Investigation of the Crystalline "V" Amylose Complexes by High-Resolution ^{13}C CP/MAS NMR Spectroscopy

R. P. Veregin and C. A. Fyfe

Guelph-Waterloo Centre for Graduate Work in Chemistry, Department of Chemistry and Biochemistry, University of Guelph, Guelph, Ontario N1G 2W1, Canada

R. H. Marchessault*

Xerox Research Centre of Canada, 2660 Speakman Drive, Mississauga, Ontario L5K 2L1, Canada. *Received March 25, 1987; Revised Manuscript Received July 8, 1987*

ABSTRACT: The ^{13}C CP/MAS NMR spectra of the "V" amylose complexes are shown to be characteristic of their crystalline structure, as has recently been shown for the "A" and "B" starch polymorphic structures. The NMR spectra are consistent with the structures suggested by the limited X-ray diffraction information available. Using the cyclodextrin inclusion complexes with their excellent single-crystal X-ray structures as models, the molecular conformation of the "V" amylose chains can be predicted. On the basis of these correlations the torsion angles ϕ_1 and ϕ_2', describing the conformation about the glycosidic linkage, and the angle χ describing the conformation of the C(6) hydoxyl are predicted. The predicted torsion angles are in good agreement with those previously suggested from X-ray fiber diffraction data. Comparison of ^{13}C CP/MAS chemical shifts for the "A" and "B" starches with those for the "V" amylose complexes suggests that pronounced differences in molecular conformation exist. The NMR results, previous X-ray fiber diffraction studies, and molecular modeling are consistent with right-handed helices for the "A" and "B" starches, and left-handed helices for "V" amylose complexes and starch in noncrystalline regions.

Introduction

Amylose and amylopectin are the two major components of starch. In amylose (1) D-anhydroglucose residues are joined by α-1,4 linkages to give a linear polysaccharide. Amylopectin has the same backbone structure but with clusters of short side chains attached via α-1,6 linkages to give a branched polymer. Natural starches crystallize in two different polymorphic forms: the cereal starches give a characteristic X-ray pattern classified as "A" and the starches of tubers yield a "B" pattern.[1] Recent surveys of the amylose crystal structure[1] and starch organization[2] have emphasized the fact that starch crystallizes as parallel-stranded sixfold double helices. The presence of water favors starch crystallinity, with both "A" and "B" structures being interpreted in terms of hydrates. When amylose is precipitated from aqueous solution with alcohols, straight chain fatty acids, or iodine,[1,3-9] it crystallizes as "V" amylose complexes. The "V" form is also obtained when amylose crystallizes from dimethyl sulfoxide (DMSO) solution.[6] As with the "A" and "B" structures, the different "V" amylose structures are identified from their characteristic X-ray diffraction patterns. Unlike the "A" and "B" starches the "V" amylose complexes crystallize as single strand sixfold helices packed antiparallel.[1]

The 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.[10] The chemical shifts obtained in these spectra are the "isotropic" values for the solid state, and thus the shifts are similar in nature to those obtained in solution. As the chemical shifts are a sensitive probe of the environment of the nuclei, they may be used for structural elucidation in the solid state in terms of both the molecular chain conformation and the intermolecular chain packing.

A number of workers have very recently shown that the ^{13}C CP/MAS NMR spectra of the "A" and "B" starches are characteristic of the two different crystalline forms.[11-15] In previous papers[11,12] we have shown that these NMR spectra are consistent with the structures suggested by X-ray diffraction analysis, and, with use of the crystalline cyclodextrin hydrates as model compounds, the molecular conformation of the starch chains could be predicted.[16] The purpose of the present work is to show that the ^{13}C CP/MAS NMR spectra of the "V" amylose complexes are also sensitive to molecular conformation, thereby providing a basis for predicting the molecular conformation.

Experimental Section

Amylose was extracted from commercial corn starch (BDH Chemicals) using the hot water method of Kerr[17] and then precipitated as single crystals by saturating the aqueous amylose solution with *n*-butyl alcohol. The precipitate was filtered and, while still "wet" with the mother liquor, was stored over saturated aqueous butanol. The amylose prepared in this way was in the form of the crystalline amylose–butanol complex V_h(BuOH). Washing the precipitate three times with *n*-butyl alcohol gave the V_a(BuOH) complex. Soaking the precipitate in *n*-butyl alcohol for 3 days followed by drying over P_2O_5 for 1 week gave the hydrated (V_h) amylose complex. Samples of the anhydrous (V_a) amylose were then prepared by drying the V_h amylose complex at 100 °C for 8 h under vacuum. Films of the amylose–DMSO complex (V_{DMSO}) were prepared by dissolving V_a amylose in DMSO (25 wt % amylose) and then drying under vacuum at 70 °C for 1 day. The hydrated starch samples have been previously described.[11,12]

The ^{13}C CP/MAS NMR 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 approximately 12 G, and spinning rates of 2–3 kHz were used in all cases. The spectra were referenced to external hexamethyldisiloxane (by substitution) and converted to TMS by adding 2.1 ppm to the measured chemical shifts. Some spectra were also obtained at 108.4 MHz on a commercial MSL-400 courtesy of Bruker Spectrospin Karlsruke, GFR.

Powder X-ray diffraction patterns were obtained at room temperature on a Rigaku diffractometer equipped with a copper X-ray source (λ = 1.542 Å). The "V" amylose complexes were characterized by their X-ray powder diffraction patterns. The V(BuOH), V_a, V_h, and V_{DMSO} structures were identified by their characteristic *d* spacings, which were in good agreement with those from the literature.[3-6] The two V(BuOH) complexes were distinguished from each other by the characteristic intensities of a number of the diffraction maxima, allowing clear identification of these two complexes.[3]

Results and Discussion

Typical ^{13}C CP/MAS NMR spectra of the "V" amylose complexes are shown in Figure 1. Also included in Figure 1 is a spectrum of a hydrated "A" starch of high crystallinity. In Figure 2 typical ^{13}C CP/MAS NMR spectra of a hydrated "B" starch of high crystallinity and a dried sample of low crystallinity are shown. The chemical shifts

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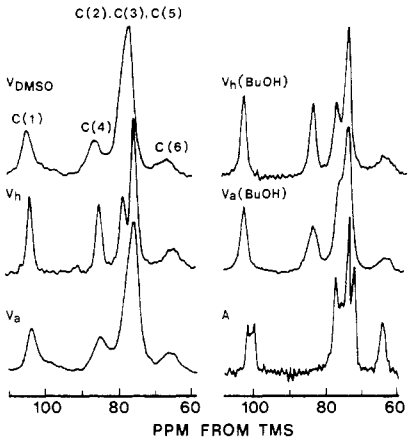


Figure 1. ^{13}C CP/MAS NMR spectra of some "V" amylose complexes. A spectrum of a fully hydrated crystalline "A" starch (Amioca starch, prepared as described in ref 12) is included for reference. The spectra of the "V" complexes are the accumulation of 20 000 scans, the "A" starch 10 000 scans. All spectra were recorded at 22.6 MHz and are plotted with −25-Hz Gaussian resolution enhancement.

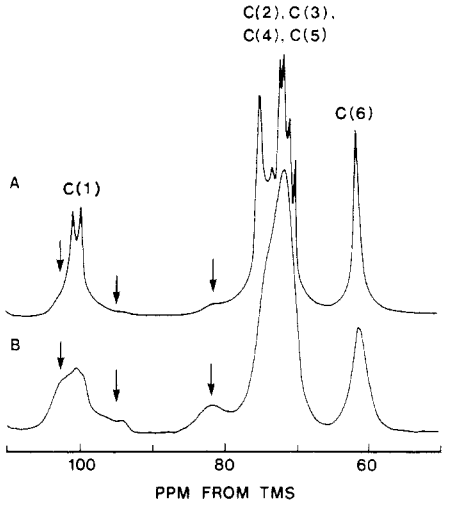
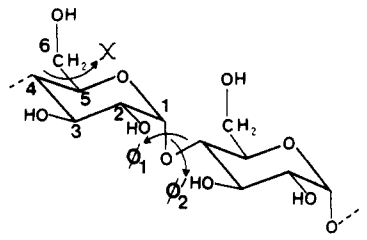


Figure 2. ^{13}C CP/MAS NMR spectra of a "B" starch (potato starch): (a) spectrum of the fully hydrated crystalline "B" starch. (b) Spectrum of the dried noncrystalline potato starch. The arrows denoted peaks assigned to C(1) and C(4) carbons in noncrystalline regions. Both samples were prepared as described in ref 12). The spectra are the accumulation of 2000 and 400 scans, respectively. They were recorded at 108.4 MHz and are plotted with no line-broadening.

of the V amyloses and the hydrated "A" and "B" starches are tabulated in Table I. The assignments of the C(1), C(4), and C(6) resonances were made based on the corresponding solution spectra.[18] The assignment of the C(2), C(3), and C(5) resonances has not been made, as ^{13}C labeling studies have shown that the correspondence of the solid-state shifts with the solution values is not straightforward.[19]

A characteristic feature of the ^{13}C CP/MAS NMR spectra of the "V" amylose complexes is that the NMR peaks are much broader than those in the highly crystalline hydrated "A" and "B" starches (see Figures 1 and 2). The crystalline "V" amylose peaks are nearly as broad as those in the dried starch of low crystallinity, and in fact C(6) is broader in the "V" amylose spectra. It has been demonstrated that in the solid-state spectra of the "A" and "B" starches,[11,12] and indeed in a number of other polysaccharides,[20] that the NMR line widths are sensitive to the local short-range order of the carbon environment. In general, the less short-range order the broader the NMR resonances, as a distribution of local molecular environments will give rise to a distribution of chemical shifts for each carbon. Thus, the "A" and "B" starches have the most short-range order, followed by the V_h and V_h(BuOH), V_a(BuOH) and V_a, V_{DMSO}, and the dried starch. In agreement with this interpretation, the X-ray diffraction maxima are narrowest for the "A" and "B" starches and become progressively broader in the above order, with the V_{DMSO} and the dried starch showing the broadest diffraction maxima. The broadening of diffraction maxima reflects a decrease in long-range order of the crystalline regions. As a general observation, C(6) shows more disorder than C(1) or C(4) in the "V" amyloses, and in fact C(6) is more disordered in the "V" amyloses than in dried starches of low crystallinity. As the amount of water that is included in the unit cell of these amyloses and starches decreases, the amount of long-range order (from X-ray diffraction) and short-range order (from NMR) also decreases. Thus the fully hydrated "A" and "B" starches may contain up to 40 molecules of water in the unit cell,[21] compared to four molecules in the V_h amylose,[9] one molecule in the V_a amylose,[7] and no water in the dried starch or the V_{DMSO} amylose.[6] It may be that the addition of water to amylose or starch allows the release of strain associated with nonoptimum hydrogen bonds, with water taking part in the hydrogen-bonding network.

Previous ^{13}C CP/MAS NMR studies of crystalline 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 β-1,4-glucan oligomers[22,24] and the C(1) resonance of starches show multiplicities that are sensitive to the crystalline structure of these carbohydrates.[12-16] Our previous work has shown that the crystalline cyclomaltose inclusion complexes are good models for the interpretation of the ^{13}C CP/MAS NMR spectra of the natural "A" and "B" starch polymorphs.[16] The cyclomaltoses are macrocyclic oligosaccharides of starch, composed of six or more α-1,4-linked D-anhydroglucose residues (**1**). The cyclomaltoses form inclusion



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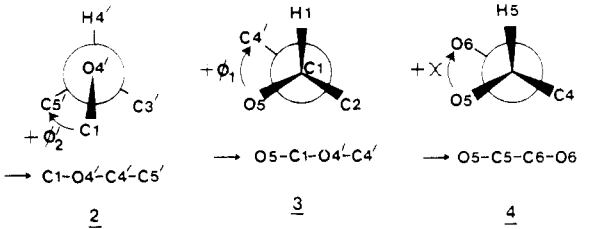
complexes with a wide variety of small molecules that can fit inside the annular cavity. The crystalline structures of a number of the inclusion complexes of cyclomaltohexaose (consisting of six residues) and cyclomaltoheptaose (consisting of seven residues) have been accurately described by using the techniques of single-crystal X-ray diffraction.[23] The conformation of the cyclomaltose molecule can be described by three angles ϕ_1, ϕ_2', and χ. The angles ϕ_2' (**2**) and ϕ_1 (**3**) describe the conformation of pairs of residues about each glycosidic linkage, and the angle χ (**4**) describes the conformation of



2 **3** **4**

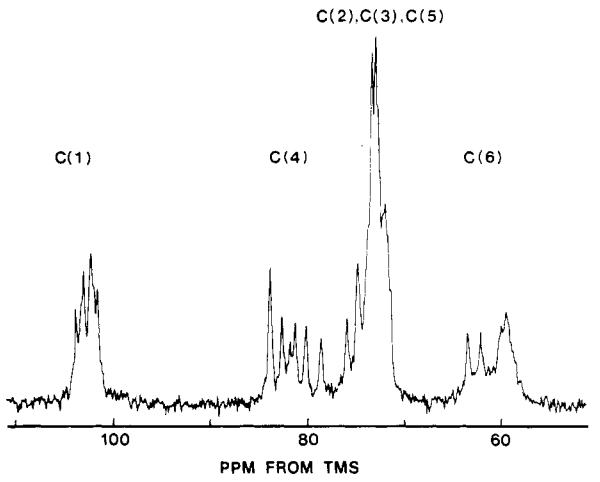


Figure 3. ^{13}C CP/MAS NMR spectrum of the cyclomaltoheptaose dodecahydrate complex. The sample was prepared as described in ref 16. The spectrum is the accumulation of 40 scans. It was recorded at 108.4 MHz and is plotted with no line broadening.

the C(6) hydroxyl in each residue. Thus, in the ^{13}C CP/MAS NMR spectrum the resonance for each chemically distinct carbon will consist of up to six peaks (one for each residue) in cyclomaltohexaose and seven peaks in cyclomaltoheptaose.

For a series of six cylomaltose inclusion compounds (the spectrum of cyclomaltoheptaose is shown in Figure 3) empirical correlations have previously been found[16] between the ^{13}C NMR solid-state chemical shifts and the torsion angles determined from single-crystal X-ray diffraction studies. In particular it was found that the C(1) and C(4) chemical shift correlate with the angles ϕ_2' and ϕ_1, respectively. To illustrate these correlations, Figures 4 and 5 show the linear correlation between the X-ray diffraction derived torsion angles ϕ_2' and ϕ_1 and the respective C(1) and C(4) resonances for cyclomaltoheptaose dodecahydrate (shown in Figure 3). The torsion angle χ has three energy minima, known as *gg*, *gt*, and *tg* (**4**). For the cyclomaltoses it was found[16] that C(6) ^{13}C shifts of 59.6–61.7 ppm corresponded to the *gg* conformation and 62.7–65.9 ppm to the *gt* conformation. These values are in accord with those proposed previously for β-1,4-glucans: 62 ppm for *gg*, 62.7–64.5 ppm for *gt*, and 66 ppm for *tg*.[24] These correlations between the ^{13}C shifts and the torsion angles from single-crystal X-ray studies of these starch oligomers have proven to be very useful in interpretation of the ^{13}C CP/MAS NMR spectra of the natural "A" and "B" starches, where only the limited information from X-ray fiber diffraction studies is available.[16] These correlations will be used below to predict the torsion angles in the "V" amylose complexes.

Recent work[11-16] has shown that the ^{13}C CP/MAS NMR spectra of the natural "A" and "B" starches and amyloses are characteristic of the polymorphic crystalline structures. In particular the multiplicities of the C(1) resonance and the chemical shifts of the C(1), C(4), and C(6) resonances are most sensitive, each allomorph giving rise to an NMR spectrum reflecting the unique symmetry of the unit cell. Thus the hydrated "A" starches give rise to a characteristic 1:1:1 triplet for C(1) and the "B" starches a 1:1 doublet, as shown in Figure 1 and 2. These characteristic features are identical in both starches and amyloses[11-16] and thus are independent of the amylopectin content, as discussed in our previous work.[12] This is consistent with amylose and amylopectin crystallizing into the same unit cells, as suggested by the similarity of the X-ray powder diffraction patterns. These multiplets are in accord with the recently

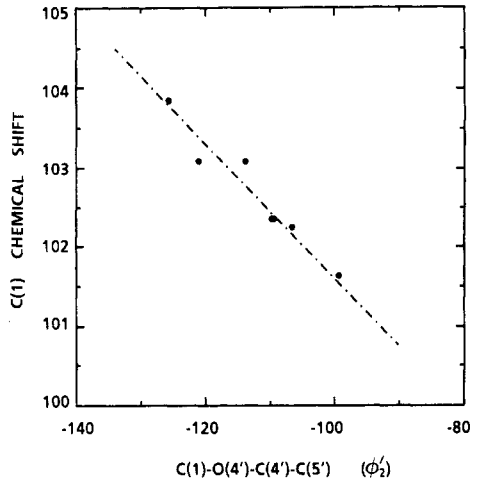


Figure 4. Correlation of solid-state ^{13}C C(1) chemical shifts of peaks from the C(1) resonance of cyclomaltoheptaose dodecahydrate with the torsion angle C(1)–O(4′)–C(4′)–C(5′) (ϕ_2'), determined from single-crystal X-ray diffraction studies.

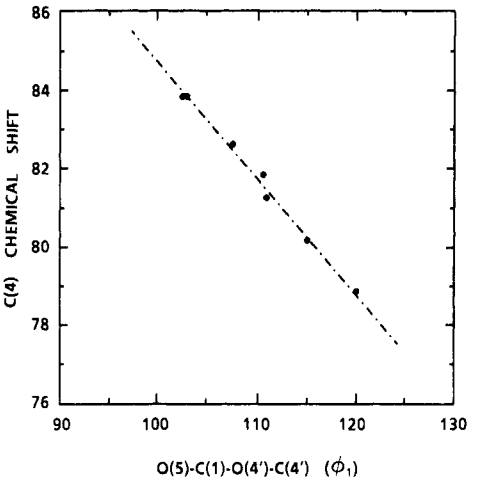


Figure 5. Correlation of solid-state ^{13}C C(4) chemical shifts of peaks from the C(4) resonance of cyclomaltoheptaose dodecahydrate with the torsion angle O(5)–C(1)–O(4′)–C(4′) (ϕ_1), determined from single-crystal X-ray diffraction studies.

proposed space groups for the "A" and "B" amyloses.[1,25,26] Both amyloses are thought to crystallize as sixfold double helices with parallel strands. The assigned $P2_1$ space group for "A" starch results in an asymmetric unit of maltotriose (the three residues in half a strand); thus there are three different environments for C(1) giving rise to three peaks of equal areas. The assigned $P3_121$ space group for the "B" amylose has the 3_1 axis down the strand, specifying two glucose residues in the asymmetric unit (or one-third of a strand); thus there are two different environments for C(1) giving two peaks of equal areas in the NMR spectrum. Note in both cases the two strands of the double helix are in identical environments.[25,26] Thus the C(1) multiplicity appears to reflect the helix conformation in the "A" and "B" starches (and amyloses) rather than an *intermolecular* effect between neighboring chains.

Using the cyclodextrin inclusion complexes as models lends further support for a predominantly *intramolecular* origin for these multiplets. In the cyclomaltoses studied the packing of the different complexes varies little, while the ^{13}C shifts correlate well with the torsion angles that determine the molecular conformation.[16] On the basis of the cyclomaltose models, there are thus two sets of conformational angles ϕ_1 and ϕ_2' in the sixfold "B" starch helix and three sets of angles ϕ_1 and ϕ_2' in the sixfold "A" starch helix, giving rise to the characteristic multiplets in the ^{13}C

CP/MAS NMR spectra. In both starches there appears to be a single conformational angle χ, giving rise to a single peak for C(6). One would also expect to see the helix conformation in the "A" and "B" starches to be reflected in multiplicities of the C(4) resonance; however, the overlap with the C(2), C(3), and C(5) resonances does not allow assignment of the C(4) resonance. Note that the large shift in the C(4) resonance in the starches compared to the cyclomaltoses suggests that the angle ϕ_1 is very different in the starches versus the cyclomaltoses.

Except for the changes in the line widths of the ^{13}C resonances due to changes in short-range order, the spectra of the different "V" amylose complexes are identical. The ^{13}C chemical shifts appear to be characteristic of the "V" structure and are very different from those in the hydrated "A" and "B" starch structures. In particular, the C(1) resonance is shifted 1–2 ppm downfield and the C(4) resonance 6–11 ppm in the "V" structure compared to the "A" or "B" structure (note that in the "A" and "B" starches C(4) cannot be assigned as it overlaps the C(2), C(3), and C(5) resonances). In addition, the C(1) peak in the "V" structure is a singlet, while it is a multiplet in the "A" and "B" structures. These characteristic features of the "V" amylose spectra appear to reflect the particular helix conformation of the "V" amylose chains. The singlets for C(1) and C(4) suggests that the helix in the "V" structure is more symmetric than that in the "A" and "B" structures, with all residues in the "V" helix occupying approximately equivalent positions, i.e. described by one set of ϕ_1 and ϕ_2' angles. However, it must be noted that the C(1) and C(4) resonances are considerably broader in the "V" complexes than in the "A" and "B" starches, which may indicate that the residues in the "V" amylose chain are in a spread of slightly different environments. In this regard, the C(6) resonance in all the "V" amyloses are very broad, suggesting that the C(6) carbons in the "V" amylose helix are in a number of environments described by a range of conformations about an average angle, χ. It may be that differences in the C(6) conformation disrupt the symmetry of the "V" helix, as is suggested by some X-ray diffraction studies.[1,7]

The concomitant narrowing of the NMR resonances and the X-ray diffraction maxima suggests that the interpretation of the NMR peak widths in terms of static disorder is the most reasonable one: decreasing short-range order correlates with decreasing long-range order. However, dynamic disorder of the polymer chains can also cause NMR line width changes, due to changes in ^{13}C T_2 relaxation times. In this case increased molecular motion would lead to narrower NMR resonances. In this view, "A" and "B" hydrated starches would be the most mobile, followed by the "V" amyloses and dehydrated starch. Water would thus be thought of as a "plasticizer", increasing the mobility of polymer chains in the crystalline regions. However, it does not seem reasonable that the most mobile material would also give rise to the greatest perfection of long-range order in the crystalline regions, as shown by X-ray diffraction. In addition, very recent ^{13}C T_1 relaxation data[14] suggests that "A" or "B" amylose in fact has very similar mobilities in the dry and hydrated states, while "V" amyloses appear to be considerably *more* mobile than "A" or "B" amylose, rather than less. If relaxation effects were dominant in NMR line widths, this predicts line width changes opposite to those observed. Thus in general, dynamic disorder does not appear to be consistent with changes in NMR linewidths and ^{13}C T_1's.

The "V" amylose complexes studied here are thought to consist of sixfold left-handed single helices, crystallizing in the $P2_12_12_1$ space group.[1] This space group does not require all six residues within a helix to be identical. However, in the V_h and V_{DMSO} structures all residues are thought to be identical, with approximately sixfold symmetry.[8,9] For the V_a complex, a three-residue asymmetric unit has been suggested on the basis of the X-ray diffraction and molecular modeling.[7] However, the three residues are thought to differ *only* in the conformation of the C(6) hydroxyl, with residues being in each of the *gg*, *gt*, and *tg* conformations. In this case the polymer chain can still be described by only one set of conformational angles, ϕ_1 and ϕ_2'. The X-ray studies are thus consistent with the single peak seen for each of the C(1) and C(4) ^{13}C resonances. The X-ray study of V_a amylose also suggests that the extreme broadness of the C(6) resonance in all of the V amyloses may be due to different conformations of the C(6) hydoxyl, as will be discussed later.

Table I
^{13}C CP/MAS NMR Chemical Shifts for the "V" Amylose Complexes and the "A" and "B" Starches[a]

complex	C(1)	C(4)	C(6)
V_{DMSO}	103.7	83.3	63.0
V_a	103.6	83.1	63.2
V_h	103.3	82.7	61.2
V_a(BuOH)	103.5	82.7	62.0
V_h(BuOH)	103.4	82.6	62.3
"A" starch	102.3		
	101.5	72–77[b]	62.8
	100.3		
"B" starch	101.4	72–77[b]	62.1
	100.4		
noncrystalline starch	103		
	95	82	62

[a] ^{13}C chemical shifts are in ppm from TMS. The precision of the shifts is estimated to be σ = 0.3 ppm. The data for "A" and "B" starches are from ref 11 and 12. [b] The ^{13}C resonance for C(4) in the "A" and "B" starches could not be assigned as it lies in the region of the C(2), C(3), and C(5) resonances.

Comparison of starch samples with low crystallinity to those with high crystallinity has allowed the assignment of resonances for C(1) and C(4) associated with noncrystalline material (see Figure 2 and Table I). The broad peak at 103 ppm and the very broad peak centered near 95 ppm have been assigned to C(1) carbons in noncrystalline regions; the broad peak at 82 ppm has been assigned to C(4) carbons in noncrystalline regions. The peaks at 103 and 82 ppm have virtually identical shifts to the C(1) and C(4) carbons, respectively, in the "V" amylose complexes. This strongly suggests that the helix conformation in the "V" amylose complexes and noncrystalline regions of starches is the same, as has been discussed by Gidley and Bociek.[15] This is an interesting conclusion, that much of the material in the noncrystalline starch has the same conformation as in the "V" structure, and will be discussed in more detail later.

On the basis of correlations of ^{13}C NMR solid-state shifts and the single-crystal X-ray diffraction torsion angles, the corresponding torsion angles have previously been predicted for the "A" and "B" starches[16] and can be extended to the "V" amylose complexes and noncrystalline starch. From the relationship between C(1) and ϕ_2' and C(4) and ϕ_1 the torsion angles describing the conformation of the residues about the glycosidic linkages in the various starch and amylose structures have been predicted. The results of this modeling are shown in Table II, along with the limited results from X-ray fiber diffraction analysis. The agreement between the torsion angles predicted from the ^{13}C shifts and the X-ray fiber diffraction studies is impressive, especially in light of the dramatic changes that are predicted in the conformations between the "V" type

Table II
Predicted Torsion Angles in Starches and Amyloses from Correlations of ^{13}C CP/MAS NMR Shifts in Cyclomaltoses and X-ray Diffraction Studies (XRD)

	predicted torsion angles			
	ϕ_2'		ϕ_1	
	NMR[a]	XRD[b]	NMR[c]	XRD[b]
V_h	−120	−128	95–110	106
V_{DMSO}	−120	−130	95–110	114
V_a	−120	−131	95–110	115
V_a(BuOH)	−120		95–110	
V_h(BuOH)	−120		95–110	
"A" starch	−106			
	−97			
	−84			
	av −96	−84	120–155	142
"B" starch	−96			
	−85			
	av −91	−84	120–155	142
noncrystalline starch	−120			
	−40 to −90		95–110	

[a] Predicted from the correlation of the C(1) chemical shift with the angle ϕ_2'. [b] Predicted from fiber X-ray diffraction studies: V_h from ref 9, V_{DMSO} from ref 8, V_a from ref 6, and "A" and "B" from ref 21 and 25. [c] Predicted from the correlation of the C(4) chemical shift with the angle ϕ_1.

structure and the "A" and "B" type structures. In the "A" and "B" starches two and three different ϕ_2' angles are predicted, respectively, as suggested by recent analysis of X-ray fiber data. However, the only calculations of the helix conformation are those of Wu and Sarko who assumed sixfold symmetry.[21,25] The NMR predictions would thus be a good starting point for a more complete modeling analysis of these starches based on X-ray diffraction.

From the correlation of C(6) with the angle χ in the cyclomaltoses the angle χ has been predicted to be *gt* in both the "A" and "B" starches. It also appears to be *gt* in the noncrystalline regions of starch, although the C(6) resonance is considerably broader in the noncrystalline material, suggesting a broader distribution of angles. In the "V" amyloses, the C(6) resonance is broader than that in the noncrystalline starch, the resonance covering the range expected for *gg*, *gt*, and *tg* conformations. If all three low-energy conformations for the exocyclic angle χ were present in the V amyloses, then three peaks would be expected in the range of 60–66 ppm in the spectrum. This would be true if exchange between conformations was slow on the NMR time scale: however, if the exchange between the different conformations was approximately equal to the ^{13}C shift differences ($\nu \approx 50$ Hz), then a single broad line would result. The observed line is very broad (at half-height its width is approximately 6 ppm) and does cover the expected range of chemical shifts. This model for the conformation of "V" amylose C(6) hydroxyl is supported by a careful analysis based on X-ray diffraction data and molecular modeling that suggests C(6) does have three different conformations in V_a amylose.[7] Indeed, the shorter T_1 relaxation time associated with C(6) compared to ring carbons in "V" amyloses is consistent with dynamic disorder for C(6), as suggested by Horii et al.[14]

The results shown in Table II have interesting implications for the helix chirality in the "A", "B", and "V" starch and amylose structures. As French has shown,[27,28] modeling right-handed "A" and "B" starch helices leads to a ϕ_1 value of 142° and a ϕ_2' value of −84°, as suggested by Wu and Sarko,[21] and consistent with the NMR predicted angles for the "A" and "B" starches. However, the modeling of left-handed "V" helices results in a ϕ_1 value of 93° and a ϕ_2' value of −150°, in general agreement with the X-ray diffraction studies[1,6,8,9] and the NMR shifts for the "V" amylose structures. The conclusion that the "A" and "B" starches crystallize as right-handed double-stranded helices while the "V" amyloses crystallize as left-handed single-stranded helices is strongly supported.

The ϕ_2' and ϕ_1 torsion angles are also predicted in Table II for the noncrystalline regions of starch. The C(1) resonance in the noncrystalline starch shows a very broad peak which yields ϕ_2' angles in the range of −40° to −90° for some of the C(1) carbons in noncrystalline regions. However, much of the material in the noncrystalline regions of starch appears to have a helix conformation very similar to that of the "V" amylose complexes (see Table II). This again suggests that most of the material in the noncrystalline regions consists of left-handed helices. [Since the analysis of the spectra of the cyclomaltoses and the "A" and "B" starches assume the major contribution to chemical shifts is intramolecular (based on helix conformation) and intermolecular effects are minor, it is not clear whether the noncrystalline regions of starch are double helical, as X-ray diffraction studies suggest for the "A" and "B" starches, or single-helical, as suggested for the "V" amylose complexes.] On hydration, X-ray diffraction studies and ^{13}C NMR studies have clearly shown that noncrystalline material is converted into crystalline material.[2,11,12,29] The present work thus suggests that in the process of hydration left-handed helices in noncrystalline material are converted into right-handed double helices in the crystalline "A" and "B" starches. This is especially interesting in the light of the fact that French has suggested that there is no low-energy path between left- and right-handed helical conformations in starches.[28] It is also interesting that much of the material in noncrystalline starch appears to have the same helix conformation as that in the crystalline "V" amylose. One possible explanation is that the right-handed helix is the stable conformation in the presence of large amounts of water in the unit cell, as in the "A" and "B" structures which may contain up to 40 water molecules. In the absence of sufficient water in the structure, as in dehydration of "A" and "B" starches or in the crystallization of the "V" amyloses (which typically contain less than four water molecules per unit cell), the left-handed helix becomes more stable.

Conclusions

The ^{13}C CP/MAS NMR spectra of the "V" amylose complexes show broader resonances than the "A" and "B" starches and for C(6) a broader resonance than that in the noncrystalline regions of starch. The NMR results and X-ray diffraction results show that the broadening is associated with decreasing short- and long-range order. The degree of perfection of ordering is associated with increasing amounts of water in the unit cell of the starch or amylose. The broadness of the C(6) resonance is suggested to be due to the presence of a number of conformations for the C(6) hydroxyl, probably in some state of dynamic exchange.

The ^{13}C CP/MAS NMR spectra of the "V" amylose complexes, the "A" and "B" starch polymorphs, and the noncrystalline regions of starch are shown to be characteristic of their differing crystalline states. The NMR spectra are interpreted in terms of helix conformation, while intermolecular packing effects are thought to be minor. The ^{13}C CP/MAS NMR spectra of these α-1,4-glucans are consistent with the structures suggested by the limited X-ray diffraction information available. The derived conformational angles for the "V" structures are in keeping with a proposed conformation for a left-handed fragment of the crystalline amylosic chain.[29] With use of

the cyclodextrin inclusion complexes with their excellent single-crystal X-ray structures as models, the molecular conformation of the residues in the helical chains can be predicted. On the basis of these empirical correlations the torsion angles ϕ_2' and ϕ_1 describing the conformation about the glycosidic linkage and the angle χ describing the conformation of the C(6) hydoxyl are predicted. The predicted torsion angles are in general agreement with those previously suggested from X-ray fiber diffraction data and suggest that the helix conformation of the "V" amyloses is different from that of the "A" and "B" starches. The molecular conformation in the noncrystalline regions of starches appears to be similar to that in "V" amylose. The NMR results, previous X-ray fiber diffraction studies, and molecular modeling are consistent with right-handed helices for the "A" and "B" starches and left-handed helices for "V" amylose complexes and starch in noncrystalline regions.

Acknowledgment. We acknowledge the financial assistance of NSERC (Canada) in the form of an operating grant (C.A.F.) and a Graduate Scholarship (R.P.V.). We also acknowledge the molecular modeling done by Dr. A. D. French and very helpful discussions regarding the X-ray diffraction data with Dr. A. Sarko and Dr. P. Zugenmaier. We acknowledge the technical assistance of C. Pasztor in obtaining the powder diffraction data. We also acknowledge Bruker Spectrospin Karlsruke, GFR, for the use of an MSL-400 NMR spectrometer.

Registry No. V_h(BuOH), 66461-46-9; V_{DMSO}, 110682-30-9; cyclomaltoheptaose dodecahydrate complex, 20986-19-0.

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Triplet Photophysical Properties of the Alternating Copolymer of 2-Vinylnaphthalene with Methyl Methacrylate

Richard D. Burkhart* and Gregory W. Haggquist

Chemistry Department, University of Nevada, Reno, Nevada 89557

Stephen E. Webber

Chemistry Department and Center for Polymer Research, University of Texas, Austin, Texas 78712. *Received April 27, 1987; Revised Manuscript Received June 22, 1987*

ABSTRACT: Delayed luminescence spectra of the subject alternating copolymer have been obtained in frozen glassy solutions at 77 K, solid films at 77 K, and fluid solutions at ambient temperature. Delayed fluorescence emission is detected in all three media. In solid films at 77 K and in fluid solutions at ambient temperature the delayed fluorescence emission consists of both monomeric and excimeric components. In frozen glassy solutions both phosphorescence and delayed fluorescence emissions are monomeric in character. Negligible phosphorescence was observed at 77 K from solid films of the copolymer which had been treated by prior heating under vacuum. As a rationale for this unusual behavior it is proposed that triplet exciton trap sites are essentially absent here and so the migratory excursions of these excitons are relatively unhindered. This leads to enhanced rates of triplet–triplet annihilation which are orders of magnitude larger than the rate of phosphorescent decay.

Introduction

In recent years a rather substantial body of evidence has been accumulated attesting to the special photophysical properties of chromophoric groups covalently bonded to the backbone of a polymer chain.[1] Vinyl aromatic polymers, for example, display some luminescence characteristics similar to their monomeric analogues but other characteristics which serve to remind us of the relatively high local chromophore concentrations and special steric arrangements which are peculiar to the polymeric species.

0024-9297/87/2220-3012$01.50/0