A High-Resolution Solid-State ^{13}C NMR Study of the Secondary Structure of Branched (1→3)-β-D-Glucans from Fungi: Evidence of Two Kinds of Conformers, Curdlan-Type Single-Helix and Laminaran-Type Triple-Helix Forms, as Manifested from the Conformation-Dependent ^{13}C Chemical Shifts

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The high-resolution ^{13}C NMR spectra of a variety of fungal branched (1→3)-β-D-glucans were recorded in a DMSO solution and a lyophilized solid in order to gain an insight into the primary and secondary structures in relation to their gel-forming property. We found that all the five lyophilized branched (1→3)-β-D-glucans examined so far, except lentinan, exhibit the laminaran-type triple-helix form, as established by the close resemblance of the ^{13}C NMR spectra among them. On the contrary, lentinan taking the curdlan-type single-helix conformation was readily converted to the triple-helix form by lyophilization after having been dissolved in a 8M urea solution (1M=1 mol dm^{-3}) and dialysis against distilled water. This finding should be compared with the similar conformational change of curdlan: the conversion by this procedure was at most 50%; annealing at 150°C, followed by slow cooling, was essential for a complete conversion. This treatment did not induce the reverse conformational change from the triple helix to the single helix. The differential behavior of gel-formation between the linear and branched (1→3)-β-D-glucans was explained in terms of the molecular conformations in the solid and gel states.

The molecular conformation or polymorphs of crystalline polysaccharides have previously been analyzed by means of X-ray diffraction studies.[1] No information, however, is provided by this diffraction method as to the conformational feature of noncrystalline polymers or such region in crystalline samples. Nevertheless, the conformations of such portions are not necessarily the completely randomly coiled form. Instead, we have previously shown that a variety of biological macromolecules, such as polypeptides, fibrous proteins,[2-6] and polysaccharides,[7-10] obtained by lyophilization take regular conformation, as is shown by the characteristic displacements of ^{13}C chemical shifts. Thus, high-resolution solid-state ^{13}C NMR spectroscopy offers an excellent means for the conformational characterization of noncrystalline as well as crystalline samples. In particular, the ^{13}C chemical shifts vary significantly (up to 8 ppm) with the respective conformation as characterized by the glycosidic torsion angles (ϕ, ψ).[11-13] Thus, this approach has been used for the conformational characterization of a number of polysaccharides, including cellulose[12-19] chitin, chitosan,[9,20,21] amylose,[7,11] starch,[22-25] and (1→3)-β-D-glucans.[8,10,26-32]

The crystallinity of (1→3)-β-D-glucans is, in many instances, low so as to give a halo X-ray diffraction pattern, unless otherwise examined samples are extensively annealed. However, this treatment could severely alter the molecular conformation. Therefore, the development of a method for the in situ conformational characterization of a starting material is essential for better understanding of the conformational feature of these glucans in relation to their physical properties. For this purpose, we previously demonstrated[10] that at least four kinds of secondary structures could be distinguished by means of solid-state ^{13}C NMR spectra. In particular, two major forms, the curdlan- or laminaran-type forms, can readily be distinguished by means of the conformation-dependent ^{13}C chemical shifts when these samples are obtained either by lyophilization or spray-drying.[10] In the preceding paper, these curdlan- and laminaran-type forms were identified as having the single- and triple-helix conformation, respectively.[33]

Previously, we found that the gel-forming ability of linear (1→3)-β-D-glucans is related to the presence of a single helix as the major conformer, and swelling does not occur from samples taking the triple helix as the major conformation. In this connection, it is surprising to note that the two kinds of branched glucans previously studied, HA-β-glucan and lentinan, take the two different above-mentioned conformations, in spite of exhibiting a similar gel-forming property.[10] Therefore, it seems to be very important to clarify the stability and ease of conformational changes in branched (1→3)-β-D-glucans.

In the present paper, we will examine the ^{13}C NMR spectra of four additional branched (1→3)-β-D-glucans in order to clarify their conformations in the solid state. We noted that all five (1→3)-β-D-glucans assume the laminaran-type triple-helix. The curdlan-type single-helical conformation of lentinan was converted to the laminaran-type triple-helix when dialyzed after dissolving in 8M urea solution.

Table 1. Characteristics of Branched (1→3)-β-D-Glucans Used

Trivial name	Physical property	Chemical analysis		Source	Ref.
		m-(m'-)res./ b-res.[a]	1.6-linkage		
T-5-N	Soft gel	2.5(1.4)[b]	0.06	*Dictyophora industiata*	36
T-4-N	Highly viscous soln.	1.5	0	*Dictyophora industiata*	35
Schizophyllan	Extremely viscous	2	0	*Schizophyllum commune*	38
Scleroglucan		2	0	*Sclerotium rolfsii*	39
HA-β-Glucan	Non-elastic gel	3	0	*Pleurotus ostreatus*	34
Lentinan	Non-elastic gel	3.4—1.5	0.2	*Lentinus edodes*	37

a) m- and m'-residues stand for 3-mono-*O*-substituted glucosyl residues, while b-residue stands for the 3,6-di-*O*-substituted glucosyl residue. b) Data in parentheses are from enzymatic analysis (C. Hara, T. Kiho, and S. Ukai, *Carbohydr. Res.*, **117**, 201 (1983)).

Experimental

Table 1 summarizes the trivial names, physical properties, degrees of branching, and sources of isolation for the variety of fungal branched (1→3)-β-D-glucans used in this study. The isolation of HA-β-glucan[34] (from *P. ostreatus* (Fr.) Quél), T-4-N[35] and T-5-N[36] (from *Dictyophora indusiata* FISH.) was described previously. Lentinan[37] (from *Lentinus edodes*) was a generous gift from Dr. G. Chihara of the National Cancer Center Research Institute. Schizophyllan[38] and its partially depolymerized sample by sonication (from *Schizophyllum commune*) were donated by Kaken Pharmaceutical Co. Ltd., Tokyo, Japan. Scleroglucan[39] (from *Sclerotium rolfsii*) was provided by Taito Co. Ltd., Tokyo, Japan. These samples were first used as received, for the measurements of the high-resolution solid-state ^{13}C NMR spectra. Then, they (200 mg) were dissolved in an 8M urea solution (5 ml), dialyzed against distilled water, and lyophilized for use for further measurements of the solid-state-NMR. Curdlan (Takeda Chemical Industries, Osaka, Japan) and GE-3[40] (by courtesy of Dr. Y. Nishikawa of Kanazawa University) were used as reference compounds for the ^{13}C NMR spectra of (1→3)-β-D- and (1→6)-β-D-glucans, respectively.

The high resolution ^{13}C NMR spectra were recorded on a Bruker CXP-300 spectrometer operated at 75.46 MHz. These samples (30 mg) were dissolved in DMSO-d_6 (1.5 ml) and contained in a 10 mm NMR tube. Single-contact 75.46 MHz ^{13}C cross polarization-magic angle spinning (CP-MAS) NMR spectra were recorded on a Bruker CXP-300 spectrometer equipped with a CP-MAS accessory. Solid lyophilized samples were placed in an Andrew-Beams type rotor machined from perdeuterated poly(methyl methacrylate), and then spun as fast as 3 kHz. 90° Pulse width was 5 μs and repetition time was 4 s. Spectra were usually accumulated 1000—2000 times. Chemical shifts were calibrated indirectly from the signal of liquid benzene (118.5 ppm) and then converted to the value from tetramethylsilane.

Results and Discussion

Primary Structure. Figure 1 illustrates the ^{13}C NMR spectra of a variety of (1→3)-β-D-glucans with different degrees of branching (see Table 1) in a DMSO-d_6 solution. As has previously been described,[31] the ^{13}C NMR spectra recorded in a DMSO solution can be ascribed to glucans taking the random-coil form and the displacements of the peaks can be considered to arise mainly from the primary sequence of the glucans. Obviously, none of these branched

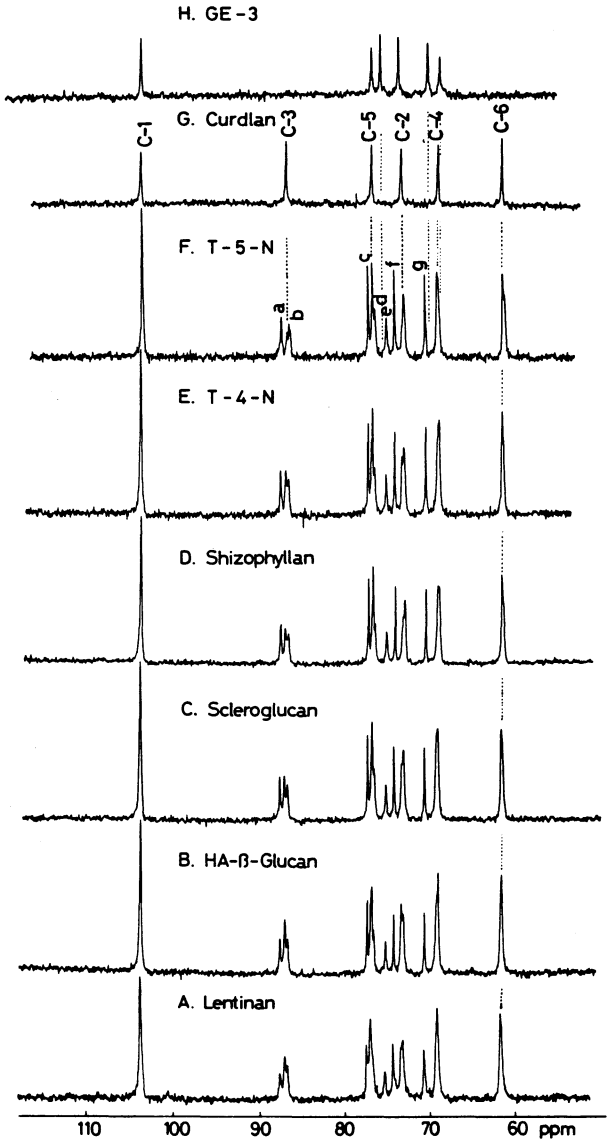

Fig. 1. High-resolution ^{13}C NMR spectra of a variety of branched (1→3)-β-D-glucans together with curdlan and GE-3 ((1→6)-β-D-glucans) in DMSO-d_6 solution (30 mg/1.5 ml DMSO-d_6).

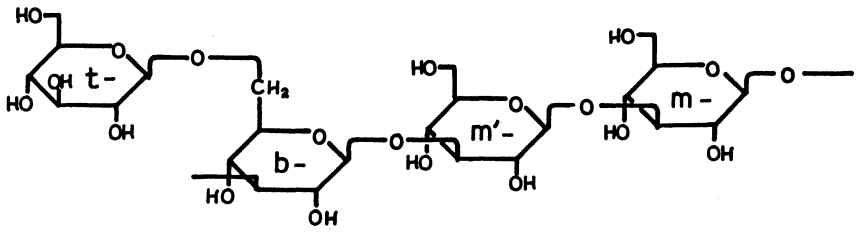

Fig. 2. Branching portion of branched (1→3)-β-D-glucans.

Table 2. The Assignment of the Peaks in the ^{13}C NMR Spectra of Various Branched (1→3)-β-D-Glucans

Peak	Chemical shift ppm	m- (or m'-) Residue	b-Residue	t-Residue
C-1	103.7	C-1	C-1′	C-1″
a	87.7	C-3 m′		
C-3	87.0	C-3 m		
b	86.4		C-3′	
c	77.4			C-3″
C-5	77.0	C-5		
d	76.7	C-5		C-5″
e	75.4		C-5′	
f	74.5			C-2″
C-2	73.8	C-2		
	73.6	C-2	C-2′	
g	70.9			C-4″
C-4	69.3	C-4	C-4′, C-6′	
C-6	61.8	C-6		C-6″

Table 3. Relative Peak Intensities of the ^{13}C NMR Spectra of Branched (1→3)-β-D-Glucans in a DMSO Solution

	Relative-peak intensity		
	C-4[a]/g	C-2[b]/f	C-3[c]/c
T-5-N	2.4	1.5	1.5
Schizophyllan	3.1	2.6	2.2
Scleroglucan	2.9	2.1	2.2
T-4-N	2.9	2.1	2.0
Lentinan	2.7	1.9	2.3
HA-β-glucan	3.2	3.1	2.6

a) The C-4 and g peaks contain C-4, C-4′, and C-6′, and C-4″, respectively. b) The C-2 and f peaks contain C-2 and C-2′, and C-2″, respectively. c) The C-3 and c peaks contain C-3 and C-3′, and C-3″, respectively.

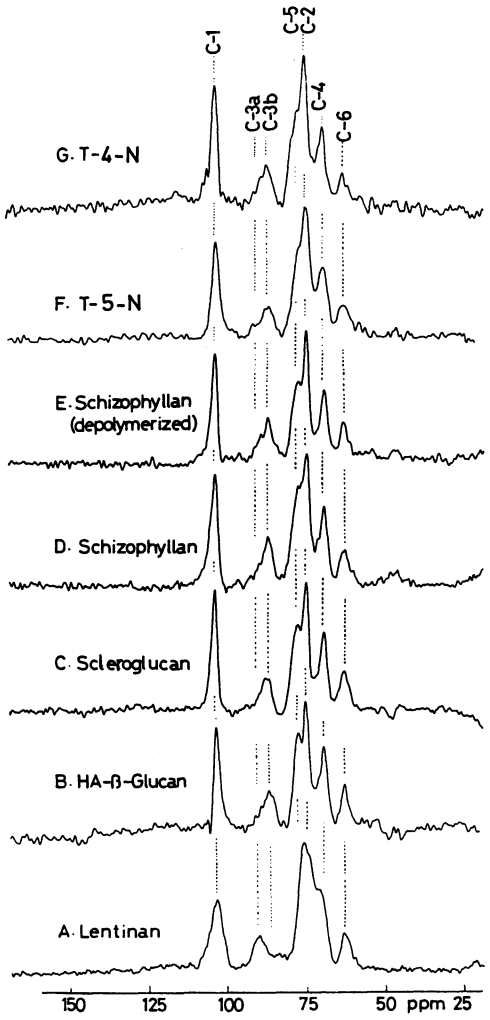

Fig. 3. 75.46 MHz High-resolution solid-state ^{13}C NMR spectra of a variety of branched (1→3)-β-D-glucans.

(1→3)-β-D-glucans possesses longer β-1,6 sequence, because no such signals can be seen as inferred from the peak-position of (1→6)-β-D-glucan (GE-3). The individual ^{13}C NMR signals were assigned to the carbons of the b-, m-(m′-), and t-residues (Fig. 2 and Table 2) on the basis of our previous assignment of the peaks for HA-β-glucan.[34] Further, it appears that two kinds of signals can be distinguished in the m- and m′-residues, as manifested by the C-3, C-5, and C-2 peaks. Further, the distinction of the peaks between m- and m′-residues is based on the data of Takeo and Tei[41] who synthesized some repeating units of branched (1→3)-β-D-glucans. The inspection of the ^{13}C NMR spectral profile leads to the classification of these polysaccharides into the following three regions: (1) HA-β-glucan, lentinan, and T-4-N, (2) scleroglucan and schizophyllan, and (3) T-5-N. In fact, the relative intensities of C-4, C-2, and C-3 with respect to those of the g, f, and c peaks (C-4″, C-2″, and C-3″, respectively, of the t-residue (side-group)), respectively, decrease in this order (Table 3). Undoubtedly, this is consistent with the degree of branching determined by the previous chemical analysis, as is summarized in Table 1.

Secondary Structure in the Solid State. Figure 3 summarizes the ^{13}C CP-MAS NMR spectra of a variety

Table 4. ^{13}C Chemical Shifts of Branched (1→3)-β-D-Glucans in the Solid State (ppm from TMS)

	Branched glucan							Linear glucan	
	Lentinan[c]		HA-β-D-glucan	Sclero-glucan	Schizo-phyllan	T-4-N	T-5-N	Curdlan[a] (triple helix)	Laminaran[b]
	Before	After							
C-1	103.2	103.5	102.9	103.3	103.5	103.3	103.2	103.6	102.9
C-2	73.3	74.0	73.6	73.8	74.2	74.0	74.1	74.3	73.9
C-3	89.3								
		86.1	85.7	86.7	86.3	85.9	85.7	86.9	85.5
C-4	69.1	68.9	67.9	67.9	68.1	68.1	68.4	68.4	67.8
C-5	75.0	76.0	76.0	76.6	76.8	76.4	76.4	77.5	76.6
C-6	61.8	62.3	61.5	61.3	61.5	61.7	61.9	61.8	61.7
		59.4							

a) Ref. 33. b) Ref. 10. c) The ^{13}C NMR spectra were recorded before and after treatment with 8M of urea.

of (1→3)-β-D-glucans in the solid state. Interestingly, all of the branched glucans, except for lentinan, give rise to a spectral pattern similar to that of laminaran and annealed curdlan adopting the triple helix (see Fig. 2 of Ref. 33), in spite of the differences in the degree of branching. This finding indicates that the manner of side-chain orientation does not affect the resulting back-bone conformation. The characteristic feature of the ^{13}C NMR pattern arising from the triple helix is that the C-3 peak is resonated at 86.4±0.5 ppm (C-3b). On the contrary, the C-3 peak of lentinan is resonated at 89.6±0.5 ppm (C-3a), characteristic of the presence of the single-helical curdlan-type form. This observation is in contrast to that of the previous X-ray diffraction study of lentinan, which suggests the presence of the triple helix.[42] This discrepancy might be, however, ascribed to the difference in the manner of sample preparation: the specimen for X-ray diffraction was obtained by fiber prepared from gel, whereas a spray-dried powder sample was used for the NMR study. In this connection, it is important to examine a plausible conformational change between spray-dried powder and lyophilized solid.

To confirm this point, we examined the conditions of the conformational change of branched (1→3)-β-D-glucans. We first dissolved these glucans in an 8M urea solution and then dialyzed it against distilled water and lyophilized to obtain solid samples. It was essential to utilize 8M urea to achieve a clear aqueous solution of branched glucans, corresponding to the completely random- coiled state, because these glucans take an ordered conformation (see Table 4). Figures 4 and 5 illustrate how the ^{13}C NMR spectra of (1→3)-β-D-glucans, taking the curdlan-type (single-helix) and laminaran-type (triple-helix) forms, respectively, are changed by treatment with 8M urea.

Interestingly, the lyophilization of lentinan resulted in a significant conformational change from the single helix to the triple-helix forms, as manifested by the displacements of the peaks from the C-3a to C-3b peak, as is indicated by the arrows, together with the characteristic change in the relative peak intensities in the C-2/C-5 peaks (Figs. 4A and B). On the contrary, the

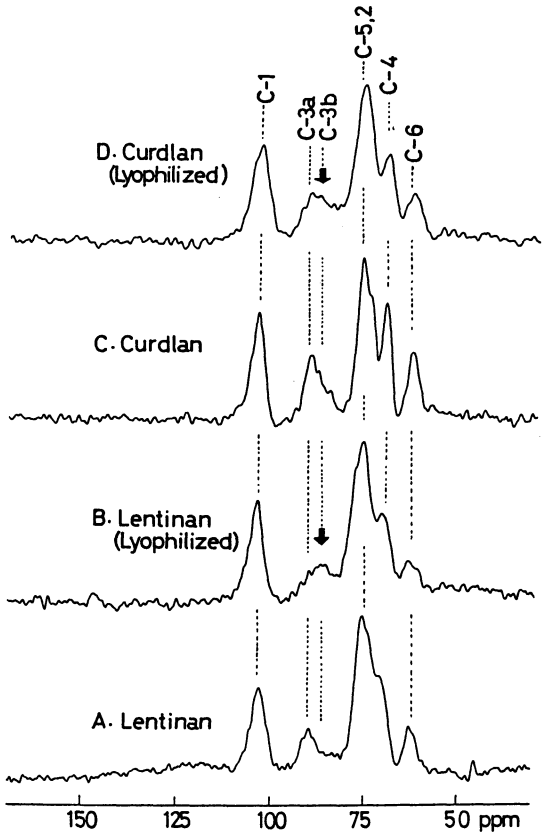

Fig. 4. Variation of high-resolution solid-state ^{13}C NMR spectra of lentinan (A and B) and curdlan (C and D) before and after treatment with 8 M urea and lyophilization. The peaks indicated by the arrows denote the peaks whose peak-intensities are varied.

extent of the conversion of curdlan is 50% at most, as judged from the relative peak-intensities between the C-3a and C-3b peaks. In fact, annealing of the sample at 150 °C, followed by slow cooling, is required for a complete conversion from the single to the triple helix, in the case of curdlan, a linear (1→3)-β-D-glucan.[33] Therefore, this distinction implies that the potential barrier to the converison from the single to the triple helix form in the linear glucan is much higher than that in the branched glucans. Accordingly, it may be

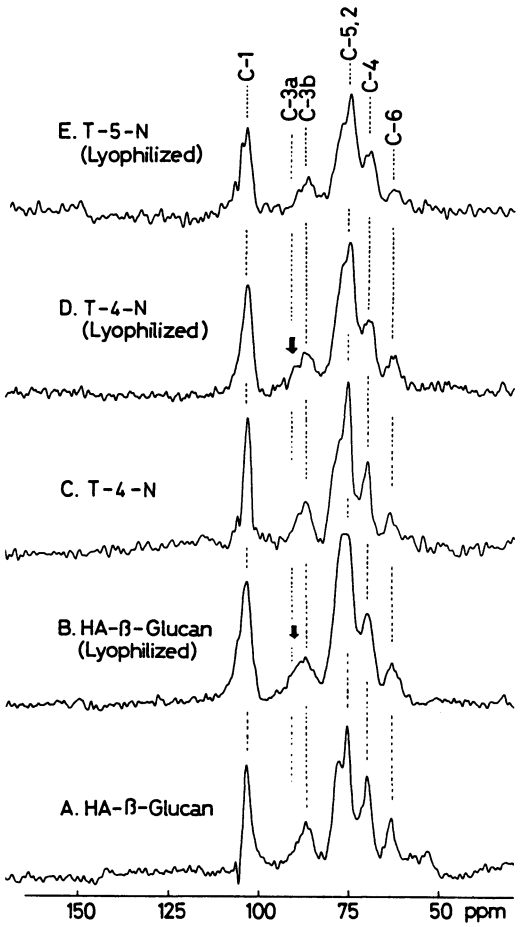

Fig. 5. Variation of high-resolution solid-state ^{13}CNMR spectra of HA-β-glucan (A and B), T-4-N (C and D) and T-5-N by treatment with 8 M urea.

concluded that the branched glucans obtained by lyophilization from the gel state take the triple-helix conformation. This result is reasonable, for branched glucans such as schizophyllan and scleroglucan take the triple helix in aqueous media[43-47] and any conformation achieved in aqueous media is usually preserved in a lyophilized solid, as has been discussed in the preceding paper.[33]

The two types of the above-mentioned conformations were also observed by Yadomae et al.[41] for branched (1→3)-β-D-glucans from *Grifora frodosa* and from *Sclerotinia sclerotiorum* IFO 9395.[48,49] Their terms of the two forms, "helix" and "native," correspond to the single and triple helix conformations, respectively, in view of the characteristic ^{13}CNMR peak-positions. They showed that milder and drastic conditions for isolation resulted in the conformations of the triple helix and single helix forms, respectively. This view seems generally to be correct in view of the presence of the triple helix in aqueous media. However, they also showed a conversion from the triple-helix conformations to the single-helix conformation by treatment with 8M urea. This finding was supported by the observation of an increased proportion of Congo Red bound to the glucans.[49] As pointed out previously, Congo Red is bound to the single-helical portion.[50]

It is now clear that the triple-helix form is the major form for lyophilized solid of the branched (1→3)-β-D-glucans, reflecting their major conformations in the gel state. On the contrary, curdlan powder contains the triple-helical region of less than 10%, as estimated from the ^{13}C NMR peak-intensity of the C-3 region.[33] This contradiction may be ascribed to apparent differences in the organization of the gel network between the linear and branched (1→3)-β-D-glucans. In the former, the major form is the single helix; the triple helix and its aggregates serve as portions of the cross-links.[28-30,33] Molecular chains other than the cross-links are sufficiently flexible, for high-resolution ^{13}C NMR signals are visible from the gel sample. In fact, the amount of the triple helix in lyophilized curdlan is less than 50%, even if the elastic gel has been annealed at 150 °C.[33]

On the contrary, gel from the branched glucan is not elastic and forms a viscous solution when the concentration is low. In addition, the ^{13}C NMR signals from the gel network are completely suppressed: the rate of molecular tumbling is longer than 10^{-6} s, for rod-like chains of the triple helix are stiffer than the native collagen fibril.[43,45] We previously pointed out that high-resolution ^{13}C NMR signals were made visible by a partial disruption of the triple helix to the single helix by adding NaOH (0.06—0.2 M).[27,28] Thus, the gel network of the branched (1→3)-β-D-glucans at a neutral pH arises mainly from the triple helix.

Concluding Remark. We have analyzed the two kinds of conformations of noncrystalline branched (1→3)-β-D-glucans in the solid state by means of the high-resolution solid-state ^{13}C NMR spectroscopy. The major conformation of the branched (1→3)-β-D-glucans is found to be the laminaran-type triple-helix on the basis of an examination of the conformation-dependent displacements of the ^{13}C chemical shifts. This obsevation is in contrast to that for the linear (1→3)-β-D-glucans, reported previously.[33] The curdlan-type single-helix conformation of spray-dried lentinan was easily converted to the laminaran-type triple helix, by the treatment with 8 M urea, followed by dialysis and lyophilization.

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References

1) H. Marchessault and A. Sarko, *Adv. Carbohydr. Chem.*, **22**, 421 (1967).

2) H. Saitô, R. Tabeta, A. Shoji, T. Ozaki, I. Ando, and T. Asakura, "Magnetic Resonance in Biology and Medicine," ed by G. Govil, C. L. Kheterapal, and A. Saran, Tata McGraw-Hill, New Delhi (1985), p. 195.
3) H. Saitô, *Magn. Reson. Chem.*, **24**, 835 (1986).
4) H. Saitô, R. Tabeta, A. Shoji, T. Ozaki, and I. Ando, *Macromolecules*, **16**, 1050 (1983).
5) H. Saitô, R. Tabeta, A. Shoji, T. Ozaki, I. Ando, and T. Miyata, *Biopolymers*, **23**, 2279 (1984).
6) T. Asakura, A. Kuzuhara, R. Tabeta, and H. Saitô, *Macromolecules*, **19**, 1841 (1985).
7) H. Saitô and R. Tabeta, *Chem. Lett.*, **1981**, 713.
8) H. Saitô, R. Tabeta, and T. Harada, *Chem. Lett.*, **1981**, 571.
9) H. Saitô, R. Tabeta, and S. Hirano, *Chem. Lett.*, **1981**, 1479.
10) H. Saitô, R. Tabeta, T. Sasaki, and Y. Yoshioka, *Bull. Chem. Soc. Jpn.*, **59**, 2093 (1986).
11) H. Saitô, G. Izumi, T. Mamizuka, S. Suzuki, and R. Tabeta, *J. Chem. Soc., Chem. Commun.*, **1982**, 1386.
12) F. Horii, A. Hirai, and R. Kitamaru, *ACS Symp. Ser.*, No. **260**, 27 (1982).
13) R. L. Dudley, C. A. Fyfe, P. J. Stephenson, Y. Deslandes, G. K. Hammer, and R. H. Marchessault, *J. Am. Chem. Soc.*, **105**, 2469 (1983).
14) R. Atalla, J. G. Gast, D. W. Sindorf, V. Bartuska, and G. E. Maciel, *J. Am. Chem. Soc.*, **102**, 3249 (1980).
15) W. L. Earl and D. L. VanderHart, *J. Am. Chem. Soc.*, **102**, 2351 (1980).
16) W. L. Earl and D. L. VanderHart, *Macromolecules*, **14**, 570 (1981).
17) G. E. Maciel, W. L. Kolodziejski, M. S. Bertran, and B. E. Dale, *Macromolecules*, **15**, 686 (1982).
18) R. H. Atalla and D. L. VanderHart, *Science*, **223**, 283 (1984).
19) C. A. Fyfe, R. L. Dudley, P. J. Stephenson, Y. Deslandes, G. K. Hammer, and R. H. Marchessault, *J. Macromol. Sci. Rev. Macromol. Chem. Phys.*, **C23**, 187 (1983).
20) H. Saitô, R. Tabeta, and S. Hirano, "Chitin and Chitosan. Proceeding of the Second International Conference on Chitin and Chitosan," ed by S. Hirano and S. Tokura, Japanese Society of Chitin and Chitosan (1982), pp. 71—76.
21) H. Saitô, R. Tabeta, and K. Ogawa, *Macromolecules*, **20**, 2424 (1987).
22) R. H. Marchessault, M. G. Taylor, C. A. Fyfe, and R. P. Veregin, *Carbohydr. Res.*, **144**, C1 (1985).
23) M. J. Gidley and S. M. Bociek, *J. Am. Chem. Soc.*, **107**, 7040 (1985).
24) B. P. Veregin, C. A. Fyfe, R. H. Marchessault, and M. G. Taylor, *Macromolecules*, **19**, 1030 (1986).
25) F. Horii, A. Hirai, and R. Kitamaru, *Macromolecules*, **19**, 930 (1986).
26) C. A. Fyfe, P. J. Stephenson, M. G. Taylor, T. L. Bluhm, Y. Deslandes, and R. H. Marchessault, *Macromolecules*, **17**, 501 (1984).
27) H. Saitô, T. Ohki, and T. Sasaki, *Carbohydr. Res.*, **74**, 227 (1979).
28) H. Saitô, *ACS Symp. Ser.*, No. **150**, 1125 (1981).
29) H. Saitô, T. Ohki, and T. Sasaki, *Biochemistry*, **16**, 908 (1977).
30) H. Saitô, E. Miyata, and T. Sasaki, *Macromolecules*, **11**, 1244 (1978).
31) H. Saitô, T. Ohki, N. Takasuka, and T. Sasaki, *Carbohydr. Res.*, **58**, 293 (1977).
32) H. Saitô, T. Ohki, Y. Yoshioka, and F. Fukuoka, *FEBS Lett.*, **68**, 15 (1976).
33) H. Saitô, R. Tabeta, M. Yokoi, and T. Erata, *Bull. Chem. Soc. Jpn.*, **60**, 4259 (1987).
34) Y. Yoshioka, R. Tabeta, H. Saitô, N. Uehara, and F. Fukuoka, *Carbohydr. Res.*, **140**, 93 (1985).
35) C. Hara, T. Kiho, and S. Ukai, *Carbohydr. Res.*, **117**, 201 (1983).
36) S. Ukai, C. Hara, and T. Kiho, *Chem. Pharm. Bull.*, **30**, 2147 (1982).
37) T. Sasaki and N. Takasuka, *Carbohydr. Res.*, **47**, 99 (1976).
38) S. Kikumoto, T. Miyajima, S. Ohkubo, and N. Komatsu, *Nippon Nogei Kagaku Kaishi*, **45**, 162 (1971).
39) T. L. Bluhm, Y. Deslandes, R. H. Marchessault, S. Perez, and M. Rinaudo, *Carbohydr. Res.*, **100**, 117 (1982).
40) Y. Nishikawa, T. Takeda, S. Shibata, and F. Fukuoka, *Chem. Pharm. Bull.*, **17**, 1910 (1969).
41) K. Takeo and S. Tei, *Carbohydr. Res.*, **145**, 293 (1986).
42) T. L. Bluhm and A. Sarko, *Can. J. Chem.*, **55**, 293 (1977).
43) T. Yanaki, T. Norisuye, and H. Fujita, *Macromolecules*, **13**, 1462 (1980).
44) T. Norisuye and H. Fujita, *J. Polym. Sci., Polym. Phys. Ed.*, **18**, 547 (1980).
45) Y. Kashiwagi, T. Norisuye, and H. Fujita, *Macromolecules*, **14**, 1220 (1981).
46) T. Yanaki, T. Kojima, and T. Norisuye, *Polym. J.*, **13**, 1135 (1981).
47) T. Yanaki and T. Norisuye, *Polym. J.*, **15**, 389 (1983).
48) N. Ohno, Y. Adachi, I. Suzuki, S. Oikawa, K. Sato, Y. Suzuki, M. Ohsawa, and T. Yadomae, *Chem. Pharm. Bull.*, **34**, 2555 (1986).
49) N. Ohno and T. Yadomae, *Carbohydr. Res.*, **159**, 293 (1987).