 © 1991 The Chemical Society of Japan Bull. Chem. Soc. Jpn., 64, 3528—3537 (1991)

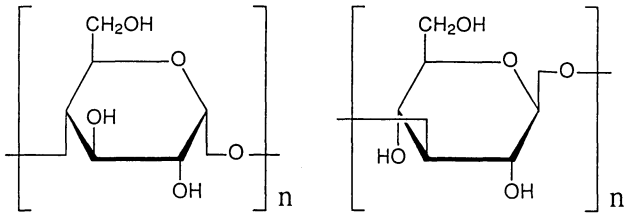
Conformational Stability of V-Amyloses and Their Hydration-Induced Conversion to B-Type Form as Studied by High-Resolution Solid-State ^{13}C NMR Spectroscopy

Hazime SAITÔ,* Junko YAMADA,† Tohru YUKUMOTO,††
Hirofumi YAJIMA,†† and Ryuichi ENDO††
Department of Life Science, Himeji Institute of Technology,
Harima Science Garden City, Kamigori-cho, Hyogo 678-12
†Biophysics Division, National Cancer Center Research Institute,
Tsukiji 5-chome, Chuo-ku, Tokyo 104
††Department of Applied Chemistry, Science University of Tokyo,
Kagurazaka, Shinjuku-ku, Tokyo 162
(Received July 19, 1991)

We have recorded ^{13}C NMR spectra of several preparations of amorphous samples, V-amyloses and their hydrates to gain insight into their relative stability and subsequent hydration-induced conformational change. It was found that the ^{13}C NMR line width of the iodine complex prepared as a precipitate in aqueous solution was the narrowest among these preparations, including the iodine complex formed from cast film. It is noteworthy that these V-amyloses are not always stable when they are equilibrated in an atmosphere of 96% R. H. in a desiccator and converted to B-type form, consistent with previous observation by Senti and Witnauer (*J. Am. Chem. Soc.*, **70**, 1438 (1948)). ^{2}H NMR line shape and spin-lattice relaxation times were measured for partially deuterated V and B-type amylose samples containing DMSO-d_6 and deuterium oxide, respectively, to explore the dynamics of the DMSO and water molecules bound to the polymer chains. It is unlikely that any drastic conformational change such as unfolding followed by refolding and plausible change of handedness is caused by such mild physical treatment as humidification. Therefore, it appears that the resulting B-type form should be ascribed to a left-handed single helix as a consequence of a slight modification of V-amylose, in contrast to the previous interpretation based on the data of X-ray diffraction and NMR studies. Gelation mechanism of amylose gel based on this view is also discussed.

It has been shown that amyloses (I) and starches ((1→4)-α-D-glucan) exhibit the following polymorphs, as analyzed mainly by X-ray diffraction study:[1,2] V-, A-, B-, and C- forms. The V-form exists as complexes with small organic molecules, water or iodine, and have in common a left-handed, single six-residue helix.[1,3–6] The A- and B-forms are found for cereal and tuber starches, respectively.[1,2] The detailed structure of the B-form is still the subject of current debate. The B-form was initially considered as a single helical conformation,[7] since the conversion of V- to B-amylose takes place on humidification.[8] Later, the structure was refined as a right-handed double helix packed in an antiparallel fashion,[9,10] following the suggestion by Kainuma and French,[11] although two sixfold helical models were proposed from the data for similar unit cell dimension.[12] The double helical model was later questioned upon taking into account the biosynthesis mechanism[13] and optical rotation.[14] The handedness of the double helix, however, was recently revised as a left-handed one on the basis of refined X-ray diffraction and optical rotation.[15–17] In addition, it was shown that a left-handed, single-stranded helix is formed by crystalline complex between maltoheptaose and glycogen phosphorylase a.[18] An antiparallel, left-handed double-helix[19] was found in the crystal structure of the polyiodide complex, a dimer of *p*-nitrophenyl-α-maltohexaose.

In addition, a number of complementary studies on the conformations of amylose and starch have been performed by ^{13}C NMR method,[17,20–30] since X-ray fiber diffraction study provides only relatively limited information. In general, consistent results between X-ray and NMR studies have been obtained for a number of samples having V-,[20,21,24,26–28] A-, and B-forms,[17,22,23,25–27] although the NMR approach is further able to afford conformational data for less crystalline sample or gels. It seems, however, essential to analyze the process of mutual conversion among the V-, A-, and B-forms to obtain additional information on this subject. In this connection, a comparative study is indispensable to reach any definite conclusion.



I II

(1→3)-β-D-Glucan (II) of high molecular weight is considered to be a pertinent reference compound for the present purpose, because it adopts a single helix similar to that of (1→4)-α-D-glucan[31–33] and because it posseses the ability to form multiple-stranded helices,[34,35] in relation to gel formation. We have previously demonstrated that (1→3)-β-D-glucan as well as (1→3)-β-D-

xylan can assume the three kinds of different conformations: single chain, single helix, and triple helix, depending upon sources and sample history.[36-42] We found that these forms can be mutually converted in a controlled manner by various types of physical treatments such as hydration, dehydration, annealing, etc. The resultant conformational changes are readily monitored by a characteristic displacement of conformation-dependent ^{13}C chemical shifts[43,44] as recorded by high-resolution solid-state ^{13}C NMR spectroscopy.

This sort of ^{13}C NMR study on polysaccharides with systematically manipulated conformations provides a simple and reliable means to distinguish a single chain/helix form from multiple-stranded chains. Such a distinction, however, is not always feasible by X-ray diffraction alone because distinguishing between multi-stranded coaxial helices and nested single helices is often one of the most difficult problems in interpreting fiber-diffraction data.[45,46] Instead, it is emphasized that the present ^{13}C NMR approach, utilizing physically converted samples in a controlled manner, is an excellent means for this purpose, as manifested from our studies on (1→3)-β-D-glucans,[36-41] (1→3)-β-D-xylan,[42] and agarose.[47]

We report here ^{13}C NMR studies on various preparations of V-amyloses and their hydration-induced conversion from V- to B-type forms to gain further insight into the relative stability of these preparations when being exposed to high humidity, handedness of the single or double helical B-type amylose, and distinction between the single- and double-stranded helices for the B-amylose and its consequence to a gelation mechanism of amylose gel.

Experimental

We used several types of amylose samples with different degree of polymerization from different commercial sources: DP 17 (EX-I, Seikagaku Kogyo, Tokyo), DP 100 (EX-III, Seikagaku Kogyo, Tokyo), DP 1000 (Aldrich Chemicals, USA). These samples were used without further purification. As starting materials for conversion study, we prepared either cast film (AF) or lyophilized samples by evaporating solvent from a DMSO solution. For the latter, an amylose film was prepared by casting a DMSO solution (dissolved in DMSO at 40 °C) on $(CH_3)_3SiCl_2$-coated flat dishes, followed by evaporation of DMSO at 50 °C. The preparation of the 1-butanol complex has been previously described.[20] The iodine complex from cast film (AFI) was prepared by placing AF for 3 days in a desiccator (96% R.H.) exposed to iodine vapor. The crystalline iodine complex was isolated as precipitate by addition of excess amount of iodine solution to amylose aqueous solution (ca. 1 g dm^{-3}), and dried over silica gel. The amount of iodine in the complexes was determined by a flask-combustion method. All of the hydrated samples were prepared by placing the samples in an atmosphere of 96% R.H. for 12 h. An annealed amylose sample was prepared by heating an aqueous suspension of V-amylose lyophilized from DMSO solution (DP 1000, Aldrich) at 155 °C in an autoclave for 15 min followed by slow cooling, in a similar manner to that of annealed curdlan.[37] As reference samples of the A- and B- forms, we used corn and potato starches commercially available (Nacalai Chemicals, Kyoto). Partialy deuterated V- or B-amylose samples containing DMSO-d_6 or deuterium oxide, respectively, were prepared by analogous manner, described above.

^{13}C NMR spectra of solid materials were recorded on a Bruker CXP-300 spectrometer (75.46 MHz) utilizing cross polarization-magic angle spinning (CP-MAS). All of samples were contained in a ceramic rotor with a double air bearing and spun as fast as 3 kHz. Pulse width, contact time and repetition time were 3.5 μs, 1 ms, and 4 s, respectively. Spectra were usually accumulated ca. 1000 times. ^{13}C chemical shifts were referred to TMS through ^{13}C chemical shift of carboxyl peak of glycine (176.03 ppm). High power ^2H NMR spectra were recorded on the same spectrometer at 46.08 MHz with a pulse sequence of quadrupole echo.[48]

X-Ray powder diffraction was measured on a Rigaku Denki RINT-1400 utilizing Cu $K\alpha$, 40 kV 200 mA. Samples were placed in a sealed capillary to preserve the relative humidity.

Results

In Fig. 1, we show ^{13}C NMR spectra of two typical examples of V-amyloses, iodine (A) and 1-butanol complexes (B). Five peaks are well resolved and assigned on the basis of previous work.[20] Exactly the same charac-

Table 1. ^{13}C Chemical Shifts of (1→4)-α-D-Glucans (ppm from TMS)

	DP 17 (EX-I)		DP 100 (EX-III)		DP 1000 (Aldrich)		
	Lyophilized from DMSO solution	Hydrate[a]	Lyophilized from DMSO solution	Hydrate[b]	Lyophilized from DMSO solution	Hydrate[b]	Iodine complex
C-1	101.5		102.2	101.8	102.1		101.0
		99.2	99.2	100.0		99.3	
		98.3		98.5		98.0	
(C-4)	81.2	—	81.4	80.3	82.3	—	79.6
C-2,3,4,5	71.4	73.7	74.1	74.0	71.3	72.6	73.7
		70.6	71.5	70.9	71.6	70.5	71.0
		69.2					
C-6	60.1	60.2	61.2	60.6	60.7	60.6	60.3

a) Hydrate sample of anhydrous powder. b) Hydrate samples which are lyophilized from DMSO solution.

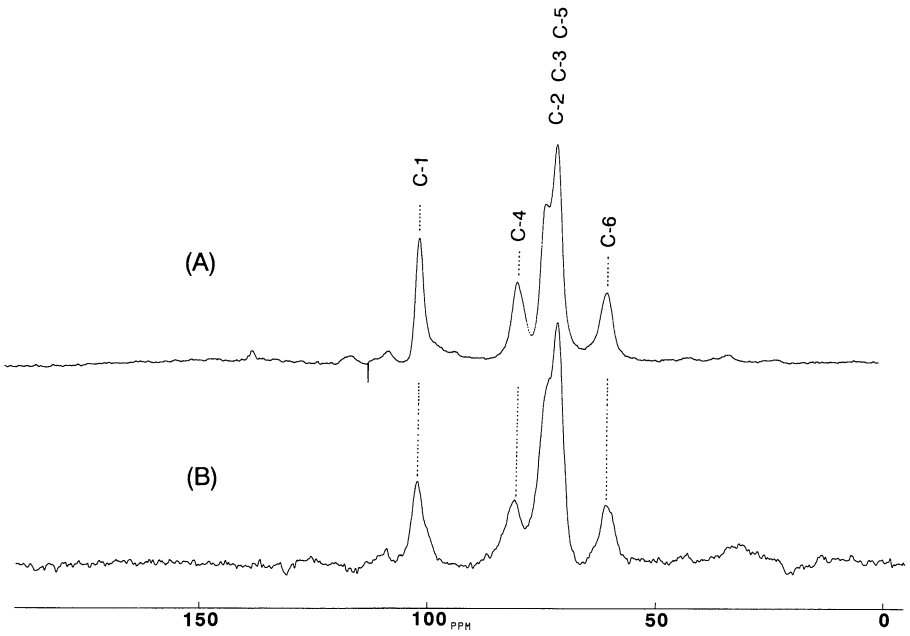


Fig. 1. ^{13}C NMR spectra of crystalline complexes of amyloses (Aldrich, DP 1000) with iodine (A) and (EX-III, DP 100) with 1-butanol (B).

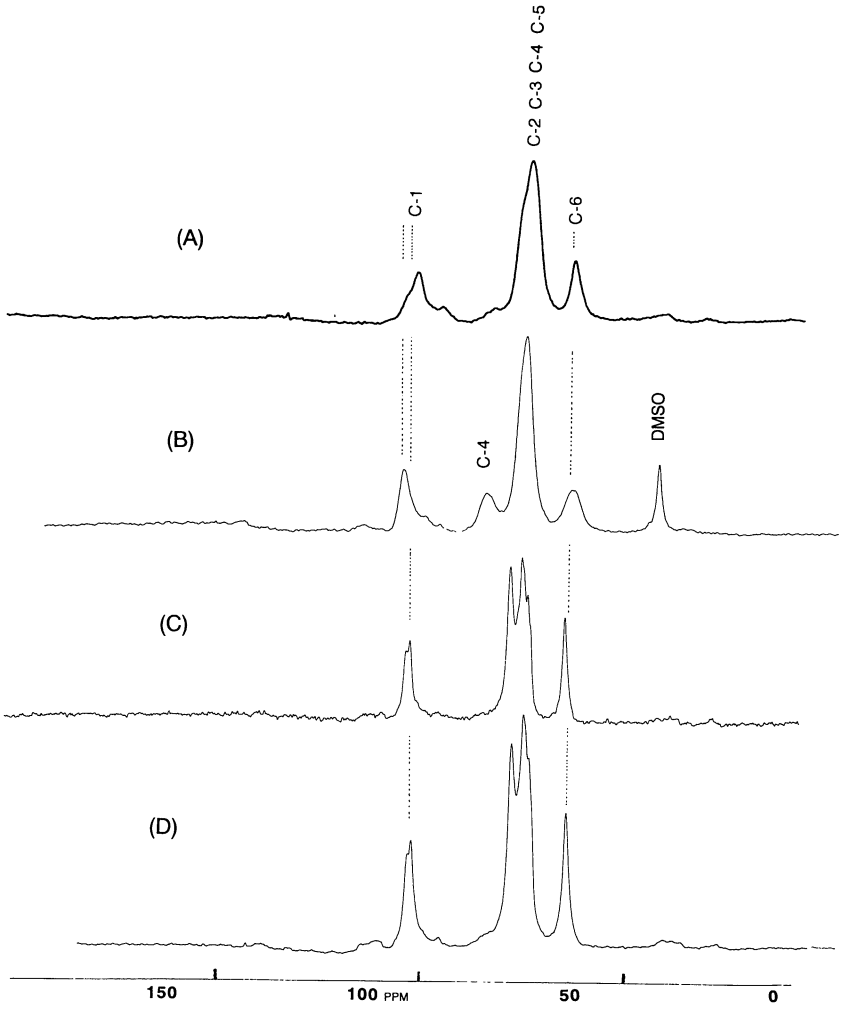


Fig. 2. ^{13}C NMR spectra of low molecular weight amylose (EX-I, DP 17) (A) anhydrous amylose powder, (B) anhydrous lyophilized powder, (C) hydrated amylose powder, and (D) hydrated iodine complex.

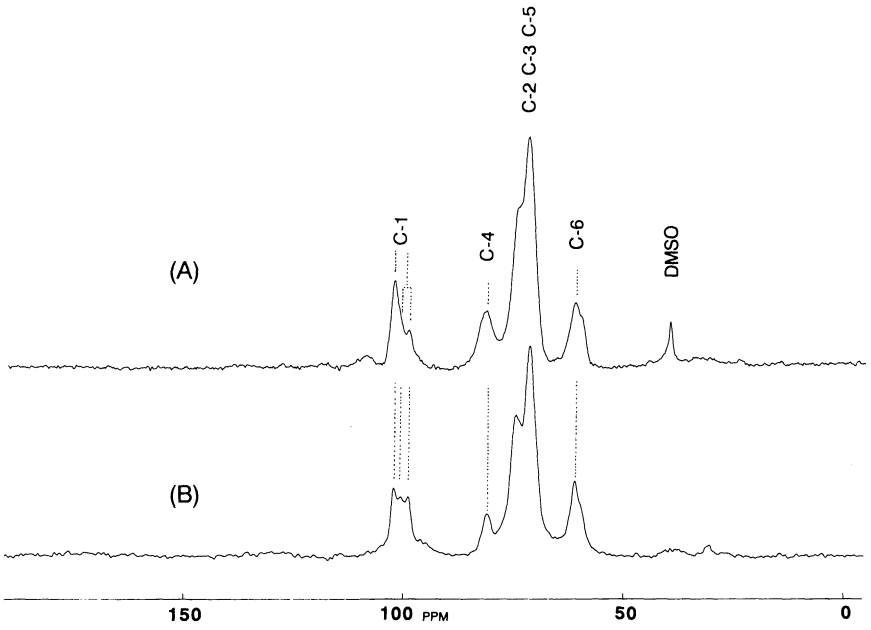


Fig. 3. ^{13}C NMR spectra of amylose with intermediate molecular weight (EX-III, DP 100) lyophilized from DMSO solution. (A) anhydrous, (B) hydrated.

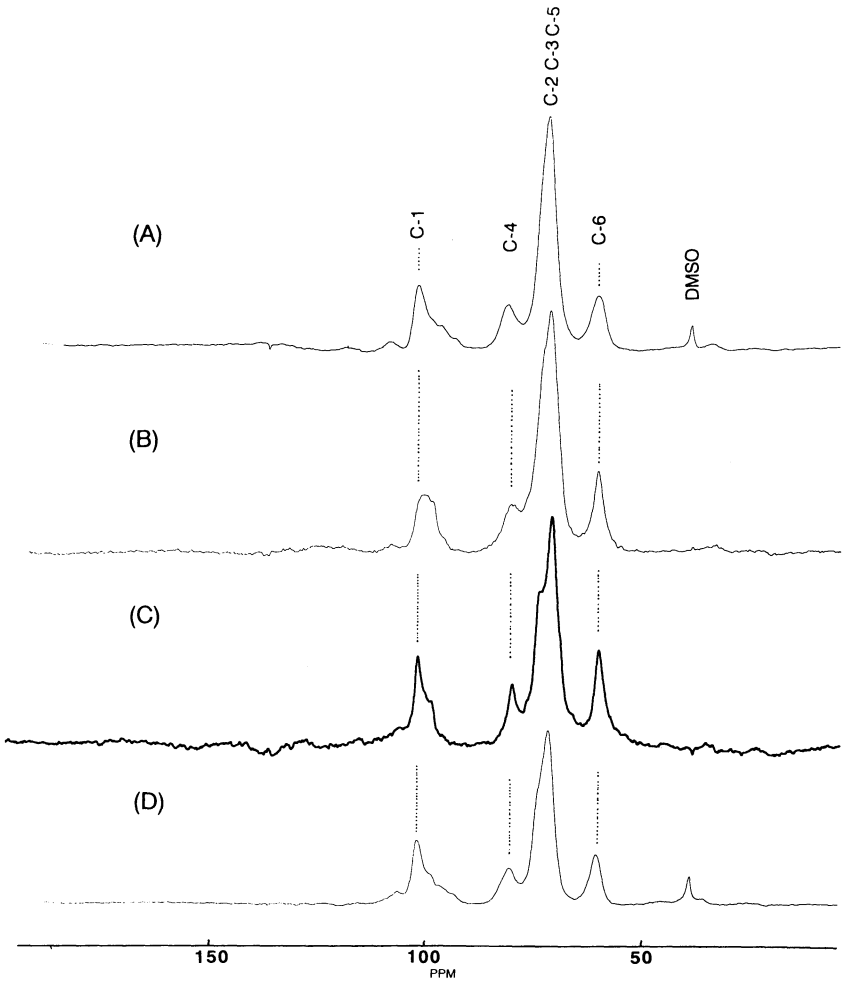


Fig. 4. ^{13}C NMR spectra of amylose with high molecular weight (Aldrich, DP 1000) lyophilized from DMSO. (A) anhydrous, (B) hydrate, (C) hydrated iodine complex and (D) anhydrous iodine complex.

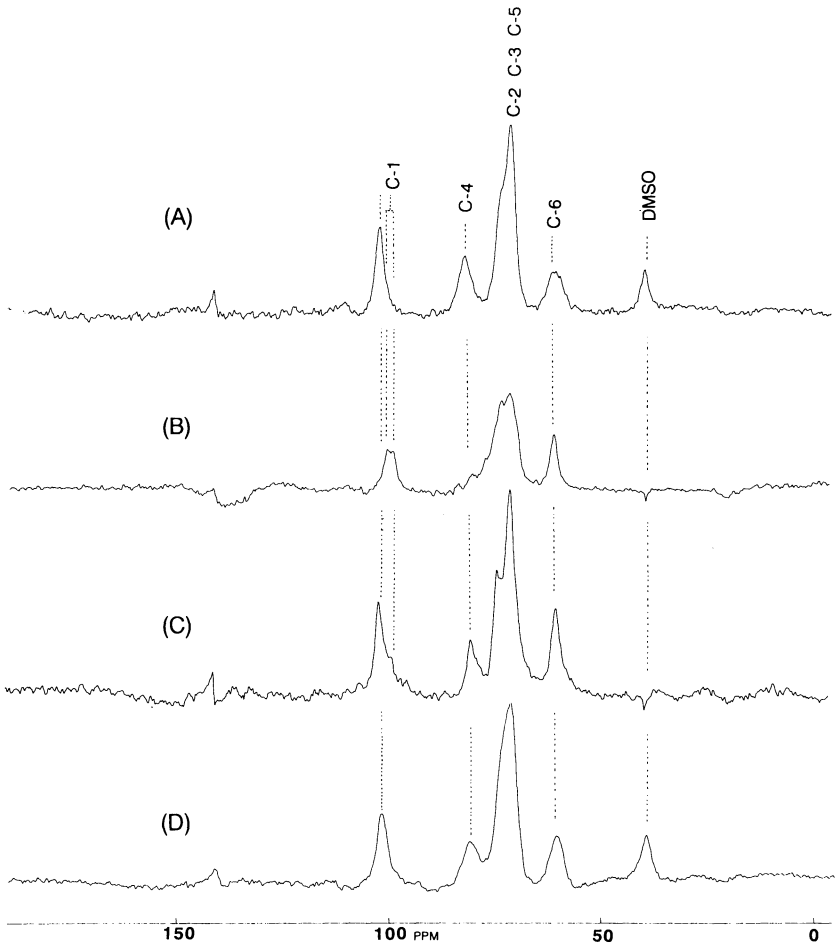


Fig. 5. ^{13}C NMR spectra of amylose film from high molecular weight sample (Aldrich, DP 1000).
(A) anhydrous, (B) hydrated, (C) hydrated iodine complex, and (D) anhydrous iodine complex.

teristic pattern was seen for both type of V-amyloses, in spite of a difference in guest molecules and molecular weight in host molecules (Aldrich, DP 1000 and EX-III, DP 100 for the former and the latter, respectively).

Figure 2 shows ^{13}C NMR spectra of amylose powder of low molecular weight (EX-I, DP 17) under various conditions. The ^{13}C chemical shifts thus obtained were summarized in Table 1. Clearly, anhydrous powder lyophilized from a DMSO solution (Fig. 2(B)) gave rise to the spectral pattern of V-form, as compared with that of the iodine complex (Fig. 1(A)), although this form might be distorted to some extent compared to that of the crystalline complex. The uppermost signal at 39 ppm is assigned to the methyl signal of DMSO molecules bound to the amylose chain. The anhydrous powder, however, has an amorphous structure distorted from the B-form, as judged from the broad envelope of the ^{13}C NMR signal in the C-1 region and the absence of the C-4 peak at 81 ppm (Fig. 2(A)). It is noteworthy that hydration of the anhydrous powder induced distinct spectral change (Fig. 2(C)). Obviously, the spectral pattern of the resulting hydrate powder (C and D) arises from that of B-amylose (doublet pattern in the C-1 region,[20–24] spectrum not shown). The spectral pattern of Fig. 2(D) is of the B-form despite being the iodine complex. The iodine content for this sample turned out to be less than 0.5%, indicating that the amount of the iodine complex (V-form) is negligible and the rest is the major B-form.

Figure 3 shows the ^{13}C NMR spectra of an amylose of an intermediate degree of polymerization (EX-III, DP 100). The C-1 peak of the lyophilized sample from DMSO solution arises from two components: the downfield singlet signal (the lowermost peak) ascribable to V-form (70%) and the upfield doublet shoulder (the middle and uppermost peak) ascribable to B-form (30%) (Fig. 3A), as estimated from comparison of the respective peak intensities. Again, the peak at 39 ppm is assigned to the methyl signal of DMSO bound to the polymer. Hydration of this sample resulted in partial conformational change from the V- to B-form, as manifested from the buildup of the upfield doublet pattern (up to 50%) in the C-1 with expense of the C-4 intensity (V-amylose) (Fig. 3B).

Table 2. Summary of Conformation Characterized by ^{13}C NMR and X-Ray Diffraction

	Anhydrous (lyophilized from DMSO solution)		Hydrate[a)]	
	NMR	X-Ray	NMR	X-Ray
DP 17 (EX-I)	V	V	B	Amorphous B[b)]
DP 100 (EX-III)	V(70%)+B(30%)	Amorphous	V(50%)+B(50%)	Amorphous
DP 1000 (Aldrich)	V	V	B	Amorphous
Iodine complex	V	V	V	V

a) Anhydrous lyophilized sample from DMSO solution was hydrated under the condition of 96% R.H. for over 12 h. b) Anhydrous powder sample was hydrated.

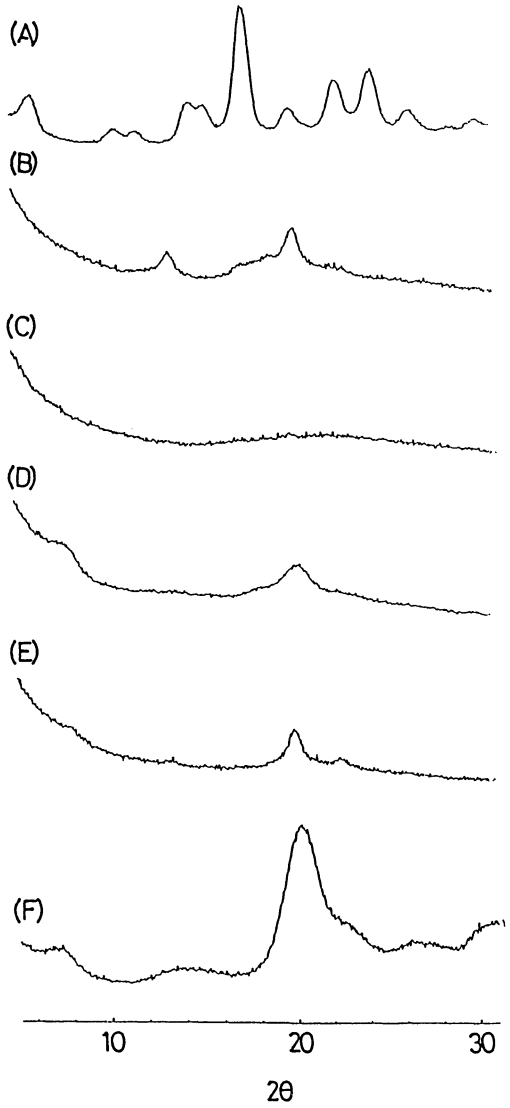


Fig. 6. Comparison of X-ray powder diffraction patterns of various types of amyloses (samples (B)—(E) correspond with the respective samples (A)—(D) of Fig. 4).
(A) hydrated amylose (DP 17) from anhydrous powder, (B) anhydrous amylose (DP 1000) lyophilized from DMSO, (C) hydrated amylose (DP 1000) lyophilized from DMSO, (D) hydrated iodine-complex (DP 1000), (E) anhydrous iodine complex (DP 1000), (F) amylose iodine complex as precipitate (DP 1000).

Similar experiments were repeated for amylose with high degree of polymerization (Aldrich, DP 1000). In contrast to the case of intermediate degree of polymerization, hydration of V-form amylose resulted in complete conversion to B-form (Figs. 4(A) and (B)). Annealing of this sample yielded a very narrow B-type spectrum comparable to that of Figure 2D, although the X-ray diffraction pattern was still a halo (spectra not shown). The iodine content in this complex is ca. 10%, as determined by the change of weight before and after the complexation. It is interesting to note that the iodine complex (Fig. 4(D)) was stable after exposure to an atmosphere of 96% R.H. (Fig. 4(C)). These experiments were also performed for samples prepared from cast film. Interestingly, the hydration-induced conformational change is incomplete but still observable as seen from the increase of the upper field doublet of the C-1 region and the decrease of the peak intensity at the C-4 for the cast film (Figs. 5(A) and (B)), in contrast to the observation for lyophilized powder (Figs. 4(A) and (B)). Again, it is found that the iodine complex prepared from a cast film is stable for the exposure to high humidity.

Typical X-ray powder diffraction patterns of the above-mentioned samples (see Fig. 4) were illustrated in Fig. 6. The resulting conformational features obtained from the X-ray and NMR studies are summarized in Table 2. The conformation revealed by X-ray diffraction is consistent with that obtained by NMR as far as V-amylose is concerned. On the other hand, the hydration-induced B-form converted from V-amylose (as judged from NMR data) turned out to be amor-

Table 3. ^{2}H Spin-Lattice Relaxation Times of DMSO-d_6 and Deuterium Oxide Bound to Amylose and Curdlan (ms)

	Temp/K	DMSO-d_6	D_2O
Amylose, V form	295	42.9	
	303	52.6	
B form	295		14.3
Curdlan, Anhydrous	295	44.2	
	303	44.8	
	314	48.5	
	323	68.3	
Hydrate	295		10.1

phous as shown by X-ray diffraction study (Table 2). The 46.08 MHz ^{2}H NMR signals of DMSO-d_6 (V-amylose) and ^2H_2O (B-amylose) were observed as single peaks with line widths of 1.9 and 2.4 kHz at ambient temperature, respectively (spectra not shown). Almost the same result was obtained for curdlan, a (1→3)-β-D-glucan as a reference compound for amylose. The ^{2}H spin-lattice relaxation times of DMSO-d_6 and D_2O are 44 and 10 ms at 22 °C, respectively, as summarized in Table 3.

Discussion

Relative Stability of V-Amyloses and Molecular Motions of Guest Molecules. The present observations clearly demonstrate that three types of amylose preparations, complexes with 1-butanol, DMSO, and iodine, exhibit similar V-type forms, consistent with the X-ray data (Fig. 6 and Table 2). In fact, previous X-ray diffraction studies showed that all V-amylose structures are almost the same with h (rise per residue) in a very narrow range from 1.32 to 1.36 Å.[1] Complexing agents such as water, small organic molecules, or iodine are found inside the helix channel on the basis of the X-ray diffraction studies.[1] Nevertheless, the relative stability of these complexes varies with the type of complexing agent: Water molecules are readily removed by lyophilization, as in the case of the amorphous form (Fig. 2(A)), and DMSO and 1-butanol (spectra not shown) molecules are readily replaced by water molecules upon exposure to high humidity as a result of a conformational change from the V- to the B-form.

For this reason, it is expected that these complexing agents are not necessarily strongly bound to the amylose chain and can undergo rapid motion in the cavity if the sizes of these molecules are smaller than that of the cavity. Such motion, if any, could be easily detected by examining how ^{2}H quadrupole splittings are averaged out by plausible molecular motions and by the extent of the ^{2}H spin-lattice relaxation times of the deuterated guest molecules.[49,50] It is expected that ^{2}H NMR signals of ^2H_2O or DMSO-d_6 complexed to amylose by forming B- or V-form are split into a doublet pattern whose separation of peaks being about 100—200 and 40 kHz (with C_3 rotation of methyl group), respectively, if they are tightly bound (static). This expectation is based on the fact that the quadrupole coupling constants, e^2qQ/h, of the former and the latter are of the order of 140—300 and 170 kHz, respectively.[49] It turned out that only a singlet signal was visible as a result of rapid isotropic averaging of the quadrupole interaction in the solid state with correlation times of the order of 10^{-6} s.[49] Also, Table 3 shows that the ^{2}H T_1 values of bound ^2H_2O and DMSO-d_6 molecules are 10 and 44 ms, respectively, at ambient temperature and are increased to 68 ms for the latter (in the case of DMSO bound to curdlan). These results indicate that the correlation times of the isotropic tumbling motions of the former and latter are in the vicinity of the T_1 minimum (10 ms),[50] namely about 10^{-8} s, and on the high temperature side of the T_1 minimum.[51]

The existence of such loosely bound guest molecules undergoing rapid isotropic motions is consistent with a view that DMSO molecules inside the cavity are readily replaced by water molecules when these samples were hydrated. Humidification of these samples results in hydration-induced conformational change. Replaced water molecules, however, are not always tightly bound to amylose chains and undergo rapid reorientation motion (correlation time being in the order of 10^{-8} s) as revealed by the present ^{2}H NMR observation, in contrast to the view from X-ray diffraction study.[16]

It is interesting to note that, in contrast, the iodine complex is stable under high humidity. This stability is indicated by the fact that these complexes are formed in the presence of water molecules. The amounts of water bound to the iodine complexes, however, are less than 50% of that of the uncomplexd amyloses.[52] The adsorbed water molecules are consumed in converting the molecular iodine in the cavity to I_3^- or I_5^- ions, which are indispensable for stabilization of the iodine complexes.[53] Consequently, approximately three iodines occupy the helix channel within one fiber repeat, but the iodines form an almost linear polyiodide chain of undetermined length.[1] This is the reason why the iodine complexes are highly stable in the presence of high humidity.

Hydration-Induced Conformational Change. It is now clear that hydration induces a conformational change of amyloses of various molecular weights from either amorphous or V- to B-type forms (Figs. 2—5), although the extent is different between powder and cast film. Here, we use the term "B-type" form instead of B-form when its X-ray diffraction pattern is a halo (Fig. 6) even if its NMR pattern exhibits the spectral feature of B-form. Nevertheless, it is emphasized that the conformation of the "B-type" form is very close to that of the B-form as far as short-range order is concerned, as will be discussed later. This observation is consistent with the previous work of Blackwell et al.[7] who previously described this hydration-induced form as B-form.

It is interesting that conversion is incomplete for amylose preparation of intermediate degree of polymerization (DP 100), as compared with that of high degree (DP 1000) or low degree of polymerization (DP 17). The conformation of amylose of intermediate molecular weight (DP 100) lyophilized from DMSO solution turned out to be a mixture of V- and B-forms. The proportion of the B-type form is increased by humidification but it is not completely converted to pure B-type form. This trend is consistent with the previous study on the effect of chain length on phase behavior and aggregation kinetics utilizing monodisperse amyloses:[54] Amyloses having chain lengths of <110 residues are found to precipitate from aqueous solution, and gela-

tion is found to predominate over precipitation for longer chains (>1100). It is probable that aggregated amylose is rather hydrophobic and can be resistant to conformational change due to hydration. Here, the following two points are noteworthy as to the conformational behavior of amylose in cast film. First, the ^{13}C NMR spectra of cast film (Fig. 5A) are generally broadened compared to those of the lyophilized powder from DMSO solution (Fig. 4A). This is readily explained on the basis that the conformation in cast film can be distorted to some extent when the liquid film was heated up to 50 °C to evaporate the DMSO. This sort of conformational distortion is accelerated when DMSO molecules inside the cavity are also removed, as manifested by the considerably reduced peak intensity of DMSO methyl signal (Fig. 5B). Second, there remains a considerable proportion of unconverted V-form (Fig. 5B) after humidification in spite of the high molecular weight sample used (DP 1000) (see also Fig. 4B). Such a distortion is not necessarily taken into account for the lyophilized sample, because DMSO molecules are removed at frozen state. It is probable that the formation of the aggregated amylose as in the case of intermediate molecular weight sample might prevent complete conformational change due to humidification.

In addition to the present observations from V- to B-change, hydration-induced conversion between amorphous and B-form has been also previously demonstrated.[17,23–27] The "amorphous" structure defined by X-ray diffraction does not necessarily indicate the presence of a completely disordered structure because there are a number of cases where secondary structure is amorphous as viewed from X-ray diffraction but is still quite ordered as viewed from the ^{13}C NMR study.[44] This situation is readily understood in that X-ray diffraction is sensitive to long range order, while NMR is sensitive to short range order. Hence, there appear several types of amorphous structures which are close to either B- or V-forms, or a mixture of both (see Table 3). For instance, the ^{13}C NMR spectrum of an anhydrous powder with a low degree of polymerization (DP 17) (Fig. 2(A)) may be ascribed to a distorted B-form. On the other hand, a number of examples have been presented for dried potato starch[23–27] whose ^{13}C NMR patterns are rather close to those of V-amylose, although dispersion of chemical shifts, especially at the C-1 region, is enormous. Accordingly, the "amorphous"→B-type or B-type→ "amorphous" conversion for samples with intermediate or high degree of polymerization can be considered as a modified V→B-type or B-type→V conversion.

The reverse B→V conversion, however, has not been realized by X-ray diffraction study. This may be one of the reasons why the single helical model of B-amylose by Blackwell et al.[7] has long been neglected. Nevertheless, it is now clear that such reverse conversion can be readily identified by solid-state NMR.[17,23–27] In addition, the B-form amyloses are completely converted to V-form by dissolving those in DMSO solution, followed by lyophilization (spectra not shown). This sort of V→B or B→V conversion is parallel with the conversion between anhydrous and hydrate state as observed for (1→3)-β-D-glucans previously reported, as summarized in Table 4.[37–41] It is emphasized that complete dissolution in aqueous solution is an essential requirement for the conversion of (1→3)-β- D-glucan from the singe helix to the triple helix.[37,39,40] This means that unfolding of the polymer chains followed by refolding in aqueous media is a necessary condition for such conversion. In this connection, it is hardly likely that simple humidification of amylose in a desiccator causes such an unfolding/refolding process leading from the single-stranded helilx (V-form) to double-stranded helix (B-form). It is therefore natural to conclude that the left-hand chirality is preserved upon humidification. The above-mentioned hydration-induced conversion is related to a change among a variety of left-handed single-stranded helices as observed for amylose, its derivative and its oligomers.[19] It is also shown that the helical parameters of an antiparallel double helix of maltohexaose are comparable to those obtained for the left-handed amylose single helix of maltoheptaose complexed with phosphorylase a.[18,19] As mentioned above, annealing of amylose at 155 °C did not induce a spectral change from the B-type form. This means that multiple-stranded helices may not be formed even after annealing followed by slow cooling. It is concluded that the B-type form thus obtained takes a left-handed single helix form, rather than the previously proposed double helix conformation, although the helical parameters should be different from those of the V-form.[18,19] Thus, it is needless to claim that the ^{13}C chemical shifts of the B-type form differ from those of V-form.

Significance of Single Helix and Gelation Mechanism. It is well recognized that biopolymer gels are usually formed by the physical association of parts of the polymer chains at regions of local order or "junction zones" to produce a continuous, three dimensional poly-

Table 4. Summary of Conformational Conversion Achieved for (1→4)-α-D- and (1→3)-β-D-Glucans

Treatment	(1→4)-α-D-Glucans	(1→3)-β-D-Glucans
Lyophilization from DMSO soln	V	Single chain
Hydration (humidification)	B	Single helix
Annealing/slow cooling	B	Triple helix

mer network.[55] So far, two different views have been presented for the gelation mechanism of amylose: Miles et al.[56] have suggested that gels are formed upon cooling molecularly entangled solutions as a result of phase separation of the polymer-rich phase, whereas Wu and Sarko[9] proposed that gelation occurs through cross-linking by double helical junction zones. As an alternative model of the former type of gelation, the presence of short segments of antiparallel double helices between single helical chains also would result in three dimensional network.[19] In principle, it is possible to distinguish which mechanism is more likely for the network structure of the amylose gel on the basis of conformational elucidation of dynamically heterogeneous network by means of variety of ^{13}C NMR technique.[24,40]

In fact, Gidley[24] showed that amylose gel contains two kinds of ^{13}C resonances: The B-type signals from motionally restricted regions as recorded by the CP-MAS technique and the signals identical to those found in aqueous solution. Obviously, the latter signals can be ascribed to the presence of mobile region in the network whose conformation is a random coil.[20] The former peaks, on the other hand, are thus ascribed to aggregated regions of the single helical chains based on our present observation, although they were ascribed to double-helical junction zone by Gidley.[17] At this point, it is worthwhile to point out that only a small amount of cross-linking agent, say 0.1—5%, is sufficient to form elastic gels, as shown by studies of chemically cross-linked gels of synthetic polymers.[57,58] To support this view, we showed that the triple-helical region as cross-links is ca. 10% at most in an elastic curdlan gel.[37,40,57] This means that the amylose gel does not necessarily consist entirely of molecular chains arising from the double-helical structure. Instead, it appears that the "solid-like" region of the amylose gel[17] might be ascribed to the presence of phase-separated aggregates of the B- type single-helical chains as cross-links. This view seems to be in favor of the gelation mechanism of Miles,[56] although alternative cross-links by the antiparallel short segments of the double-helices[19] could also exist. The latter could not be easily distinguished from the aggregated single helices, because the resulting helical parameters are comparable to those of the single-stranded helices.[19]

References

1) A. Sarko and P. Zugenmaier, *ACS Symp. Ser.*, **141**, 459 (1980).

2) D. French, "Starch: Chemistry and Technology," 2nd ed, ed by R. L. Whistler, J. N. BeMiller, and E. D. Paschall, Academic Press, New York (1984), p. 183.

3) R. E. Rundle, *J. Am. Chem. Soc.*, **69**, 1769 (1947).

4) A. D. French and H. F. Zobel, *Biopolymers,* **5**, 457 (1987).

5) W. Winter and A. Sarko, *Biopolymers,* **11**, 849 (1972).

6) B. Zaslow, V. G. Murphy, and A. D. French, *Biopolymers,* **13**, 779 (1974).

7) J. Blackwell, A. Sarko, and R. H. Marchessault, *J. Mol. Biol.,* **42**, 379 (1969).

8) F. R. Senti and L. P. Witnauer, *J. Am. Chem. Soc.,* **70**, 1438 (1948).

9) H. C. H. Wu and A. Sarko, *Carbohydr. Res.,* **61**, 7 (1978).

10) H. C. H. Wu and A. Sarko, *Carbohydr. Res.,* **61**, 27 (1978).

11) K. Kainuma and D. French, *Biopolymers,* **11**, 2241 (1972).

12) R. Cleven, C. van der Berg, and L. van der Plas, *Starch/Stärke,* **30**, 223 (1978).

13) D. A. Rees, *Pure Appl. Chem.,* **53**, 1 (1981).

14) D. L. Wild and J. M. V. Blanshard, *Carbohydr. Polym.,* **6**, 121 (1986).

15) A. Imberty, H. Chanzy, S. Perez, and A. Buleon, *J. Mol. Biol.,* **201**, 365 (1988).

16) A. Imberty and S. Perez, *Biopolymers,* **27**, 1205 (1988).

17) M. J. Gidley, *Macromolecules,* **22**, 351 (1989).

18) E. Goldsmith, S. Sprang, and R. Fletterrick, *J. Mol. Biol.,* **156**, 411 (1982).

19) W. Hinrichs, G. Buttner, M. Steifa, Ch. Betzel, V. Zabel, B. Pfannemuller, and W. Saenger, *Science,* **238**, 205 (1987).

20) H. Saitô and R. Tabeta, *Chem. Lett.,* **1981**, 713.

21) H. Saitô, G. Izumi, T. Mamizuka, S. Suzuki, and R. Tabeta, *J. Chem. Soc., Chem. Commun.,* **1982**, 1386.

22) R. H. Marchessault, M. G. Taylor, C. A. Fyfe, and R. P. Veregin, *Carbohydr. Res.,* **144**, Cl (1985).

23) R. P. Veregin, C. A. Fyfe, R. H. Marchessault, and M. G. Taylor, *Macromolecules,* **19**, 1030 (1986).

24) R. P. Veregin, C. A. Fyfe, and R. H. Marchessault, *Macromolecules,* **20**, 3007 (1987).

25) F. Horii, A. Hirai, and R. Kitamaru, *Macromolecules,* **19**, 930 (1986).

26) F. Horii, H. Yamamoto, A. Hirai, and R. Kitamaru, *Carbohydr. Res.,* **160**, 29 (1987).

27) M. J. Gidley and S. M. Bociek *J. Am. Chem. Soc.,* **107**, 7040 (1985).

28) M. J. Gidley and S. M. Bociek, *Carbohydr. Res.,* **183**, 126 (1988).

29) S. F. Tanner, S. G. Ring, M. A. Whittam, and P. S. Belton, *Int. J. Biol. Macromol.,* **19**, 219 (1987).

30) J. M. Hewitt, M. Linder, S. Perez, and A. Buleon, *Carbohydr. Res.,* **154**, 1 (1986).

31) R. H. Marchessault and Y. Deslandes, *ACS Symp. Ser.,* **126**, 221 (1980).

32) H. Takeda, N. Yasuoka, N. Kasai, and T. Harada, *Polym. J. (Tokyo),* **10**, 355 (1978).

33) K. Okuyama, M. Otsubo, Y. Fukuzawa, M. Ozawa, T. Harada, and N. Kasai, *Carbohydr. Res.,* in press.

34) T. L. Bluhm and A. Sarko, *Can. J. Chem.,* **55**, 293 (1977).

35) T. L. Bluhm and A. Sarko, *Carbohydr. Res.,* **54**, 125 (1977).

36) H. Saitô, R. Tabeta, T. Sasaki, and Y. Yoshioka, *Bull. Chem. Soc. Jpn.,* **59**, 1957 (1986).

37) H. Saitô, R. Tabeta, M. Yokoi, and T. Erata, *Bull. Chem. Soc. Jpn.,* **60**, 4259 (1987).

38) H. Saitô and M. Yokoi, *Bull. Chem. Soc. Jpn.,* **62**, 1957 (1989).

39) H. Saitô, M. Yokoi, and Y. Yoshioka, *Macromolecules,* **22**, 3892 (1989).

40) H. Saitô, Y. Yoshioka, M. Yokoi, and J. Yamada, *Biopolymers,* **19**, 1689 (1990).
41) H. Saitô, Y. Yoshioka, N. Uehara, J. Aketagawa, S. Tanaka, and Y. Shibata, *Carbohydr. Res.,* **217**, 181 (1991).
42) H. Saitô, J. Yamada, Y. Yoshioka, Y. Shibata, and T. Erata, *Biopolymers,* **31**, 933 (1991).
43) H. Saitô, *Magn. Reson. Chem.,* **24**, 835 (1986).
44) H. Saitô and I. Ando, *Annu. Rep. NMR Spectrosc.,* **21**, 209 (1989).
45) S. Arnott and A. K. Mitra, "Molecular Biophysics of the Extracelluar Matrix," ed by S. Arnott, D. A. Rees, and E. R. Morris, Humane Press, New York(1981), Chap. 3.
46) S. A. Foord and E. D. T. Atkins, *Biopolymers,* **28**, 1345 (1989).
47) H. Saitô, M. Yokoi, and J. Yamada, *Carbohydr. Res.,* **199**, 1 (1990).
48) J. H. Davis, K. R. Jeffrey, M. Bloom, M. I. Valic, and T. P. Higgs, *Chem. Phys. Lett.,* **42**, 390 (1976).
49) H. H. Mantsch, H. Saitô, and I. C. P. Smith, *Prog. Nucl. Magn. Reson. Spectrosc.,* **11**, 211 (1978).
50) J. H. Davis, *Biochim. Biophys. Acta,* **737**, 117 (1983).
51) It is unlikely that conformational change of host molecules occurs within the temperature range studied (295—323 K), because backbone of polysaccharides is generally rigid as judged from the data of ^{13}C spin-lattice relaxation times of the laboratory frame for (1→3)-β-D-glucans and β-xylan (Refs. 38, 39, and 42).
52) H. Yajima, T. Otsuka, and R. Endo, *Rep. Prog. Polym. Phys. Jpn.,* **31**, 679 (1988).
53) R. C. Teitelbaum, S. L. Ruby, and T. J. Marks, *J. Am. Chem. Soc.,* **102**, 3322 (1980); H. Yajima, T. Nishimura, T. Ishii, and T. Handa, *Carbohydr. Res.,* **163**, 155 (1987).
54) M. J. Gidley and P. V. Bulpin, *Macromolecules,* **22**, 341 (1989).
55) P. J. Flory, *Faraday Discuss. Chem. Soc.,* **57**, 7 (1974).
56) M. J. Miles, V. J. Morris, and S. G. Ring, *Carbohydr. Res.,* **135**, 257 (1985).
57) H. Saitô, *ACS Symp. Ser.,* **150**, 125 (1981).
58) K. Yokota, A. Abe, S. Hosaka, I. Sakai, and H. Saitô, *Macromolecules,* **11**, 95 (1978).