High-Resolution ^{13}C NMR Study of (1→3)-β-D-Glucans in the Solid State: DMSO-Induced Conformational Change and Conformational Characterization by Spin-Relaxation Measurements

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High-resolution ^{13}C NMR spectra of a variety of (1→3)-β-D-glucans were recorded in the solid state after lyophilization from DMSO solution in order to study resulting conformations free from the triple-helix conformation. Lyophilization from DMSO solution caused conformational change of laminaran from *Laminaria species* ($\overline{Dp}$=38) from the triple helix to the oligomer-type form (form I). On the contrary, single-chain conformation (form II) of high molecular-weight curdlan was unchanged before and after lyophilization from DMSO solution. Further, DMSO induced conformational change of both single-chain (form II) and triple-helical (form III) conformations of acid-degraded curdlan ($\overline{Dp}$=14) to the form I. These findings support our view that the single-chain conformation is a major form in curdlan powder. We found that these three forms are distinguished by either the ^{13}C spin-lattice relaxation times (T_1^C's) of the laboratory frame or proton spin-lattice relaxation times in the rotating frame ($T_{1\rho}^H$). In particular, the form I was distinguishable from others by its prolonged T_1^C values (3.5—12 s) of C-6 carbon. In addition, the $T_{1\rho}^H$ of the triple helix of annealed curdlan was one order of magnitude longer than that of the single chain, reflecting their difference in the manner of molecular packing.

Linear or branched (1→3)-β-D-glucans function as structural components in cell walls of plant cell or microorganism and also act as reserve polysaccharides.[1] Such function depends on secondary structure of polysaccharides, single or triple helix or others, which is strongly affected by a number of structural and physical parameters such as the degree of polymerization and/or of branching, the extent of annealing, etc. In this connection, it appears that conformational elucidation of these polysaccharides in the solid state is essential for better understanding in their function as well as solution properties. However, conformational characterization by X-ray diffraction is not feasible for noncrystalline samples which are in many instances important in relation to their solution/gel properties.

For this purpose, we have demonstrated that high-resolution solid-state ^{13}C NMR spectroscopy can be conveniently used as an alternative means for characterization of secondary structures of a number of molecular systems,[2-4] because this method is equally applied to noncrystalline as well as crystalline substances. In fact, we demonstrated that three types of conformations, form I (laminaripentaose or laminariheptaose), form II (curdlan-type) and form III (laminarn-type), are readily distinguishable for (1→3)-β-D-glucans in the solid, on the basis of the conformation-dependent displacements of ^{13}C chemical shifts (up to 8 ppm).[5-7] Most of samples adopting these conformations were obtained by lyophilization or spray-drying, except for the annealed curdlan. Nevertheless, we found that conformations of the forms I and II are not always completely disordered as anticipated, because the C-3 ^{13}C NMR peaks of these forms (δ 91.2±0.4 and 89.6±0.5 for the forms I and II, respectively) are significantly displaced downfield by 5—6 ppm as compared from those of disordered state achieved in aqueous solution (δ 84.5).[5] This finding is in contrast to the case of amorphous (1→4)-α-D-glucans whose C-1 peak is so broad and shows a remarkably large chemical shift range (δ 94—106). Further, the weighted average of amorphous C-1 shift is similar to the chemical shift in aqueous solution.[8] Accordingly, these forms should be considered as locally ordered conformations as judged by ^{13}C NMR spectroscopy, which is sensitive to change of the local conformations, although they are disordered as determined by X-ray diffraction.

In this connection, we previously proposed the existence of *single*-helix[9] conformation in (1→3)-β-D-glucans of higher molecular-weight (curdlan and pachyman) obtained by spray-drying or lyophilization,[5,6] although this form might not be always straightforwardly acceptable, as an energetically stable form on the basis of calculation of molecular mechanics.[10] It is easily recognized, however, that this sort of form could be isolated as anhydrous form as a result of lyophilization. The evaluation of relative proportion of such single chains is very important to clarify molecular architecture of their resulting gel networks and gelation mechanism.[11-16] Then, it is expected that single-stranded chains can be obtained if (1→3)-β-D-glucans dissolved in DMSO solution are lyophilized, because DMSO molecules tend to disperse the triple-helical chains into single chains and to yield conformations free from the triple-helix form. As a result, direct evidence as to the existence of the single chain is available by means of the conformation-dependent ^{13}C chemical shifts.

In the present article, we aimed to study conformational change of various types of (1→3)-β-D-glucans lyophilized from DMSO solution. In addition, we measured ^{13}C spin-lattice relaxation times in the laboratory frame (T_1^C's)[17] and proton spin-lattice relaxation time in the rotating frame ($T_{1\rho}^H$'s)[18] to gain

information as to dynamic features of these three kinds of conformations.

Experimental

Curdlan powder ($\overline{Dp}$=540) was provided by Takeda Chemical Industries, Osaka, Japan. Laminaran from *Laminaria species* ($\overline{Dp}$=38) was purchased from Nutritional Biochemistry Corporation, Ohio, USA. Laminaran from *Eisenia araborea* was purchased from Nakarai Chemical Industry, Kyoto, Japan (Lot. No. M6G2275). Preparation of acid-degraded curdlan, $\overline{Dp}$=14, was described in our previous paper.[5] Laminaripentaose and laminariheptaose were purchased from Seikagaku Kogyo, Tokyo (Lot No. 407191). Lentinan and HA-β-glucan, branched (1→3)-β-D-glucans,[5] were generous gift from Dr. G. Chihara and Dr. Y. Yoshioka of this Institute, respectively. Lyophilized solid samples from DMSO solution were prepared by dissolving the glucans in DMSO or DMSO-d_6, followed by lyophilization in vacuo.

High-resolution ^{13}C NMR spectra in the solid state were recorded on a Bruker CXP-300 spectrometer by means of cross polarization-magic angle spinning (CP-MAS) method. Samples were contained in a ceramic rotor used for the double air-bearing type MAS system and spun as fast as 3—3.5 kHz. The duration of 90° pulse was 4 μs. The contact, sampling and repetition times were 1 ms, 20—30 ms, and 4 s, respectively. The ^{13}C chemical shifts were referred to TMS through the peak position of the glycine carboxyl group (δ 176.0).

The ^{13}C spin-lattice relaxation times were measured by the method of cross polarization enhancement, with an inversion of proton-spin temperature.[17] The peak intensity $M_{net}(t)$ at the delay time t after turning off the ^{13}C H_1 field decays exponentially from its initial value, $2M_{cp}(0)$ as shown by Eq. 1,

$$M_{net}(t)=2M_{cp}(0)\exp(-t/T_1^C) \quad (1)$$

Thus, the T_1^C values were obtained by a semilog plot of the peak-intensity against the delay time t. Carbon-resolved proton spin-lattice relaxation times in the rotating frame ($T_{1\rho}^H$'s) were obtained from the exponential decrease in ^{13}C NMR peak-intensity of longer contact time.[18] Namely, the ^{13}C peak-intensity $M(t)$ is given by

$$M(t)\approx M_0\exp(-t/T_{1\rho}^H)/(1-T_{CH}/T_{1\rho}^H) \quad (2)$$

where M_0 and T_{CH} stand for the maximum peak-intensity and cross polarization relaxation time, respectively. Thus, we obtained the $T_{1\rho}^H$ values from a semilog plot of $M(t)$ vs. t.

The proton spin-lattice relaxation times of the laboratory frame (T_1^H's) were measured by the pulse sequence via ^{13}C CP-MAS, in which 180° pulse and variable delay time were inserted prior to standard cross polarization.[19] So, the T_1^H values were obtained by a semilog plot of ^{13}C NMR intensity vs. t, as in a similar manner to the inversion-recovery measurement in liquid state.

Results

Figure 1 shows that the C-3 ^{13}C NMR peak of laminaran from *Laminaria species* lyophilized from DMSO solution (δ 90.8) (Fig. 1B) is displaced downfield by 3.9 ppm from that of untreated sample[6] (δ 86.9; form III, triple helix) (Fig. 1A). These ^{13}C chemical shifts of (1→3)-β-D-glucans in the solid state were assigned on the basis of the data recorded in DMSO-d_6 solution, as summarized in Table 1. The relative peak-positions

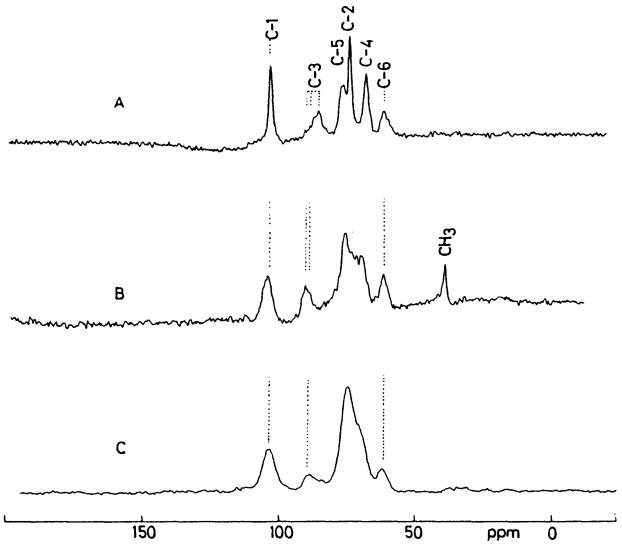

Fig. 1. ^{13}C NMR spectra of laminarans taking different conformations in the solid state.
(A) laminaran ($\overline{Dp}$=38) from *Laminaria species* (from Ref. 6), (B) laminaran ($\overline{Dp}$=38); lyophilized from DMSO solution, and (C) laminaran from *Eisenia araborea*.

Table 1. ^{13}C Chemical Shifts of (1→3)-β-D-Glucans in Various Conformations (ppm from TMS)[a]

	Form I (oligomer-type)			Form II (single chain)		Form III (triple helix)		DMSO
	Laminari-heptaose	Curdlan ($\overline{Dp}$ 14) from DMSO	Laminaran from DMSO	Curdlan from DMSO	Curdlan powder[b]	Annealed curdlan[b]	Laminaran[b]	soln.
C-1	104.0	103.8	104.7	103.8	104.1	103.7	103.4	103.7
C-2	72.9	72.4	c	71.1[d,e]	74.0[d,e]	74.3	74.3	73.8
C-3	91.3	90.7	90.8	89.1	89.7	87.1	86.9	87.0
C-4	69.7	69.1	71.0	67.1	69.3	68.6	68.4	69.3
C-5	76.4	76.1	75.7	75.0[d]	76.1[d]	78.0	76.7	77.0
C-6	61.5	61.8	62.1	61.0	62.3	62.2	62.1	61.8
CH_3	—	38.9	39.9	39.5	—	—	—	39.6

a) Estimated error ±0.3 ppm. b) Data taken from Ref. 6. c) Not well resolved from the C-5 peak.
d) Assignment reversible. e) Estimated error ±1.5 ppm.

of the C-5 and C-2 carbons in the form III were previously confirmed by measurements of the ^{13}C spin-lattice relaxation times of the laboratory frame,[6] although these are obscured in the form II due to overlap of peaks. The C-3 NMR peak-position of laminaran lyophilized from DMSO solution is very close to that of the form II (single-chain conformation; δ 89.6±0.5[5]) but is resonated slightly at a low-field position (90.8 ppm), corresponding to that of the form I[5,6] (δ 91.2±0.4) (Table 1). In contrast to the above-mentioned water-soluble species, the ^{13}C NMR peak-positions of water-insoluble laminaran from *Eisenia araborea* (Fig. 1C) are similar to those of curdlan powder (form II; Fig. 2B), although the individual peaks of the former were considerably broadened as compared with those of the latter, reflecting the enhancd extent of disorder. Figure 2 illustrates the ^{13}C NMR spectra of curdlan and its acid-degraded fraction ($\overline{Dp}$=14) lyophilized from DMSO solution, together with those of annealed curdlan (at 180°) and curdlan powder as reported previously.[5,6] Again, the ^{13}C NMR spectrum of acid-degraded fraction ($\overline{Dp}$=14) lyophilized from DMSO solution is found to be very similar to that of laminaripentaose or laminariheptaose (form I), as judged from the C-3 peak-positions and the splitting pattern of the C-5, C-2, and C-4 peaks (Fig. 2D and 2E). However, the ^{13}C NMR pattern of curdlan powder (high molecular-weight) is unchanged

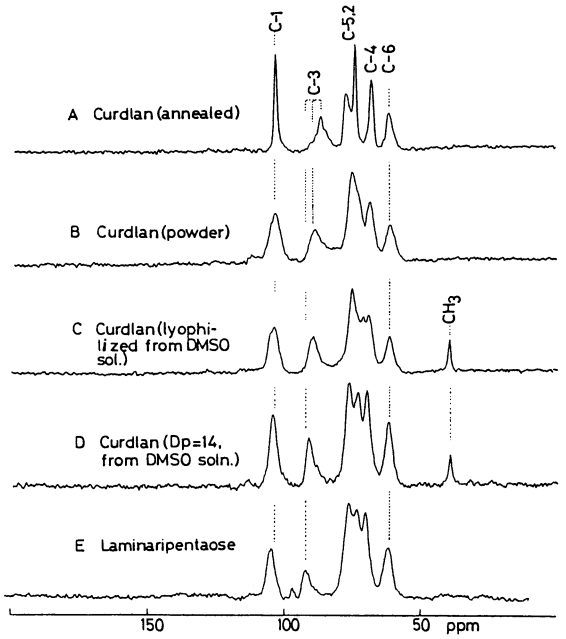

Fig. 2. ^{13}C NMR spectra of a variety of (1→3)-β-D-glucans in the solid state. (A) Annealed curdlan at 180° (Ref. 6), (B) curdlan powder (Ref. 6), (C) curdlan lyophilized from DMSO solution, (D) acid-degraded curdlan ($\overline{Dp}$=14) lyophilized from DMSO solution, and (E) laminaripentaose.

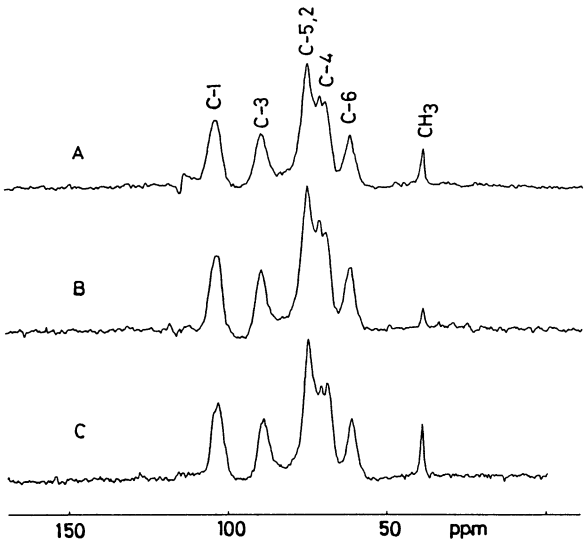

Fig. 3. ^{13}C NMR of curdlan lyophilized from DMSO-d_6 solution (A and B) and from DMSO solution (C). Contact time; 2 ms for A, 1 ms for B and C.

Table 2. DMSO-Induced Conformational Change of (1→3)-β-D-Glucans[a]

	$\overline{Dp}$	Conformation before and after DMSO treatment	
		Before[b]	After
Curdlan	540	Single chain (90%) + Triple helix (10%)	Single chain
Laminaran	38	Triple helix	Single chain
Curdlan	14	Single chain (60%) + Triple helix (40%)	Form I

a) Conformations based on the displacement of the C-3 ^{13}C chemical shifts. b) Data taken from Refs. 5 and 6.

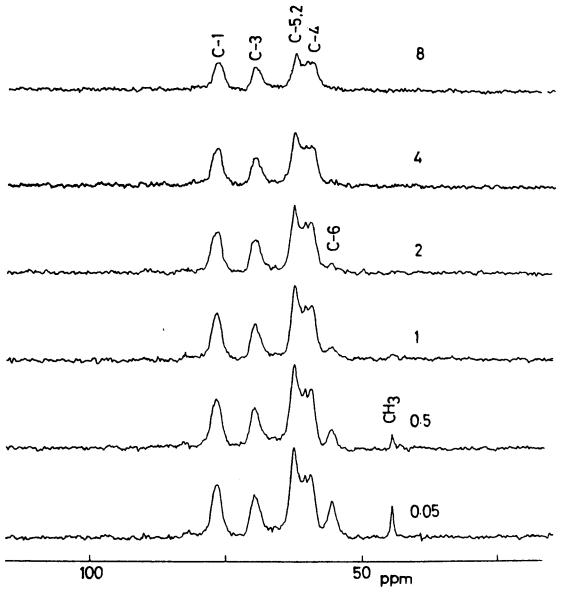

Fig. 4. Stacked plot of ^{13}C NMR peak-intensities of curdlan lyophilized from DMSO, obtained by cross polarization for the T_1^C measurements. Delay times (s) are given in the right-hand side.

before and after lyophilization from DMSO solution (form II, Figs. 2B and 2C). Obviously, the ^{13}C NMR pattern of the triple-helix (form III) is readily distinguishable from those of the forms I and II. The DMSO-induced conformational change of (1→3)-β-D-glucans as described above was summarized in Table 2.

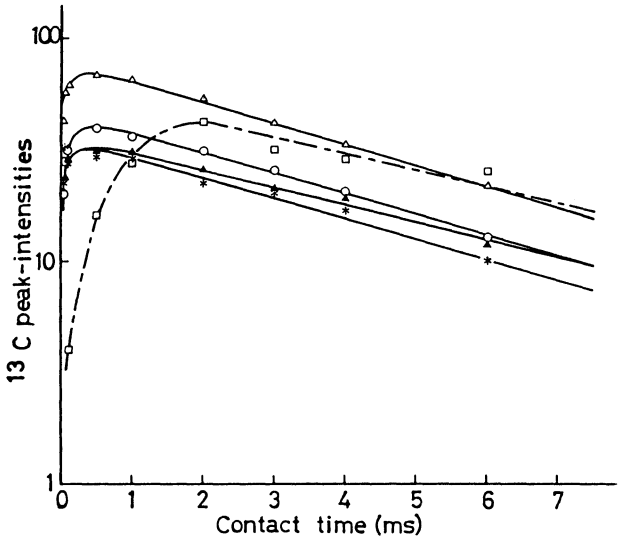

Fig. 5. Plot of the ^{13}C peak-intensities of curdlan lyophilized from DMSO against the contact time: (○) C-1; (*) C-3; (△) C-5,2; (●) C-6; (□) CH_3

As demonstrated in Fig. 3, the methyl ^{13}C NMR signal of perdeuterated DMSO is clearly visible (with approximate 30% peak-intensity as compared with that of protonated DMSO: see Figs. 3B and C) in spite of the absence of nearby protons. The peak-intensity of the methyl signal is enhanced by increasing the contact time from 1 ms to 2 ms (Fig. 3A). Figure 4 illustrates a typical stacked plot of the ^{13}C NMR spectra of curdlan lyophilized from DMSO solution for measurements of the ^{13}C spin-lattice relaxation times. It is evident from Fig. 4 that the methyl, C-6 and other ^{13}C signals decay with different time constant. The T_1^C values based on these stacked plots were summarized in Table 3.

Figure 5 illustrates a typical plot of the ^{13}C peak-intensity vs. the contact time for curdlan lyophilized from DMSO solution. The maximum peak-intensity of curdlan polymer was achieved at the contact time of 500 μs, whereas the maximum peak of DMSO was at 2 ms. Carbon-resolved $T_{1\rho}^H$ and T_1^H values were summarized in Table 4.

Discussion

Conformation of (1→3)-β-D-Glucans Lyophilized from DMSO Solution. Linear (1→3)-β-D-glucans are not soluble in aqueous media when the degree of polymerization ($\overline{Dp}$) is larger than 14.[20] This is a

Table 3. ^{13}C Spin-Lattice Relaxation Times (T_1^C's) in the Laboratory Frame of (1→3)-β-D-Glucans in the Solid State (s)

	Form I (oligomer-type)			Form II(single chain)		Form III (triple helix)
	Oligomers			Curdlan		Annealed curdlan[c]
	n=5	n=6	$\bar{n}$=14[b]	Powder[c]	from DMSO soln.	
C-1	32	17	22	14	15	16
C-2	25[a]	11[a]	15[a]	10	11	18
C-3	38	15	29	11	16	16
C-4	34	17	22	7.8	10	8.2
C-5	30[a]	14[a]	15[a]	10	9.7	9.3
C-6	12	3.5	4.2	1.0	1.6	1.2
CH_3 (DMSO)	—	—	0.6	—	0.4	—

a) Interchangeable. b) Lyophilized from DMSO solution. c) Data taken from Ref. 6.

Table 4. Proton Spin-Lattice Relaxation Times in the Laboratory (T_1^H's) and Rotating Frame ($T_{1\rho}^H$'s)

	Single chain (form II)				Triple helix (form III)		
	Curdlan from DMSO		Curdlan powder	Lentinan powder	Annealed curdlan		HA-β-gluca
	$T_{1\rho}^H$/ms	T_1^H/s	$T_{1\rho}^H$/ms	$T_{1\rho}^H$/ms	$T_{1\rho}^H$/ms	T_1^H/s	$T_{1\rho}^H$/ms
H-1	4.9	0.34	3.9	4.7	31	0.51	7.2
H-2	4.8[a]	0.34	3.3	4.4	34	0.51	8.4
H-3	4.8	0.31	4.3	4.5	29	0.48	7.8
H-4	4.8	0.33	4.5	4.4	31	0.52	7.7
H-5	5.7[a]	0.34	3.3	4.4	36	0.62	8.4
H-6	5.6	0.30	4.8	5.7	37	0.48	8.2
$(CH_3)_2SO$	6.6	0.28	—	—	—	—	—

a) Assignment interchangeable.

consequence of adopting regular secondary structure which causes aggregation of polymer chains in aqueous media. In particular, we previously showed that (1→3)-β-D-glucans with $\overline{Dp}$=14—131 take considerable fraction of the triple helix conformation (ca. 40%).[5] On the contrary, relative fraction of the triple helix is reduced to less than 10%[5] for the glucans with $\overline{Dp}$ larger than ca. 200, which possess gel-formig ability.[20] Branched glucans such as lentinan and schizophyllan, on the other hand, form soft gel when the glucans of higher concentration are dissolved in aqueous media.[21] In any cases, ^{13}C NMR peak-intensity of these gel samples is suppressed partially or completely,[21] depending on the extent of polymer chains participated in formation of the triple helix.

On the other hand, full peak-area is observed in DMSO solution due to complete disruption of the triple-helix conformation.[22] As a result, the high-field shoulder or peak at δ 87.0 arising from the triple-helix coformation (form III) (approximately 10, 40, and 100% contribution for curdlan, acid-degraded fraction ($\overline{Dp}$=14), and laminaran, respectively) disappeared almost completely for preparations lyophilized from DMSO solution. In fact, (1→3)-β-D-glucans take effectively disordered conformation in DMSO solution, as substantiated from the displacement of peaks as well as relaxation parameters.[22] This view was also supported by the data of light scattering, viscosity and refractometry as applied to DMSO solution of schizophyllan, a branched glucan.[23,24]

Nevertheless, it is interesting to note that the lyophilized samples from DMSO solution do not necessarily take completly disordered conformation but adopt local regular secondary forms, either the forms I or II, depending on their chain-lengths, as judged from the peak-position of the C-3 signal which exhibits conformation-dependent displacement (see Figs. 1 and 2).[3-7] In general, lyophilization results in retaining ordered solution conformation in the solid state, if solvent molecules do not play dominant role to adopt ordered conformation in solution, as demonstrated previously for a number of sequential polypeptides and silk fibroin.[25,26] On the contrary, the present finding indicates that rather regular secondary structure (at least as viewed as a local conformation) is formed during the course of freezing followed by lyophilization, even if solution conformation is completely disordered as in DMSO solution. This situation is not unexpected, because conformational freezing around the minimum conformational energy could occur during the freezing step of lyophilization. Similar result was obtained for formation of silk I-like form by lyophilization from disordered aqueous fibroin solution (unpublished). As summarized in Table 2, achieved conformations differ as in the single chain or form I, depending on $\overline{Dp}$ of the glucans. It is emphasized that the latter form is also single-stranded by taking the condition for formation into account. In any cases, the present result demonstrates that DMSO causes conformational change of (1→3)-β-D-glucans from the triple helix to the form I or form II (single chain) These findings clearly support our view about the existence of the single chains in gel-forming linear glucans such as curdlan. On the contrary, the reverse conformational change from the single chain to triple helix is associated with hydration or hydration/annealing of samples, as illustrated schematically in Fig. 6. In other word, it is seen that the present conformational transitions are mainly caused by either hydration or dehydration process.

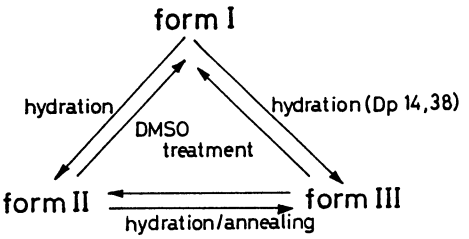

Fig. 6. Schematic representation of conformational changes.

It is interesting that DMSO molecules are always present in lyophilized samples from DMSO solution (less than 1/2 DMSO molecules per single residue), as seen by the methyl ^{13}C NMR signal from DMSO (Figs. 1—3). This is again true for the ^{13}C NMR peak of DMSO-d_6, in spite of the absence of nearby proton. This means that build-up of the DMSO signal is caused by intermolecular cross-polarization with protons from the glucan due to short proton-carbon distance (the order of 10 Å or less).[27]

Conformational Characterization by Spin-Lattice Relaxation Times. As pointed out previously, noncrystalline samples characterized by X-ray diffraction are not necessarily motionally disordered, because there appears no distinction of the ^{13}C T_1 values between curdlan powder and annealed curdlan[6] (see Table 3). In many instances, it appears that the presence of methyl or hydroxymethyl groups, undergoing rapid reorientational motions with correlation time at the order of 10^{-8} s, strongly affects the spin-lattice relaxation times of nearby carbons due to dipolar coupling, instead of slow overall motions ineffective to the T_1 process of the laboratory frame.[6,28-30] In fact, very long T_1^C values (50—800 s) were reported for highly crystalline cellulose samples where there exists no rapid reorientation in the C-6 hydroxymethyl groups.[31,32] Thus, the T_1^C values of the C-4 and C-5 carbons in the single-chain (form II) and triple-helical (form III) conformations are appreciably shortened by the dipolar couplings to the C-6 hydroxymethyl protons undergoing rapid internal rotation (Table 3),[6] since the extent of the dipolar couplings is proportional to r^{-6} where r is the interatomic distance. On the contrary, the corresponding C-6 T_1^C values in the oligomer-type form (form I) (3.5—12 s) are much

longer than those (1.0—1.6 s) of the above-mentioned polymers adopting the forms II and III. Therefore, the T_1^C values of the oligomers are generally longer than those of the polymer samples. For this, it is tempted to explain that this is simply caused by difference in molecular packing. However, these two types of samples were not obtained by crystallization but by lyophilization. Thus, it is reasonable to assume that conformation of the olilgomer-type (form I) is altered, especially at the site of C-6 hydroxymethyl group, from the single-chain conformation. In this connection, it is worthwhile to take into account the plausible conformational diversity at C-6 as viewed from the C-6 ^{13}C chemical shifts which are sensitive to C-6 conformation (i.e. tg, gt, or gg).[33,34] No such differences, however, are found for the spectra and therefore there is no evidence for conformational diversity at C-6. Thus, the longer T_1^C values for the oligomers may reflect a closer-packed, more immobile local structure.

Interestingly, very short ^{13}C spin-lattice relaxation times (0.4—0.6 s) arose from the methyl carbons of DMSO molecules which undergo rapid rotational reorientation in the solid state (Table 3). It is rather surprising to note, however, that the presence of such rapid motion of the methyl groups in DMSO is not effective for shortening of the T_1^C's of nearby carbons from the glucans as a fast relaxation center for dipolar couplings. In fact, there appear no significant differences in the T_1^C values between samples with and without DMSO. The most probable explanation is that DMSO molecules are not closely located at a short (2—3 Å) distance to any of carbons in the glucans, as an effective center for further shortening of the T_1^C values, even if they are bound to either the hydroxyl groups at the C-2, C-4, or C-5 carbons.

In contrast, only averaged values are observed for both proton spin-lattice relaxation times in the laboratory and rotational frame due to efficient spin diffusion[35] (Table 4). The $T_{1\rho}^H$ values of the single-chain (1→3)-β-D-glucans are almost the same among three kinds of glucans (4—5 ms), irrespective of differences in the primary structures. However, the $T_{1\rho}^H$'s of triple-helical annealed curdlan are almost one order of magnitude longer than those of the single-chain conformation. In the case of a branched glucan adopting the triple helix (HA-β-glucan), the $T_{1\rho}^H$ values are not substantially prolonged as in the annealed curdlan but are still prolonged as compared with those of the single-chain glucans (Table 4). Such distinction seems to be accounted for by differences in molecular packing: the latter molecules are not crystalline but lyophilized. In general, the T_1^H's are sensitive to spatially dependent proton-proton spin diffusion as well as spectral density of ^1H dipolar modulation. In this connection, it appears that the above-mentioned difference between the triple and single helix is a consequence of differential motional state rather than spin-diffusion process.

Conclusion

We have shown that (1→3)-β-D-glucans lyophilized from DMSO solution undergo substantial conformational change from the triple helix to single-chain or oligomer-type form, depending on their chain lengths. The present finding supports our view about the existence of the single chains in gel-forming high molecular-weight (1→3)-β-D-glucans. Further, we found that three types of conformations are distinguishable by either ^{13}C spin-lattice relaxation times in the laboratory frame or proton spin-lattice relaxation times in the rotating frame.

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References

1) J. J. Marshall, *Adv. Carbohydr. Chem. Biochem.*, **30**, 257 (1974).

2) H. Saitô, R. Tabeta, A. Shoji, T. Ozaki, I. Ando, and T. Asakura, "Magnetic Resonance in Biology and Medicine," ed by G. Govil and C.L. Kheterapal, and A. Saran, Tata McGraw-Hill, New Delhi (1985), pp. 195—215.

3) H. Saitô, *Magn. Reson. Chem.*, **24**, 835 (1986).

4) H. Saitô and I. Ando, *Ann. Rep. NMR Spectrosc.*, **21**, 209 (1989).

5) H.Saitô, R. Tabeta, T. Sasaki, and Y. Yoshioka, *Bull. Chem. Soc. Jpn.*, **59**, 2093 (1986).

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

7) H. Saitô, R. Tabeta, Y. Yoshioka, C. Hara, T. Kiho, and S. Ukai, *Bull. Chem. Soc. Jpn.*, **60**, 4267 (1987).

8) M. J. Gidley and S. M. Bociek, *J. Am. Chem. Soc.*, **110**, 3820 (1988).

9) In our previous series of papers,[5-7] form II conformation was ascribed to the "single helix" form based on the similarity of peak positions between the powder and gel sample. However, we found on the basis of ^{13}C NMR data that the single-helix conformation as seen in the gel state is the same as that of the "swollen form" (A. J. Stipanovic and P. J. Giammatteo, "Industrial Polysaccharides: Genetic Engineering, Structure/Property Relations and Applications," ed by M. Yalpani, Elsevier, Amsterdam, (1987), p. 281), rather than the form II (to be published). For this reason, it is more appropriate to ascribe the form II to "single chain" form.

10) R. H. Marchessault and Y. Deslandes, *Carbohydr. Res.*, **75**, 231 (1979).

11) H. Saitô, "Solution Properties of Polysaccharides," ed. by D. A. Brant, ACS Symp. Ser. No. 150, pp. 125—147 (1981).

12) H. Saitô, T. Ohki, and T. Sasaki, *Biochemistry*, **16**, 908 (1977).

13) H. Saitô, E. Miyata, and T. Sasaki, *Macromolecules*, **11**, 1244 (1978).

14) N. Kasai and T. Harada, "Fiber Diffraction Methods," ed. by A. D. French and K. H. Gardner, ACS Symp. Ser. No. 141, pp. 363—383 (1980).

15) W. S. Fulton and E. T. Atkins, "Fiber Diffraction Methods," ed. by A. D. French and K. H. Gardner, ACS Symp. Ser. No. 141, pp. 385—410 (1980).
16) It was shown that intense ^{13}CNMR signals are visible (70—80%) in curdlan gel at the temperature of swelling (54 °C; translucent low set gel) by conventional high-resolution spectrometer (Ref. 11). The conformation of such a portion is not a random coil but an ordered form (single helix) with increased flexibilty of chains as viewed from the significant displacements of the C-3 signals from those of aqueous solution and condition of acquisition of spectra, in contrast to the cases of a variety of gels formed by chemically cross-linked synthetic polymers. This means that major conformation of starting curdlan powder is not the triple-helical but single-chain form (form II), in contrast to the expectation based on the previous X-ray diffraction studies (Refs. 10 and 15). In fact, conversion from the single to triple helix is at most 50%, even if annealing of the gel sample is made at 150—180° and resulting samples were cooled rapidly (Ref. 6).
17) D. A. Torchia, *J. Magn. Reson.*, **30**, 613 (1978).
18) E. O. Stejskal, J. Schaefer, M. D. Sefcik, and R. A. McKay, *Macromolecules*, **14**, 275 (1981).
19) M. J. Sullivan and G. E. Maciel, *Anal. Chem.*, **52**, 1615 (1982).
20) H. Saitô, E. Miyata, and T. Sasaki, *Macromolecules*, **11**, 1244 (1978).
21) H. Saitô, T. Ohki, and T. Sasaki, *Carbohydr. Res.*, **74**, 227 (1979).
22) H. Saitô, T. Ohki, and T. Sasaki, *Biochemistry*, **16**, 908 (1977).
23) Y. Kashiwagi, T. Norisuye, and H. Fujita, *Macromolecules*, **14**, 1220 (1981).
24) T. Sato, T. Norisuye, and H. Fujita, *Macromolecules*, **16**, 185 (1983)
25) H. Saitô, R. Tabeta, T. Asakura, Y. Iwanaga, A. Shoji, T. Ozaki, and I. Ando, *Macromolecules*, **17**, 1405 (1984).
26) H. Saitô, R. Tabeta, A. Shoji, T. Ozaki, T. Ando, and T. Miyata, *Biopolymers*, **23**, 2279 (1984).
27) J. Schaefer, M. D. Sefcik, E. O. Stejskal, and R. A. McKay, *Macromolecules*, **14**, 188 (1981).
28) H. Saitô and R. Tabeta, "Peptide Chemistry 1987," ed by T. Shiba and S. Sakakibara, Protein Research Foundation, Osaka, pp. 53—58 (1988).
29) A. Naito, S. Ganapathy, K. Akasaka, and C. A. McDowell, *J. Magn. Reson.*, **54**, 235 (1983).
30) R. Teeaar and E. Lippmaa, *Polym. Bull.*, **12**, 315 (1984).
31) F. Horii, A. Hirai, and R. Kitamaru, *J. Carbohydr. Chem.*, **3**, 641 (1984).
32) D. L. VanderHart, *J. Magn. Reson.*, **72**, 13 (1987).
33) H. Saitô, G. Izumi, T. Mamizuka, S. Suzuki, and R. Tabeta, *J. Chem. Soc., Chem. Commun.*, **1982**, 1386.
34) F. Horii, A. Hirai, and R. Kitamaru, *Polym. Bull.*, **10**, 357 (1983).
35) A. Abragam, "The Principle of Nuclear Magnetism," Claredon Press, Oxford(1963), Chap. V.