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Crystallinity and structuring role of water in native and recrystallized starches by ^{13}C CP-MAS NMR spectroscopy 1: Spectral decomposition

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Abstract

Amorphous, native, and recrystallized starches were studied by ^{13}C CP-MAS NMR spectroscopy with respect to their behavior with hydration. The study of space groups, associated to crystalline polymorphs (B2 and $P6_1$ for A and B forms, respectively), provided decomposition rules for the spectral part due to crystalline phases. Moreover, the subtraction of a standard amorphous spectrum apparently showed the existence of three phases in native starches (amorphous, partially-ordered and crystalline) and only two in spherulitic crystals (partially-ordered and crystalline). The proportion of each phase was estimated at two different hydration levels. The amount of crystalline phase was compared to the degree of crystallinity as evaluated by wide angle X-ray scattering. The NMR spectral changes with hydration could be interpreted in terms of two complementary roles of water molecules, i.e. structuring and plasticizing.

Keywords: Crystalline polymorphs; Spherulitic crystals; Hydration

1. Introduction

Starch, the major energy reserve of higher plants, is a mixture of two main components: amylose, a linear or slightly branched (1 → 4)-α-D-glucan and amylopectin, a highly branched macromolecule consisting of (1 → 4)-α-D-glucan short chains linked through α-(1 → 6) linkages. All starches are biosynthesized as semi-crystalline granules containing densely packed polysaccharides with a little amount of water included. The crystallinity (about 20–45%; Zobel, 1988; Gernat, Radosta, Anger & Damaschun, 1993) and polymorphism of the native starches, as revealed by X-ray diffraction analysis, have been recognized since long, and three main forms A, B and V have been reported (Katz & van Itallie, 1930; French & Murphy, 1977; Duprat, Galland, Guilbot, Mercier & Robin, 1980; Sarko & Zugenmaier, 1980; French, 1984; Buleon, Colonna, Planchot & Ball, 1998). The A polymorph occurs frequently in cereal starches, while the B polymorph is characteristic of tuber and amylose-rich starches. The V polymorph essentially results from the complexation of amylose with compounds, such as iodine, alcohols or lipids, and is rarely detected as a crystalline material in native starches (Gernat et al., 1993; Morrison, Law, & Snape, 1993). The crystalline regions of starch granules can be isolated by mild acid hydrolysis using hydrochloric or sulphuric acid ('lintners' or 'naegelis'), such residues with high crystallinity (Buleon, Bizot, Delage & Pontoire, 1987) have been frequently studied as models to elucidate the structure and properties of the crystalline parts of starch granules. These residues were also used for recrystallization of A- and B-type spherocrystals (Helbert, Chanzy, Planchot, Buleon & Colonna, 1993; Planchot, Colonna & Buleon, 1997). Amylopectin is usually thought to be the dominating component in the crystalline regions of starch granules (French, 1984; Jenkins & Donald, 1995), while amylose can be recrystallized from solution in A, B or V form (Buleon, Duprat, Booy & Chanzy, 1984). This ability was used to determine the three-dimensional (3D) structures of these different polymorphs, and to investigate the structure of crystalline domains in native starches. In A and B forms, a double helical conformation has been proposed for amylose chains by Wu and Sarko (1978a,b), and more recently by Imberty, Chanzy, Perez, Buleon and Tran (1988) and Imberty and Perez (1988). In the A-type structure, left-handed parallel-stranded double helices are packed in the monoclinic space group B2 (Imberty et al.,

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1988). In the B-type structure, however, the double helices are packed in a hexagonal unit cell with the $P6_1$ space group (Imberty & Perez, 1988).

Water plays many significant roles in determining the properties of starch. Water below the gelatinization (melting + dissolution) temperature domain acts essentially as a plasticizer (Bizot, Buleon, Mouhous-Riou & Multon, 1997) while, at higher temperatures, it becomes a solvent (Moates, Noel, Parker & Ring, 1997). Water is essential to the crystallinity of starch based glucans (Buleon, Bizot, Delage & Multon, 1982; Buleon et al., 1987) as it permits rearrangements by plasticization of amorphous areas and the buildup of crystalline hydrate lattices of different stoichiometries depending on the polymorphic type (Imberty, Buleon, Tran & Perez, 1991; Rappenecker & Zugenmaier, 1981).

Solid state NMR has proved to be a powerful tool for characterizing some degrees of molecular order such as helicity in the structure of starchy substrates (Gidley & Bociek, 1985; Veregin, Fyfe, Marchessault & Taylor, 1986; Horii, Yamamoto, Hirai, & Kitamaru, 1987; Singh, Ali & Divakar, 1993; Morgan, Furneaux & Larsen, 1995). The multiplicities and the chemical shifts assigned to C1 atoms have been correlated for a long time to the glycosidic conformation and polymorphism of starch (Hewitt, Linder, Perez & Buleon, 1986; Veregin et al., 1986; Horii et al., 1987) and other polysaccharides (Jarvis, 1994; Isogai, Usuda, Kato, Uruy & Atalla, 1989). The influence of hydration on the ordering of starch chains and decrease of the spectral bandwidth was also studied (Tanner, Ring, Whittam & Belton, 1987; Horii et al., 1987; Cheetham & Tao, 1998b). The NMR data have been used to identify, and sometimes quantify the crystalline order of native, hydrolyzed or gelatinized starches (Willenbucher, Tomka & Müller, 1992), but there is still some debate about the exact level of structure reached through this technique, i.e. the degree of helicity (molecular order) or the fraction of helices packed with respect to crystalline order (Gidley & Bociek, 1985), and the quantitative character of the measurements.

This article aims at presenting an improved analysis of native and recrystallized starches CPMAS spectra, focusing mainly on the crystalline and amorphous components of the spectra and their changes with hydration. It is the first step of a general study dedicated to the identification of local orders and local mobilities within polysaccharide matrices.

2. Experimental

2.1. Material

Waxy maize and potato starches were donated by Roquette Frères (Lestrem, France).

Lintnerized potato starch was prepared by mild acid hydrolysis (HCl 2.2 N, 35°C, 35 d) as described previously (Robin, Mercier, Charbonniere & Guilbot, 1974). A- and B-type amylose spherocrystals were prepared from lintnerized potato starch dissolved in water and precipitated in water ethanol mixtures upon slow cooling, as described earlier (Planchot, Colonna & Buleon, 1997).

Extruded potato starch was considered as a standard for amorphous starch. Conditions were: $T_{max} = 120$°C, 30% H_2O w.b., specific mechanical energy = 142 Wh/kg.

2.2. Methods

2.2.1. Water content adjustment

The water content of all samples was adjusted via a vapor phase isopiestic equilibration over saturated salt solutions (Multon & Bizot, 1991; Robinson & Stokes, 1959) delivering relative partial water vapor pressures of 0.53, 0.75 and 0.90 using $Mg(NO_3)_2$, NaCl and $BaCl_2$, respectively at 25 ± 0.1°C. If thermodynamic equilibrium is obtained, these relative partial pressures are considered to be equivalent to the water activity (a_w) in both salt and starch phases. Average water contents deduced from sorption isotherms of former studies are expressed in % weight basis (w.b.) where necessary.

2.2.2. X-ray diffraction measurements

Samples (5–20 mg) were sealed between two aluminum foils, to prevent any significant change in water content during the measurement. Diffraction diagrams were recorded using Inel (France) X-ray equipment operating at 40 kV and 30 mA. CuKα_1 radiation ($\lambda = 0.15405$ nm) was selected using a quartz monochromator. A curved position sensitive detector (Inel CPS120) was used to monitor the diffracted intensities using 2 h exposure periods. Relative crystallinity was determined by the method of Wakelin, Virgin and Crystal (1959), dry extruded potato starch and hydrated recrystallized amylose were used as amorphous and crystalline standards, respectively. All diffractograms were normalized at the same total area under the scattering curve over the Bragg angle range 3–30° (2θ).

2.2.3. Calorimetric measurements

The glass transition temperatures $T_g^{1/2}$ of the amorphous fraction of native starches were evaluated by differential scanning calorimetry (DSC) as previously described (Bizot, Le Bail, Leroux, Davy, Roger & Buleon, 1997) for bulk phases. $T_g^{1/2}$ is taken at half maximum heat capacity increment for a 3°/min heating rate.

2.2.4. Solid state NMR experiments

The CP-MAS ^{13}C NMR spectra were recorded on a Bruker MSL 300 equipped with CP-MAS (cross-polarization magic-angle-spinning) accessories. Dipolar decoupling was systematically used during the acquisition sequence. The samples were spun at a rate of 7.5 kHz at room temperature in a 4 mm ZrO_2 rotor, the accumulation of 3072 and

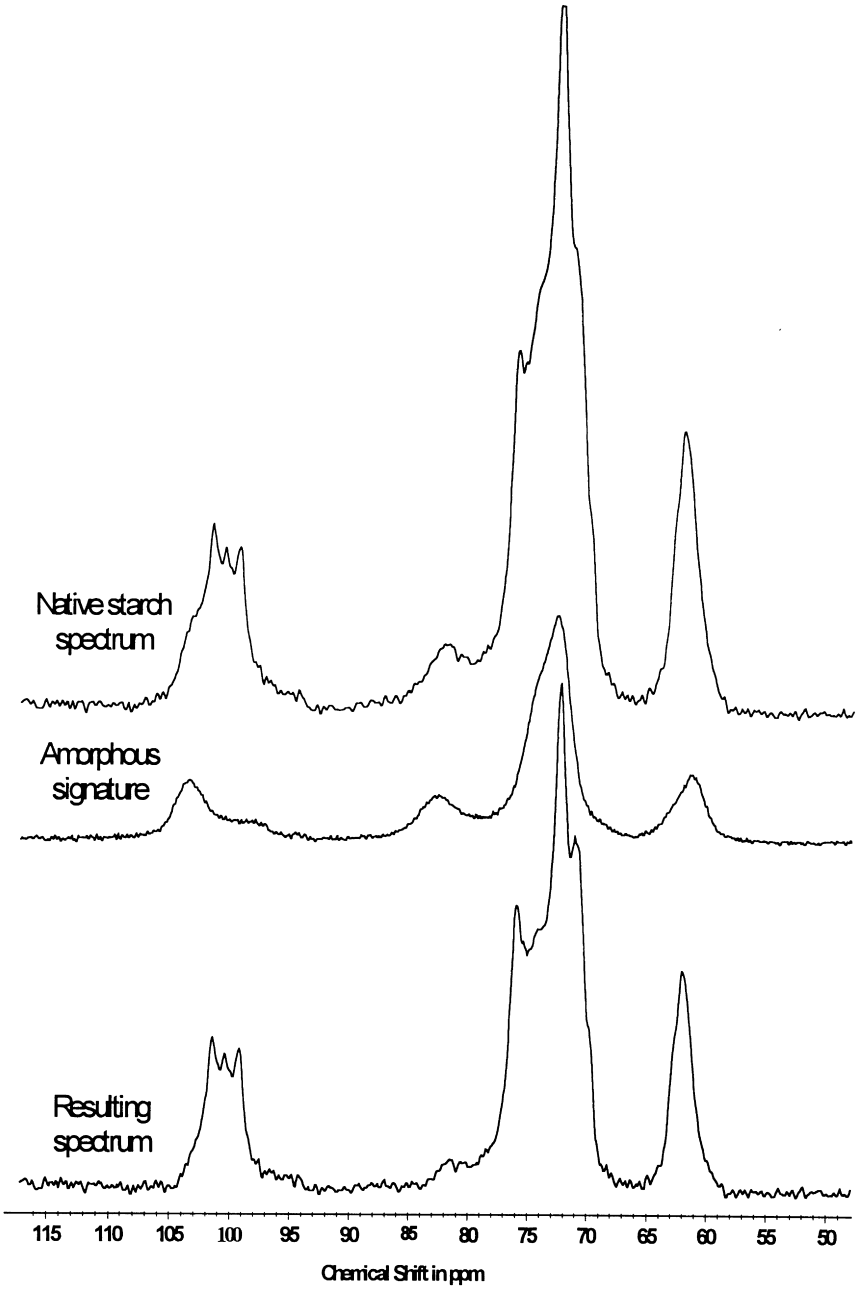

Fig. 1. Stripping of the amorphous signature (extruded starch) from native A-type starch ^{13}C CP-MAS NMR spectrum and resulting spectrum. The empirically optimized subtraction coefficient is obtained when a negligible intensity is reached at 84 and 105 ppm. Hydration level is assumed to be identical for both products conditioned at $a_w = 0.90$.

2048 scans were used for native starches and spherulitic crystals, respectively, to obtain a satisfactory signal to noise ratio. For all hydrations, a repetition time of 2 s appeared to be sufficient and the optimal contact time was chosen in the order of 1.25 ms after probing the range 0.75–2.25 ms. Spectra were referenced using the high field resonance of adamantane (29.5 ppm).

2.2.5. Spectral analysis

In order to decompose the spectra into identified signatures we rely on the following rules or hypotheses:

- The chemical shift of each C1 carbon depends on the screening of the magnetic field by local electron densities essentially related to conformational angles (Veregin et al., 1986; Gidley & Bociek, 1988). This has tentatively been related to ab initio calculations (Durran, Howlin, Webb & Gidley, 1995).
- The probability density of conformations reflects a Boltzmann's statistical weighing of energy maps of glycosidic linkages (Gagnaire, Perez & Tran, 1982) altered by mean field forces present in condensed phase environments and/or partial hydration. Narrow lines

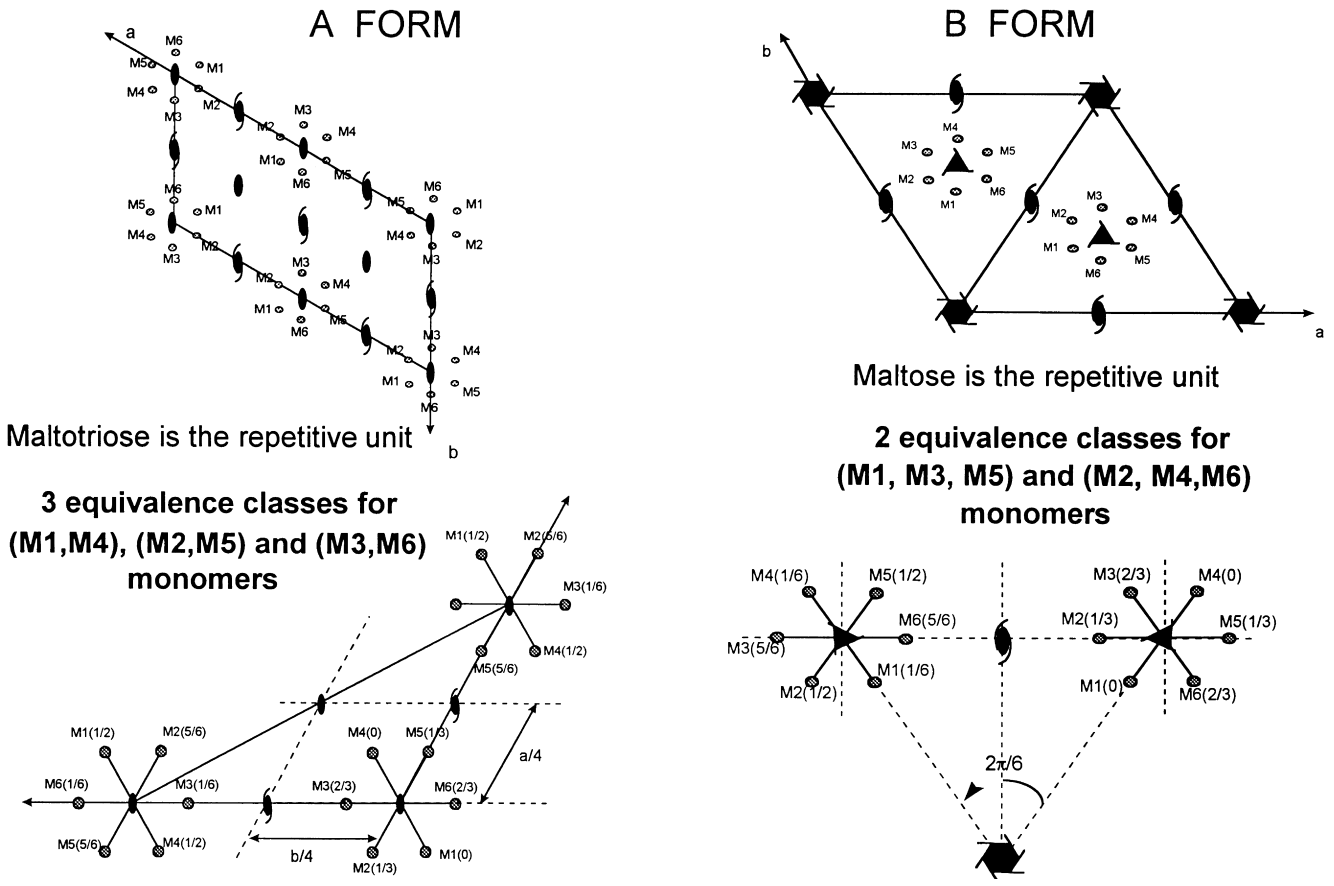

Fig. 2. Crystallographic positions of double helices and constituting monomers in the unit cell, and representation of the space group and corresponding operations of symmetry for A- and B-type structures.

indicate narrow statistical distributions (Gidley & Bociek, 1988).

- Regularly arranged conformations of crystalline packed chains correspond to narrow resonance frequency C1 bands with multiplicities determined by space group equivalent symmetry classes (Veregin et al., 1986; Horii et al., 1987).
- As no hydrogen bond linkage can exist to stabilize the (1–6) branching points (Gidley, Cooke & Ward-Smith, 1993), they are expected to correspond to a wide distribution of chemical shifts of C1 carbons.
- The more exotic conformations leading to more displaced (less probable) chemical shifts are assumed to belong to constrained and/or entangled single chains belonging to the main amorphous fraction.
- As far as the quantitative character of the reported measurements is concerned, we consider this to be satisfactory based on the following arguments:

1. For each phase studied, the local environments of each carbon C_i ($i = 1$ to 6) are taken as quasi identical, thus the efficiency of cross polarization will essentially depend on the motional averaging of the heteronuclear dipolar interaction.
2. The glass transition temperature of the more flexible chains within semi-crystalline granular structures could approach our working temperature (290 K) for samples prepared at higher relative humidity (90%), as suggested by calorimetric measurements of similarly hydrated amylopectin supposedly representative of the amorphous growth ring. This argument has already been put forward by Morgan et al. (1995) and Cheetham and Tao (1998a). However, the characteristic time scales of the two methods differ significantly (10^{-6} to 10^{-3} s for NMR and 10^{+2} for DSC), so that the glass transition temperature relevant for NMR probably lies about 20° above that for DSC. In addition, two spectra recorded at 290 and 210 K ($T_g^{1/2} - 80$ K) for native potato starch gave identical intensities, thus confirming our guess. Finally, this fact is further supported by the constant intensity ratio between the C2-3-4-5 and the 83 ppm resonances specifically attributed to amorphous single helical conformations over the whole 0.75–2.25 ms contact time range, in agreement with Gidley's findings (Gidley & Bociek, 1985), but yet illustrating the different behavior of the carbon C6. Thus, the amorphous and crystalline regions behave similarly with respect to their solid state nature as probed by this NMR experiment.

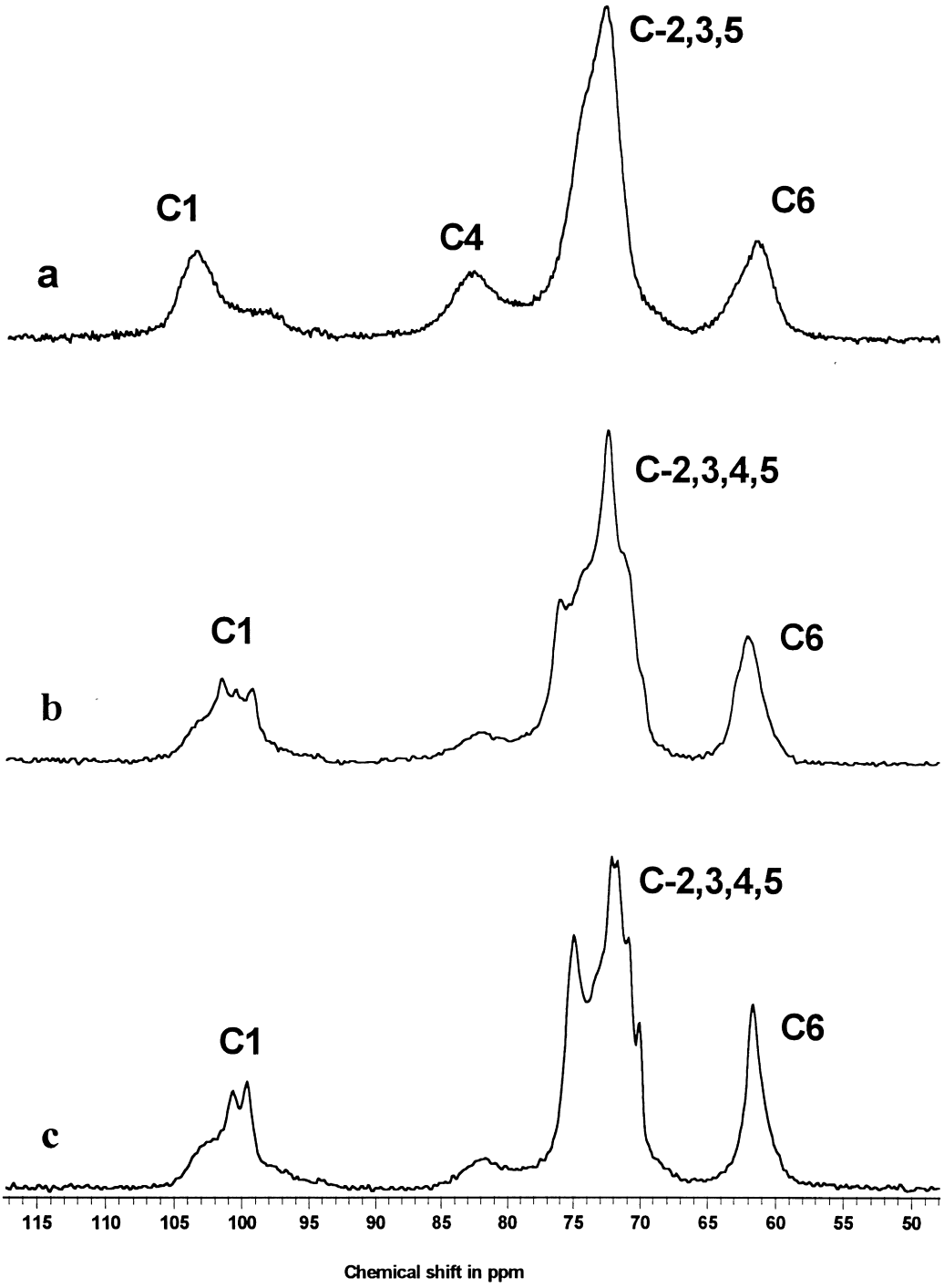

Fig. 3. ^{13}C CP-MAS NMR spectra of starchy substrates after rehydration at $a_w = 0.9$: (a) extruded potato starch; (b) native waxy maize starch (A-type); (c) native potato starch (B-type).

3. Still another argument refers to the optimal contact time leading to maximum intensities and which may have a common value on top of a broad enough plateau (within the 0.75–2.25 ms range) for both native and extruded products.

Consequently, in the present approach CP-MAS is considered to be sufficiently quantitative to allow the decomposition of spectra in a linear combination of individual phase signatures.

Within this framework, we have attempted to adapt the treatment of wide angle X-ray scattering (WAXS) spectra (Wakelin et al., 1959; Gernat et al., 1993) to CP-MAS (as used by Willenbucher et al., 1992) and determine in a preliminary step the fraction of amorphous contribution by subtracting the standard amorphous signature from a

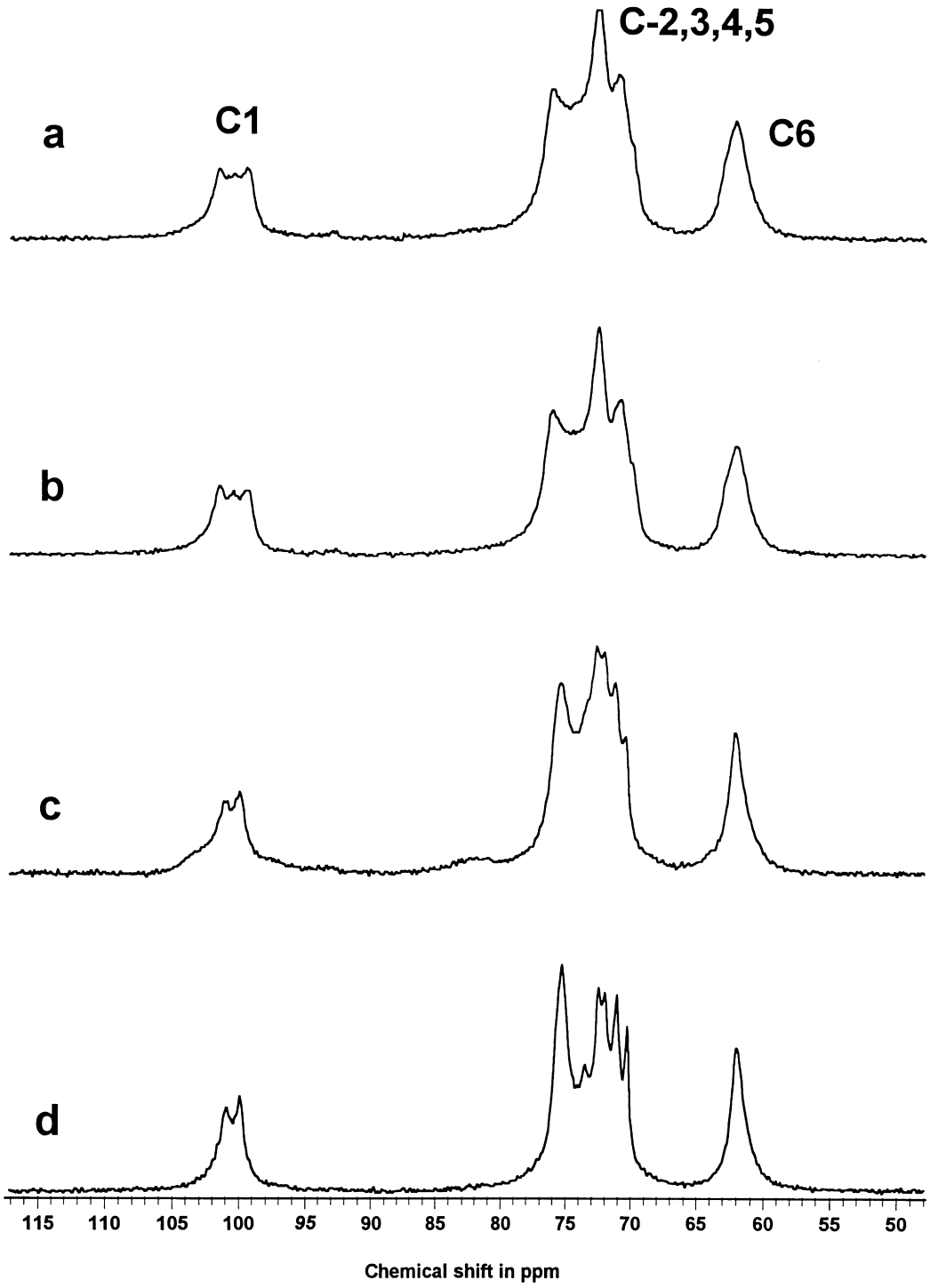

Fig. 4. ^{13}C CP-MAS NMR spectra of amylose spherulitic crystals after water sorption at $a_w = 0.75$ and 0.9: (a) A-type, $a_w = 0.75$; (b) A-type, $a_w = 0.9$; (c) B-type, $a_w = 0.75$; (d) B-type $a_w = 0.9$.

hydrated native spectrum obtained after conditioning at identical relative humidity. Ignoring the exact water content within each granular domain, we consider that amorphous domains are similarly hydrated in their various environments as suggested by the small differences between sorption isotherms of native and destructurized starch (Bizot, Buleon, Mouhous-Riou & Multon, 1985) although this may not be general (J. Mitchell, personal communication). An ad hoc subtraction ratio was obtained when zero intensity was reached for the difference spectrum at 105 and 84 ppm. An example is given in Fig. 1 for A-type starch hydrated at $a_w = 0.90$. The same technique was applied to A- and B-type spherulitic crystals. Further, to quantitatively determine the average conformational

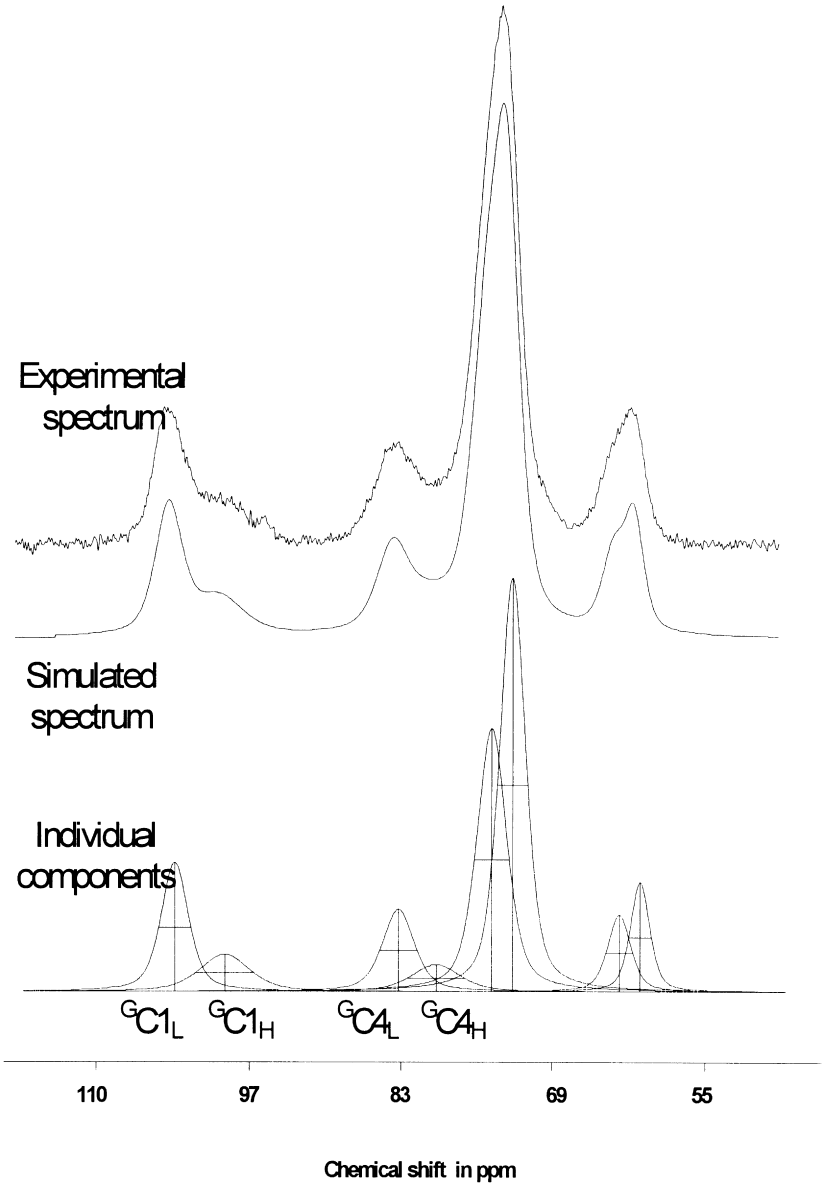

Fig. 5. Spectral decomposition of ^{13}C CP-MAS NMR lines of amorphous starch after conditioning at $a_w = 0.53$.

order and crystalline content from the spectra two main strategies are possible:

- use conformationally known structures or highly crystalline standards as for cellulose.
- attribute elementary narrow line components contributing to complex envelopes in agreement with crystalline symmetry rules.

In addition to the first traditional approach, we investigate the second possibility although limited to the clearly assignable C1 chemical shift range. This is justified by the fact that, in this isolated range, the correlation between chain conformation and glycosidic torsion angles is more straightforward. We used two softwares intended to simulate NMR spectra: 1DWINNMR (Bruker) and an extended version of WINFIT (Massiot, Thiele & Germanus, 1994). However, in the present stage of analysis only empirical and visual criteria were used for data fitting of native starches and spherulitic crystals. In any case, the type of curve used for fitting was a linear combination (50/50) of lorentzian and gaussian profiles which gave excellent agreement as shown in the following figures.

Thus, applying Veregin's ideas (Veregin et al., 1986) to A- and B-type polymorphic structures, the following rules involving a possible coupling of the different carbon atoms in the polymer were used for decomposition:

- As a result of crystallographic packing constraints, two sets of established symmetry rules were applied, these correspond to A- and B-type space groups, i.e. C2 (B2 with *c* as unique axis—Imberty et al., 1988) and $P6_1$ (Imberty & Perez, 1988), respectively. This yields two

Table 1
Position, width at half height and relative integrated intensity of the lines resulting from the spectral decomposition of amorphous extruded potato starch conditioned at $a_w = 0.53$ and 0.90

Extruded potato starch		$a_w = 0.53$	$a_w = 0.90$
$^{G}C1_L$	δ (± 0,1 ppm)	103,3	103,3
	ω (± 0,1 ppm)	2,9	2,7
	I% (± 1%)	11	12
$^{G}C1_H$	δ (± 0,1 ppm)	98,8	99,1
	ω (± 0,1 ppm)	5,3	4,2
	I% (± 1%)	6	5
$^{G}C4_L$	δ (± 0,1 ppm)	83	82,6
	ω (± 0,1 ppm)	3,4	3,4
	I% (± 1%)	8	10
$^{G}C4_H$	δ (± 0,1 ppm)	(79.6)	78.3
	ω (± 0,1 ppm)	(5.2)	4.2
	I% (± 1%)	(4)	3

equivalence classes for A-type and three for B-type. Fig. 2 gives a detailed 3D account of the symmetry operations applied to glucose monomers M*i*, belonging to double helices. In A-type structure, the crystallographic two-fold axes within the double helices allows us to relate the monomers M1 and M4, M2 and M5, M3 and M6 inside each double helix. Moreover, other two-fold axes link the same monomers from one double helix to the next. Therefore, the six monomers per turn are classified in three equivalence classes: (M1,M4), (M2,M5) and (M3,M6). Similarly, using the crystallographic 3_1 and 2_1 axes defined in B-type structure, two equivalence classes, (M1,M3,M5) and (M2,M4,M6), can be identified. Consequently, three different (1 → 4)-α linkages exist in A form and two in B form. Within the chains supporting crystallographic packing constraints, the glycosidic linkages correspond to a limited number of Φ and Ψ torsion angles, resulting in C1 and C4 lines of spectra to be a triplet with intensities 1:1:1 for A form and a doublet with intensities 1:1 for B form. Thus, when selecting the isotropic chemical shift component by Magic Angle Spinning, powder NMR spectra are expected to reflect crystallographic symmetries of their crystalline components. Then, the crystallographic equivalence between monomer is also a NMR equivalence. Moreover, NMR being a local probe technique, ordering will be detected at a shorter range than by WAXS. However, band splitting being governed by packing constraints, multiplet spectral signatures are not attributed to a general 'molecular order' (helicity) but to crystallinity itself. Purely well crystallized double helices are considered to yield narrow distributions of conformational angles associated to their respective chemical shift, while non-crystallographically constrained chain ends (as well as defective chains at unpacked lateral boundaries or internal defects) should be sufficiently distorted on an average so as not to show up at similar chemical shifts.

3. Results and discussion

Standard CP/MAS spectra for amorphous and semi-crystalline A- and B-type starches at identical relative humidity are shown in Fig. 3, while those recorded for A- and B-type spherulitic crystals at two hydration levels ($a_w = 0.75$ and 0.90) are presented in Fig. 4. The spectrum of amorphous starch was very similar to that shown by Gidley and Bociek (1985) from gelatinized and ethanol precipitated potato starch. The C1 and C6 lines were in the 99–103 and 59–64 ppm ranges, respectively, the C2, C3, C5 were not well separated and were centered on 73 ppm. By analogy to starch solution spectra (Colson, Jennings & Smith, 1974), the peak at 83 ppm was assigned to the amorphous C4 carbons. The different resonances and C1 multiplicities around 100 ppm observed for the semi-crystalline substrates agree with both the earlier works (Veregin et al., 1986; Horii et al., 1987) and structural arguments presented before.

The spectra of spherulitic crystals presented a higher resolution than those of native starches but with the same C1 multiplicities. The unordered contribution in the C1 and C4 regions was very weak except for B-type spherulitic crystals hydrated at $a_w = 0.75$. Notably, B-type spherulitic crystals showed a higher sensitivity to the hydration level as the resolution is significantly improved at higher hydration (Fig. 4(d)). This tendency appears quite reasonable with regard to the stability of the hydrated B-type structure as this refers to the saturation of hydration columns and the loss of chain–chain correlations once the branched topology is disrupted.

In an attempt to identify the signatures of the different phases, we have applied a spectral decomposition with the minimum number of individual resonances possible. We introduced the following labeling (nomenclature). Each resonance was denoted by a superscript prefix related to the morphology (G = glassy, N = native, S = spherulitic crystals) and the crystalline type (A or B) when necessary, then a carbon number (here limited to C1 and C4) and a relative position subscript (L for low field or left, M for middle, H for high field, D for doublet and T for triplet, I for intermediate or interfacial material). Such an intermediate amorphous material may, however, retain more local order than the interlamellar rigid amorphous fraction (Kalika, Gibson, Quiram & Register, 1998) considered in synthetic polymers because of the residual correlation between chains engaged in double helical topology even when distorted such as by incomplete hydration. Therefore, $^{N,B}C1_D$ refers to the doublet of B-type native starch spectrum, while $^{G}C4_L$ refers to the low field component in the C4 band of the amorphous spectrum.

In Fig. 5, the amorphous spectrum ($a_w = 0.53$, water content = 11.6%) is thus decomposed into eight resonances. A similar procedure was applied to the same sample after conditioning at $a_w = 0.9$ (% H_2O w.b. = 21%) (Fig. 3(a)). The results are summarized in Table 1 where C1 and C4 resonances are characterized by their position δ (chemical

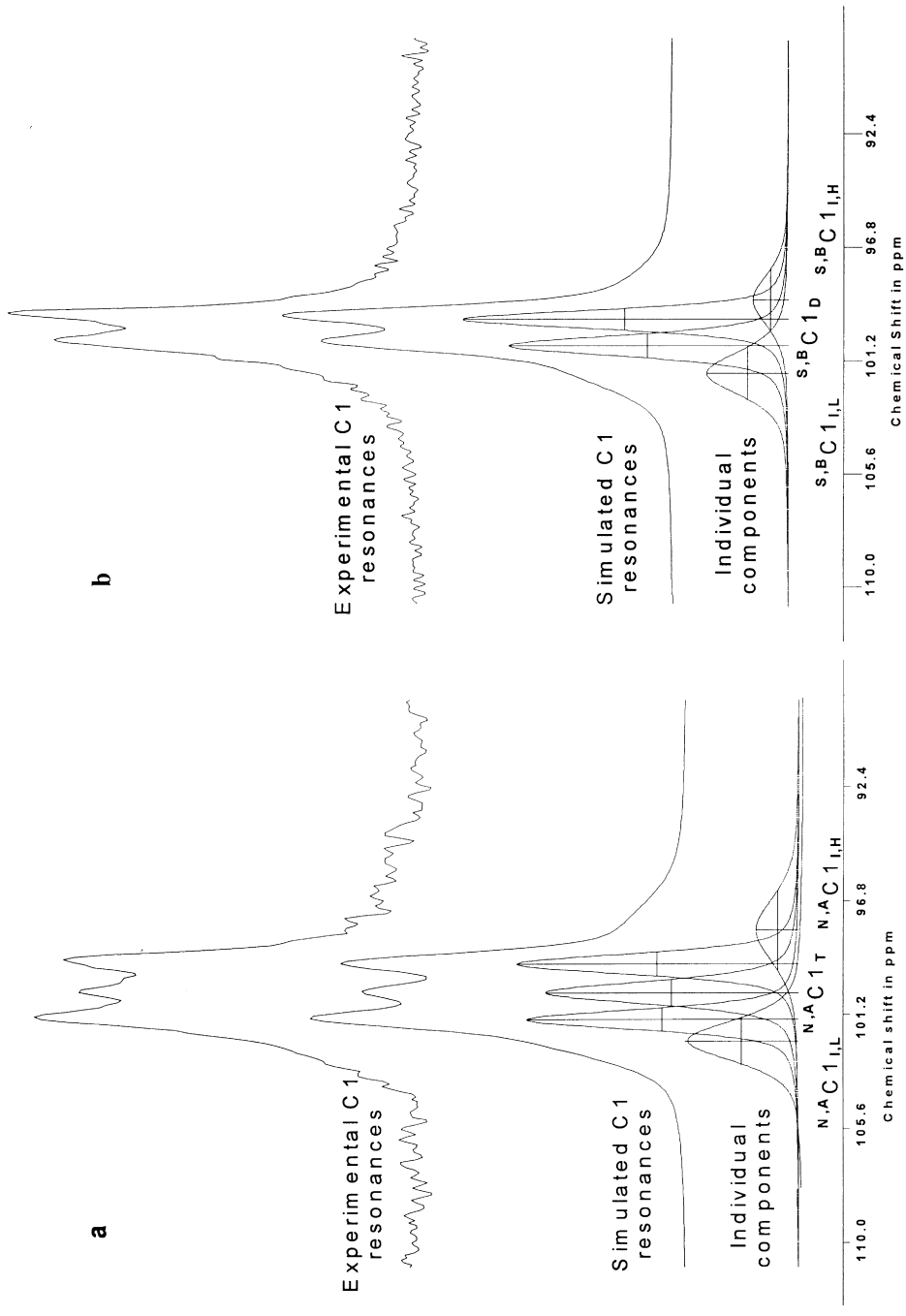

Fig. 6. Spectral decomposition of the C1 lines; (a) native waxy maize starch (A-type, $a_w = 0.9$); (b) amylose spherulitic crystals (B-type, $a_w = 0.90$) after subtraction of the amorphous contribution.

Table 2
Position, width at half height and relative integrated intensity of the lines resulting from the spectral decomposition in the C1 region of A- and B-type native starches (a_w = 0.53 and 0.9) after subtraction of the amorphous contribution

Native starches		$a_w = 0.53$	$a_w = 0.90$
$^{N,A}C1_{I,L}$	δ (± 0,5 ppm)	102,4	102,3
	ω (± 0,5 ppm)	2,4	1,9
	I% (± 5%)	11	14
$^{N,B}C1_{I,L}$	δ (± 0,5 ppm)	102,1	101,9
	ω (± 0,5 ppm)	2,1	2,5
	I% (± 5%)	14	25
$^{N,A}C1_{T}$	δ (± 0,1 ppm)	101,5	101,4
	ω (± 0,1 ppm)	1,0	0,9
	I%	12	16
	δ (± 0,1 ppm)	100,4	100,3
	ω (± 0,1 ppm)	1,0	1,0
	I%	12	17
	δ (± 0,1 ppm)	99,4	99,2
	ω (± 0,1 ppm)	1,0	0,9
	I%	12	17
	Total intensity (± 5%)	36 ± 5	50 ± 5
$^{N,B}C1_{D}$	δ (± 0,1 ppm)	100,9	100,6
	ω (± 0,1 ppm)	1,2	0,9
	I%	14	19
	δ (± 0,1 ppm)	99,9	99,6
	ω (± 0,1 ppm)	1,2	0,8
	I%	15	19
	Total intensity (± 5%)	29 ± 5	38 ± 5
$^{N,A}C1_{I,H}$	δ (± 0,5 ppm)	97,5	97,9
	ω (± 0,5 ppm)	4,0	3,1
	I% (± 5%)	11	8
$^{N,B}C1_{I,H}$	δ (± 0,5 ppm)	98,1	98,1
	ω (± 0,5 ppm)	4,4	3,9
	I% (± 5%)	14	17

shift), width at half maximum ω, and partial relative intensity to the total spectrum I%. The errors were estimated from averaging of 5–10 repeated decomposition procedures with different initial guess values. While low field (shielded) components $^{G}C1_{L}$ and $^{G}C4_{L}$ are negligibly affected by hydration, the $^{G}C1_{H}$ shoulder and, to a lesser extent, the $^{G}C4_{H}$ hidden speculative component are more sensitive. Relying on a correlation between the mean value and width of the distribution of chemical shifts (Durran et al., 1995), we infer that two main populations of conformations coexist. Among the 95% of (1–4) glycosidic linkages present in native starches, 2/3 would correspond to the low field relatively narrow distribution of stable conformations and 1/3 to a higher field (i.e. possibly more extended and less shielded) wider distribution of conformations sensitive to hydration (narrowing of the distribution). In their initial studies, Horii et al. (1987) attributed the 83 ppm $^{G}C4_{L}$ (and consequently $^{G}C1_{L}$) to a single helical conformation, keeping in line with the V-type amylose spectra (Gidley & Bociek, 1985; Gidley & Bociek, 1988; Morgan et al., 1995; Cheetham & Tao, 1998b).

In Fig. 6, examples of a detailed decomposition for C1

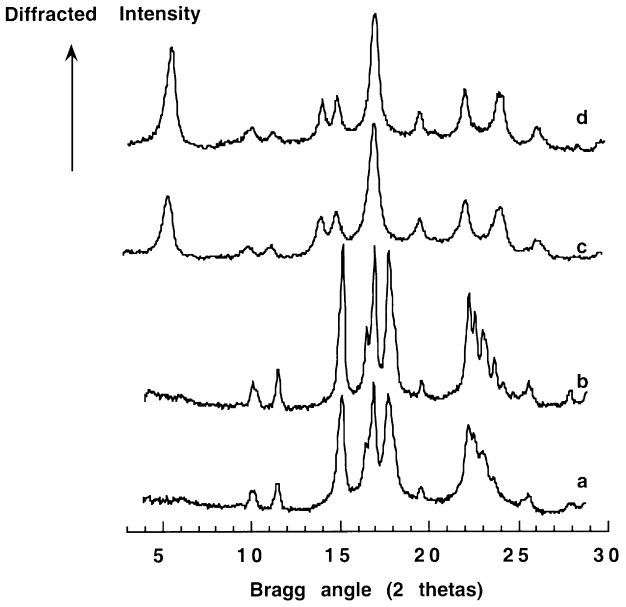

Fig. 7. X ray diffraction diagrams of amylose spherulitic crystals after water sorption at two water activity levels: (a) A-type, a_w = 0.75; (b) A-type a_w = 0.9; (c) B-type a_w = 0.75; (d) B-type a_w = 0.9.

resonances are presented for A-type native starch and B-type spherulitic crystals (a_w = 0.90). The results obtained on the different substrates studied are summarized in Table 2 (native starches) and three (spherulitic crystals) where I% is the partial intensity relative to the total C1 resonances and not relative to the total spectrum as in Table 1. As mentioned in the experimental part, all C1 resonances belonging to linear chains with packing constraints in agreement with A- or B-type crystalline lattice are expected to yield triplet or doublet of even intensities. This argument is reinforced by the existence of chain duplexes (see Imberty et al., 1988; Imberty & Perez, 1988) which should equalize the statistical weight of each component, whatever the chain length involved.

It was not possible to subtract a standard amorphous signature from the spectra of spherulitic crystals hydrated at identical relative humidity, because the amount of this phase was too limited or non-existent in these samples. In each case, once the doublet or triplet bands were fitted, two low and high field components ($^{N,A}C1_{I,L}$, $^{N,A}C1_{I,H}$, $^{S,B}C1_{I,L}$ and $^{S,B}C1_{I,H}$) were isolated (Fig. 6) and denoted 'I' for interfacial material. The corresponding bandwidth was much larger than that for crystalline components, but slightly smaller than that of the amorphous components (Table 2). This residual component was considered as non-crystalline. It was similar for both A and B crystalline types. Therefore, the corresponding conformations should be closely related in the two structures. 'Interfacial' $^{N}C1_{I,L}$ resonances and $^{N}C1_{D\ or\ T}$ crystalline multiplets were more affected by hydration for B-type starches than A-type, the partial relative intensities increase with water content while the width of the crystalline doublet was more reduced for similar

Table 3
Position, width at half height and relative integrated intensity of the lines resulting from the spectral decomposition in the C1 region of A- and B-type amylose spherulistic crystals ($a_w = 0.75$ and 0.9)

Spherulitic crystals		$a_w = 0.75$	$a_w = 0.90$
$^{S,A}C1_{I,L}$	δ (± 0,5 ppm)	102,5	102,5
	ω (± 0,5 ppm)	2,9	2,5
	I% (± 3%)	20	19
$^{S,B}C1_{I,L}$	δ (± 0,5 ppm)	102,4	101,9
	ω (± 0,5 ppm)	2,9	2,0
	I% (± 3%)	28	20
$^{S,A}C1_{T}$	δ (± 0,1 ppm)	101,4	101,5
	ω (± 0,1 ppm)	1,0	1,0
	I%	24	24
	δ (± 0,1 ppm)	100,3	100,4
	ω (± 0,1 ppm)	1,1	1,0
	I%	23	22
	δ (± 0,1 ppm)	99,3	99,3
	ω (± 0,1 ppm)	1,0	1,0
	I%	24	25
	Total intensity (± 3%)	71	71
$^{S,B}C1_{D}$	δ (± 0,1 ppm)	101,0	100,9
	ω (± 0,1 ppm)	1,1	1,0
	I%	27	34
	δ (± 0,1 ppm)	99,9	99,8
	ω (± 0,1 ppm)	1,0	0,9
	I%	27	35
	Total intensity (± 3%)	54	69
$^{S,A}C1_{I,H}$	δ (± 0,5 ppm)	98,8	99
	ω (± 0,5 ppm)	1,9	2,1
	I% (± 3%)	9	10
$^{S,B}C1_{I,H}$	δ (± 0,5 ppm)	98,4	99,1
	ω (± 0,5 ppm)	3,7	2,5
	I% (± 3%)	18	11

conditions, (Table 2). For B-type spherulitic crystals, the trend was similar for the intensity increase of the crystalline doublet, but opposite for the relative intensity of $^{S,B}C1_{I,L}$ and $^{S,B}C1_{I,H,}$ suggesting some interconversion of interfacial chains into more regularly packed chains. The width narrowing of $^{N,B}C1_{D}$ is consistent with a narrower distribution of glycosidic linkages due to the structuring role of water.

Only a minor evolution for A-type materials (particularly for spherulitic crystals) was observed at increasing water content, whatever the bands. This probably means that the amount of water present at $a_w = 0.75$ was already sufficient to complete solvent annealing at the distance probed by NMR while X-ray spectra (Fig. 7) may still be improved. Finally, the similar width and position of $^{N,A}C1_{I,L}$, $^{N,B}C1_{I,L}$ compared to $^{S,A}C1_{I,L}$, $^{S,B}C1_{I,L}$ could indicate that the corresponding conformations are probably not related to the morphology but to more local structural features such as for example inherent crystal defects along the DP 15 amylopectin branches.

In order to establish a comparison of crystallinity of the different substrates studied, the (i) NMR values derived by amorphous background subtraction $^{G}C1_{L\&H}$ (amorphous standard spectrum) as well as the interfacial amorphous component $^{N}C1_{IL\&H}$, and (ii) those determined by X-ray

Table 4
Amount of crystalline phase determined by ^{13}C CP-MAS NMR and degree of crystallinity determined by WAXS on native and recrystallized starchy substrates after conditioning at different water activities

	a_w	CP-MAS NMR		WAXS	
		0.53	0.90	0.53	0.90
Native starches	A-type	36 ± 5%	50 ± 5%	37 ± 5%	43 ± 5%
	B-type	29 ± 5%	38 ± 5%	23 ± 5%	53 ± 5%
	a_w	0.75	0.90	0.75	0.90
Spherulitic crystals	A-type	71 ± 3%	71 ± 3%	80 ± 5%	91 ± 5%
	B-type	54 ± 3%	69 ± 3%	53 ± 5%	77 ± 5%

diffraction are presented in Table 4. The general trend is similar to the results of Cheetham and Tao (1998b), who suggest that amylopectin crystallites are more stable in the absence of distorting amylose chains (such as in waxy maize amylopectin). However, their crystallinity method may underestimate the values for B-type at higher hydration because of an non-normalized difference and we differ on this particular point. As it was not possible to subtract the amorphous background contribution from the spectra of spherulitic crystals, we have considered to have essentially interfacial (or rigid interlamellar) amorphous fraction at the boundaries in these highly crystalline substrates. This is consistent with the similar position and width observed for lines $^{N,A}C1_{I,L}$, $^{N,B}C1_{I,L}$ and $^{S,A}C1_{I,L}$, $^{S,B}C1_{I,L}$ (Tables 2 and 3). The values obtained for spherulitic crystals were much higher than for native starches (up to 90% for A-type spherulitic crystals versus 43% for native A-type at $a_w = 0.90$). The values calculated from NMR and WAXS were very similar, except at higher water contents ($a_w = 0.90$), possibly where ordering at higher water content affected the chain regularity differently as seen by the two techniques. Moreover, in contrast to native and hydrolyzed starches which are well documented (Buleon et al., 1982; Cleven, Van den Berg & Van der Plas 1978; Buleon et al., 1987), the structuring role of water in spherulitic crystals may be different in the absence of longitudinal chain correlations. Fig. 7 shows X-ray diffraction diagrams of A and B-type spherulitic crystals after conditioning at $a_w = 0.75$ and 0.90. The increase of crystallinity upon rehydration of B-type crystals was revealed mainly by the intensity increase of the 100 reflexion ($2\theta = 5.6°$) and to a lesser extent by a slight lowering of the base line, while in A-type spherulitic crystals, rehydration leads to a general enhancement of band intensities over the entire range of diffraction angles used. This behavior is different from that usually observed for A-type native starches and could be related to the specific morphology of these crystals (Helbert et al., 1993). This improvement of crystallinity detected by WAXS was not evidenced by NMR data in contrast to what occurred for B-type substrates (Table 4). These differences could be related to hydration of the unit cell of each crystalline type (4 and 27% for A and B, respectively), intra-crystalline hydration being completed at a much lower relative

humidity during sorption for A-type than for B-type. For B-type spherulitic crystals, as improvement of the crystallinity between $a_w = 0.75$ and 0.90 still appeared essentially on 100 reflexion ($2\theta \cong 5°$) which corresponds to the inter-helical distance, it is conceivable that the amount of crystalline phase found by NMR was also enhanced as the ordering in helices is improved in this water content range. Considering the NMR data, for A-type native starch there was insignificant change in the amount of interfacial phase (Table 2) upon hydration which could mean that only some amorphous phase recrystallized (the amorphous contents determined by NMR decreases from 43 to 28%) or that a moving boundary layer of constant thickness surrounds the crystallites in which case some irregular double helices should be pre-existing. For native potato starch, both crystalline and interfacial phases ($^{N,B}C1_{I,L}$) were improved simultaneously with a crystallinity increase as evaluated by NMR which is smaller than that by WAXS even if the amorphous content as shown by NMR decreased from 43 to 20%. Lastly, the amount of interfacial phase of B-type spherulitic crystals disappearing upon hydration was equal to the amount of crystalline phase appearing (Table 3), therefore the crystallinity improvement corresponds to ordering of interfacial elements.

The response of the two techniques to structural evolutions upon rehydration seems to be quite different and stems from the different characteristic distance each technique is sensitive to. One may thus consider that CP-MAS NMR detects structural improvement within and at the boundaries of each microcrystals, while WAXS can be more sensitive to the alignment of crystallites as observed especially for highly hydrated spherulitic crystals.

4. Conclusion

The experiments described in this article show that it is possible to reveal and quantify three structural phases in native A- and B-type starches (amorphous background, partially-ordered interfacial and crystalline). Such a partition was obtained after subtraction of an amorphous spectrum recorded from hydrated extruded potato starch and decomposition of crystalline signatures taking into account the space groups of A and B structures. The spectrum of the amorphous standard was also shown to contain two types of α(1–4) linkages, one of which may correspond to the reported chemical shift of single helical conformation. For A- and B-type spherulitic crystals, the amount of amorphous background was too limited to be subtracted and only two phases (crystalline and partially-ordered interfacial) were quantified. Considering the space group and symmetry constraints in the crystalline phase yielding distinct (C1) and overlapping (C4) multiplets, it was assumed that we may better identify a strict crystallinity value rather than a degree of helicity. The structural evolution induced by hydration changes between $a_w = 0.53$ and 0.90 was studied by both WAXS and NMR. Even if, in spite of the relative character of the crystallinity measurements, the results obtained at lower a_w were similar, the values obtained at $a_w = 0.90$, and therefore, their evolution upon rehydration did reveal some differences. This could be attributed to differences in both the morphology of the substrates and the scale range of order detected by the two techniques. Further studies are underway to check whether the signature of amorphous starch is sensitive to preparation conditions and to improve the technique for spectral analysis by using other substrates with well defined helical conformations and/or crystallinity.

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