July, 1986] © *1986 The Chemical Society of Japan* *Bull. Chem. Soc. Jpn., 59, 2093—2101 (1986)* 2093

A High-Resolution Solid-State ^{13}C NMR Study of (1→3)-β-D-Glucans from Various Sources. Conformational Characterization as Viewed from the Conformation-Dependent ^{13}C Chemical Shifts and Its Consequence to Gelation Property

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(Received February 27, 1986)

High-resolution solid-state ^{13}C NMR spectra of a variety of (1→3)-β-D-glucans in the solid state were recorded by means of cross polarization-magic angle spinning (CP-MAS) NMR spectroscopy. We found that the C-1 and C-3 peak-positions of these glucans are significantly displaced (up to 8 ppm) depending upon variety of samples used. Interestingly, these glucans obtained by lyophilization or spray-drying possess regular secondary structures as detected by displacements of the conformation-dependent ^{13}C chemical shifts. In fact, at least four kinds of molecular conformations were distinguishable by ^{13}C NMR spectroscopy. Gel-forming ability of linear (1→3)-β-D-glucans, however, was related to the presence of a single particular conformation without involving any other form. By contrast, gel-formation was noted for a branched glucan whose conformation is similar to that of a linear glucan without gel-forming ability. Further, conformation of all these linear and branched (1→3)-β-D-glucans turned out to be the same when solid samples were prepared by lyophilization from DMSO solution.

It is well known that linear and branched (1→3)-β-D-glucans (Fig. 1) function as structural components in the cell walls of many plants and microorganism and also act as reserve polysaccharides.[1] Physical properties of (1→3)-β-D-glucans isolated from various sources are thus widely different: low molecular weight laminaran is water-soluble glucan, while pachyman (from *Poria cocos*) is not; curdlan (from *Alcaligenes faecalis*) is known to form elastic gel when aqueous suspension is heated at a temperature above 54 °C.[2,3] On the contrary, some fungal branched (1→3)-β-D-glucans such as lentinan (from *Lentinus edodes*) and schizophyllan (from *Schizophyllum commune*) form nonelastic gel without heating.[4] Our previous high-resolution ^{13}C NMR studies showed that these gel-structures are composed of rather flexible single-helical chains, a part of which being associated to give physical cross links such as multiple-helical junction-zones or these aggregates.[5–9] On the contrary, acid-degraded lower molecular weight fractions of these glucans ($\overline{\mathrm{DP}}_n$<20 for linear glucans) are freely soluble in water.[6–8] In this connection, it is interesting to note that anti-tumor activity of these glucans is related to their solution properties:[10,11] higher molecular weight fractions with gel-forming ability are biologically active but water-soluble fraction is not.

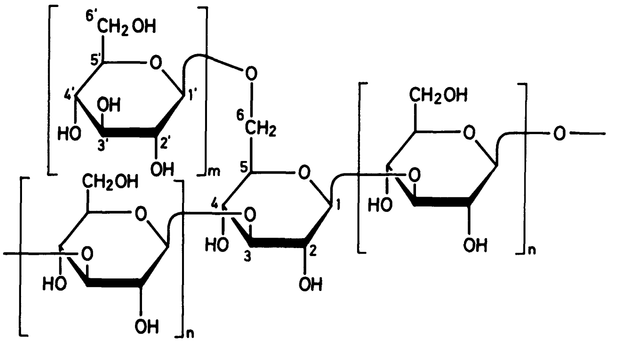

Fig. 1. Primary structures of linear and branched (1→3)-β-D-glucans: m=0, $\overline{\mathrm{DP}}_n$=540, curdran; m=0, $\overline{\mathrm{DP}}_n$=38, laminaran; m=1, n=50, pachyman; m=1, n=2.5, lentinan; m=1, n=3, HA β-glucan.

Accordingly, it appears that such a physical property might be also related to a secondary or tertiary structure of these glucans in addition to the degree of branching. As to the former, however, no detailed analysis for this purpose has been made, because these glucans obtained as spray-dried powder or lyophilized solid are naturally not at crystalline state and no X-ray diffraction study can be applied without extensive annealing of samples.[13,14] In addition, it is not yet clear whether or not glucans thus obtained do take any regular secondary structure. In order to reveal conformational features of noncrystalline as well as crystalline samples, high-resolution solid-state ^{13}C NMR spectroscopy has recently proven to be very useful for a various types of molecular systems.[15,16] The displacements of ^{13}C chemical shifts of backbone carbons (up to 8 ppm) are thus well related to variation of nearby torsion angles of respective carbons under consideration. In particular, the ^{13}C chemical shifts of the C-1 and C-X carbons of various types of glucans at the glycosidic linkages are well related to the torsion angles of the C-1–O$_{\mathrm{gly}}$ (ϕ) and C-X–O$_{\mathrm{gly}}$ (ψ), respectively.[17,18] Thus, conformational features of cellulose,[19–26] chitin,[27,28] (1→3)-β-D-,[29,30] and (1→4)-α-D-glucans[31] have been analyzed by the data of high-resolution

^{13}C NMR spectroscopy.

In this paper, we aimed to examine secondary structures of a variety of (1→3)-β-D-glucans from various sources by means of cross polarization-magic angle spinning (CP-MAS) ^{13}C NMR spectroscopy. First of all, we found that these glucans take various kinds of regular secondary structures, as viewed from the linewidths and displacements of ^{13}C NMR peaks, depending upon molecular weights and a manner of sample preparation. In fact, at least four kinds of different conformations can be distinguished by displacements of the C-1 and C-3 ^{13}C chemical shifts: form I (curdlan-type), form II (laminaran-type), form III (laminaripentaose-type), and form IV (DMSO-adduct). Then, it is found that paramylon granules which is over 90% crystalline is not consisting of molecular chains with a single conformation but a composite of several conformers. Further, conformational features of these glucans in the gel state were examined by observation of high resolution ^{13}C NMR spectra.

Experimental

Materials. Table 1 summarizes trivial names, degree of polymerization, physical properties, and sources of isolation for a variety of (1→3)-β-D-glucans studied in this paper. Lower-molecular-weight (1→3)-β-D-glucans were prepared from curdlan ($\overline{DP}_n$=540) by hydrolysis with formic acid or sulfuric acid by the method previously described.[10] Gel-filtration patterns of these preparations ($\overline{DP}_n$=14, 20, 82, and 131) on a Sephadex G-150 or Sepharose CL-4B column each gave a symmetrical peak. Curdlan with $\overline{DP}_n$=380 was obtained from the culture filtrate of *Alcaligenes faecalis* under different fermentation time. Numerically averaged degree of polymerization ($\overline{DP}_n$) was determined by the method of Manners et al.[32] The above-mentioned glucans were supplied by Takeda Chemical Industries, Osaka, Japan. Pachyman[33] was obtained from Sclerotium of *Poria cocos wolf* (Bukuryu) purchased from Shihira Shoten Ltd., Tokyo (by courtesy of Dr. G. Chihara of this Institute). Paramylon[34] (from *Euglena gracilis*) was a generous gift from Professor B. A. Stone of La Trobe University, Australia. Lentinan[35] was a generous gift from Dr. G. Chihara. Laminaran with $\overline{DP}_n$=38 (as determined by gel-filtration chromatography) was purchased from Nutritional Biochemical Corporation, Ohio, USA. Fractionated lower oligomers of (1→3)-β-D-glucans, laminaritriose, laminaripentaose, and laminariheptaose (Lot No. 407191), were purchased from Seikagaku Kogyo, Ltd., Tokyo, Japan. Isolation of fungal (1→3)-β-D-glucan, HA β-glucan (from *P. ostreatus (Fr.)* Quél.),[36] was reported previously. Some of these glucans were once dissolved in DMSO solution and lyophilized to obtain samples of DMSO-adduct. Curdlan gel was obtained by heating aqueous suspension (20 mg) at a temperature at 60 °C and cooling at an ambient temperature. Gels of fungal (1→3)-β-D-glucans were obtained by hydration of samples (20 mg) with excess water. To prepare gel samples for high resolution ^{13}C NMR spectroscopy, a few drops of concd NaOH solution were added stepwise to achieve 0.05 M.

^{13}C CP-MAS NMR Spectroscopy. Single contact 75.46 MHz ^{13}C CP-MAS NMR spectra were recorded on a Bruker CXP-300 spectrometer equipped with a CP-MAS accessory. Solid samples were contained in an Andrew–Beams type rotor machined from perdeuterated poly (methyl methacrylate), and spun as fast as 3—4 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 (128.5 ppm) and converted to the value from tetramethylsilane.

Table 1. Characteristics of (1→3)-β-D-Glucan Samples Used

Sample No.	Trivial Name	Appearance	Linear or branched	$\overline{DP}_n$[a)]	Solution properties in aqueous medium	Sources
1	Curdlan	Powder	Linear	540	Elastic gel	*Alcaligenes faecalis*
2	Curdlan	Powder	Linear	380	Elastic gel	*Alcaligenes faecalis*
3	Pachyman	Powder	Linear	250	Nonelastic gel	*Poria cocos*
4	Laminaran	Powder	Linear	38[b)]	Not soluble	*Laminaria species*
5	Paramylon	Granule	Linear	—	Not soluble	*Euglena gracilis*
6	Lower-molecular-weight curdlans					
6a		Powder	Linear	14	Soluble	
6b		Powder	Linear	20	Soluble	
6c		Powder	Linear	82	Not soluble	
6d		Powder	Linear	131	Not soluble	
7	Laminaritriose	Lyophilized	Linear	3[c)]	Soluble	
8	Laminaripentaose	Lyophilized	Linear	5[c)]	Soluble	
9	Laminariheptaose	Lyophilized	Linear	7[c)]	Soluble	
10	Lentinan	Powder	Branched	—	Nonelastic gel	*Lentinus edodes*
11	HA β-glucan	Lyophilized	Branched	—	Nonelastic gel	*Pleurotus ostreatus*(Fr.) Quél.

a) Determined by the method of Manners et al. b) By gel filtration chromatography. c) Single species.

Results

Figure 2 shows the ^{13}C CP–MAS NMR spectra of four representative linear (1→3)-β-D-glucans in the solid state. These glucans are classified into three groups as viewed from peak-positions of the C-3 (and C-2,5) carbons (Table 2): (1) pachyman and curdlan giving the C-3a peak at 89.6±0.3 ppm, (2) laminaran showing the C-3b peak (at 85.5 ppm) (in addition to a minor peak of the C-3a (89.6 ppm)), and (3) paramylon exhibiting the three peaks, the aforementioned C-3a, and C-3b, and an additional C-3c (82.4 ppm). The spread of the C-3 NMR signals in paramylon (C-3a -C-3c) turned out to be about 8 ppm, as shown in Table 2. The C-1 peak, on the other hand, appears as a singlet in most cases, but as a doublet peak in paramylon. Next, we illustrate the ^{13}C CP–MAS NMR spectra of branched (1→3)-β-D-glucans isolated from various fungi in the solid state (Fig. 3). Here, the presence of two distinct peaks is evident in the C-3 region: the C-3a peak position of lentinan is exactly the same as that of curdlan or pachyman within the experimental error (±0.3 ppm), whereas the C-3b peak position of HA β-glucan is identical to that of laminaran (Table 2). Again, the spectral profile of the C-2 and C-5 region of HA β-glucan resembles that of laminaran (Fig. 2). In Fig. 4, we demonstrate how the ^{13}C NMR peak profiles are varied with their numerically averaged degree of polymerization ($\overline{DP}_n$) of acid-degraded curdlan. Apparently, the glucans with $\overline{DP}_n$ 380 exhibit

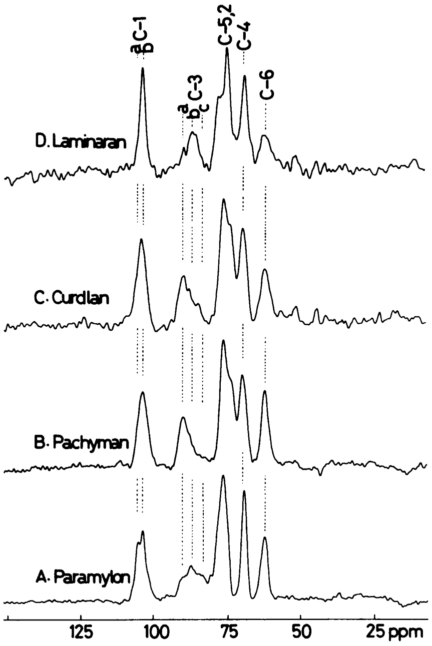

Fig. 2. 75.46 MHz ^{13}C CP–MAS NMR spectra of linear (1→3)-β-D-glucans in the solid state. A. paramylon, B. pachyman, C. curdlan, D. laminaran.

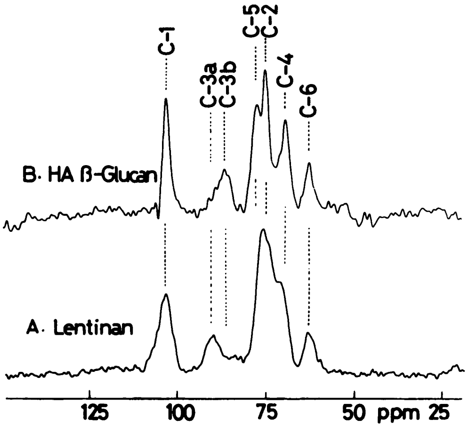

Fig. 3. 75.46 MHz ^{13}C CP–MAS NMR spectra of branched (1→3)-β-D-glucans in the solid state. A. lentinan, B. HA β-glucan. Number of transients is about 1000.

Table 2. ^{13}C Chemical Shifts of (1→3)-β-D-Glucans in the Solid State[a)]

	Form I		Form II			Form III		Paramylon
	Laminariheptaose	Laminaripentaose	Curdlan	Pachyman	Lentinan	Laminaran	HA β-glucan	
C-1	104.5	104.3	104.5	103.6	103.2	102.9	102.9	104.9
								103.3
C-2	72.9	73.0	73.5	73.0	73.3	73.9	73.6	75.6
C-3	91.3	91.1						90.2
			89.8	89.3	89.3			
						85.5	85.7	86.4
								82.4
C-4	69.8	69.4	68.8	69.6	69.1	67.8	67.9	68.6
C-5	76.1	75.8	75.5	75.5	75.0	76.6	76.0	75.6
C-6	61.5	61.6	61.8	61.6	61.8	61.7	61.5	61.7

a) ppm from TMS. experimental error ±0.3 ppm.

significant amount of the C-3b signals, although the peak height of the C-3a peak is higher than that of the C-3b peak.

In Figure 5, we summarized the ^{13}C CP-MAS NMR spectra of lower-molecular-weight oligomers, laminaritriose, laminaripentaose and laminariheptaose. Interestingly, all the ^{13}C NMR signals of these oligomers are identical within the experimental error (±0.3 ppm) by going from trimer to heptamer, except for the ^{13}C NMR signals arising from anomeric carbons of the terminal residues (peaks C-1a, and C-3a). The C-3 ^{13}C NMR peak positions of these oligomers (91 ppm) are significantly displaced downfield by about 1—2 ppm as compared with the corresponding peaks of curdlan, pachyman and lentinan, although the other carbon signals are identical to those of curdlan.

Further, Figure 6 summarizes the ^{13}C CP-MAS NMR spectra of a variety of (1→3)-β-D-glucans lyophilized from DMSO solution. The most notable feature is that DMSO molecule is incorporated into these polysaccharides as a quantitative manner (1/2 DMSO molecule per single residue), as manifested from the observed peak intensities. The linewidth of the C-3 peak as well as others is rather small for the samples prepared from DMSO solution as compared with that of samples lyophilized from aqueous medium. In addition, the peak positions of the C-3 signals were distinct from those of other preparations (Table 3) as shown in Figs. 1—3. On the contrary,

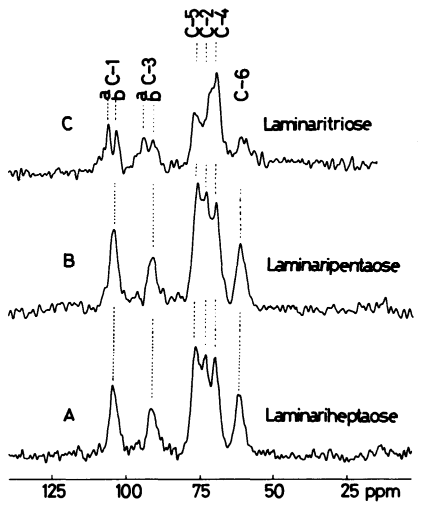

Fig. 5. 75.46 MHz ^{13}C CP-MAS NMR spectra of fractionated oligomers in the solid state. A. laminariheptaose, B. laminaripentaose, and C. laminaritriose.

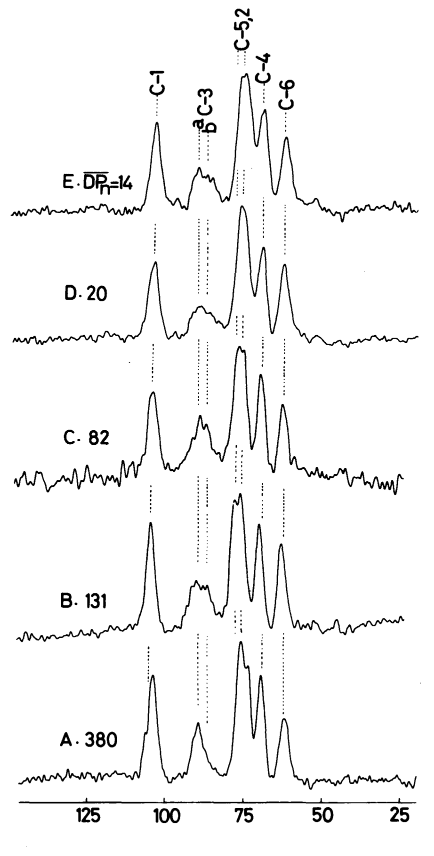

Fig. 4. 75.46 MHz ^{13}C CP-MAS NMR spectra of acid-degraded (1→3)-β-D-glucans and curdlan. $\overline{\mathrm{DP}}_n$: A, 380; B, 131; C, 82; D, 20; and E, 14.

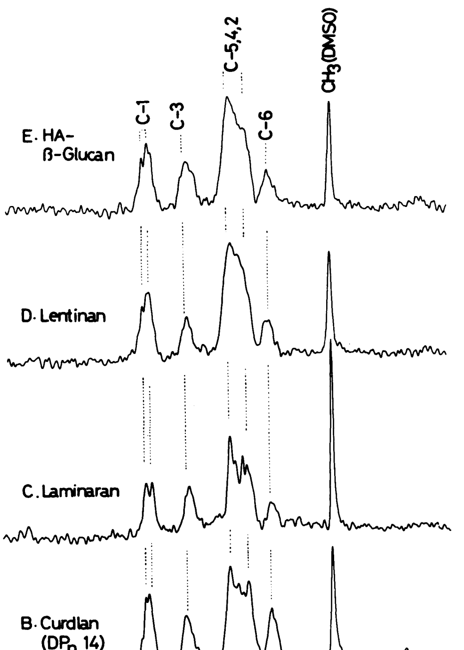

Fig. 6. 75.46 MHz ^{13}C CP-MAS NMR spectra of a variety of (1→3)-β-D-glucans lyophilized from DMSO solution in the solid state. A. curdlan, B. curdlan ($\overline{\mathrm{DP}}_n$ = 14), C. laminaran, D. lentinan, and E. HA β-glucan.

Table 3. ^{13}C Chemical Shifts of (1→3)-β-D-Glucans Lyophilized from DMSO Solution[a)]

	Curdlan ($\overline{\mathrm{DP}}_n=540$)	Curdlan ($\overline{\mathrm{DP}}_n=14$)	Laminaran	Lentinan	HA β-glucan
C-1	106.3	105.5	105.5	105.7	105.9
	104.5	103.9	103.4	104.2	104.1
					102.7
C-2	71.5	73.5	71.1	—	72.6
C-3	90.3	91.2	90.2	89.7	90.1
C-4	68.8	69.0	69.1	—	69.0
C-5	75.5	75.7	75.6	74.4	75.2
C-6	61.0	61.4	61.0	61.4	61.7
CH_3 (DMSO)	39.5	39.4	39.6	39.5	39.5

a) ppm from TMS.

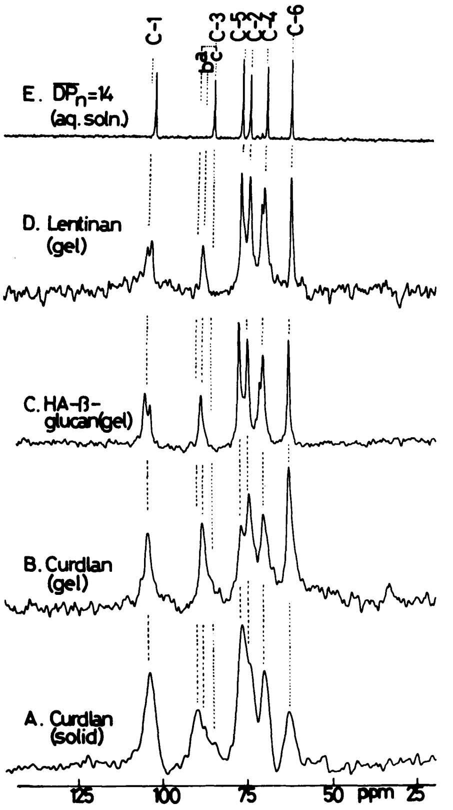

Fig. 7. Comparison of the ^{13}C NMR spectra of (1→3)-β-D-glucans among solid, gel and solution.

the C-1 signals are in many instances split into two separate peaks.

Finally, we recorded the ^{13}C NMR spectra of these (1→3)-β-D-glucans in the presence of excess water, as shown in Fig. 7. Interestingly, the ^{13}C NMR peaks of elastic gel of curdlan were well resolved at this higher frequency (75 MHz), in contrast to our previous observation at lower frequency (25 MHz).[6,7] Nevertheless, no ^{13}C NMR signals could be seen at neutral pH, in the case of nonelastic gels consisting of branched (1→3)-β-D-glucans. Consistent with our previous observation, ^{13}C NMR signals from these gel samples became visible when small amount of NaOH (less than 0.2 M) was added. Again, the ^{13}C NMR chemical shifts of these samples were found to be identical among various types of (1→3)-β-D-glucans such as curdlan, lentinan and HA β-glucan, in the gel state (Table 4), although conformations of these glucans in the solid state are quite different. It appears that the peak position of the C-3 carbon in the gel state is slightly displaced upfield (2 ppm) as compared with that of the solid state. On the contrary, these peaks are more distinct from those of the homogeneous aqueous solution ($\overline{\mathrm{DP}}_n=14$).

Table 4. ^{13}C Chemical shifts of (1→3)-β-D-Glucans in the Gel and Solution State (ppm from TMS)

	Gel			soln
	Curdlan	HA β-glucan	Lentinan	
C-1	104.0	104.5, 102.9	104.3, 102.9	102.7
C-2	73.5	73.4	73.5	73.5
C-3	87.5	87.5	87.5	84.5
C-4	69.1	68.9	69.1, 70.0	68.4
C-5	75.9	76.0	76.1	75.8
C-6	61.4	61.1	61.2	61.0

Discussion

Conformational Features in the Solid State. As previously demonstrated, ^{13}C chemical shifts of backbone carbons for a various types of molecular systems such as polysaccharides, polypeptides, and ionophores are significantly displaced (up to 8 ppm) together with their local conformations as described by a set of nearby torsion angles.[15,16,37–41] The advantage of ^{13}C NMR spectroscopy over other method is that conformational analysis can be done equally for noncrystalline as well as crystalline molecules. For polysaccharides, it is useful to examine displacements of ^{13}C NMR signals of carbons at the glycosidic linkages (C-1 and C-X carbons), because these ^{13}C signals are sensitively varied with any conformational change.[17,18,25] In fact, the maximal splitting of the C-3 chemical shifts of (1→3)-β-D-glucans is found to be as large as 8 ppm for paramylon (Table 2).

Interestingly, curdlan, pachyman, and lentinan gave rise to almost identical spectral features (Fig. 2) and these ^{13}C chemical shifts are identical within the experimental error (±0.3 ppm) (Table 2) in spite of differences in the primary structure. In particular, curdlan is completely a linear glucan containing no other linkage than β-(1→3)-linkages with $\overline{\mathrm{DP}}_n$=540, while pachyman ($\overline{\mathrm{DP}}_n$=250) contains a branch for every 50 β-(1→3)-linked glucose residues and one or two internal β-(1→6)-linkages. Lentinan, on the other hand, is a branched (1→3)-β-D-glucan with two branches for every five residues. In a similar manner, two different kinds of glucans, laminaran (linear) and HA β-glucan (branched), gave identical spectral patterns whose peak positions of these two glucans are the same within the experimental error but distinct from those of the glucans as mentioned above (see Figs. 2 and 3, and also Table 2). These data indicate that the presence (or the degree of) of branching at the C-6 position does not affect secondary structures of these branched glucans in the solid state as compared with that of the linear glucans, although this factor plays dominant role in gelation conditions of the former.[5,8] In addition, the ^{13}C NMR peak positions are the same among lower molecular oligomers, laminaritriose, laminaripentaose and laminariheptaose (Fig. 5 and Table 2). Further, the ^{13}C chemical shifts are identical among lyophilized samples from DMSO solution (Table 3). These findings strongly indicate that (1→3)-β-D-glucans so far studied take regular ordered conformations in spite of lacking in any treatment to improve the degree of crystallinity. Otherwise, it is expected that very broad envelope of ^{13}C NMR signals whose linewidths are as large as 8 ppm should be observed, because random coil form in the solid state is considered to be superposition of any conceivable several kinds of conformations.[42]

It is now possible to claim that at least four types of conformations for these (1→3)-β-D-glucans can be distinguished by the aforementioned characteristic displacements of the C-1 and C-3 peaks due to the conformation-dependent ^{13}C chemical shifts. For the sake of convenience, we tentatively classified conformations of these polysaccharides into the following four forms, I—IV, as viewed from the C-1 and C-3 peak-positions (Tables 2 and 3). Naturally, spectral patterns of the C-2 and C-5 peaks also could be varied to some extent by going from one form to another. On the basis of the aforementioned discussion, it is now clear that paramylon is not consisting of a single conformation but is a composite of at least three kinds of different conformations, as judged from the C-1 and C-3 ^{13}C chemical shifts (Table 2): form I (laminaripentaose-type), form III (laminaran-type) and an additional conformation giving rise to the peak at 82.4 ppm for the C-3 carbon. In fact, Marchessault et al. found that these split signals were converted to a single peak as a result of conformational change due to hydration.[30] Obviously, this kind of conformational heterogeneity seems to be essential to achieve better crystalline packing of paramylon granules, by analogy with the cases of higher crystalline samples as in cellulose[19–26] and polyethylene.[43,44] In this connection, crystallinity of paramylon granules is highest (≈90%) among a series of (1→3)-β-D-glucans.[45] By contrast, crystallinity of curdlan powder is only 30%[45] which does not require the presence of conformations more than the single species, as judged from the ^{13}C NMR signals.

In this connection, it is worthwhile to point out that conformation of native curdlan obtained as surface matter stripped from the colonies grown on solid culture of *Alcaligenes faecalis* is essentially the same as that of alkaline regenerated curdlan reported herein, as judged from the similarity of the ^{13}C NMR spectra,[29] in contrast to the similar pair of native and regenerated celluloses.[19–26]

The C-3 ^{13}C NMR signals of laminariheptaose and laminaripentaose (form I) are resonated at the lowermost region (91.2±0.2 ppm) and are clearly distinguishable from those of other three forms as shown in Tables 2 and 3. In fact, this peak is displaced downfield by 1.6 ppm with respect to that of curdlan and pachyman (form II)(89.6±0.3 ppm). For this reason, conformation of higher-molecular-weight (1→3)-β-D-glucan ($\overline{\mathrm{DP}}_n$=250), curdlan and pachyman, is not always identical to that of lower-molecular-weight glucans (DP$_n$=3, 5, and 7). Instead, the torsion angles ψ might be different to some extent as viewed from the difference in the C-3 signals, although the ϕ angle might be the same as indicated in the similarity of the C-1 signals among these glucans.

On the other hand, the C-1 and C-3 ^{13}C chemical shifts of laminaran and HA β-glucan are resonated at 103.2 and 85.7 ppm (C-3b of Figure 3), respectively, and these peak positions are resonated significantly at higher field, as compared with those of the forms I and II (Table 2). The presence of this form is ascribed to its lower molecular weight in the case of linear (1→3)-β-D-glucans as in laminaran ($\overline{\mathrm{DP}}_n$=38). This view was further confirmed by the fact that lower-molecular-weight acid-degraded curdlans, whose $\overline{\mathrm{DP}}_n$'s are between 14 and 131, contain significant amount of this form (40—10%), as seen from Fig. 8 where relative proportion of the form II (C-3a) is plotted against $\overline{\mathrm{DP}}_n$'s (thus, contribution of the form III (C-3b) is 100-contribution of the form II %). In a similar manner, paramylon does not form a gel. Accordingly, lower-molecular-weight (1→3)-β-D-glucans

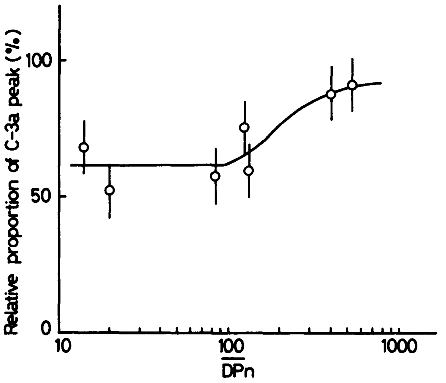

Fig. 8. A plot of the relative proportion of the C-3a peak (form II) against $\overline{\mathrm{DP}}_n$.

taking either the forms I or III do not have gel-forming ability. In this connection, it is probable that molecular weight of HA β-glucan is rather low as in laminaran on the basis of the C-3 ^{13}C peak position (Fig. 3). Nevertheless, molecular weight of this sample estimated from a study of gel permeation chromatography is an order of 10^6 (unpublished finding). As to molecular weights of branched glucans, however, caution must be exercised about reliability of data, because aggregation phenomenon leading to infinite gel network at neutral pH or lower alkaline (<0.2 M NaOH) state would make apparent molecular weight significantly overestimated (infinite for gel!). To make situation more complicated, gelation condition as a function of molecular weight is strongly modified for branched glucans as compared with that of linear glucans. In particular, a lower-molecular-weight fraction of lentinan (fraction IV, M.W. 16200) was found to be gel-forming in spite of its lower molecular weight.[8] An explanation for this is that number of physical cross links is effectively increased for branched glucans.[8]

Alternatively, Marchessault et al.[30] suggested that the differences in the degree of hydration might strongly modify the spectral patterns of (1→3)-β-D-glucans as described above. This view, however, is not necessary true for an interpretation of the conformational heterogeneity as encountered for a variety of samples listed in Table 1, because there appears no distinct change about the degree of hydration among the samples of lyophilization or spray-drying, which were kept long time at an ambient temperature. It is also worthwhile to consider the difference in the degree of branching between lentinan and HA β-glucan. Lentinan has two branches for every five D-glucose residues,[11] while HA β-glucan has one branch for every four D-glucose residues[36] (see Fig. 1). Accordingly, there appears no direct relation between the degree of branching and molecular conformations so far elucidated.

As described already, lyophilized samples from DMSO solution take a regular conformation as manifested from observation of relatively narrow ^{13}C NMR signals. In particular, the C-1 ^{13}C signals of these samples are split into doublet signal at 105.8 and 104.0 ppm (Fig. 6). As an interpretation of this splitting, it is natural to postulate that there exist two kinds of different conformers in this form (form IV), although peak splitting of the C-3 ^{13}C signal is not pronounced. Alternatively, conformation of two chains is identical but splitting of peak is distinguishable by the effect of hydrogen bonding to one of chains with DMSO molecule.

Conformations in the Gel State. It appears that the ^{13}C NMR linewidths of the gel samples recorded at 75 MHz are much narrowed and excellent spectral separation is achieved in this study of higher frequency (especially at the region of C-2, 4, and 5) (Fig. 7). The observed linewidths in this case, however, are about 100 Hz and the extent of line narrowing as compared with the data of 25 MHz is about 30% for the C-1 and C-3 signals. This observation might be mainly ascribed to a plausible reduction in the number of effective cross links formed by multiple helical junction zones or aggregate of these regions, because the present spectra were recorded at a lower concentration (20 mg ml^{-1}) (one fifth of those of low frequency measurements). Naturally, the ^{13}C chemical shifts of gel samples in this study are in good agreement with our previous data.[4–9] However, more accurate data about the ^{13}C chemical shifts were obtained in this study because of much improved separation of peaks. Thus, it is now clear that the ^{13}C NMR peaks of curdlan gel are displaced downfield by 1.3 and 3.0 ppm for the C-1 and C-3 carbons, respectively, from those of water-soluble low-molecular-weight glucan taking random coil conformation, but the rest of peaks is unchanged by going from the gel to random-coil form (Table 4).

In spite of obvious differences in the conformations between curdlan (and lentinan) and HA β-glucan in the solid state, we found that exactly the same spectral features were observed between the two samples in the gel state (Fig. 7). In our previous papers,[5,29] we concluded that conformation of flexible molecular chains in curdlan, which is visible by high resolution ^{13}C NMR spectroscopy, is identical to that of the solid state. However, closer examination of the ^{13}C chemical shifts of these gel samples (Tables 2 and 4) shows that the C-3 ^{13}C chemical shifts of the gel samples appear at the midpoint of those of forms II and III, although no appreciable change was noted for the rest of peaks. The displacements of peaks in each case are 2—3 ppm and can be regarded as significant change, even if contribution of a plausible experimental error

(<1 ppm) is taken into account.[46] This finding implies that a sort of conformational rearrangement in association with hydration is required to form gel when solid samples of linear glucans with form II or of branched glucans with form II or III were used. It is conceivable that conformational change would be induced when these samples are hydrated to some extent. Consistent with this view, existence of polymorphic structures depending on humidity or hydration was demonstrated for curdlan by means of X-ray diffraction study.[12,30]

Concluding Remarks.

We found that (1→3)-β-D-glucans obtained either from lyophilization or spray-drying take regular secondary conformations as viewed from high-resolution solid-state ^{13}C NMR spectroscopy, in spite of inherent poor crystallinity. Obviously, this is the major advantage of the ^{13}C NMR spectroscopy as a new means of conformational elucidation complementary to X-ray diffraction. It is demonstrated that these secondary forms can be classified into at least four forms, on the basis of the conformation-dependent displacements of peaks at the C-1 and C-3 carbons. As a result, we concluded that paramylon with crystallinity of over 90% is not consisting of molecular chains of single conformation but a composite of at least three different conformers. In addition, we found that gelation property of linear glucans is related to the presence of particular conformation in the solid state. This view, however, must be modified to some extent in branched glucans, because gelation condition is not simple as in linear glucans.

We are grateful to Takeda Chemical Industries for providing us with curdlan and its acid-degraded samples, Dr. Goro Chihara for gift of lentinan and pachyman, and Professor B. A. Stone for gift of paramylon.

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46) In this connection, it is important to consider a plausible contribution from the difference in molecular packing, by going from the solid to gel state, to the displacements of the ^{13}C chemical shifts (2—3 ppm for the C-3 carbon). As a means to estimate the extent of molecular packing, VanderHart compared the ^{13}C chemical shifts of *n*-alkane of different crystalline structure but the same molecular conformation and showed a separation of peaks 1.3 ppm in the resonance position of the internal methylene resonance.[47] In a same manner, Sorita et al. noticed the difference of the ^{13}C chemical shift due to molecular packing as large as 0.9 ppm for the system of *cyclo*- and *n*-alkanes.[48] Accordingly, it is obvious that such a contribution by molecular packing, if any, cannot well explain the observed displacements of peaks. Moreover, it is pointed out that significant displacements of peaks by going from the solid to gel state is limited to the C-3 peak alone, and the rest of peaks is unchanged within the experimental error. Therefore, it is reasonable to ascribe the significant displacements of peaks to a plausible conformational change, as treated in the present article.

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