crystallinity. As we showed in the preceding X-ray powder diffraction study the degree of crystallinity of A- and B-type starches increases with increasing hydration (Cheetham and Tao, 1997?). This helical crystalline material exhibits lower chemical shift anisotropy, and importantly, is visible to the CP/MAS experiment.

(ii) The amorphous material made more mobile as a result of hydration is invisible to the CP/MAS experiment. As pointed out by Morgan et al. (1995), cross-polarisation depends on $^{1}H/^{13}C$ heteronuclear dipolar coupling. If these couplings are averaged because of fast molecular motion, cross-polarisation does not occur.

(2) Hydration induces the appearance of the characteristic C-1 multiplicities of A- and B-type maize starches, and reveals the presence of V-amylose in high amylose samples. WMS and MSA belong to the A-type. Three narrow resonances at about 101, 100, 99 ppm are superimposed on a broad hump in the anomeric carbon region of these samples. The resolution of these three peaks increased with an increase in hydration. In B-type starches (MSC–MSE), the general spectral features of hydrated samples are similar to those of potato starch (B-type) reported by Marchessault et al. (1985) and crystalline B-type α-(1-4) glucan (Gidley and Bociek, 1988). However, we observed three peaks in the C-1 region rather than the two peaks reported by those authors. The singlet peak at ~102.5 ppm derives from amorphous parts of starch, almost certainly from V-amylose (see later). The upfield doublet (99–100 ppm) is ascribed to the B-form. MSB, despite having a C type X-ray powder diffraction pattern (Cheetham and Tao, 1998), has a C-1 splitting pattern similar to those in MSC–MSE, indicating that B-type is actually predominant in this sample.

Multiplicity effects in starch double-helical polymorphs have been attributed to helix packing symmetry effects, i.e. to the difference in packing of helical chains in the unit cells of A- and B-amylose (Gidley and Bociek, 1985; Horii et al., 1987; Gidley and Bociek, 1988). In the A-type structure, the presence of a twofold packing symmetry leads three different environments for C-1 sites, hence three peaks appear in the A-type spectrum. In the B-type structure, the threefold packing symmetry provides two different environments for C-1 (Gidley and Bociek, 1988). There is no evidence for multiple carbon resonances in single helical starch glucans such as V-amylose. The resonances characteristic of V-amylose are reported to appear at 103 ppm (singlet, C-1), ≈ 82 ppm (singlet C-4) and ≈ 75 ppm (Gidley and Bociek, 1988; Morgan et al., 1995). From careful examination of spectra of MSB to MSE at 30% moisture (Fig. 3), of the V6 amylose complex with sodium palmitate (Gidley and Bociek, 1988) and of the subspectrum of the amylose–lipid inclusion complex of wheat starch (Morgan et al., 1995), we conclude that the high-field shoulder at ≈ 61.1 ppm is also due to the presence of V-amylose.

(3) Hydration leads to the reduction of amorphous feature peaks at 95 and 82 ppm. The relative intensity of C-1 (98–101 ppm) to C-2, C-3, C-5 (70–75 ppm) resonances is basically preserved. The resonance at 61.4 ppm (C-6) increases simultaneously in intensity, but the magnitude of this change differed across the range of maize starches studied.

Comparing all the spectra of hydrated maize starch samples, A-type starches with high amylopectin levels such as WMS and MSA seem the most sensitive to the degree of hydration (Fig. 3). When the moisture content is ~30%, the shoulders at ~103 ppm and 95 ppm, and the peak at 82 ppm, (non-crystalline material) almost fully disappear compared with that of ~10% moisture sample.

In the case of high-amylose starches (MSB–MSE), hydration decreases the intensity of the C-4 peak at 82 ppm only slightly, and the low-field shoulder becomes a distinct singlet at 102.5 ppm as the overall C-1 line-widths decrease. We interpret this as the emergence of V-amylose resonances at 102.5 and 82 ppm from the broad background spectrum as hydration progressively renders the amorphous regions invisible to CP/MAS, i.e. V-amylose is present in

significant amounts in the native granules. In support of this, it was found that the 102.5 ppm peak area (V-amylose) relative to the doublet at 100/99 ppm (B-type) increases progressively from MSB to MSE, indicating an increase in V-amylose levels with increasing amylose content. Unfortunately the accuracy of the present data for this trend is not high. A correlation coefficient (r) of 0.969 was obtained when we plotted amylose contents of MSB–MSE vs their respective lipid contents (determined by Soxhlet extraction with 95% ethanol). Lipid contents determined from the area of the NMR peak at 31 ppm (Morrison et al., 1993a, b) gave a correlation of 0.94 when plotted vs amylose content. These results are consistent with the NMR results, in that the V-amylose (i.e. amylose–lipid complex) content is likely to increase in parallel with the amylose and lipid levels. We plan further experiments to confirm the proposed trend, e.g. by obtaining enhanced resolution of the amylose–lipid complexes as described by Morgan et al. (1995).

3.3. The effect of iodination on the ^{13}C NMR spectra of maize starch

The ^{13}C NMR spectra of amylodextrin and amylodextrin–triiodide aqueous solutions show obvious differences. The chemical shifts of C-1 and C-4 of the latter move downfield and are broader than the former. This result was explained as conformational transition from random coil of amylodextrin to helix of amylodextrin–triiodide (Jane and Robyt, 1985). Unlike the spectral behaviour of the amylodextrin–triiodide complex in solution, the chemical shifts and peak shapes of spectra of the native and respective iodine-treated starches in the solid state are similar for all the carbon resonances (Fig. 4), suggesting that there is no large change in amylose helical structure. This result is in agreement with the conclusion of Gidley and Bociek (1988) that ^{13}C chemical shifts are not sensitive to expansion of the amylose helix, from a six- to a sevenfold repeat to accommodate more bulky molecules.

Although no large differences were observed, the minor spectral differences which occurred are significant (Fig. 4). The first is the number of resolved signals for C-1. For WMS, the same spectra were obtained before and after iodination. Thus iodination has little effect on the short range order of WMS, which is in agreement with the results found by X-ray diffraction (see Cheetham and Tao, 1998). However, ^{13}C CP/MAS spectra of iodinated MSA and MSE show a slight increase in the intensity of signals for C-1 (~103 ppm) and C-4 (~82 ppm), which we have attributed to the V-amylose complex. Significantly, the effect is greater for MSE than for MSA, while WMS is unaffected.

Another characteristic change after iodination is the increase in the line-width of the C-6 resonance (at ~61.1 ppm) in MSA and MSE. In the case of WMS, no obvious line-width change was observed. The line-width of C-6 resonances shows the greatest change among all signal peaks after iodination. This is consistent with the proposal by Veregin et al. (1987) that C-6 shows more disorder than C-1 or C-4 in V-amylose. Increased disorder at C-6 following iodination could be a result of the conformational flexibility of hydroxymethyl groups at the exterior of the amylose helix. As the helix is induced by diffusing iodine, the hydroxymethyl groups would be the most likely to be disturbed by steric interactions with surrounding chains, and be distributed between the gg, gt and tg conformations.

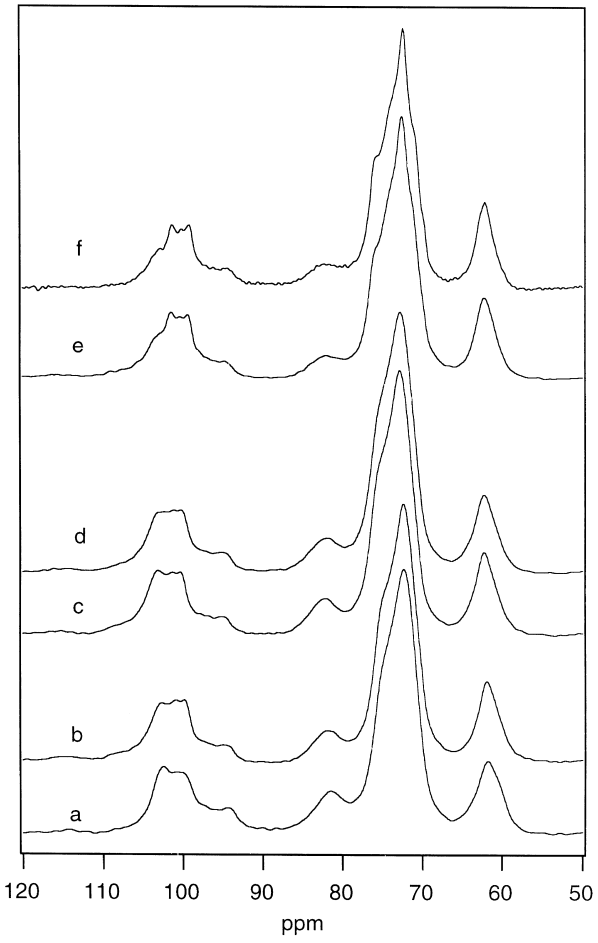

Fig. 4. The effect of iodination on the ^{13}C CP/MAS NMR spectra of maize starches with different amylose contents. a, MSE–iodine; b, MSE; c, MSA–iodine; d, MSA e, WMS–iodine; f, WMS.

4. Conclusions

Carbon-13 CP/MAS NMR spectra of hydrated high amylose maize starches reveal the presence of lipid–amylose complexes (V-type amylose). The amount of complex present is approximately proportional to the amylose content of the sample. The degree of crystallinity of the granule is inversely related to the amylose content,

confirming that crystallinity is a feature of amylopectin rather than amylose. Amylose tends to disrupt the order in starch crystallites. This appears to be the case for V-type amylose to a greater extent than for less conformationally ordered amylose. Such a proposal is supported by the effects of absorbed iodine, which induces an increase in V-type amylose, with a concomitant loss in degree of crystallinity. The iodine effect is not observed in waxy maize starch, showing that the double-helical amylopectin in crystallites is not affected per se by iodine. Conformationally disordered amylose must be in intimate contact with crystalline amylopectin. When iodine is added, the ordered V-type amylose induced from this disordered amylose must be of such dimensions and in such a location as to disrupt existing crystalline structural order.

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