Characterization of the Crystalline A and B Starch Polymorphs and Investigation of Starch Crystallization by High-Resolution ^{13}C CP/MAS NMR

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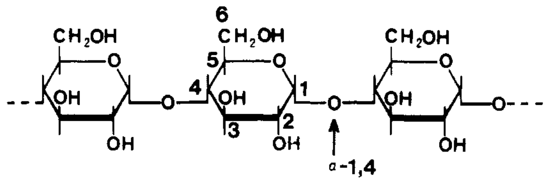
ABSTRACT: High-resolution ^{13}C CP/MAS NMR spectra have been obtained for a series of starches, providing considerable information on the crystalline structure. The spectra show a dramatic narrowing of the ^{13}C resonances upon hydration, due to an increase in the short-range order in the crystalline regions, and a concomitant loss of intensity for resonances arising from noncrystalline regions. The NMR spectra give characteristic spectra for the two natural starch polymorphs, the "A" and "B" starches. The ^{13}C NMR resonance for C(1) in both forms has a multiplet structure that is related to the crystalline structure. For "A" starches C(1) gives rise to a triplet, while for "B" starches C(1) gives rise to a doublet, in agreement with recent X-ray diffraction results proposing three glucose units and two glucose units in the respective asymmetric units. Hydration of starches with high amylose content yields increased amounts of less crystalline material, in comparison to starches with high amylopectin content. This supports the concept that amylopectin is the predominant crystalline component in starches.

Introduction

Starch is composed of two polysaccharides: amylose and amylopectin, both polymers consisting of linked D-anhydroglucose residues. In amylose (1) the residues are joined by α-1,4 linkages to give a linear polymer. In the large branched amylopectin molecule (2), side chains are grafted to the linear α-1,4 polymer by a single α-1,6 linkage at intervals of 20 units or so. These branches occur in clusters like the string elements of a tassle. This unique branching is referred to as a racemose structure. The crystalline X-ray patterns of starch have been known for many years from the early work of Katz and Van Italie;[1] however, their interpretation in terms of crystalline organization has been slow to develop. Natural starches occur in a number of different polymorphic forms.[2] The cereal starches give an X-ray pattern classed as "A", and the starches of tubers yield a "B" pattern. Recent surveys of amylose crystal structures[2] and starch organization[3] have emphasized the fact that the most probable mode for crystallization of starch is a double helix and that the uniquely shaped amylopectin molecule, with its clusters of short branches, is the *predominant* crystalline component. Indeed, starches composed solely of amylopectin crystallize as well as or better than those containing amylose. The presence of water favors starch crystallinity, with both "A" and "B" structures being interpreted in terms of hydrates. However, dehydration does not destroy the crystalline organization as such.[4]



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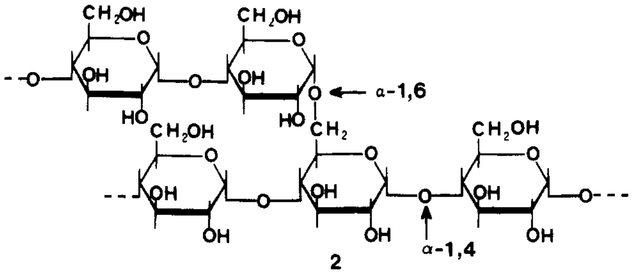

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The recently developed techniques of ^{13}C cross-polarization and magic-angle spinning (CP/MAS) nuclear magnetic resonance (NMR) spectroscopy yield high-resolution NMR spectra in the solid state.[5] The chemical shifts obtained in these spectra are the "isotropic" values for the solid state. These shifts are thus similar in nature to those obtained in solution and may be used for structural elucidation in terms of both molecular and crystal structures. In general, noncrystalline compounds such as glassy polymers give broad resonances, as the distribution of local molecular environments gives rise to a broad distribution of chemical shifts for each carbon. Crystalline compounds give rise to much narrower resonances due to the extreme regularity of the crystalline environment. Although the CP/MAS spectra of solids yield "isotropic" shifts as in solution, the solid-state spectra can be more complex due to inequivalences that may be induced between "equivalent" nuclei by the solid state. There may be a problem in interpretation of these complex spectra but much information of the lattice interactions in the solid may in principle be obtained, as will be discussed later in more detail.

The purpose of the present work, as a continuation of a previous communication,[6] is to demonstrate how ^{13}C CP/MAS NMR can be used to characterize the solid-state structures of the starch polymorphs and can add to our understanding of starch crystallization.

Experimental Section

Starch samples were obtained from a variety of commercial sources. The lintner starch was kindly provided by Dr. Alain Buleon of the Institut de Recherche Agronomique, Nantes, France, and was prepared from potato starch by removal of ~35% by weight of noncrystalline material through room-temperature

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hydrolysis with 4 N HCl. The nageli starch was kindly provided by Prof. Robitt of Iowa State University and was prepared according to ref 4. The hydrated materials were obtained by maintaining the starch samples at 100% relative humidity for periods varying from days to weeks. The dried materials were obtained by drying over P_2O_5.

The ^{13}C CP/MAS spectra were obtained at 22.6 MHz on a Bruker CXP-100 spectrometer using a home-built probe and room-temperature spinning apparatus. Spin-locking and decoupling fields of ~12 G and spinning rates of ~2.5 kHz were used. All CP spectra were obtained with a 1-ms contact time and a 1-s recycle delay. Spectra obtained with ^{13}C 90° pulses had a 30-s recycle delay. The spectra were referenced to external hexamethyldisiloxane (by substitution) and the reference was converted to tetramethylsilane (Me_4Si) by adding 2.1 ppm to the measured chemical shifts.

Powder X-ray diffraction measurements were obtained at room temperature on a Rigaku diffractometer equipped with a cobalt X-ray source.

Results and Discussion

Previous X-ray diffraction results have shown the importance of water in the crystallization of starches.[3,4] These studies show a marked increase in peak resolution and intensity upon hydration, associated with increasing order in the crystalline regions and with decreasing noncrystalline content.

The dramatic effect of hydration on the ^{13}C CP/MAS spectra of starches is shown in Figure 1. The spectra show typical results for a cereal starch and for a tuber starch when dried and when carefully hydrated by conditioning at 100% relative humidity for a period of weeks. A marked improvement in the sharpness of the peaks was noted both in the X-ray patterns and in the NMR spectra as the conditioning proceeded, to the limiting situations shown in the figure. The similar behavior of the X-ray and NMR spectra suggests that the dramatic narrowing of the ^{13}C resonances on hydration is associated with an increase of local short-range ordering concurrent with longer range effects producing crystalline material as reflected by the diffraction data. This effect has been previously observed in ^{13}C CP/MAS studies of hydrated solid (1→3)-*β*-D-glucans.[7]

However, care must be taken in the interpretation of the ^{13}C CP/MAS NMR results, as there are two possible explanations for the narrowing of the ^{13}C resonances. Crystalline materials generally show narrow resonances due to the extreme regularity of the crystalline environment, while in noncrystalline materials the distribution of local molecular environments gives rise to a distribution of chemical shifts. For example, in solid polyethylene there are two peaks in the ^{13}C NMR spectrum, one arising from the crystalline regions of the polymer, the other from noncrystalline regions.[8] In that study the contributions to the observed line widths for these resonances were estimated. The contribution due to the distribution of chemical shifts was small for the resonance from the crystalline material, giving rise to a very narrow resonance, and much larger for the resonance of the noncrystalline material, giving a much broader peak. By the use of this reasoning, the narrowing of the starch resonances would be ascribed to an increase in short-range order in the crystalline regions. However, line narrowing can also be caused by fast molecular motions. Solid *cis*-polybutadiene is elastomeric at room temperature and the molecular motion of the chains is sufficient to average the chemical shift anisotropy (CSA) (sharp resonances are observed with CP alone on static samples) and the proton–carbon dipolar interactions (sharp resonances are observed with a conventional "solution" proton decoupling field).[9] Figure 2 shows the ^{13}C NMR spectra of a solid hydrated starch obtained under various experimental conditions. Although the CP/MAS spectrum gives extremely narrow lines, CP alone on a static sample gives only a broad envelope due to the overlapping "rigid" CSA patterns. Under "solution" NMR conditions, no spectrum is obtained, indicating that the proton–carbon dipolar interactions have not been averaged by molecular motions.

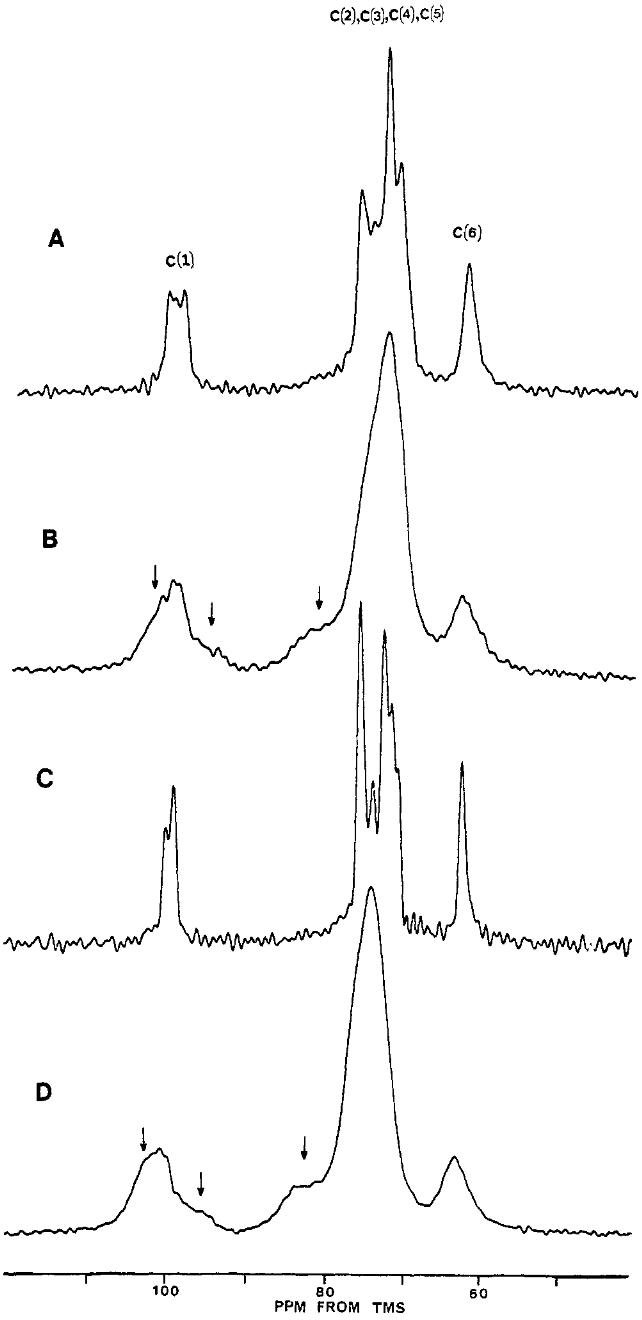

Figure 1. ^{13}C CP/MAS spectra of (A) amioca starch, a cereal starch hydrated at 100% relative humidity, (B) amioca dried over P_2O_5, (C) potato starch, a tuber starch hydrated at 100% relative humidity, and (D) potato starch dried over P_2O_5. All spectra are the accumulation of approximately 10 000 scans and are plotted with −25-Hz Gaussian resolution enhancement.

Thus the narrowing of the ^{13}C resonances of starch on hydration is not due to fast molecular motion but to increased short-range order in the crystalline regions of the hydrated starch, in agreement with the X-ray diffraction data showing line narrowing on hydration.[3,4]

On drying of the starches, the ^{13}C CP/MAS spectra (see Figure 1) show a general broadening of all resonances compared to those of the hydrated samples, and additional

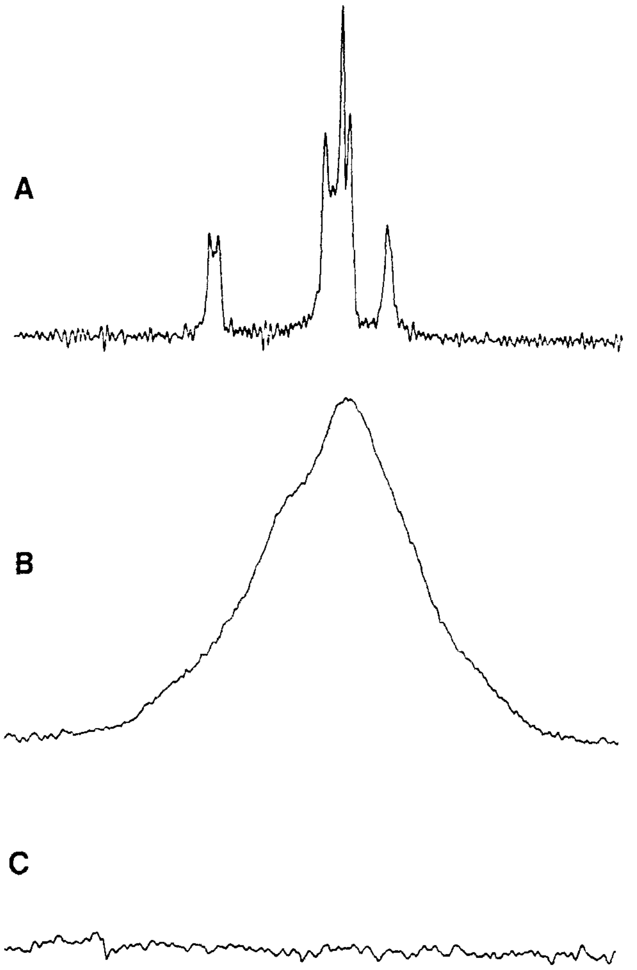

Figure 2. ^{13}C NMR spectra of nageli amylodextrin hydrated at 100% relative humidity: (A) CP/MAS spectrum; (B) CP spectrum on a static sample; (C) spectrum obtained with conventional "solution" proton decoupling fields.

broad resonances appear. The two broad shoulders that appear at δ 103 and 95 are thought to arise from noncrystalline material for C(1) carbons, and the broad resonance at δ 82 arises from noncrystalline material for C(4) carbons. These resonances are very broad due to the distribution of chemical shifts in the noncrystalline regions of the starch. Then the disappearance of these broad resonances on hydration is due to a decrease in the amount of noncrystalline material in the starch. However, spectra obtained with the CP pulse sequence are not necessarily quantitative.[22] Thus this observed loss of peak intensity could be the result of a number of variables, including fast molecular motion in the noncrystalline regions. To rule out this possibility, spectra were obtained by using ^{13}C 90° pulses (instead of CP) with high-power decoupling and magic-angle spinning. The results of this experiment and the CP experiment are identical, showing a large decrease in intensity of resonances arising from noncrystalline material on hydration of the starch. Thus the ^{13}C CP/MAS spectra confirm a large decrease in the amount of noncrystalline material, consistent with the observed increase in the intensity of the X-ray diffraction pattern on hydration.[3,4]

As mentioned previously, the natural starches can be classified into two groups, the "A" and the "B" starches, on the basis of X-ray diffraction data. Both forms are thought to crystallize as double helixes with parallel strands but differ in unit cell type, water disposition, and duplex packing.[10] A similar classification of starches can be made on the basis of their ^{13}C CP/MAS spectra as shown in Table I for a series of starches. Representative ^{13}C CP/MAS spectra for the "A" and "B" classes of starch are shown in Figure 3. The "A" starches all show a characteristic triplet for C(1), while the "B" starches show a characteristic doublet. In addition, the chemical shift of C(6) is different for each of the starch polymorphs. Thus the ^{13}C CP/MAS spectra are sensitive to the crystalline structure of starches, each polymorph giving rise to a characteristic NMR spectrum reflecting the unique packing in the unit cell.

Table I
^{13}C Chemical Shifts of Crystalline Amyloses

starch polymorph	^{13}C chemical shifts^{a} C(1)	C(6)
nageli (A)	102.7, 101.6, 100.8	62.9
amioca (A)	102.4, 101.5, 100.4	62.9
topioca (A)	102.2, 101.1, 100.0	62.7
cornstarch (A)	102.0, 101.9, 99.8	62.5
lintner (B)	101.6, 100.7	62.3
potato (B)	101.3, 100.2	62.1
pea (B)	100.9, 99.8	61.8
amylon (B)	101.7, 100.7	62.2
av of A^{b}	102.3 (0.3), 101.5 (0.4), 100.3 (0.4)	62.8 (0.2)
av of B^{b}	101.4 (0.4), 100.4 (0.4)	62.1 (0.2)

[a] ^{13}C chemical shifts in ppm relative to tetramethylsilane. [b] Values in parentheses are standard deviations.

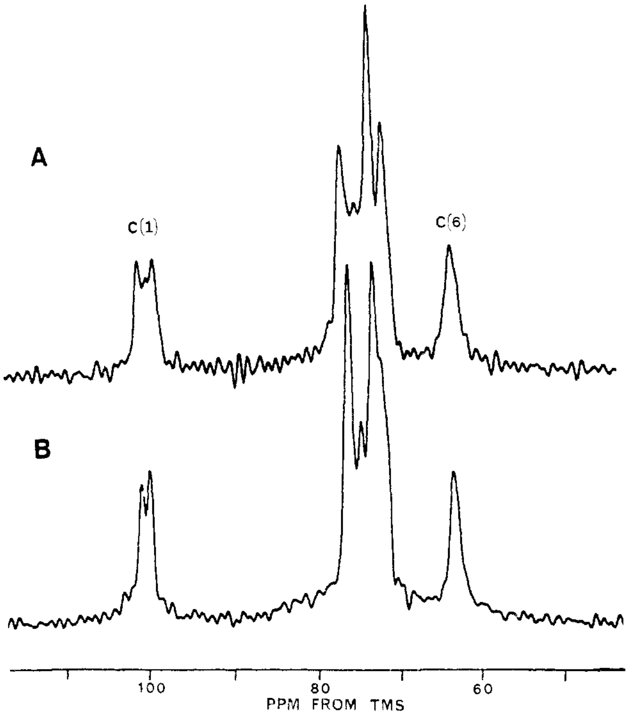

Figure 3. ^{13}C CP/MAS spectra of starches hydrated at 100% relative humidity: (A) nageli amylodextrin (an "A" starch); (B) lintner starch (a "B" starch). The spectra were the accumulation of approximately 10000 scans and are plotted with −25-Hz Gaussian resolution enhancement.

The interpretation of the multiplet splittings may be complicated. There are two possible reasons why multiplet peaks may be observed in solid-state NMR spectra, both due to effects of the crystal lattice. First, the packing in the solid could reduce the symmetry of the molecule by inducing either a fixed conformation (as for 1,4-dimethoxybenzene[11]) or a site symmetry of the lattice that is lower than the molecular symmetry (as for the heptamethylbenzenium ion[12]). In either case the result would be that more than one signal would be observed for *some* reso-

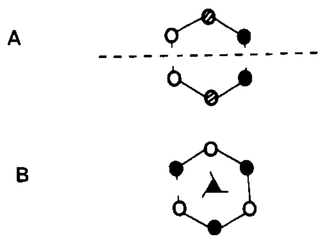

Figure 4. Schematic diagrams of the asymmetric unit of (A) "A" starch with space group $P2_1$, showing the maltotriose repeat unit, and (B) "B" starch with space group $P3_121$, showing 3_1 screw axis and maltobiose repeat unit. The view shown is down the helix axis, with only one of the two strands of the double helix shown, as the two helices are identical.

nances. This effect is ruled out here as the amylose monomer has no symmetry. The second possibility is that the unit cell of amylose might contain a number of crystallographically inequivalent sites (whatever the symmetry of each site), in principle yielding multiplet signals for *all* resonances (as for 2,4-dinitrotoluene[13]). These effects have been observed for carbohydrates[14] and carbohydrate polymers such as cellulose,[15] although there is still much debate concerning the detailed interpretation of these multiplets. A simple rule would appear to be that the number of signals should be directly related to the number of carbons in the asymmetric unit. The ^{13}C NMR spectra of crystalline α- and β-methylxylosides follow this rule. The spectrum of the β-anomer shows a single resonance for each carbon, while that of the α-anomer shows two resonances per carbon. These observations correlate well with the X-ray diffraction data, which shows that the β-anomer contains a single molecule in the asymmetric unit and the α-anomer contains two molecules.[16] The ^{13}C NMR spectra of cyclohexaamylose hexahydrate and cycloheptaamylose heptahydrate support this rule as well, showing a number of overlapping resonances that are resolved for C(4) to exactly the number of C(4) carbons in the asymmetric unit.[17] However, the ^{13}C CP/MAS spectra of lactulose illustrate the need for care in the application of this rule.[18] These spectra show three signals for the C(1) resonance, which from NMR data might be interpreted as three different polymorphic forms. X-ray diffraction data from a single crystal show that all three forms in fact coexist wihtin a single crystal, occupying random sitings in the lattice.

Previous ^{13}C NMR studies of the crystalline structure of carbohydrates have shown that the ^{13}C resonances of the carbons involved in the glycosidic linkage are particularly sensitive probes of the lattice structures. The C(1) and C(4) resonances in cellulose and its $\beta(1\rightarrow4)$-glucan oligomers show multiplicities that are sensitive to the crystalline structures of these carbohydrates.[21] In the case of the crystalline starches, the C(1) signal shows a triplet for the "A" form, and a doublet for the "B" form. This is in accord with the recently proposed space groups for the "A" and "B" starches[2] as shown schematically in Figure 4, illustrating the asymmetric unit in each case. The assigned $P2_1$ space group for "A" starch has the 2_1 axis perpendicular to the six residue per turn strands of the double helix, with maltotriose (or one-half of a strand) as the asymmetric unit and the two strands of the double helix related by a twofold rotation axis. Thus there are three different environments for each of the carbon atoms, in agreement with the observation of three signals for C(1) in the NMR spectra of "A" starches. For "B" starches, the assigned space group $P3_121$ has the 3_1 axis down the strand, specifying two glucose residues in the asymmetric unit (or one-third of a strand). In this case, there are two different environments, in agreement with the observation of two signals for C(1) in the NMR spectra of all "B" starches. If this interpretation is correct, then the ratio of peak areas in the C(1) triplet in "A" starch should be 1:1:1, and in the C(1) doublet in "B" starch should be 1:1. The series of starches studied here show ratios of intensities that are in general agreement with this requirement, although there are variations in some samples. However, it is difficult to obtain quantitative intensities in these spectra for a number of reasons. First, there are a number of resonances, arising from varying amounts of noncrystalline material, that lie under the resonances arising from the crystalline regions. Second, the CP experiment does not in general yield quantitative peak intensities. This latter effect, however, should be less important than the former under the experimental conditions used.[22]

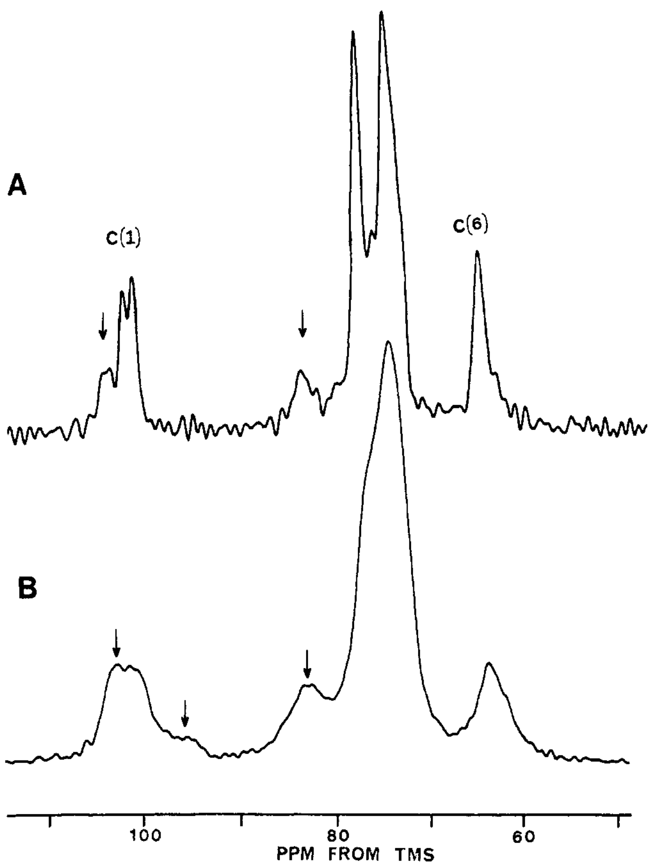

Figure 5. ^{13}C CP/MAS spectra of Amylon VII, a "B" starch with approximately 70% amylose content: (A) spectrum of sample hydrated at 100% relative humidity, (B) spectrum of sample dried over P_2O_5. The spectra are the accumulation of approximately 10 000 scans and are plotted with −25-Hz Gaussian resolution enhancement.

The C(6) resonance of starch also appears to be characteristic of the particular polymorph. The C(6) resonance of the "A" starch is broader and has a different chemical shift than the "B" starch. This may be due to the presence of a distribution of C(6) rotamer populations for the "A" starch[19] compared with a single rotameric component for the "B" starch.[20]

Since starches with high amylose content tend to be less crystalline than starches composed solely of amylopectin, the effect of amylose content on the ^{13}C CP/MAS spectra is of interest. The spectra of hydrated corn starch (an "A" starch containing about 25% amylose) and of hydrated Amylon VII (a "B" starch containing about 70% amylose) show additional resonances for C(1) and C(4). The spectra of dried and hydrated Amylon VII are shown in Figure 5.

As noted before (see Figure 1) the dried starches show extra broad resonances at δ 103 and 95 for C(1) and δ 82 for C(4) associated with noncrystalline material. In starches with low amylose content (see Figures 1 and 3) these resonances disappear on hydration, presumably due to conversion of the noncrystalline material into crystalline material as a result of the presence of water. In starches with high amylose content, these resonances do not disappear completely on hydration. On hydration of Amylon VII (see Figure 5) the broad resonance at δ 95 does disappear and the broad resonances at δ 103 and 82 decrease considerably in intensity. In addition, these latter two resonances appear to narrow considerably, suggesting that the short-range order in these noncrystalline regions has increased, although the breadth of these resonances is still considerably larger than those of the crystalline resonances. Thus hydration of high-amylose starches appears to leave a larger proportion of C(1) and C(4) carbons in a less crystalline environment than does hydration of high-amylopectin starches. This is important because it tends to support the concept that amylose, the linear component of starch, is less responsive to induced crystallization by water treatment than is the highly branched amylopectin. This surprising result is probably due to the unique "racemose" architecture of branching[3] in the latter, which enables crystallization, whereas branching in the usual dendritic architecture would be a hindrance to crystallization. Except for the appearance of resonances arising from C(1) and C(4) carbons in a less crystalline environment, the ^{13}C CP/MAS spectra of hydrated starches with high amylose content are identical with those of starches with high amylopectin content (compare Figures 5A and 3B). The same characteristic spectra for "A" and "B" starches (see Table I) are observed for all starches, independent of the amylose-to-amylopectin content. This observation strongly suggests that the local environment in the crystalline regions of "A" and "B" starches is very similar to that in "A" and "B" amyloses, respectively. Thus, although some questions have arisen regarding the relationship between the structures of amyloses compared to those of the corresponding "A" and "B" starches,[3] the CP/MAS spectra strongly suggest that the structures are similar in terms of their local environments.

Conclusions

High-resolution ^{13}C CP/MAS NMR spectra of starches show a dramatic narrowing of the ^{13}C resonances upon hydration and a concomitant loss of intensity for the resonances arising from noncrystalline regions. The narrowing of these resonances is not due to an increase in molecular motion but rather to an increase in short-range order in the crystalline regions. These results are in general agreement with X-ray diffraction data. Both the NMR spectra and X-ray diffraction data give characteristic spectra for the two natural starch polymorphs, the "A" and "B" starches. The ^{13}C NMR resonance for C(1) in both forms has a multiplet structure that is related to the crystalline structure. For "A" starches, C(1) gives rise to a triplet in agreement with recent X-ray diffraction data proposing maltotriose as the asymmetric unit. For "B" starches, C(1) gives a doublet, in agreement with the proposed structure, which has two glucose units in the asymmetric unit. Hydration of starches with high amylose content yields increased amounts of less crystalline material in comparison to starches with high amylopectin content. This supports the idea that amylopectin is the predominant crystalline component in starches.

The technique of ^{13}C CP/MAS NMR provides considerable information on the crystalline structure of starches. Because starch X-ray patterns contain relatively limited information, the ^{13}C CP/MAS technique promises to provide a valuable complementary tool in the field of starch structure research.

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