^{13}C CP/MAS NMR Studies of Amylose Inclusion Complexes, Cyclodextrins, and the Amorphous Phase of Starch Granules: Relationships between Glycosidic Linkage Conformation and Solid-State ^{13}C Chemical Shifts

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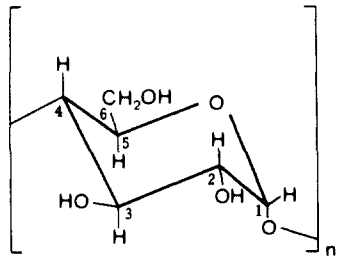
Abstract: In order to characterize molecular conformations within starch granules and to examine the relationships between polysaccharide conformation and solid state ^{13}C chemical shifts, a range of polymeric and oligomeric α-(1→4) glucans has been examined by cross polarization and magic angle spinning (CP/MAS) ^{13}C NMR spectroscopy. Single helical amylose (polymeric α-(1→4) glucan) polymorphs with various molecular inclusions as well as α- and β-cyclodextrin hydrates have been studied and their ^{13}C CP/MAS spectral features compared with those of both double helical and amorphous α-(1→4) glucans. Spectra of single helical amyloses show similar features irrespective of the nature of the included molecule and have only one resolved signal for each carbon site consistent with the nearly hexagonal packing of sixfold helices as characterized by X-ray diffraction. Cyclodextrin hydrates show resolved C-1 and C-4 resonances from each of the six (α-cyclodextrin) or seven (β-cyclodextrin) α-(1→4)-linked glucose residues present in the macrocycle. Chemical shift ranges in cyclodextrins are closely similar to those of single helical amyloses with the exception of one C-1 and one C-4 resonance in α-cyclodextrin which are at unusually high field and assigned to sites adjacent to a conformationally strained glycosidic bond. A comparison of solution chemical shifts with weighted averages of solid-state shifts suggests that β-cyclodextrin adopts glycosidic solution conformations similar to those found in the crystalline state but that α-cyclodextrin may be slightly more expanded in solution than in the crystalline state. Line widths in the α-(1→4) glucans studied can be rationalized in terms of crystalline perfection, and signal multiplicity arises through either intramolecular conformational effects (α- and β-cyclodextrin) or considerations of packing symmetry (double helical α-(1→4) glucans). The wide range of chemical shifts observed for C-1 and C-4 sites together with the essentially constant chemical shifts for other sites suggests that C-1 and C-4 chemical shifts are primarily determined by glycosidic linkage conformation. Correlations are found between C-1 chemical shifts and the sum of the moduli of the two torsion angles (ϕ and ψ) describing rotation about the glycosidic bonds as well as with the modulus of ψ. Both correlations accurately predict the range and qualitatively predict the distribution of chemical shifts found for amorphous α-(1→4) glucans assuming the equiprobable occurrence of all allowed glycosidic conformations. Similarities in C-1 and C-4 chemical shifts for single helical amyloses and amorphous materials show that starch granule amorphous phases contain a significant fraction of single-helix-like local conformations. This observation is consistent with the presence of α-(1→4) glucan/lipid inclusion complexes within starch granules.

Starch is produced in nature by higher plants as a carbohydrate energy reserve. It contains branched (amylopectin) and largely linear (amylose) (1→4)-α-D-glucans (I) and is always stored in



I

the form of granules. Despite numerous studies,[1-3] the molecular organization within starch granules is incompletely understood. Granule size and morphology vary widely with botanical source, but certain granule properties are universal. Thus, all granules are insoluble in cold water, contain partially hydrated and densely packed polysaccharides, and are semicrystalline (as judged by X-ray diffraction).[1-3] Two types of X-ray diffraction patterns, A and B, are commonly observed in granular starches whilst a third type (C) is considered to be a mixture of A- and B-types. From an X-ray diffraction analysis of amylose fibers, Wu and Sarko[4,5] showed that both crystalline types were due to ordered arrays of double helices. Quantitative analyses of X-ray powder diffraction patterns show that such long-range ordering accounts for only 20–40% of starch granule structure.[6,7] There is no information available on the molecular conformation of polysaccharides in the residual noncrystalline portion of starch granules (the amorphous phase).

In addition to α-glucans, all amylose-containing cereal starches (but not root, tuber, or legume starches) contain a small (<1.2%) but significant percentage of monoacyl lipids, predominantly free fatty acids and lysophospholipids.[8,9] In vitro, such lipids readily associate with the amylose component of starch both in solution[10] and in the solid state[11,12] to form complexes known as V-type structures. Similar structures are formed in the presence of a wide variety of other complexing agents (e.g., iodine, alcohols, ketones, etc.).[13,14] It is not known whether such V-type complexes are formed with lipids in vivo.[1-3,8,9]

X-ray diffraction analyses of V-amylose complexes[15-19] have

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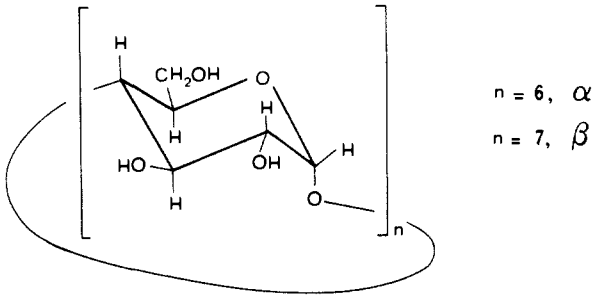
shown that the amylose is present as a single, left-handed helix with the complexing agent included in the helical channel. Most complexes have a six residue helix repeat[12,15-20] but bulkier complexing agents are thought to cause an expansion to a seven-[12,20-23] or eightfold[24] helix. A general feature of amylose V-conformations is the presence of a relatively hydrophobic helix interior which plays a major role in stabilizing inclusion complexes with organic molecules. This feature is also found in cyclodextrins II, cyclic



II

(1→4)-α-D-glucans which may be regarded as oligomeric analogues of polymeric V-amylose structures. Inclusion complexes of cyclodextrins and derivatives have recently been intensively studied,[25-27] not least because they have proved useful as models for enzyme active sites.[27]

In order to determine whether V-amylose or cyclodextrin-like conformations are important in native starch granules, a noninvasive molecular probe is required. Traditionally, the only noninvasive structural probe of starches has been X-ray diffraction which provides valuable information on crystalline character but is not sensitive to less ordered molecular architecture. We have recently shown,[28] however, that cross polarization and magic angle spinning (CP/MAS) solid state ^{13}C NMR spectroscopy provides a powerful noninvasive approach to the molecular analysis of starch-related structures. This technique is a probe of structure at the molecular level and therefore complements the information on long-range ordering obtained from X-ray diffraction.

Our initial study[28] showed that ^{13}C CP/MAS spectra of granular starches can be accounted for by a combination of spectral features due to model crystalline (A- or B-type as appropriate) and amorphous materials. Furthermore, the relative proportion of crystalline and amorphous model spectra needed to accurately duplicate an observed starch spectrum leads directly to an estimate of double helix content: values obtained for starch granules ranged from 38 to 53%.[28] The residual amorphous (i.e., nondouble helical) fraction of starches therefore accounts for 47–62% of the total granule polysaccharide content.

We now report the results of a comparative ^{13}C CP/MAS NMR study of V-amylose complexes, cyclodextrins, and granular starches. We compare the spectral features of single-helical V-polymorphs and the conformationally restrained cyclodextrins. We discuss the possible conformational origins of chemical shift effects and attempt to characterize the molecular structures present within the amorphous phase of starch granules. A preliminary account of part of this work has been reported.[29]

Experimental Section

Amylose was either obtained commercially (potato amylose:Sigma) or synthesized using potato phosphorylase.[30] All starches, complexing agents, and cyclodextrins were obtained commercially.

V-amylose inclusion complexes were prepared by dissolving amylose (0.5 g) in deionized water (40 mL) in a sealed tube at 160 °C for 20 min, cooling the solution to 80–90 °C, and adding one of the following complexing agents: sodium palmitate (0.5 g), hexanoic acid (0.5 g), *n*-butanol (10 mL), *tert*-butyl alcohol (150 mL, prewarmed to 75 °C), or 1-naphthol (1.2 g). These mixtures were allowed to cool slowly, and after 3–5 days the resultant precipitates were recovered by filtration, washed with a little water, air dried, and gently ground to a powder. In the case of the amylose–palmitate mixture, a surface precipitate of solidified fat formed on initial cooling and was removed prior to filtration. V-type amylose containing no organic inclusion was prepared by precipitation of amylose from aqueous solution with 4 volumes of ethanol, recovery of the precipitate by filtration, followed by extensive washing with ethanol and then diethyl ether, and finally air drying. A-type α-(1→4) glucan was obtained by crystallization of maltododecaose (i.e., 12 residue oligomer) from 35% w/v aqueous solution.[31] B-type material was obtained as a crystalline precipitate on cooling a 1% w/v aqueous solution of nearly monodisperse synthesized[30] amylose with a chain length of ~40 residues.

X-ray powder diffraction patterns (obtained as described previously[28]) were recorded for all preparations both before and after being examined by NMR in order to characterize the polymorphic form. For all samples diffraction patterns were identical before and after NMR analysis. A- and B-type material had similar patterns to those reported previously.[31] Amylose complexes with sodium palmitate, hexanoic acid, and *n*-butanol, and V-amylose containing no organic inclusion all showed diffraction peaks at 20, 13, and 8 $2\theta°$ characteristic[12,20] of the expected sixfold single helix structure (V_6). Inclusion complexes of *tert*-butyl alcohol showed diffraction peaks at 18.5, 12.5, and 7 $2\theta°$ as expected[12,20] for the sevenfold helical polymorph (V_7), and 1-naphthol complexes showed diffraction peaks at 22 and 17 $2\theta°$ characteristic of a V_8 structure.[24,32] α-Cyclodextrin was recrystallized from water at three different temperatures (10, 20, and 30 °C) and two concentrations (15 and 30% w/v). All crystallized α-cyclodextrins had identical X-ray diffraction patterns with major peaks at ~12, 14, 15.5, and 21.5 2θ as found by Szejtli.[26] As the same crystalline form was repeatedly obtained and is identical with that previously obtained,[26] it is reasonable to assume that it is the hexahydrate form (I),[33-35] which is known to be produced preferentially during aqueous crystallization.[35] β-Cyclodextrin was recrystallized from water at 10–30 °C and 5–15% w/v. All crystalline materials had the same X-ray diffraction pattern which was essentially identical with that obtained[25] for the most common hydrate (originally designated as the dodecahydrate[36] but later redesignated as the undecahydrate[37]).

Solid-state ^{13}C CP/MAS NMR spectra were obtained at 75.46 MHz on a Bruker CXP-300 spectrometer operating at 303 K by using either an "Andrews"-type probe head or a double bearing (DB/MAS) probe head. Spectra are referenced to external Me_4Si via the low field resonance of adamantane (38.6 ppm[38]). At least 1000 scans were averaged for each spectrum. A single contact time of 1 ms, a spectral width of 30 KHz, and line broadenings of 10–20 Hz were routinely used. For the double bearing probe head (with which all cyclodextrin spectra were acquired), a spinning rate of 4 KHz and spin locking and ^1H decoupling fields of ~80 KHz (20 G) were employed. Other parameters with the double bearing probe head were as follows: acquisition time 140 ms, recycle delay 4 s, time domain points 8 K, transform size 32 K. For the "Andrews" probe head parameters are as follows: spinning rate 3–3.5 KHz, spin locking and ^1H decoupling field ~50 KHz, acquisition time 30 ms, recycle delay 2 s, time domain points 2 K, transform size 8 K.

Results

1. V-Amylose Structures. A range of crystalline V-amylose materials were prepared as described above and characterized by

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Table I. ^{13}C Chemical Shift Values (ppm) for α-(1→4) Glucans in Solid and Solution States

material[a]	C-1	C-4	C-3	C-2,5	C-6
V_6 amylose (sodium palmitate complex)	103.6	82.2	75.9	72.7	61.0
V_6 amylose (hexanoic acid complex)	103.9	82.7	75.9	72.9	61.9
V_6 amylose hydrate	103.5	83.2	75.7	73.1	62.2
V_6 amylose (butanol complex)	104.1	83.5	75.8	73.5	62.0
V_7 amylose (*tert*-butyl alcohol complex)	103.9	83.2	75.7	72.9	61.9
V_8 amylose (1-naphthol complex)	104.9	84.1	75.4	73.4	61.7
α-cyclodextrin hydrate	102.1[b,c] (102.9)[e]	81.4[b,d] (82.1)[f]	71–76	71–76	61.6[b]
α-cyclodextrin (aqueous soln)	102.6	82.4	74.5	72–73	61.7
β-cyclodextrin hydrate	103.2[b,g]	82.2[b,h]	71–76	71–76	61.1[b]
β-cyclodextrin (aqueous soln)	103.1	82.3	74.3	72–73	61.7
A-type α-(1→4) glucan	100.4[b,i]	76.0	70–75	70–75	62.1
B-type α-(1→4) glucan	100.4[b,j]	76.2	70–75	70–75	62.3
amylose (aqueous soln)	100.9	78.6	74.6	72–73	61.9

[a] Solid except where specified. [b] Weighted average value. [c] Individual chemical shift values of 103.8, 103.2, 102.8 (2), 102.0, and 98.1 ppm. [d] Individual chemical shift values of 83.1 (2), 82.0, 81.9, 80.6, and 77.7 ppm. [e] Weighted average excluding peak at 98.1 ppm. [f] Weighted average excluding peak at 77.7 ppm. [g] Individual chemical shift values of 104.5, 103.8, 103.7, 103.1, 102.8, 102.7, and 101.9 ppm. [h] Individual chemical shift values of 84.8, 83.7, 82.6, 81.9 (2), 81.3, and 78.8 ppm. [i] Individual chemical shift values of 101.5, 100.4, and 99.3 ppm. [j] Individual chemical shift values of 100.9 and 100.0 ppm.

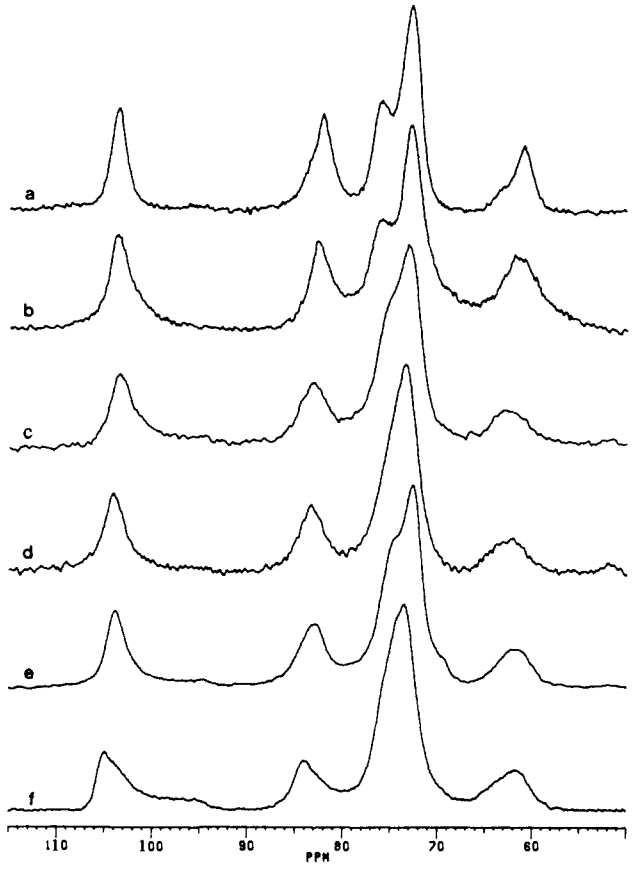

Figure 1. ^{13}C CP/MAS NMR spectra of V amyloses formed in the presence of (a) sodium palmitate, (b) hexanoic acid, (c) water, (d) butanol, (e) *tert*-butyl alcohol, and (f) 1-naphthol. Signal intensity between 93 and 101 ppm in (f) indicates the presence of some amorphous material (Figure 5a). Included organic molecules give rise to signals in the expected chemical shift ranges.

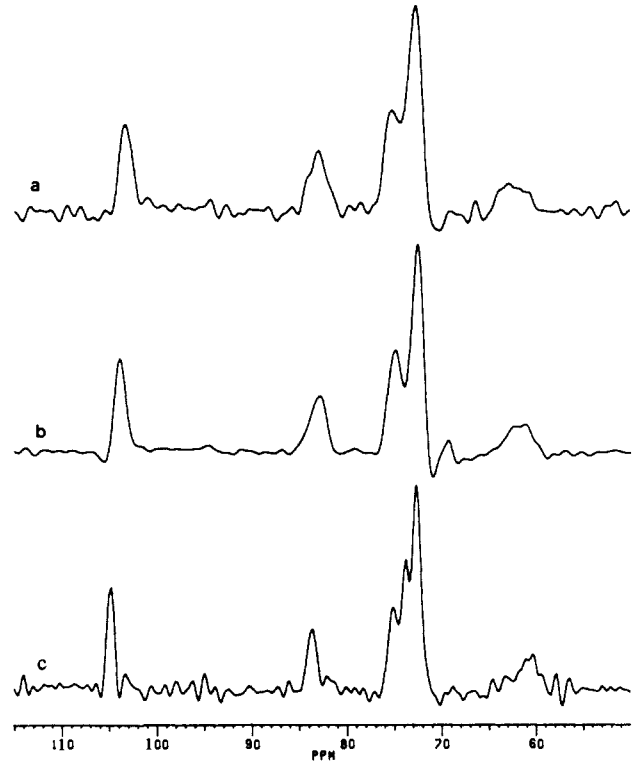

Figure 2. Resolution enhanced (LB = −200, GB = 0.2) spectra of V amyloses (a) sodium palmitate V_6 complex (see Figure 1a), (b) *tert*-butyl alcohol V_7 complex (see Figure 1e), and (c) 1-naphthol V_8 complex (see Figure 1f).

X-ray powder diffraction. All preparations were of the expected polymorphic form, i.e., V_6 in the absence of a complexing agent and in the presence of sodium palmitate, hexanoic acid, and *n*-butanol; V_7 for *tert*-butyl alcohol complexes, and V_8 for 1-naphthol complexes. ^{13}C CP/MAS NMR spectra of these materials are presented in Figure 1. Essentially identical X-ray diffraction patterns and ^{13}C CP/MAS spectra were obtained for complexes prepared from natural (potato) lightly branched[39] amylose and strictly linear[30] synthetic amylose. All ^{13}C CP/MAS spectra showed resolved resonances of equal intensity in the ranges 102–105, 82–84, and 60–64 ppm which may be assigned to C-1, C-4, and C-6 sites by comparison with solution chemical shifts for (1→4)-α-D-glucans.[40,41] A further signal at 75–76 ppm is also resolved for the two lipid complexes (Figure 1a,b) and is apparent following resolution enhancement of other V-type structures (Figure 2). By analogy with solution chemical shifts,[40] we tentatively assign this signal to C-3 sites: further evidence for this assignment is given later. Apart from resolution of the signal at 75–76 ppm which is presumably dependent on line widths, all V_6 materials have closely similar spectral features (Figure 1a–d) showing that amylose solid-state chemical shifts for this polymorph are not significantly affected by the nature of the complexing agent or even the absence of any organic inclusion (Figure 1c). V_7 complexes also have very similar spectral features (Figures 1e and 2b) to those of V_6 amyloses (Figures 1a–d and 2a) demonstrating

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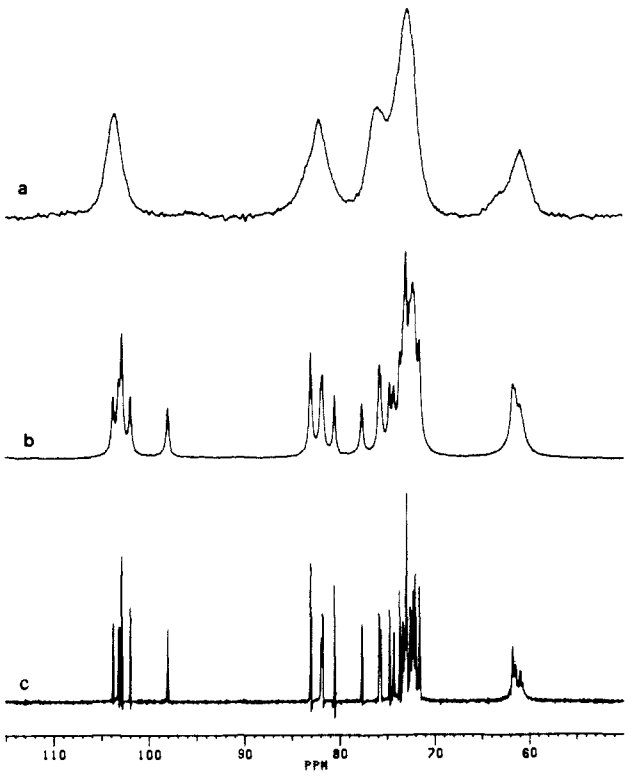

Figure 3. ^{13}C CP/MAS NMR spectra of (a) sodium palmitate–amylose V_6 complex, (b) α-cyclodextrin hexahydrate LB = 10 Hz, and (c) α-cyclodextrin hexahydrate, LB = −15 Hz, GB = 0.5.

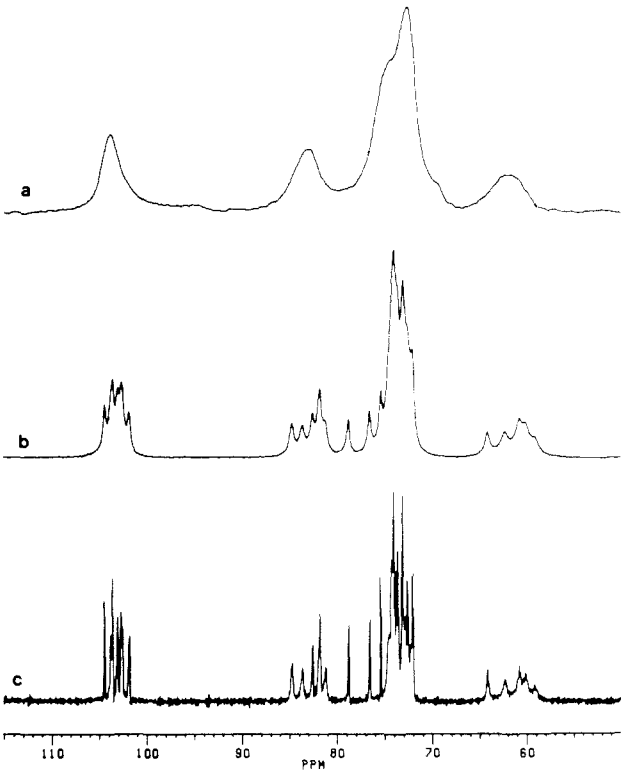

Figure 4. ^{13}C CP/MAS NMR spectra of (a) *tert*-butyl alcohol–amylose V_7 complex, (b) β-cyclodextrin undecahydrate, LB = 10 Hz, and (c) β-cyclodextrin undecahydrate, LB = −20 Hz, GB = 0.5.

that ^{13}C chemical shifts are not sensitive to expansion of the amylose helix from a six- to a sevenfold repeat to accommodate more bulky molecules such as *tert*-butyl alcohol. Significant chemical shift differences are observed, however, for the V_8 1-naphthol complex (Figures 1f and 2c). Thus C-1 and C-4 signals are shifted ~1 ppm downfield relative to V_6 and V_7 structures, whereas C-6 and C-2,3,5 signals at 61–62 and 71–76 ppm, respectively, are closely similar for all three V-type polymorphs. Chemical shift values for all resolved signals in V-amylose spectra are given in Table I.

2. α- and β-Cyclodextrins. Single-helical V-amylose structures are closely related to the cyclodextrin family of oligosaccharides which contain α-(1→4)-linked glucose residues constrained in a macrocyclic structure II. It would be of interest to examine how closely the conformationally well-defined cyclodextrins act as models for polymeric V-structures containing the same number of glucose residues in one turn of a helix. We have therefore compared the spectral features of α- and β-cyclodextrin hydrates with V_6 and V_7 amylose polymorphs, respectively.

The most obvious difference between ^{13}C CP/MAS spectra of cyclodextrins and V-amyloses (Figures 3 and 4) is the number of resolved signals. V-amylose spectra contain no more than one broad signal for each carbon site, but cyclodextrin spectra show resolution of a large number of signals. Integration of cyclodextrin spectra shows that C-1 signal intensity (between 97 and 106 ppm) is exactly 25% of C-2,3,4,5 intensity (between 69 and 87 ppm). Signals in the ranges 77–84 ppm and 78–86 ppm for α- and β-cyclodextrin, respectively, have total intensities equal to C-1 signal intensity and can therefore be assigned to C-4 sites. C-6 signal intensity for both α- and β-cyclodextrin is ~85% of C-1 intensity indicating different relaxation behavior. This is not surprising as C-6 is a methylene carbon, whereas all other sites are methine.[42]

Following resolution enhancement (Figures 3 and 4), C-1 and C-4 signals for essentially each glucose residue within the macrocycle can be resolved for both α- and β-cyclodextrin. Thus for β-cyclodextrin (Figure 4), seven C-1 resonances are clearly resolved, and six C-4 signals are resolved with one signal (81.9 ppm) accounting for two sites. Similarly, for α-cyclodextrin (Figure 3), C-1 and C-4 resonances can each be resolved into five signals with peaks at 102.8 and 83.1 ppm accounting for two sites each.

Although ^{13}C CP/MAS NMR spectra of cyclodextrins have been reported before, only limited resolution has previously been achieved.[43-45] The marked improvement in resolution in the present study cannot be ascribed to the ^{13}C operating frequency (75 MHz) or the ^{1}H decoupling power (20 G) employed as these are similar to those used in previous studies. It is more likely that increased resolution was obtained by accurate setting of the magic angle and/or the use of a double bearing probe head.

A comparison of ^{13}C CP/MAS spectra of α-cyclodextrin and V_6 amylose (Figure 3) shows that, with the exception of the high field C-1 and C-4 resonances (see Discussion), chemical shift values are similar for the two materials. β-Cyclodextrin and V_7 amylose (Figure 4) also show similar chemical shift values for equivalent sites although the range of C-4 and C-6 chemical shifts is greater for the cyclodextrin. Average C-1 and C-4 shifts for β-cyclodextrin and, ignoring highfield resonances, α-cyclodextrin are closely similar to V_6 and V_7 amylose chemical shifts (Table I). As C-1 and C-4 chemical shift values for solid V-amyloses are similar to those of cyclodextrins in both solid and solution states (Table I), it seems reasonable to suppose that C-3 resonances for V-amyloses should be shifted downfield from C-2 and C-5 resonances as in cyclodextrin solutions ($C_{2,5}$ 72–73, C_3 ~74–75 ppm, Table I). This provides further support for our assignment of the signal at 75–76 ppm in V-amylose spectra (Figures 1 and 2) to C-3 sites.

3. Comparison of α-(1→4) Glucan Polymorphs and Amorphous Starches. We have previously described the ^{13}C CP/MAS spectra of both common double helical polymorphs (A- and B-type) and amorphous preparations of α-(1→4) glucans.[28] Figure 5 shows a comparison of ^{13}C CP/MAS spectra of amorphous α-(1→4)

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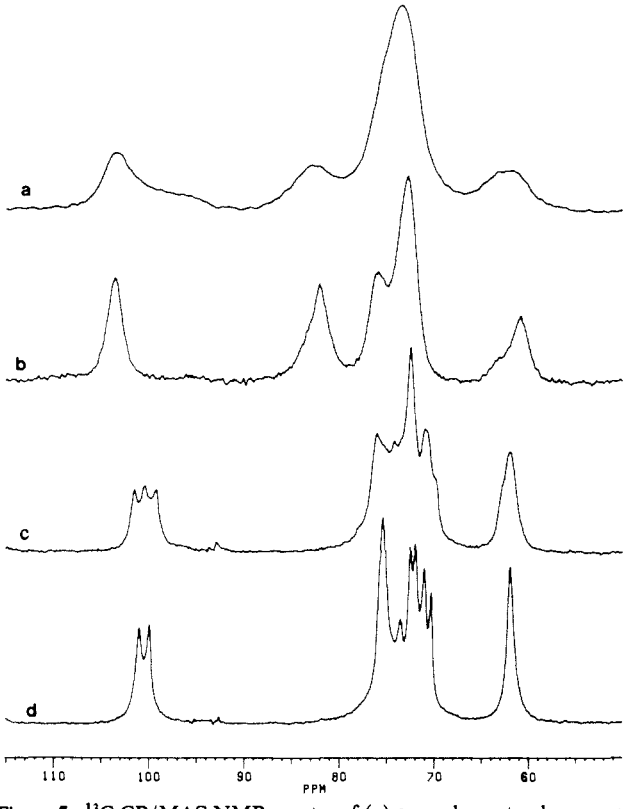

Figure 5. ^{13}C CP/MAS NMR spectra of (a) amorphous starch prepared by ethanol precipitation of gelatinized maize starch, (b) V_6 amylose complex with sodium palmitate, (c) crystalline A-type α-(1→4) glucan, and (d) crystalline B-type α-(1→4) glucan.

glucan and single helical (V_6-type) and double helical (A- and B-type) crystalline polymorphs. All spectra have signals in the ranges 60–64 ppm and 70–76 ppm which may be assigned to C-6 and C-2,3,5 sites, respectively. Signals from C-1 and C-4 sites, however, show significant chemical shift variations (Figure 5 and Table I). Double helical materials (Figure 5 (parts c and d)) have C-1 and C-4 resonances in the ranges 99.3–101.5 and 76–77 ppm, respectively, whereas C-1 and C-4 sites in single helical materials (Figures 1 and 5b) give rise to signals at 103–104 and 82–84 ppm, respectively. Amorphous material (Figure 5a) shows broader spectral features than ordered structures (Figures 5b–d) as might be expected. C-1 sites in amorphous material give rise to chemical shifts covering a wide range (94–106 ppm) with most intensity between 101 and 105 ppm; the C-4 signal is centered at 82–84 ppm (Figure 5a). The major C-1 and C-4 signal intensity for amorphous material is at similar chemical shifts to C-1 and C-4 signals in V-type structures (Figure 5 (parts a and b)).

Discussion

1. α- and β-Cyclodextrin Hydrates. Molecular structures have been reported for several cyclodextrin hydrates crystallized from aqueous solution.[34,35,37,46] In our hands, aqueous recrystallization under a variety of temperature and concentration conditions led to single polymorphs for both α- and β-cyclodextrin. We therefore assign their structures as those polymorphs which have previously been found to be produced preferentially by aqueous crystallization, i.e., α-cyclodextrin hexahydrate form I[33-35] and β-cyclodextrin undecahydrate.[37] These assignments were confirmed by X-ray powder diffraction measurements. Other less readily produced crystalline hydrates (e.g., α-cyclodextrin hexahydrate form II[35] and β-cyclodextrin dodecahydrate[46]) differ mainly in packing arrangements, individual macrocycle conformation being very similar to the more common polymorphs.[35,46] The crystalline molecular structures of α-cyclodextrin hexahydrate (I) and β-cyclodextrin undecahydrate determined by X-ray diffraction[33,36] show significant differences. β-Cyclodextrin adopts an open, nearly symmetrical macrocyclic conformation,[36] whereas α-cyclodextrin exists in a partially "collapsed" conformation[33] involving an unusual high-energy linkage conformation between two of the glucose residues. In the presence of a suitable complexing agent, the α-cyclodextrin macrocycle expands to a nearly symmetrical hexagonal structure thereby relieving the strained linkage conformation.[33] On the basis of these observations, an "induced-fit" mechanism for α-cyclodextrin complexation has been proposed.[33] In the presence of 1.2 M aqueous $BaCl_2$, α-cyclodextrin crystallizes to form a nearly symmetrical structure[47] containing more water than the hexahydrate.[47] This structure may be regarded as an α-cyclodextrin–water complex. For β-cyclodextrin, macrocyclic conformations are similar in the presence and absence of complexing agents, so a similar mechanism cannot be invoked in this case.[36] It is of interest to examine whether the high-energy conformation adopted by one of the glycosidic linkages in uncomplexed α-cyclodextrin is reflected in chemical shift values. It is indeed found that one C-1 and one C-4 resonance (98.1 and 77.7 ppm, respectively) are significantly shifted relative to other C-1 and C-4 signals (Figure 3). These shifted signals are not present in spectra of nearly symmetrical α-cyclodextrin complexes with benzoic acid or *p*-nitrophenol[48] or α-cyclodextrin crystallized in the presence of 1.2 M $BaCl_2$,[48] thus providing evidence for their assignment to the C-1 and C-4 sites adjacent to the high-energy glycosidic linkage in α-cyclodextrin hexahydrate.

The spectra shown in Figures 3c and 4c suggest that for both C-1 and C-4 sites, there are six and seven discrete resonances for α-cyclodextrin hexahydrate (I) and β-cyclodextrin undecahydrate, respectively. This is in line with the general principle[49] that ^{13}C solid-state multiplicities reflect the number of inequivalent sites in a crystal unit cell,[49] as the asymmetric unit in each case is the individual macrocycle.[33,36] The observed signal multiplicities therefore most likely reflect intramolecular effects. In view of the single high field shifted C-1 and C-4 resonances in α-cyclodextrin hexahydrate (I) which correspond to a single unusual glycosidic linkage conformation, conformational effects seem to be the most important determinant of chemical shift differences for C-1 and C-4 sites. Other possible chemical shift effects (e.g., due to specific hydrogen bonding) appear to be secondary in importance.

In aqueous solutions of α- and β-cyclodextrins, single resonances are observed for C-1 and C-4 sites due to conformational averaging. A comparison of solid- and solution-state conformations can be made by considering a weighted average of solid-state chemical shifts with observed values in solution (Table I). Such a comparison for β-cyclodextrin shows essentially identical average chemical shift values for C-1 and C-4 signals in solution and solid states (Table I) suggesting that glycosidic linkage conformations in solution are similar to those found in the crystal structure.[36] For α-cyclodextrin, average solid-state C-1 and C-4 chemical shifts (102.1 and 81.4 ppm, respectively) are to high field of solution values (102.6 and 82.4 ppm). Solution values are more closely approximated, however, if averages of solid-state chemical shifts are taken (102.9 and 82.1 ppm) which disregard the high field signals assigned to sites adjacent to the conformationally strained glycosidic linkage. Although such relatively small chemical shift variations between solid and solution states may not be completely reliable, our observations suggest the possibility that α-cyclodextrin exists in solution in a more expanded conformation than the hexahydrate characterized by X-ray crystallography[36] and more closely resembles the "water-complex" structure which can be obtained on crystallization from 1.2 M $BaCl_2$.[47]

2. Line Widths and Multiplicity Effects. A comparison of the ^{13}C CP/MAS spectra of amylose polymorphs (A, B, and V) and cyclodextrins (α and β) shows significant variations in both signal

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widths and the number of resonances for each carbon site (Figures 3–5). We will discuss each of these features in turn.

Assuming that dipole–dipole and chemical shift anisotropy effects are efficiently removed by high-power ^1H decoupling and magic angle spinning, respectively, residual line widths in ^{13}C CP/MAS spectra reflect the distribution of isotropic chemical shifts for each carbon resonance.[50] Amorphous materials are expected to have broader resonances than ordered structures and increasing crystalline perfection is expected to result in narrower signals.[50,51] In this study we have examined amorphous material, ordered polymeric structures (V-type), ordered oligosaccharide structures (A- and B-type), and highly crystalline cyclodextrins. As might be expected, line widths were found to increase through the series, cyclodextrin < A- and B-type oligosaccharides < V-type polymers < amorphous material: for C-1 and C-4 signals, approximate line widths at half height ($\nu_{1/2}$) are 10–20, ~50, 100–150, and >200 Hz, respectively. Line widths for α-cyclodextrin ($\nu_{1/2} \sim 10$ Hz) are less than for β-cyclodextrin ($\nu_{1/2} \sim 20$ Hz) presumably reflecting a greater crystalline perfection in the smaller, more compact hexasaccharide macrocycle. It may be possible to obtain narrower line widths for oligomeric and polymeric α-(1→4) glucans through annealing of ordered structures by careful hydration, as has been shown for β-(1→3) glucans.[52]

The appearance of multiple resonances in ^{13}C CP/MAS spectra where only one signal would be observed in solution-state spectra reflects the presence of inequivalent sites within a solid sample. In general these can arise through conformational (intramolecular) and/or crystal packing (intermolecular) interactions. In principle, each inequivalent site in a crystallographic unit cell might be expected to give rise to a separate signal.[49] In practice, sufficient resolution might not be achieved as chemical shift differences may be small compared with line widths.

A- and B-type α-(1→4) glucan polymorphs have asymmetric units which contain three and two glucose residues, respectively,[49,53] and have been found to have triplet and doublet C-1 signals, respectively.[28,49,54] This effect is shown in Figures 5 (parts c and d): the spectra obtained have greater signal resolution than those previously reported[28,49,54] which probably reflects the use of nearly monodisperse α-(1→4) glucan samples. With this increased resolution, multiple signals are apparent for C-2-5 sites as well as C-1 (Figure 5 (parts c and d)). In particular, doublet pairs at 72.5, 71.9 ppm and 71.0, 70.3 ppm are resolved in the B-type spectrum. From integration, these signals account for two carbon sites and may be assigned to C-2,3 or 5 sites as glycosidic C-4 sites would be to lower field. The observation of doublets of comparable splitting to that of C-1 for C-2,3,5 sites suggests that the chemical shift effect responsible is of similar magnitude at all these sites. The observed multiplicities could be due to either intramolecular (conformational) or intermolecular (helix packing) effects. If an intramolecular mechanism is responsible, this would lead to the expectation that conformation-induced variations in chemical shifts should be similar at C-1, 2, 3, and 5 sites as similar doublet splittings at these sites are observed in B-type amylose (Figure 5d). We show later, however, that dispersions of C-1 and C-4 chemical shifts for a range of α-(1→4) glucan conformations are much greater than the total dispersion of C-2, 3, 5 shifts suggesting that conformational effects are largely localized at C-1 and C-4 glycosidic sites. The doublets observed in the B-type spectrum are therefore difficult to reconcile totally with a conformational effect. However, if the multiplicity effect is due to helix packing (intermolecular) interactions, splittings of similar magnitude at different glucose sites would not be unexpected. The observed spectral features (Figure 5d) are therefore consistent with our previous proposal[28] that multiplicity effects in double helical α-(1→4) glucan polymorphs reflect the symmetry of helix packing, i.e., intermolecular effects. Thus, in the six residue repeats of both double helical structures, the twofold packing symmetry of the A-type structure leads to three nonequivalent glucose residues and a triplet resonance (Figure 5c), and the threefold packing symmetry of the B-type structure gives rise to a C-1 doublet (Figure 5d).

In contrast to double helical structures, there is no evidence for multiple resonances from carbon sites in single helical α-(1→4) glucans (Figures 1 and 2). X-ray diffraction analysis of V-amylose fibers has been carried out in the presence of a number of complexing agents, e.g., water,[15,18] iodine,[17] and butanol.[19] All of these structures contain sixfold helix repeats of essentially equivalent glucose residues packed in a nearly hexagonal array. For perfect sixfold packing of six residue helices, only one signal for each carbon site would be expected in the ^{13}C CP/MAS spectrum. The slight deviation from sixfold packing symmetry in V-amylose structures is apparently insufficient to lead to multiple resonances in any of the V-type materials examined.

^{13}C CP/MAS spectra of α- and β-cyclodextrin hydrates show six and seven signals, respectively, for both C-1 and C-4 sites as well as evidence of multiple resonances for other carbon sites (Figures 3 and 4). The correspondence between the number of C-1 and C-4 resonances and the number of glucose residues in each cyclodextrin strongly suggests that the origin of multiple resonances is intramolecular (conformational), i.e., each C-1 and C-4 site in the macrocycle gives rise to a discrete resonance. α-(1→4) glucans therefore provide examples of solid-state structures which have ^{13}C CP/MAS spectra showing only one resonance per site (V-type) and structures exhibiting multiple resonances due to both interhelix interactions (A- and B-type) and intramolecular conformational effects (α- and β-cyclodextrin hydrates).

3. Possible Conformational Origins of Chemical Shift Effects. Having obtained ^{13}C CP/MAS spectra for a range of α-(1→4) glucans, we are in a position to examine the possible origins of chemical shift effects. As can be seen from Table I, several carbon sites have very similar chemical shifts in all materials studied. For instance, C-6 resonances occur at 61–63 ppm in each of the materials examined. Furthermore, resonances assigned to C-2,3 and -5 sites are always observed in the range 70–76 ppm suggesting only minor chemical shift variations at these sites for different α-(1→4) glucans. By contrast, C-1 and C-4 resonances occur over much wider ranges: C-1 sites (including those in amorphous material) show signal intensity between 94 and 106 ppm, and C-4 resonances appear between 76 and 86 ppm. As chemical shifts of C-1 and C-4 sites but not C-2,3,5 and -6 sites show wide variations, it is reasonable to propose that features associated with the α-(1→4) glycosidic linkage play a dominant role in determining C-1 and C-4 chemical shifts in solid α-(1→4) glucans. Other chemical shift effects may also be important but are unlikely to be of sufficient magnitude to give rise to such large (10–12 ppm) shift ranges. For example, the intermolecular interactions which are proposed to be the origin of multiple C-1 signals for double helical structures[28] cover a chemical shift range of 2.2 ppm (Figure 5), i.e., only 18% of the total observed range. As A- and B-type materials have similar chemical shifts (Figure 5) and are thought to contain conformationally similar double helices,[4,5] it seems likely that the intermolecular splitting interactions in these polymorphs are secondary effects on a chemical shift which is primarily determined by glycosidic linkage conformation.

All of the ordered α-(1→4) glucans examined have been studied previously by X-ray diffraction. Detailed structures from both X-ray and neutron diffraction analysis of single crystals are available for α-cyclodextrin hexahydrate form (I)[33,34] and β-cyclodextrin undecahydrate.[36,37] Numerous fiber diffraction analyses of V-type amyloses have been reported: all structures so far proposed are left-handed single helices with essentially identical chain conformations.[15-18] The double-helical structures of A- and B-type polymorphs have proved more elusive, the only detailed structural work being that of Wu and Sarko.[4,5] These studies showed that double helices were parallel-stranded and that A-

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Table II. Comparison of Glycosidic Linkage Conformation with ^{13}C CP/MAS Chemical Shifts for Cyclodextrins and α-(1→4) Glucan Polymorphs

structure	C-1 (ppm)	C-4 (ppm)	bridge angle, θ^a (deg)	torsion angle ϕ^b (deg)	torsion angle ψ^c (deg)	distance H_1, H_4 (Å)	$\lvert\phi\rvert + \lvert\psi\rvert$
V_6^d	103.5–104.1	82.2–83.5	118.6	−14.4	−7.5	2.10	21.9
V_6^e	103.5–104.1	82.2–83.5	119.3	−4.0	−11.0	2.10	15.0
V_6^f	103.5–104.1	81.2–83.5	120.5	−5.7	−9.8	2.13	15.5
β-cyclodextrin[g]	101.9–104.5	78.8–84.8	116.0–118.9	0 – (−17)	(−5) – 20	2.01–2.08	11–24.5
α-cyclodextrin[h]	102.0–103.8	80.6–83.1	116.2–119.4	(−7) – (−30)	(−4) – (−16)	2.00–2.19	19–34
α-cyclodextrin[i]	98.1	77.7	118.2	−30.8	50.6	2.32	81.4
A[j]	99.3–101.5	76.0	105	−25 ± 5	−32 ± 5	2.0 ± 0.1	~57
B[j]	100.0–100.9	76.2	105	−25 ± 5	−32 ± 5	2.0 ± 0.1	~57

[a] Glycosidic valence angle C_1–O_1–C_4, see Figure 6. [b] Defined by $|H_1$–C_1–O_1–$C_4|$, ref 15. [c] Defined by $|H_4$–C_4–O_1–$C_1|$, see Figure 6. [d] Hydrated V-amylose, ref 18. [e] Partially hydrated V-amylose, ref 15. [f] V-amylose formed in the presence of dimethyl sulfoxide, ref 16. [g] From ref 36. [h] From ref 33 for all sites and linkages except those associated with the conformationally anomalous glycosidic linkage. [i] From ref 33 for the anomalous glycosidic linkage. [j] From ref 4 for the most probable left-handed helix.[55]

and B-type polymorphic differences were largely due to helix-packing arrangements (i.e., individual double helices are similar in the two polymorphs). On the basis of refinement of model structures against X-ray diffraction intensities, right-handed helices were considered to be more likely than left-handed helices.[4,5] However, on the basis of optical rotation measurements, Rees[55] has suggested that amylose forms a left-handed double helix in gels. We have reinvestigated[56] the optical rotation of amylose solutions, aggregates, and gels and have confirmed and extended the observations of Rees.[55] Thus optical rotation at 589 nm ($[\alpha]_D$) is found to be 185 ± 5° for a range of amyloses during the initial stages of aggregation (i.e., before turbidity precludes optical measurements) which leads eventually to ordered precipitates and gels showing B-type X-ray diffraction patterns. By monitoring the aggregation process by using other techniques such as turbidimetry, rheology, and high resolution NMR signal area, the early stages of aggregation which can be monitored by optical rotation are found to be part of a steady increase in aggregation until a B-type diffraction pattern is apparent.[56] The implication of these results is that the helical structure adopted during the initial stages of aggregation ($[\alpha]_D \sim 185°$) is the same as that which forms the basis of the extended ordered arrays which give rise to a B-type diffraction pattern. The observed optical rotation is essentially identical with that predicted[57] (190°) for the most likely left-handed double helix[4,55] and is significantly different from that predicted for the suggested[4] right-handed double helix structure (74°).[55] As optical rotation values for many ordered polysaccharide structures have been successfully predicted[55] and the quality of diffraction data available at present is not sufficient to determine the helix chirality unambiguously,[4,5] we suggest that a left-handed helical structure is the more likely for B-type α-(1→4) glucan. As A-type structures can be produced from B-type materials (α-(1→4) glucans of DP 13-16)[31] by careful reduction of water content without passing through an amorphous state,[56] we assign a similar helix structure to A-type material.

In addition to the α-(1→4) glucans used in the present study, many organic complexes of cyclodextrins have been studied by X-ray diffraction[25] and ^{13}C CP/MAS NMR.[43-45] Although accurate three-dimensional structures have been elucidated by X-ray diffraction, there seems to be no general approach to the assignment of NMR signals to specific C-1 and C-4 sites. The presence of organic inclusions in the cyclodextrin complexes may also lead to specific chemical shift effects through, e.g., dipolar, hydrogen bonding, or ring current interactions which would be difficult to disentangle from conformational effects on cyclodextrin chemical shifts.[58] For these reasons, we have not included organic cyclodextrin inclusion complexes in this study.

There are three major determinants of α-(1→4) glycosidic linkage geometry (Figure 6), namely the (C-1)–(O-1)–(C-4′)

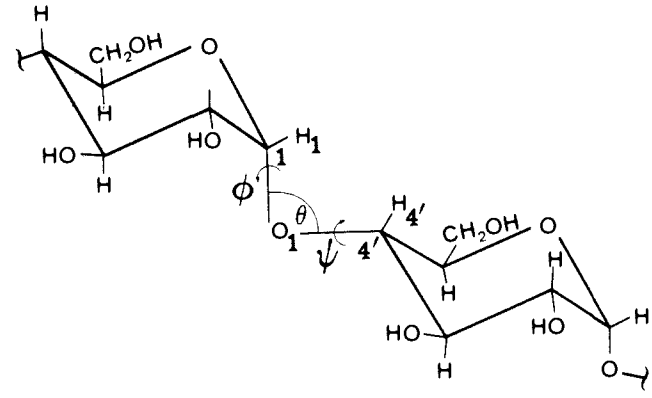

Figure 6. Conformational features associated with the glycosidic linkage in α-(1→4) glucans. The orientation of adjacent residues and hence overall polymer conformation is largely determined by the two torsion angles, φ and ψ, and the valence angle, θ. Torsion angle φ is described by (H-1)–(C-1)–(O-1)–(C4′), and angle ψ is described by (H-4′)–(C-4)–(O-1)–(C-1).

bridge angle (θ) and two torsion angles (φ and ψ): bond lengths show only minor variations in the α-(1→4) glucan structures that have been determined by X-ray diffraction. In an attempt to correlate chemical shifts with glycosidic conformation, we have compared various conformational features determined by X-ray diffraction with solid-state ^{13}C chemical shifts (Table II).

Horii et al.[59] have suggested that C-1 and C-4 solid-state chemical shifts in β-(1→4) glucans are correlated with torsion angles φ and ψ, respectively. We find no such correlation for α-(1→4) glucans (Table II); e.g., for α-cyclodextrin, there are two glycosidic linkages with $\phi \sim -30°$ which have widely different C-1 chemical shifts (98.1 and 102.0–103.8 ppm). Another ^{13}C chemical shift effect which is thought to be important in carbohydrates[60] is the interaction of protons on 1,3 carbon sites[61] (β effect). This effect could be important in α-(1→4) glycosidic linkages through interaction of H-1 and H-4′ and may depend on interproton distance and/or whether the two protons are eclipsed ($\phi-\psi = 0$) or staggered, etc. We find though that there is no correlation between C-1 or C-4 chemical shifts and either (H-1)–(H-4′) distance or φ–ψ (Table II). For example, A- and B-type structures have similar values of both φ–ψ and (H-1)–(H-4′) distance as V-type structures but have widely different C-1 and C-4 chemical shifts.

Two significant correlations are found, however, between C-1 (but not C-4) chemical shifts and conformational parameters. The first is with the sum of the moduli of φ and ψ ($|\phi| + |\psi|$, Table II): this is shown in Figure 7. The parameter $|\phi| + |\psi|$ is a measure of the deviation of H-1 and H-4′ from the plane defined by C-1, O-1, and C-4′. When $\phi = \psi = 0$, H-1 and H-4′ are both

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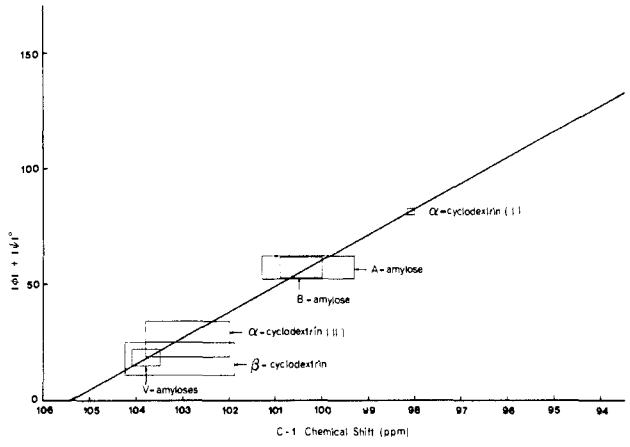

Figure 7. Representation of the correlation between C-1 chemical shift and the sum of the moduli of the two glycosidic torsion angles, $|\phi| + |\psi|$ in deg. The range of chemical shifts and torsion angle sums (determined by CP/MAS NMR and X-ray diffraction, respectively) is represented by boxes for each material. An uncertainty of ±5° is ascribed to $|\phi| + |\psi|$ values for A- and B-type double helices (ref 4 and 5). α-Cyclodextrin I and II refer to the high-energy glycosidic linkage and the remainder of the molecule, respectively.

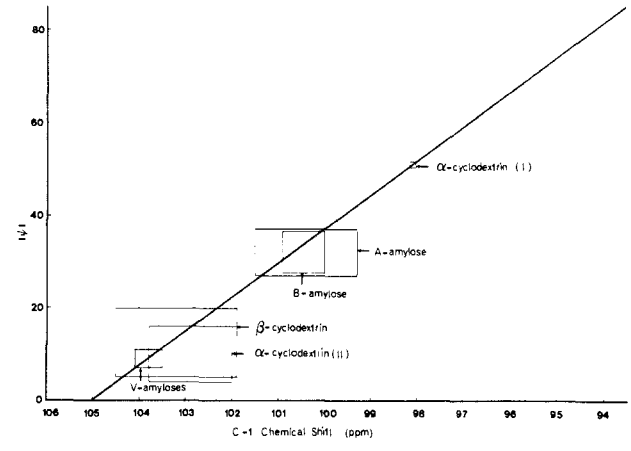

Figure 8. Correlation of C-1 chemical shifts with the modulus of ψ in deg, constructed as for Figure 7.

in the plane: on variation of ϕ (or ψ), H-1 (or H-4′) moves out of the plane by an amount dependent on the modulus but not the sign of ϕ (or ψ). The sum of the moduli of ϕ and ψ therefore represents the extent of noncoplanarity of H-1, C-1, O-1, C-4′, and H-4′. The second correlation of α-(1-4) glucan C-1 chemical shifts is with the modulus of ψ, as shown in Figure 8. Torsion angle ψ describes rotation about the O-1, C-4′ bond (Figure 6) and hence determines the relative orientation of atoms around C-4′ with respect to C-1. By contrast, variation in ϕ does not alter the relative positions of C-1 and atoms of the glycosidically linked residue. Torsion angle ϕ would therefore not be expected to be an important conformational determinant of C-1 chemical shifts.

In order to establish whether either of these two chemical shift conformation correlations (Figures 7 and 8) is important, we examined the C-1 spectra of amorphous α-(1→4) glucans. C-1 sites in amorphous starch preparations (Figure 5a) show a remarkably large chemical shift range (94–106 ppm) reflecting a wide diversity of local conformations. As the weighted average amorphous C-1 chemical shift (~101.5) is similar to the chemical shift in aqueous solution (100.9,[50] 101.0[63]), it seems reasonable to assume that amorphous solid samples contain similar conformations to those which are motionally averaged in aqueous solution.[64] If this is so, then the proposed correlations of C-1

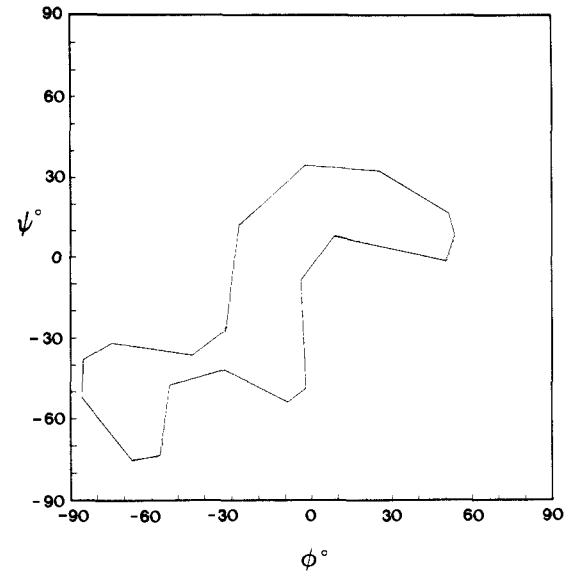

Figure 9. The region of ϕ, ψ space which is "allowed" for α-(1→4) glucans, adapted from ref 65.

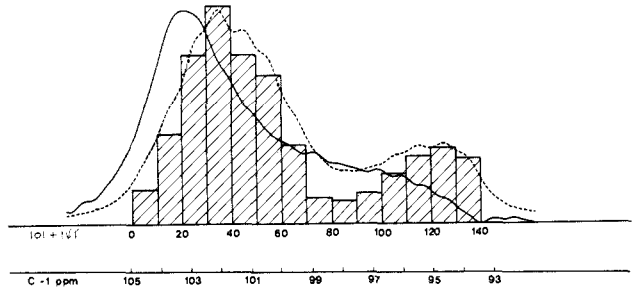

Figure 10. The relative abundance of conformations having $|\phi| + |\psi|$ values in 10° ranges assuming equal occurrence of all allowed (Figure 9) conformations, presented as a histogram. The dashed line is a predicted C-1 spectrum for amorphous α-(1→4) glucan generated by superimposing Lorentzian lines of 100 Hz width at half height corresponding to each 10° range of $|\phi| + |\psi|$. Chemical shifts were obtained from Figure 7 for the midpoint of each 10° range, and the relative intensity for each Lorentzian signal was obtained from the histogram. The observed spectrum of amorphous starch material is shown by a solid line.

chemical shift with $|\phi| + |\psi|$ or $|\psi|$ could be tested by generating a predicted C-1 signal based on the probability of finding various values of $|\phi| + |\psi|$ or $|\psi|$ in amorphous α-(1→4) glucans. Such a test can be carried out by using the results of conformational energy calculations in ϕ,ψ space. The energy diagram for α-(1→4)-linked glucose residues as a function of ϕ and ψ shows a sharp division between allowed (low-energy) and disallowed (high-energy) conformations.[65,66] Gagnaire et al.[65] have suggested the simplifying assumption that all allowed conformations occur with equal probability. Applying this assumption and by using the allowed conformational space calculated by Gagnaire et al.[65] (Figure 9), we have estimated the percentage of all conformations lying within each 10° range of $|\phi| + |\psi|$ and each 5° range of $|\psi|$. The results of this analysis for $|\phi| + |\psi|$ are presented as a histogram (Figure 10). Taking predicted chemical shift values (Figure 7) for the midpoint of each of the 14 10° ranges of $|\phi| + |\psi|$, we have generated a predicted C-1 spectrum by superimposing 14 Lorentzian lines of 100-Hz width. The result (Figure

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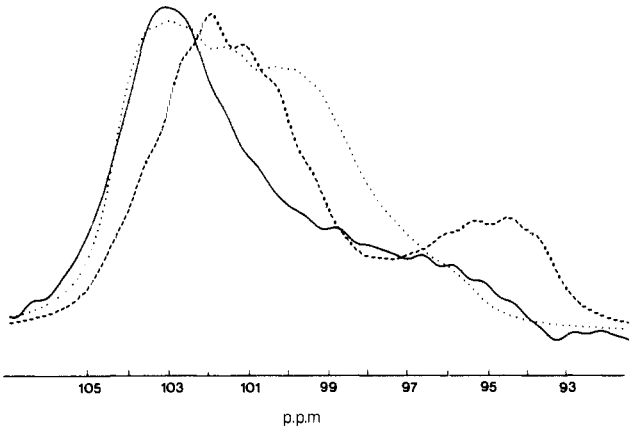

Figure 11. Predicted C-1 spectra assuming the equiprobable occurrence of all allowed conformations and based on correlations with $|\phi| + |\psi|$ (dashed line) and $|\psi|$ (dotted line), obtained by superimposing Lorentzian lines of 100-Hz width at half height corresponding to each of the 14 10° ranges of $|\phi| + |\psi|$ and the 15 5° ranges of $|\psi|$. Appropriate chemical shift values for each Lorentzian were obtained from Figures 7 and 8. The observed C-1 spectrum of amorphous starch is shown by a solid line.

10, dashed line) can be compared with the observed C-1 spectrum of amorphous starch (Figure 10, solid line). A similar spectral simulation was also carried out by using predicted populations for the 15 allowed 5° ranges of $|\psi|$. The result is shown in Figure 11 (dotted line) together with the observed spectrum (solid line) and the simulated spectrum from Figure 10 (dashed line). Although the correlation with $|\psi|$ accurately predicts the peak maximum and the chemical shift range, predicted signal intensity in the range 99–101 ppm is greater than that observed. The correlation of C-1 shifts with $|\phi| + |\psi|$ predicts the general shape of the observed signal but suggests a peak maximum ~1 ppm to higher field than that observed (Figure 11). This difference in the predicted peak chemical shift (Figure 11, dashed line), and the observed value (Figure 11, solid line) could reflect a nonequiprobable distribution of conformations. In particular, the stable V-type helical conformations may be favored over other local conformations, thereby leading to the observed signal intensity (Figure 11, solid line) in the range 103–104 ppm (Figure 1, Table I).

The agreement between the observed amorphous C-1 signal and the two spectra predicted on the basis of conformational energy calculations and empirical chemical shift/conformation correlations (Figures 7 and 8) is encouraging. It is particularly noteworthy that the range of chemical shifts is accurately predicted from the range of allowed conformations (Figure 9) by both of the observed correlations (Figure 11). However, as no single correlation was found to be obviously most appropriate, the exact nature of the conformation/chemical shift relationship for polysaccharides remains to be established through work on other model systems and/or theoretical calculations of anticipated shifts. Since this work was completed and consistent with the present findings, Veregin et al.[67] have reported that C-1 chemical shifts for a variety of cyclodextrin complexes could be correlated with X-ray derived structures without assigning individual resonances if torsion angle ψ (ϕ'_2 in the nonmenclature of ref 67) is an important chemical shift determinant.

Although conformational features seem to be the major C-1 chemical shift determinant, other secondary effects may also be significant. This is obviously true in double-helical structures for which chemical shift ranges of up to 2.2 ppm are found (Table I) for sites adjacent to linkages of identical conformation.[4,5] These secondary shifts probably arise from interhelix interactions, but the chemical shift mechanism is not clear at present. The lack of correlation between C-4 chemical shifts and individual conformational parameters (Table II) suggests that two or more conformational effects and/or other effects such as hydrogen bonding are important in determining C-4 chemical shifts in α-(1→4) glucans. This is consistent with the finding that no single conformation/chemical shift correlation could be found for C-4 resonances in a range of cyclodextrin inclusion complexes.[67]

4. On the Amorphous Phase of Starch Granules. All native starches examined to date[28,48] have ^{13}C CP/MAS spectra which can be accurately duplicated by taking an appropriate spectral combination of model crystalline (A- or B-type as appropriate) and amorphous materials.[28] This shows that α-(1→4) glucans in the amorphous phase of starch granules adopt similar conformations regardless of botanical source and that the range of conformation is very similar to those in amorphous preparations in vitro, i.e., the amorphous phase of starch granules is truly amorphous. Furthermore, the results shown in Figure 11 suggest that all allowed (Figure 9) conformations are present. The similarity of ^{13}C CP/MAS spectra of amorphous phases from starches containing ~100% amylopectin (e.g., waxy maize) and up to 70% amylose (amylomaize) shows that the low (typically 4–5%) degree of branching in amylopectin does not greatly affect the distribution of local conformations. These observations suggest a universality of local conformational features within starch granules that has not hitherto been suspected. Botanical variations in the supramolecular organization of α-(1→4) glucans within starch granules, however, cannot be ruled out.

^{13}C CP/MAS spectra of model amorphous materials (Figure 5a) are notably different than those of model double helical materials (Figures 5 (parts c and d)) but show marked similarities to spectra of single helical amyloses (Figure 5b). In particular (Figure 5 (parts a and b)), C-4 chemical shifts are very similar, and amorphous materials have substantial (~40%) C-1 signal intensity in the range of the V-type C-1 signal (102–104 ppm). This suggests that a substantial portion of the amorphous phase of starch granules consists of local conformations similar to those characteristic of V-type structures.

Although the linear α-(1→4) glucan amylose (but not the branched amylopectin) forms V-type inclusion complexes with lipids in vitro, it is not known whether such complexes exist in vivo. However, our observation of significant V-type local conformations in granules together with the recently demonstrated correlation between lipid and amylose contents for a range of cereal starches[9] tends to suggest that lipids are indeed present as inclusions in helical amyloses within starch granules. As V-type X-ray diffraction patterns have never been observed for native starches though, significant long range ordering of single helices does not occur.

Conclusions

Solid oligomeric and polymeric α-(1→4) glucans show a wide diversity of ^{13}C CP/MAS NMR spectral features (chemical shifts, line widths, multiplicities). Conformational features associated with rotation about the glycosidic bonds are suggested to be the major determinant of C-1 and C-4 chemical shifts. Two possible correlations of C-1 chemical shift with glycosidic torsion angles ($|\psi|$ or $|\phi| + |\psi|$) are indicated. Both correlations lead to the successful modelling of the observed C-1 spectrum of amorphous starches. Line widths in ^{13}C CP/MAS spectra increase through the range: crystalline cyclodextrins < ordered oligomers < ordered polymers < amorphous material. Multiplicity effects (particularly at C-1) in A-, B-, and V-amylose polymorphs can be rationalized in terms of helix-packing symmetry. α- and β-Cyclodextrin hydrates show resolved resonances for each C-1 and C-4 site within the 6-(α) or 7-(β) residue macrocycle.

Six- and sevenfold V-amylose helical inclusion complexes show similar features in the carbohydrate region of the ^{13}C CP/MAS spectrum irrespective of the nature of the included species. β-Cyclodextrin hydrate has similar C-1 and C-4 chemical shift ranges to V-amyloses. Weighted averages of C-1 and C-4 chemical shifts for β-cyclodextrin undecahydrate are essentially identical with chemical shifts in solution, suggesting similar conformations in solid and solution states. In α-cyclodextrin hexahydrate high field C-1 and C-4 signals are assigned to sites

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adjacent to a high-energy glycosidic linkage. Apart from these signals, chemical shift ranges are similar to those of V-amyloses. Solution state C-1 and C-4 chemical shifts for α-cyclodextrin are closely matched if averages of solid-state shifts are taken which ignore the sites adjacent to the high-energy linkage. This suggests that α-cyclodextrin might adopt a more expanded conformation in solution compared to the solid state. The marked similarity between ^{13}C CP/MAS spectra of amorphous starches and V-type amylose complexes provides evidence for the presence of amylose/lipid inclusion complexes in starch granules.

Registry No. α-Cyclodextrin, 10016-20-3; α-cyclodextrin hydrate, 51211-51-9; β-cyclodextrin, 7585-39-9; β-cyclodextrin hydrate, 68168-23-0; α-(1→4)glucan, 9051-96-1; amylose, 9005-82-7; starch, 9005-25-8.

Structural Factors Controlling the Aggregation of Lithium Phenolates in Weakly Polar Aprotic Solvents

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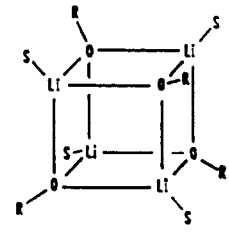
Abstract: The structures of lithium 4-fluoro-, 4-chloro, 2- and 4-bromo-, 4-(trifluoromethyl)-, 4-methoxy-, 2-methyl-, 2-ethyl-, 2-*n*-propyl-, 2-isopropyl-, 2-*tert*-butyl-, 2-(methoxymethyl)-, 3,5-dimethyl-, 3,5-dimethyl-4-methoxy-, 4-chloro-3,5-dimethyl-, 3,5-diethyl-, and 3,5-dimethoxyphenolates in solution in weakly polar, aprotic solvents such as pyridine, tetrahydrofuran, dimethoxyethane, 1,3-dioxolane, diethyl ether, 2,6-lutidine, and triethylamine have been established by ^{13}C NMR spectroscopy. Para substituents influence the equilibrium between dimer and tetramer through their effect on the basicity of the anion. *o*-Alkyl substituents promote dimer formation through steric effects in the order of their steric bulk. The 2-methoxymethyl group stabilizes the tetramer. Dimers are favored relative to tetramers by a combination of high Lewis basicity and low steric demand of the solvent. A number of lithium phenolates in 1,3-dioxolane exist as hexamers at low temperatures. The following pairs of values for ΔH (kcal mol^{-1}) and ΔS (cal mol^{-1} K^{-1}) are found for 2 dimer $\rightleftharpoons$ tetramer: lithium 4-bromophenolate (THF), 4.4 ± 0.5, 24 ± 2; 3,5-dimethylphenolate (pyridine), 6.6 ± 0.2, 33 ± 1; 2-isopropylphenolate (THF), 7.5 ± 0.5, 38 ± 2. For tetramer $\rightleftharpoons$ $^2/_3$ hexamer the values for 3,5-dimethoxyphenolate (dioxolane) are −4.7 ± 0.3 and −20 ± 1.7. ^7Li quadrupole splitting constants have been determined for several dimers and tetramers.

Many important synthetic methodologies involve the reactions of organic lithium salts with electrophiles in weakly polar aprotic solvents, particularly ethers. The nucleophiles in these reactions are usually ambident anions (e.g. enolate,[1] enamide,[2] heterosubstituted allyl[3]), the corresponding lithium salts of which are contact ion pairs or ion-pair aggregates. There is evidence that such ion-pair aggregates can function as true reactants,[4-6] and it is, therefore, probable that the degree of aggregation influences reactivity and regio- and stereochemistry. An understanding of the structural factors that control the degree of aggregation together with some knowledge of the thermodynamics of aggregation equilibria are, therefore, prerequisites for any mechanistic studies of this important group of reactions.

The main driving force for aggregation in weakly polar solvents is, of course, the maximization of electrostatic interactions between cations and anions. Aggregation, however, will generally occur at the expense of solvation of the ions, which, for weakly polar *donor* solvents, will principally involve the lithium cation. The overall process may, therefore, be viewed as a competition between anions and solvent for the available coordination sites (usually three or four) around the lithium cation and will thus reflect their Lewis basicities. In addition to considerations of intrinsic basicity, steric factors involving solvent and anion will be important. It might also be supposed that the number of lone pairs on the donor atoms of the anion could effect aggregation since in the cubic tetramer **1**, for example, a total of three lone pairs can be directed



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toward three cations. This does not appear to be a dominant factor, however, since alkyllithium compounds often have the structure **1** in ether solvents.[7] Finally, solvation, which is associated with the loss of translational freedom of small molecules, is a process with a substantial negative entropy change so that increased solvation at the expense of aggregation will be favored by lower temperatures.

We present here a study of lithium phenolates undertaken in order to provide insight into the various factors affecting aggregation. Phenolates are ideal for this purpose in that a series of compounds having a wide range of electronic and steric effects

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