  Bull. Chem. Soc. Jpn., 60, 4259—4266 (1987)

A High-Resolution Solid-State ^{13}C NMR Study of the Secondary Structure of Linear (1→3)-β-D-Glucans: A Conformational Elucidation of Noncrystalline and Crystalline Forms by Means of Conformation-Dependent ^{13}C Chemical Shifts

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(Received June 19, 1987)

The high-resolution solid-state ^{13}C NMR spectra of annealed curdlan, a (1→3)-β-D-glucan, were recorded in order to obtain reference data on the ^{13}C chemical shifts of the triple helix form, which would be useful for the conformational elucidation of two distinct noncrystalline forms, curdlan- and laminaran-types. It was found that the C-3 peak of crystalline annealed glucan is substantially displaced from that of noncrystalline starting powder, reflecting the conformational change. The laminaran-type conformation, which is found in either laminaran or a number of fungal branched glucans, was identical with the triple helix, judging from the similarity in the ^{13}C NMR spectra, although the linewidths of the former are much larger than those of the latter. The curdlan-type form, on the other hand, was ascribed to the single helix form on the basis of the previous data on high-resolution ^{13}C NMR. In addition, the ^{13}C NMR spectra of crystalline paramylon showed a number of additional signals other than the peaks of triple-helical annealed curdlan, in spite of the similarity in the X-ray diffraction patterns.

It has been previously shown that the solution properties, including the gelation behavior, of a number of (1→3)-β-D-glucans vary greatly with various parameters, such as the degree of branching, the molecular weight, and the manner of annealing.[1,2] Undoubtedly, such properties might be closely related to the secondary or tertiary structures of these polysaccharides in the solid, fully or partially hydrated, and unhydrated portions of gels, and a (fully hydrated) aqueous solution.

A fully hydrated aqueous solution of a linear (1→3)-β-D-glucan (Fig. 1) is achieved either at a higher alkaline solution (>0.2 M NaOH (1 M=1 mol dm^{-3})) or at neutral pH for lower molecular-weight glucan[3,4] and the relevant molecular conformation of this state is effectively random-coiled, as may be seen from the displacements of the ^{13}C chemical shifts as well as the relaxation parameters. The molecular architecture of the gel state formed by a linear (1→3)-β-D-glucan (curdlan) (Fig. 1) is , however, very complicated. It is considered to consist of the following two or three regions: fully or partially hydrated and unhydrated portions. In particular, the former, which is not detected by X-ray diffraction, gives rise to well-resolved isotropic ^{13}C NMR signals.[2-5] The rest of the immobilized unhydrated region, which is not detected by means of high-resolution NMR, can be characterized by the triple[7-10] or single helices[11] and their dehydrated aggregates by means of X-ray diffraction.

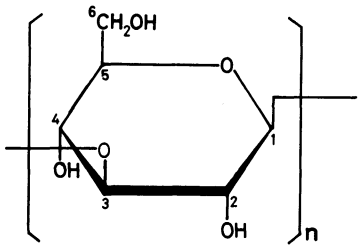

Fig. 1. Primary structure of a linear (1→3)-β-D-glucan. Curdlan (from *Alcaligenes faecalis*), n=540; laminaran (from *Porio cocos*), n=38; paramylon (from *Euglena gracila*).

The conformational feature of curdlan powder used as the starting material of gel formation or of the lyophilized sample from the gel cannot, however, be analyzed by the use of the X-ray diffraction method because of its extremely poor crystallinity: alternative means must be utilized. In fact, only 30% of the former is crystalline.[12] Nevertheless, our previous finding, based on the ^{13}C NMR data, showed that spray-dried or lyophilized (1→3)-β-D-glucans from various sources in the solid state do not necessarily take a disordered conformation,[13,14] but two kinds of distinct, regular secondary structures, curdlan-type and laminaran-type forms, as revealed by the conformation-dependent ^{13}C chemical shifts.[15,16] This finding is based on the fact that the ^{13}C chemical shifts of the C-1 and C-X carbons at the glycosidic linkage of various types of glucans are closely related to the torsion angles of C-1-O_{gly} (ϕ) and C-X-O_{gly} (ψ), respectively.[17-19] Hence, the conformation of the noncrystalline region can be as well characterized by this NMR technique as that of the crystalline portion.

In the present paper, we recorded the ^{13}C NMR spectra of several types of curdlan samples annealed at various temperatures, together with two additional linear (1→3)-β-D-glucans, laminaran and paramylon (Fig. 1), in order to clarify these conformations further. As a result, we were able to ascribe the curdlan- and laminaran-type conformations[14] of a variety of noncrystalline (1→3)-β-D-glucans to the single- and triple-helical conformations, respectively. It was further

shown, on the basis of the ^{13}C spin-lattice relaxation data, that the molecular organization of the noncrystalline portion was not dynamically disordered as compared with that of the crystalline samples.

Experimental

Materials. Curdlan powder was provided by Takeda Chemical Industries Co., Ltd., Osaka. Laminaran was purchased from Nutritional Biochemistry Corporation, Ohio, USA. Paramylon was a generous gift from Professor B. A. Stone of La Trobe University, Australia. The annealed curdlan samples were prepared by a manner similar to that described previously.[7] That is, an aqueous suspension of curdlan powder was heated at various temperatures (80—180 °C) in a sealed bomb. The resulting gel samples were cooled either slowly or rapidly, together with dehydration by means of air-drying or immersion in a methanol solution. The densities of the resultant samples were measured by means of floatation in a xylene-carbon tetrachloride. The crystallinities were estimated from the X-ray powder patterns at room humidity (40% r. h.) by the use of a Rigaku Geigerflex X-ray diffractometer RAD IIIA, employing Ni-filtered Cu $K\alpha$ radiation generated at 40 kV and 30 mA.

Method. The high-resolution solid state ^{13}C NMR spectra at 75.46 MHz were recorded on a Bruker CXP-300 spectrometer by means of the cross polarization-magic angle spinning (CP-MAS) method. Powdered samples were placed in an alumina rotor used for the double air-bearing-type MAS system and spun as fast as 3—3.5 kHz. The duration of the 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 (176.0 ppm). The ^{13}C spin-lattice relaxation times (T_{1C}'s) were measured by the method of cross polarization enhancement, with an inversion of the proton-spin temperature.[20]

Results

Figures 2 and 3 show the ^{13}C CP-MAS NMR spectra of annealed curdlan at various temperatures, together with those of laminaran, curdlan powder, and paramylon. Figures 4A and B illustrate some X-ray powder diffraction patterns of annealed curdlan under different conditions (Table 1) and of laminaran, respectively. The powder X-ray diffraction patterns of Samples 1 and 2 and of paramylon[9] (data not shown) exhibit high crystallinity and coincide with that of the triple-helical conformation, previously analyzed.[7-9] On the other hand, the diffraction patterns of Samples 3, 5, and 6 and also of laminaran were very diffusive because of their lower crystallinity. The extent of crystallinity from the X-ray diffraction is parallel with the magnitude of the density, as is summarized in Table 1.

It is noteworthy that Samples 1 and 2 gave the narrowest NMR signals (about 1 ppm linewidths) among all the samples examined herein except for paramylon. In parallel with this observation, the crystallinity of these two samples is found to be significantly improved by the annealing, as is shown by the X-ray powder diffraction (Fig. 4A) and density data (Table 1). The ^{13}C NMR peaks of C-3 and C-2/C-5 carbons are also displaced from those of the starting curdlan powder (Table 2).

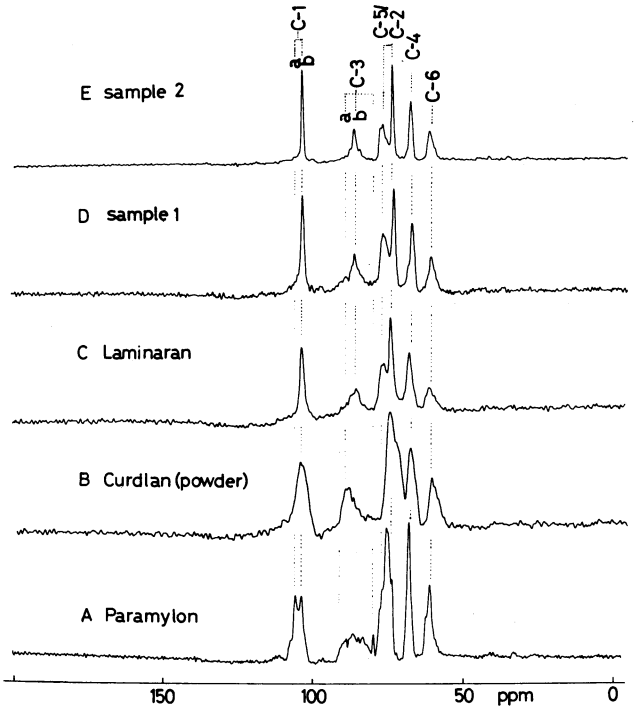

Fig. 2. 75.46 MHz ^{13}C CP-MAS NMR spectra of some linear (1→3)-β-D-glucans in the solid state.
A: Paramylon, B: curdlan powder, C: laminaran, D: Sample 1 (annealed curdlan at 180 °C, followed by slow cooling), and E: Sample 2 (annealed curdlan at 150 °C, followed by slow cooling).

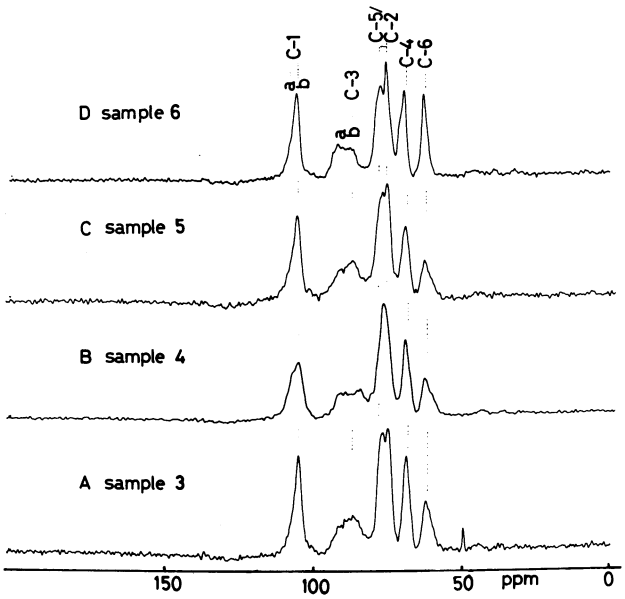

Fig. 3. 75.46 MHz ^{13}C CP-MAS NMR spectra of annealed curdlan, followed by rapid cooling (A—C).
A: Sample 3 (annealing at 180 °C), B: Sample 4 (annealing at 150 °C), C: Sample 5 (annealing at 120 °C), and D: Sample 6 (annealing at 80 °C, followed by slow cooling).

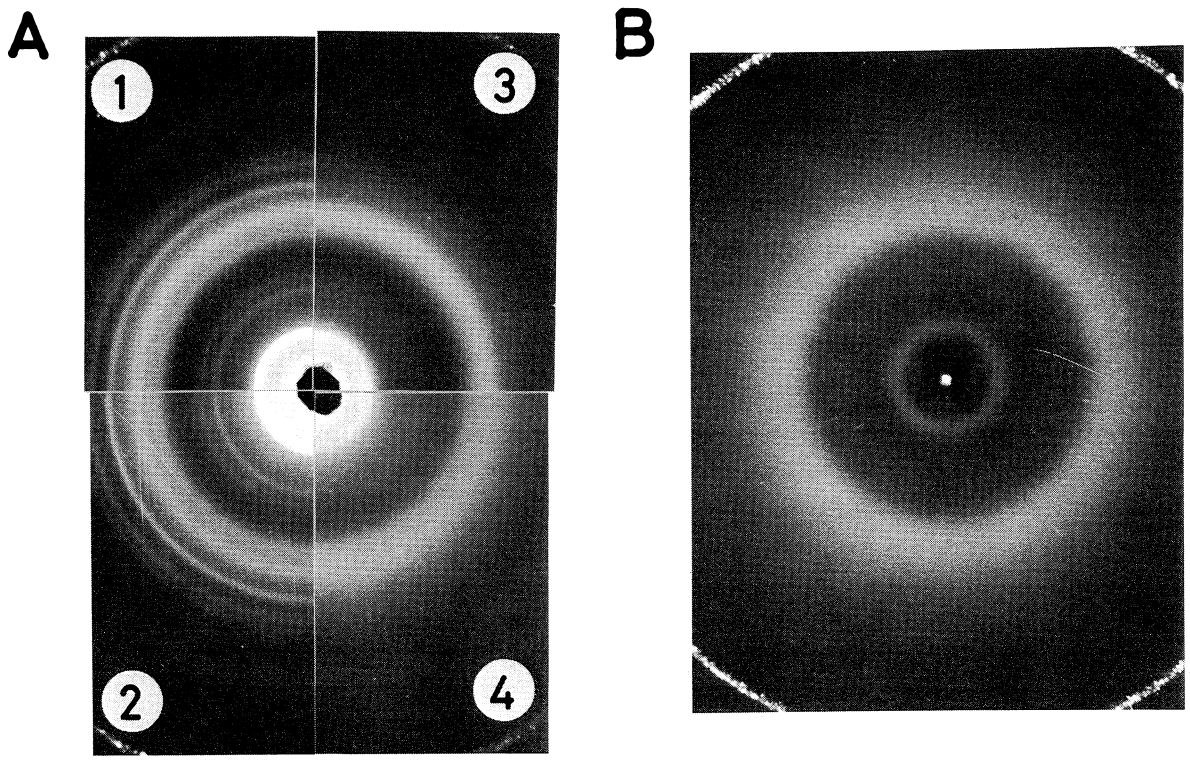

Fig. 4. X-Ray powder diffraction patterns of some (1→3)-β-D-glucans in the solid state. A: Annealed curdlan: 1; Sample 1, 2; Sample 2, 3; Sample 3, and 4; Sample 5. B: Laminaran.

Table 1. Characteristics of Annealed Curdlan together with Paramylon

Sample	Condition for sample preparation		Density	Crystallinity (by X-ray diffraction)
	Annealing temp/°C	After annealing	g cm^{-3}	
1	180	Slowly cooled/air-dried	1.49	High[a]
2	150	Slowly cooled/MeOH-dehydrated	1.49	High
3	180	Rapidly cooled/MeOH-dehydrated	1.45	Low
4	150	Rapidly cooled/air-dried	1.49	High
5	120	Rapidly cooled/MeOH-dehydrated	1.48	Low
6	80	Slowly cooled/MeOH-dehydrated	1.46	Very low
Curdlan powder			1.44[b]	Very low
Paramylon			1.53[b]	High

a) Slightly lower than that of Samples 2 and 4. b) Ref. 12.

Table 2. ^{13}C Chemical Shifts (ppm from TMS) of Annealed (1→3)-β-D-Glucans and Related Polysaccharides

		Annealed sample						Untreated sample		
		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Laminaran	Curdlan	Paramylon
C-1	a				105.6					105.7
	b	103.7	103.6	103.5	103.5	103.7	103.7	103.4	104.1	103.8
C-2		74.3	74.3	74.5	76.0[a]	74.6[a]	74.3[a]	74.3	74.0	76.2
C-3	a			90.4	89.8	89.7	90.1		89.7	89.5
	b	87.1	86.9	85.9		86.6	85.0	86.9		
					83.8					80.4
C-4		68.6	68.4	68.7	69.2	68.6	68.4	68.4	69.3	68.9
C-5		78.0	78.4	78.4	76.0[a]	76.0[a]	76.3[a]	76.7	76.1	76.2
			77.5							
C-6		62.2	61.8	62.2	62.5	62.7	61.9	62.1	62.3	61.7
										63.0

a) The peak assignment may be interchangeable.

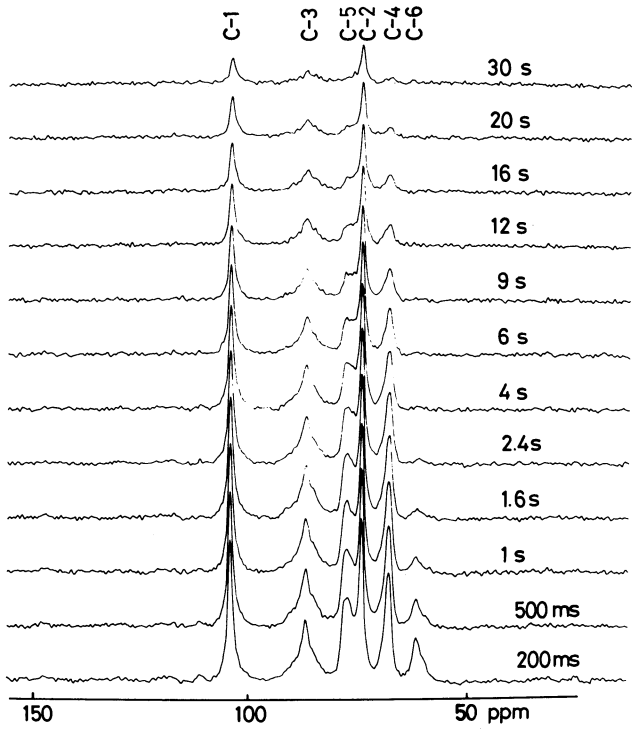

Fig. 5. Stacked plot of ^{13}C NMR peak intensities of annealed curdlan obtained by cross polarization for the T_{1C} measurements. Delay times between two 90° pulses are indicated in the right-hand side. Number of transients is 100.

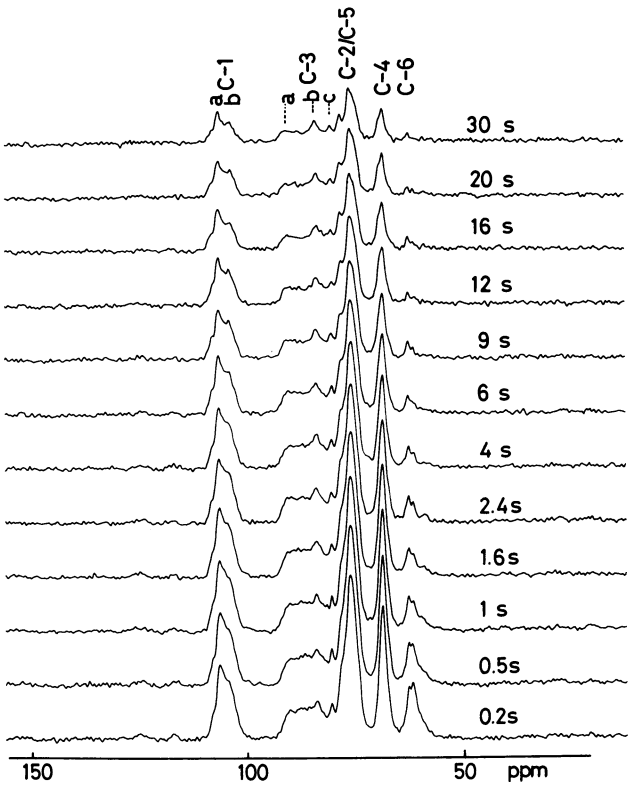

Fig. 6. Stacked plot of ^{13}C NMR peak intensities of paramylon obtained by cross polarization for the T_{1C} measurements. Delay times between two 90° pulses are indicated in the right-hand side. Number of transients is 100.

Surprisingly, the ^{13}C NMR pattern of laminaran is very similar to that of the annealed curdlan (Samples 1 and 2) in spite of its poor crystallinity, as may be seen from the X-ray diffraction data (Fig. 4B). The conformational change in curdlan from the noncrystalline to the crystalline form, however, is not complete when annealed samples at 150 and 120 °C were cooled rapidly (Samples 3 and 5). As a result, the C-3 NMR peak consists of two kinds of contributions: the peak from unconverted noncrystalline powder (C-3a) and that from converted crystalline species (C-3b). Further, it is interesting to note that the high-field shoulder of the C-3 peak in Sample 4 is resonated at 83.8 ppm, much higher than that of most annealed samples (86—87 ppm). In addition, the C-1 peaks appear to be split into doublet, 105.6 and 103.5 ppm. These data show that the ^{13}C NMR spectral pattern of Sample 4 resembles that of paramylon. Crystallinity and density of Sample 4 are found to be very high in spite of the broadened linewidths of its ^{13}C NMR pattern.

Figures 5 and 6 illustrate some typical examples of a stacked plot of the ^{13}C NMR spectra of annealed curdlan and paramylon, respectively, for measurements of the ^{13}C spin-lattice relaxation times (T_{1C}'s) by cross-polarization.[20] In this pulse sequence, the peak intensity at the delay time t after turning off the ^{13}C H_1 field, $M_{net}(t)$, decays exponentially from its initial value, $2M_{cp}(0)$, as is shown by Eq. 1,

Table 3. ^{13}C Spin-Lattice Relaxation Times of Some (1→3)-β-Glucans (s)[a]

	Curdlan		Laminaran	Paramylon
	Powder	Annealed[b]		
C-1	14	16	26	31(a),[c] 30(b)[c]
C-2	10[d]	18	24	22[d]
C-3	11	16	20	26(a),[c] 34(b),[c] 32(c)[c]
C-4	7.8	8.2	13	12
C-5	10[d]	9.3	10	22[d]
C-6	1.0	1.2	1.7	6.3

a) Estimated error ±15%. b) Sample 2 (see Table 1). c) Letters in parentheses denote the peak indicated in Fig. 6. d) Not distinguishable.

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

Thus, the T_{1C} values were obtained from a plot of log $M_{net}(t)$ vs. t, as is summarized in Table 3. Interestingly, it appears that the T_{1C} values of the annealed curdlan and laminaran can be classified into the following three groups: peaks with short (1.2—1.7 s for C-6), intermediate (8—13 s for C-4 and C-5), and long (16—26 s for C-1, C-2, and C-3) relaxation times. The existence of such a step in the relaxation times could be related to the ease of spin diffusion process with the hydroxymethyl group which undergoes rapid internal reorientation process. There appears to be no signifi-

cant differences in the T_{1C} values of curdlan between the noncrystalline and crystalline forms. The T_{1C} values of laminaran and paramylon are, however, significantly longer than those of curdlan. In particular, the C-6 T_{1C} of paramylon is 5—6 times as long as those of the curdlan samples (Table 3). Further, we found that the T_{1C} values differ among the three peaks, C-3a, b and c (see Fig. 6 and Table 3).

Discussion

Triple Helix: Laminaran-Type Form. The sharp ^{13}C NMR pattern of annealed curdlan at 150—180 °C (Figs. 2D and E) is straightforwardly ascribed to the triple-helical conformation,[7-9] in view of its characteristic X-ray powder-diffraction patterns (Fig. 4A). The above-mentioned pronounced peak narrowing (ca. 1 ppm) is obviously caused by the improved crystallinity of samples due to annealing in the presence of water, as is consistent with our previous observation of annealed chitosan.[21] The achievement of peak-narrowing as well as conformational change is not sufficient when annealed samples at 120—180 °C are cooled rapidly (Samples 3 and 5, see Fig. 3). In fact, the magnitude of the conformational change to the triple helix is, at most, 50% at the time of annealing (150—180 °C); additonal conversion was achieved by a slow crystallization process, together with concomitant dehydration during the course of the slow cooling.

Subsequently, we found that the laminaran-type conformation[14] found in laminaran and fungal branched glucans is identified with the same triple-helical conformation in view of the similarity in ^{13}C NMR patterns between laminaran and annealed curdlan (Samples 1 and 2) (Fig. 2C—E). This finding is in contrast to the observation by means of X-ray diffraction: laminaran exhibits a poor crystallinity, judging from the X-ray diffraction (Fig. 4B), whereas annealed curdlan (Samples 1 and 2) exhibits a high crystallinity and its diffraction pattern is obviously different from that of laminaran (Fig. 3A). Nevertheless, the present ^{13}C NMR data show that the overall conformation of noncrystalline laminaran is the same as that of the triple-helix form of annealed curdlan, but the presence of conformations whose torsion angles are slightly deviated from those of the most stable triple-helical conformation results in line-broadening. In addition, it is pointed out that a number of fungal branched (1→3)-β-D-glucans take a triple-helical conformation similar to that of laminaran, judging from the similarity of the ^{13}C NMR spectra, as discussed in the previous[14] and subsequent papers.[22]

Single Helix: Curdlan-Type Form. No information is available from X-ray diffraction as to the conformation of the curdlan-type form arising from the noncrystalline portion of the powder sample. In fact, curdlan powder accounts for only about 30% crystalline portion as estimated from density-measurements.[12] Nevertheless, the conformation of the remaining major portion (about 70%) is not always completely disordered, because the C-3 ^{13}C NMR peaks of this form from various samples are always resonated at the same position (89.6±0.5 ppm), which is clearly distinguishable from that of the above-mentioned triple-helix conformation (86.4±0.5 ppm). It should be emphasized that this form corresponds to the *native* conformation of curdlan, directly isolated from solid cultures of *Alcaligenes faecalis*.[13] In a similar manner, we previously showed, on the basis of the ^{13}C chemical shifts, that the molecular conformation of the disordered or random-coiled form, as judged by the X-ray diffraction, of silk fibroin is very similar to that of the crystalline silk I form.[23,24] This discrepancy may arise from the fact that conformational analysis by means of X-ray diffraction requires regular arrangement of the molecules under consideration with the neighboring molecules or interstrands, whereas the present ^{13}C NMR approach is able to afford the conformational characterization of individual molecules, irrespective of the manner of the arrangement of the neighboring molecules.

As has been demonstrated previously, intense high-resolution ^{13}C NMR signals of curdlan gel are visible (70—80%)[5] at the temperature of swelling (54 °C; low-set gel), but the peak-intensities are substantially decreased by annealing at temperatures higher than 54 °C, together with the development of the turbidity. Obviously, the portion which gives rise to the high-resolution NMR signals corresponds with the fully hydrated region, so as to give freedom of isotropic molecular motion with the correlation times of an order of 10^{-9} s.[2-4] This portion turned out to be an ordered conformation by the characteristic displacement of the C-3 ^{13}C chemical shifts[3,4] as well as by optical rotatary dispersion, intrinsic viscosity, flow birefringence, and shift of absorption maximum with Congo Red.[25-27] We previously ascribed this ordered conformation to a single helix, in view of the ease of swelling in aqueous media, resulting in the formation of a fully hydrated region able to give high-resolution ^{13}C NMR signals.[2-4,28] The relative proportion of such a fully hydrated portion (65—80%),[2] as observed by high-resolution ^{13}C NMR, at the temperature of swelling, is very close to the estimated proportion of the noncrystalline region (about 70% by density measurement[12]). Thus, it is natural to consider that the conformation of the noncrystalline portion of curdlan powder arises from this single helix. This view is based on premise that the conformation achieved in aqueous media could be preserved in lyophilized samples, because no further heat treatment required for conformational transition is given. In supporting this view, we previously found that the conformations of silk I and (L-Ala-Gly)$_n$ II achieved in an aqueous solution are also retained in lyophilized solids, judging

from the ^{13}C NMR data.[23] In addition, we showed previously that lyophilized (1→3)-β-D-glucans gave the same peak positions, depending on their molecular weight or on the solvents used.[36] In this connection, it should be taken into account that the single helix form, which is an energetically favored conformation in the fully hydrated state, is not always favored in the solid. For this reason, the conformation achieved in a lyophilized or spray-dried sample should not be considered to be the most stable conformation, as established by the calculation of the conformational energy.[12]

Interestingly, the ^{13}C NMR spectra of Samples 3, 5, and 6 (Figs. 3A, C, and D, respectively) are very similar to those of the acid-degraded lower-molecular-weight fraction (DP_n 14—131) of curdlan (see Fig. 4 of Ref. 14). Obviously, in these samples the relative proportion of the triple-helix form is increased to about 50%, as compared with that (about 10% from the peak area) of the starting curdlan powder.[12] These samples do not any longer possess gel-forming ability, because there are very few portions left able to swell because of the increased proportion of the triple helix. These results clearly show that gel-forming ability of (1→3)-β-D-glucan is simply related to the relative proportion of the triple helix. As for the gelation behavior of branched (1→3)-β-D-glulcans, however, we will discuss it in more detail in the subsequent paper.[22]

Paramylon. It has been shown that the X-ray powder diffraction pattern of paramylon is very similar to that of the hydrate triple helix of curdlan.[9] Unexpectedly, however, the ^{13}C NMR spectrum of paramylon can not be explained simply by the peak profile of the triple-helical curdlan alone, although the presence of the ^{13}C NMR peaks from the latter can easily be recognized. Instead, the presence of the conformational heterogeneity of this paramylon is well demonstrated by the spreads of several C-3 carbon signals, ranging from 89.5 to 80.4 ppm, their maximum separation being 9.1 ppm, in addition to the observation of the doublet C-1 peaks. This view is also confirmed by the observation of the differential behavior of the ^{13}C spin-lattice relaxation times among the peaks ascribed to the same carbons (C-3 or C-5/C-2 peaks) of paramylon (see Fig. 6 and Table 3). Further, it appears that the C-2/C-5 peaks consist of the superposition of several kinds of peaks, indicating the presence of a conformational heterogeneity. It is obvious that Fig. 2 also contains peaks from the curdlan-type form. Therefore, it is conceivable that highly crystalline paramylon (see the density data in Table 1) contains conformers which are essential for relaxing conformational strain, in addition to the usual triple helix. In this connection, it is interesting that a paramylon-like conformation was produced by annealing at 150 °C, followed by rapid cooling (Sample 4; Table 1 and Fig. 3B), although the crystalline packing of paramylon which is produced at the time of biosynthesis can not be well reproduced by a simple annealing procedure.

^{13}C Spin-Lattice Relaxation Data. The observed relaxation times, ranging from 8 to 34 s, indicate that these polysaccharides lack molecular motion whose correlation time of an order of 10^{-8} s effective in shortening the T_{1C} values. Instead, the spin-lattice relaxation of this magnitude may be mainly affected by the ^{13}C spin diffusion.[37] The presence of the fast rotational motion of the hydroxymethyl group leads to the effective ^{13}C T_{1C} relaxation pathways coupled with spin exchange. Then, it is expected that the ^{13}C T_{1C}'s of the C-4 and C-5 carbons are subsequently reduced as a result of the spin exchange with the C-6 hydroxylmethyl group, because the rate of the spin exchange is proportional to r^{-6} where r is the interatomic distance. On the contrary, longer T_{1C} values observed in paramylon could be interpreted in terms of lack of efficient relaxation pathways due to plausible restricted rotation of the hydroxymethyl group. This is an additional piece of evidence which suggests that the conformation of paramylon is different from that of the triple-helical curdlan.

Therefore, it appears that these ^{13}C T_{1C} values reflect the manner of crystalline packing, effective in the spin diffusion process. In fact, the present ^{13}C T_{1C} values of (1→3)-β-D-glucans are much shorter than those of the crystalline component of cellulose[38,39] but longer than those of the noncrystalline component.[38] No such a distinction can, however, be observed in the present cases. In fact, noncrystalline samples characterized by X-ray diffraction are not necessarily motionally disordered. On the contrary, it has been shown that the T_{1C}'s of the amorphous portion of polyethylene or polyoxymethylene (50—200 ms) are much shorter than those of crystalline portion (20—2000 s).[40-42] The observed reduction of the former can be explained mainly in terms of the presence of segmental motions in the solid.

Concluding Remarks. The molecular conformations of noncrystalline polysaccharides do not necessarily take a disordered form, although this view cannot be clarified by X-ray diffraction study. Nevertheless, we previously showed that two distinct types of conformations of (1→3)-β-D-glucans, the curdlan- and laminaran-type forms, are distinguishable for a number of noncrystalline solid samples by an examination of the characteristic displacements of the C-3 ^{13}C chemical shifts. As an illustrative example, we attempted to reveal the conformations of these two forms with the aid of a high-resolution NMR study of curdlan gel and a high-resolution solid-state ^{13}C NMR study of annealed curdlan. Thus, the curdlan- and laminaran-type forms were ascribed to single and triple helix conformations, respectively. Finally, we pointed out that the conformation of paramylon can not be accounted for simply by the presence of the triple helix of annealed curdlan alone, in view of the

spreads of the ^{13}C chemical shifts and differences in the spin-lattice relaxation times.

The authors wish to thank Dr. Kozo Ogawa, Radiation Center of Osaka Prefecture, for his preparation of the annealed samples, and measurements of the density and the X-ray diffraction, and for his stimulating discussion. The authors are also grateful to Professor B. A. Stone for a generous gift of paramylon.

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5) In the gel state, ^{13}C NMR peaks are usually suppressed to some extent because of the restricted molecular motions caused by the presence of cross links. The observed maximum peak intensities of swollen gels were 65—80% as referred to the peak intensities of fully hydrated 0.22M NaOH solution (Ref. 2). Further heating of the gel sample (80 °C) resulted in the subsequent reduction of the peak intensities to about 20% in parallel with the development of turbidity and a shrinkage of the gel sample. A similar extent of peak intensities (20—50%) was noted at an ambient temperature. In this connection, it is worthwhile to recall that the introduction of 0.1 and 1 mol% cross links in a chemically cross-linked synthetic polymer resulted in the reduction of peak intensities to 55 and 35%, respectively (Ref. 6).

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28) A decade ago,[3,4] we proposed that the ^{13}C NMR observation in gels is solely limited to the rather flexible *single* helical portion (the correlation time being in the order of 10^{-8} s) where molecular chains are fully exposed to the diluent and the peak areas of the triple-helical portion are lost under high-resolution NMR conditions. In fact, it is very difficult to account for the presence of the peak arising from the triple helix (86.4±0.4 ppm) in the ^{13}C NMR signal of elastic gel (88.7 ppm). Furthermore, we found that all of peak-areas were suppressed[29,30] when high-resolution ^{13}C NMR spectra of branched (1→3)-β-D-glucans taking the triple-helix conformation[31-33] were recorded in aqeuous media. Undoubtedly, such a suppression of peaks arose from an insufficient averaging of the C-H dipolar and/or chemical shift anisotropy interactions due to the presence of a slow tumbling motion of the stiff, rod-like molecules of the triple helix. In this connection, Yanaki et al. showed that the triple helix of schizophyllan, a branched (1→3)-β-D-glucan, is stiffer than that of native collagen, for the persistent chain length of the former (200±30 nm) is larger than that of the collagen triple helix (130 nm).[32,33] The preservation of chemical shift anisotropy as large as 50—140 ppm in native collagen[34,35] makes impossible the observation of the ^{13}C NMR spectra by means of high-resolution spectrometer. It may also be pointed out that the ease of the aggregation of the rod-like triple helix of curdlan, as manifested by syneris or dehydration, which are not seen in the triple helices of branched glucans, leads to an additional suppression of the NMR peaks.

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