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A ^{13}C-NUCLEAR MAGNETIC RESONANCE STUDY OF POLYSACCHARIDE GELS. MOLECULAR ARCHITECTURE IN THE GELS CONSISTING OF FUNGAL, BRANCHED (1 → 3)-β-D-GLUCANS (LENTINAN AND SCHIZOPHYLLAN) AS MANIFESTED BY CONFORMATIONAL CHANGES INDUCED BY SODIUM HYDROXIDE

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ABSTRACT

To gain further insight into the architecture of the gel network of some branched (1 → 3)-β-D-glucans, a ^{13}C-n.m.r. study of sodium hydroxide-induced, conformational change was performed. The branched D-glucans examined were lentinan from *Lentinus edodes*, a lower-molecular-weight fraction thereof, and schizophyllan from *Schizophyllum commune*; these (1 → 3)-β-D-glucans have two branches for every five D-glucopyranosyl residues (lentinan), or one for every three or four (schizophyllan) at O-6. In contrast to the gel of linear (1 → 3)-β-D-glucan (curdlan), all of the ^{13}C signals due to the β-D-(1 → 3)-linked D-glucosyl residues were completely suppressed in the gel state. As the peak intensity and line width of the ^{13}C-resonance peaks for the gel state are strongly influenced by the degree of cross-linking, such a complete loss of the peak areas can be explained in terms of a higher degree of cross-linking than that of the linear D-glucans. As demonstrated previously, the cross-links involve physical association of the helical segments, such as the double- or triple-stranded helices. These helix forms were found to be converted, at 0.2M sodium hydroxide, into the random-coil form (gel-to-sol transition), which gives rise to full peak-areas, because of complete breaking of the physical cross-links. Also, in contrast to the linear D-glucan, such helix–coil transition of the branched D-glucans proceeded in a noncooperative way: the peak intensity and line width gradually changed with the concentration of sodium hydroxide. This behavior is best interpreted in terms of distribution of the various degrees of cross-linking. Some loose cross-links are readily broken in the lower range of concentration of alkali (0.09M), and others are resistant until complete conversion into the random coil occurs (0.2M). This result is consistent with the view that the primary structure of the branched (1 → 3)-β-D-glucans is highly branched, as in a tree-like structure.

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INTRODUCTION

Recently, it has been demonstrated that ^{13}C-nuclear magnetic resonance (n.m.r.) spectroscopy is a very powerful tool for studying the architecture of the network of various types of gels, such as those of polysaccharides[1–4] and chemically cross-linked gels[5,6]. Apparently, in such gels, all parts of the molecules do not always contribute to the ^{13}C-resonance signals: considerable proportions of the peak areas are lost[1–4,6], depending upon the extent of the cross-linking[4,6], either by covalent bonds chemically introduced, or by physical association of constituent polymers. Such an apparent loss of the peak areas could be ascribed to the regions of the cross-links and to segments in their vicinity, their rate of molecular tumbling being too slow to give high-resolution ^{13}C-signals. In some instances, ^{13}C-n.m.r. peaks of the backbone were completely lost, as with the gels of *ι*-carageenan[7] and branched (1 → 3)-*β*-D-glucans[1,3]. For the latter, however, we reported that the ^{13}C-resonance peaks characteristic of the helix form (downfield displacement of the C-1 and C-3 signals by 3.0 and 2.8 p.p.m. with respect to the random-coil conformation, at pH 7) can be clearly seen in the presence of low concentrations[1,3] of alkali. For this reason, the cross-links of the branched (1 → 3)-*β*-D-glucans might be essentially the same as those in the linear (1 → 3)-*β*-D-glucan[2], the structure of which is composed of multiple-stranded helices. The presence of single and triple helices was recently confirmed by X-ray diffraction studies of curdlan and lentinan in the fiber state[8–11].

Thus, such a complete loss of the peak areas in the spectra of gels of the branched D-glucans might be explained in terms of the presence of a higher degree of cross-linking compared with that of the linear D-glucan, as the peak intensity and line width of the ^{13}C-resonance signals could be strongly influenced by the degree of the cross-linking[4,6]. In order to delineate the architecture of the gel network, we have undertaken a ^{13}C-n.m.r. study of sodium hydroxide-induced, helix–coil transition of the branched D-glucans, because stepwise addition of sodium hydroxide is a very effective means of successively breaking the physical cross-links. There exist various degrees of cross-linking in the branched D-glucans: some loosely held cross-links are broken in the presence of low concentrations ($<$0.09M) of alkali, and others are resistant until complete conversion into the random coil occurs. This observation is consistent with the view that the backbone is highly branched, as in a tree-like structure.

EXPERIMENTAL

Materials. — Materials used were branched (1 → 3)-*β*-D-glucans isolated from fungi. Lentinan[12,13] (from *Lentinus edodes*), having a backbone consisting of *β*-D-(1 → 3)-linked D-glucosyl residues containing branch points (two for every five D-glucosyl residues) at O-6 for *β*-D-(1 → 6)-linked (8%) and *β*-D-(1 → 3)-linked D-glucosyl residues, was provided by Dr. G. Chihara of this Institute. Low-molecular-weight D-glucan from lentinan (fraction IV) was prepared with formic acid as described[13]. Schizophyllan[14] (from *Schizophyllum commune*), which has a β-D-(1 → 3)-linked backbone carrying at O-6 a single D-glucosyl group, was a gift of Dr. S. Kikumoto of Taito Co., Ltd. These samples did not give aqueous solutions (100 mg/mL) at neutral pH, but formed soft gels. In contrast to curdlan 13140 [a bacterial, linear (1 → 3)-β-D-glucan], no elasticity was gained, even if these branched D-glucans were heated at temperatures up to 70° and cooled to room temperature.

^{13}C-N.m.r. spectroscopy. — ^{13}C-N.m.r. spectra were recorded with a JEOL PFT-100/EC-100 spectrometer operating at 25.03 MHz. The 90° pulse, requiring 20 μs, was used to accumulate the free-induction decays, with a repetition time of 0.6 s. A delay time (250 μs) was introduced between the end of the 90° pulse and the acquisition of the first data-point. All spectra were recorded by using 4K data points and a spectral width of 4 kHz. ^{13}C-Chemical shifts are expressed in p.p.m. downfield from external tetramethylsilane. The line width was taken as full width at half-height in an expanded spectrum, with an estimated error of ±10 to ±20%. The spin–lattice relaxation-times were obtained by using the pulse sequence of 180°–t–90°, with an estimated error of ±10%. Nuclear Overhauser enhancements (nOe values; maximum value, 3) were measured by the gated-decoupling method (±15%). To monitor the conformational transition, conc. sodium hydroxide solution was added stepwise to samples contained in 10-mm (o.d.) sample-tubes.

Interaction with Congo Red. — The complex-formation of the branched D-glucans (1 mg/mL) with Congo Red (38μM) was evaluated from the shift in the visible absorption maximum of Congo Red induced by the presence of D-glucans in 0.1M sodium hydroxide at 20°.

RESULTS AND DISCUSSION

A. *Conformational change of lentinan and its low-molecular-weight fraction*

Lentinan. — The ^{13}C-n.m.r. peaks of the β-D-(1 → 3)-linked D-glucosyl residues of lentinan in the neutral, gel state were completely suppressed (see Fig. 1A), due to the immobilization of the molecular chain, mainly as a result of the presence of physical cross-links[3]. The less-intense signals in Fig. 1A were ascribed to the β-D-(1 → 6)-linked D-glucosyl residues (8%; full peak-areas being observed[3]), present as a minor component located in a more flexible position. The ^{13}C-signals of the major, β-D-(1 → 3)-glucosidic linkages, however, were revealed on stepwise addition of sodium hydroxide solution to the gel sample (see Figs. 1B–1F). In Fig. 2 are plotted the ^{13}C-peak positions both of the β-D-(1 → 3)- and β-D-(1 → 6)-linked D-glucosyl residues against the concentration of sodium hydroxide, as the ^{13}C-shifts of the former (C-1 and C-3) are very sensitive to sodium hydroxide-induced, conformational change[2]. Most of the peaks arising from both the β-D-(1 → 3)- and the β-D-(1 → 6)-linked D-glucosyl residues, except for C-3 and C-6 of the β-D-(1 → 3)-glucosidic linkages, might overlie each other, in view of the ^{13}C-chemical shifts of each component[3]. Fortunately, however, the C-1 signal of the β-D-(1 → 3)-linked D-glucosyl residues (peak a) can be readily distinguished from that of the β-D-(1 → 6)-linked D-glucosyl

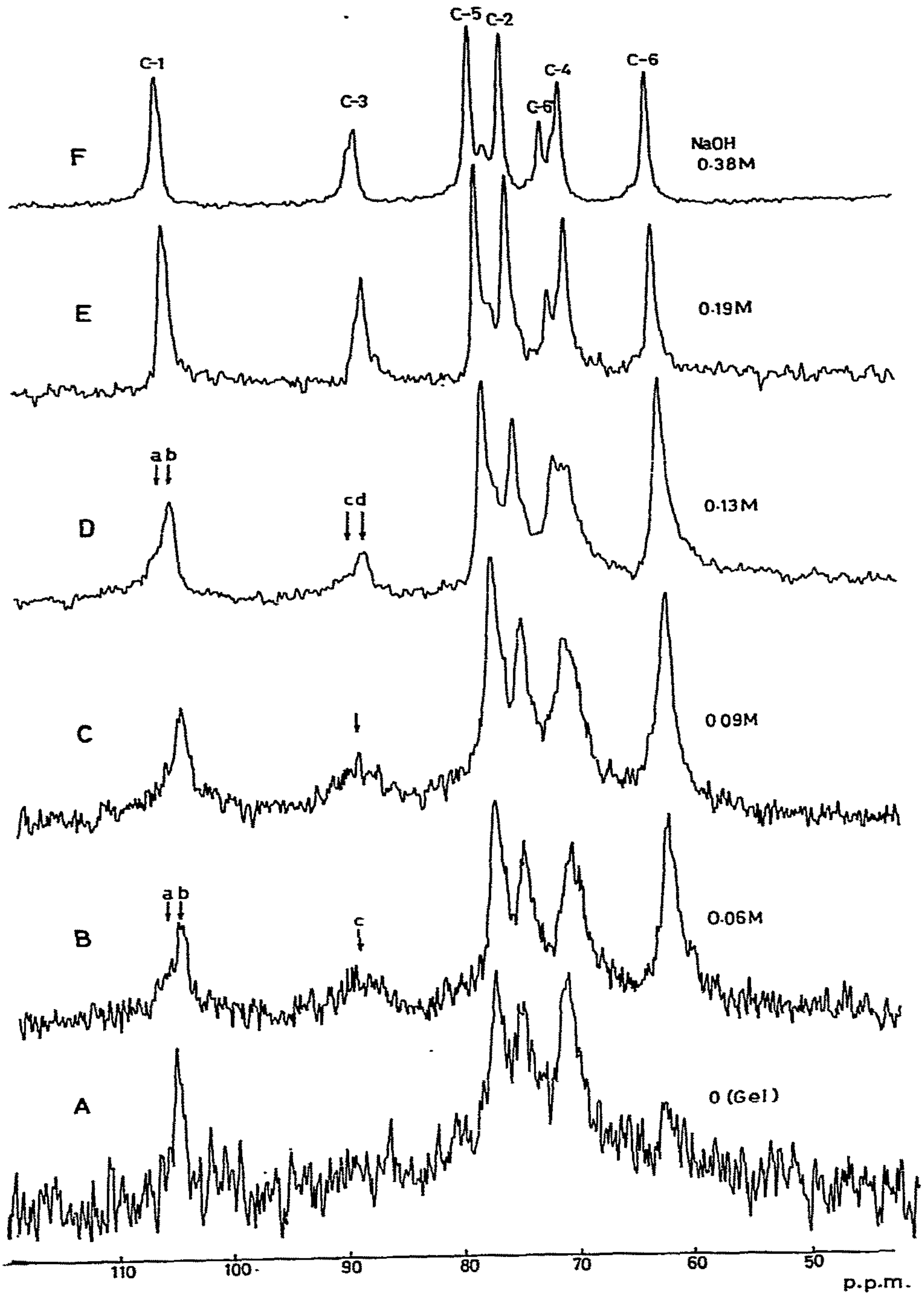

Fig. 1. ^{13}C-N.m.r. spectra of lentinan in the gel state, and the changes with concentration of sodium hydroxide. [A, Neutral (82,000 accumulations); B, 0.06M NaOH (48,500 accumulations); C, 0.09M NaOH (103,500 accumulations); D, 0.13M NaOH (78,000 accumulations); E, 0.19M NaOH (65,000 accumulations); and F, 0.38M NaOH (77,000 accumulations).]

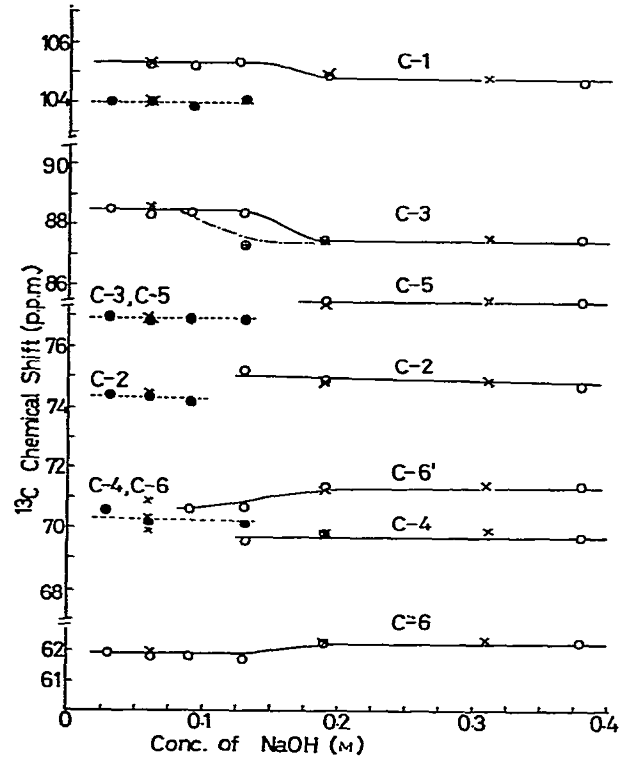

Fig. 2. Changes in the ^{13}C-chemical shifts of lentinan and fraction IV *vs.* the concentration of sodium hydroxide (the peak marked C-6′ being ascribed to C-6 of the branch points). [β-D-(1 → 3)-Glucosyl residues, —O—: lentinan; —⊕—: narrow component of lentinan at 0.13M NaOH (peak d of Fig. 1D); —×—: fraction IV. β-D-(1 → 6)-Glucosyl residues, ... ●...: lentinan; ... ×...: fraction IV.]

residues (peak b), by utilizing larger differences in the line widths in the gel states (0.06M to 0.19M NaOH) (see Fig. 1). It is also emphasized that the ^{13}C-signals of β-D-(1 → 6)-linked D-glucosyl residues are buried under the rather intense signals of the β-D-(1 → 3)-linked D-glucosyl residues for the spectra recorded at concentrations of sodium hydroxide higher than 0.13M, as may be seen from the discontinuity in the plot of the ^{13}C-peaks (see Fig. 2), because of low abundance (8%) of the former.

The ^{13}C-chemical shifts found for C-1 and C-3 of the β-D-(1 → 3)-linked D-glucosyl residue are in good agreement with those of the linear (1 → 3)-β-D-glucan (curdlan), both at the lower (0.03–0.13M) and higher (0.19 and 0.38M) concentrations[2] of alkali. Therefore, it was concluded that the conformation of the β-D-(1 → 3)-linked D-glucosyl residues of lentinan is the helix in the lower (including neutral state), and the random-coil form in the higher, concentration of alkali. Thus, between 0.13 and 0.19M NaOH, the displacements of the ^{13}C-peak positions of C-1 and C-3 may be ascribed to sodium hydroxide-induced, helix–coil transition (gel–sol transition also, macroscopically). Interestingly, such a transition is complete at almost the same concentration (0.2M) of sodium hydroxide for both the linear[2] and the branched D-glucans. At an intermediate stage of the helix–coil transition (0.13M; see Fig. 1D), however, an asymmetrical peak-profile appears for C-3, which can be resolved into two components, the broad (peak c) and the narrow (peak d). The former and latter peaks are readily assigned to the helical and random-coil forms, respectively, in view of the position of the particular peak. This observation indicates that there exists some proportion of lentinan that is readily convertible into the random-coil form at lower alkaline concentrations, in contrast to the case of the linear D-glucan.

In parallel with this observation, the line width of the C-3 signal of the β-D-(1 → 3)-linked D-glucosyl residues decreases gradually with the concentration of sodium hydroxide, as a result of a broad, helix–coil transition (see Fig. 3). Interestingly, this behavior is very similar to the change of the absorption maximum of Congo Red complexed with lentinan, as is shown in Fig. 4. Furthermore, it is likely that the peak-intensities of the β-D-(1 → 3)-linked D-glucosyl residues also gradually increase with the concentration of sodium hydroxide*. This situation may be more clearly seen in a plot of the relative peak-intensities of C-1 and C-3 (with respect to those observed at 0.38M NaOH) against the concentration of sodium hydroxide (see Fig. 5). Again, the change of the relative peak-intensities agrees very well, qualitatively, with that of the line width (see Fig 3) and of the absorption maximum of Congo Red (see Fig. 4). The changes of these three characteristics of the conformational transition for the branched (1 → 3)-β-D-glucan are in distinct contrast to those of the linear (1 → 3)-β-D-glucan: for the latter, all of these parameters exhibit[2,15] abrupt changes at 0.22M

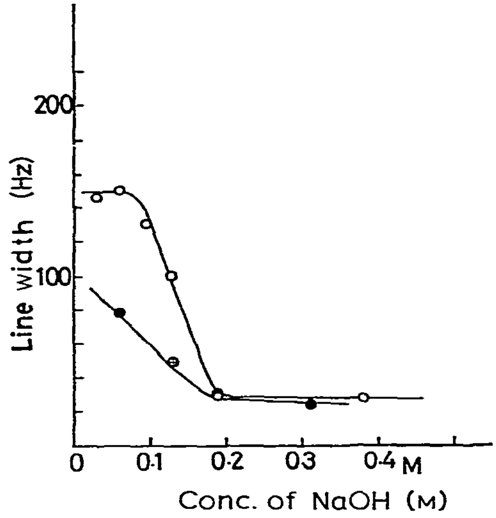

Fig. 3. A plot of the ^{13}C-n.m.r. line-widths (C-3) of lentinan and fraction IV *vs.* the concentration of sodium hydroxide. [○: Lentinan, ⊕: narrow component of lentinan (peak d), ●: fraction IV.]

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*An attempt was made to take into account a difference in the nOe values between the helix (1.1) and the random-coil (1.5) conformations. These values are taken from the experimental value obtained for solution in dimethyl sulfoxide (random-coil) (see Table I) and from the value for the linear (1 → 3)-β-D-glucan (helix form)[2].

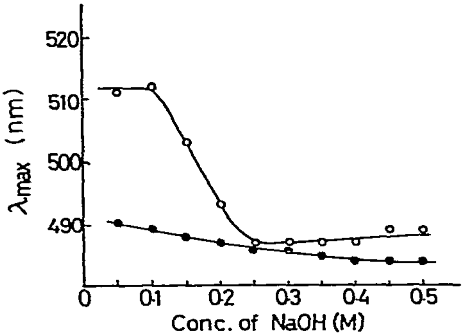

Fig. 4. A plot of the absorption maximum of Congo Red complexed with lentinan *vs.* the concentration of sodium hydroxide. [○: In the presence of lentinan; ●: in the absence of lentinan.]

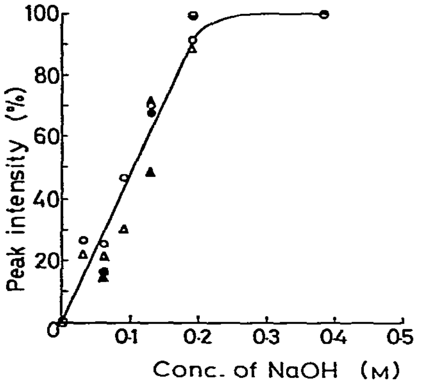

Fig. 5. A plot of the relative peak-intensities of C-1 and C-3 of β-D-(1 → 3)-linked D-glucosyl residues for lentinan and schizophyllan with respect to those at 0.38M NaOH (lentinan) and 0.19M NaOH (schizophyllan) *vs.* the concentration of sodium hydroxide. (Lentinan, △: C-1; ○: C-3; and ◓: reference. Schizophyllan ▲: C-1; ●: C-3; and ◒: reference.)

NaOH; presumably, this is due to a difference in the molecular architecture of the gel network between the linear and branched D-glucans, as will be discussed in more detail later.

Previously, we had shown that the shift of the absorption maximum of Congo Red attached to (1 → 3)-β-D-glucans is characteristic of the presence of the single-helix conformation[2,4]. This view is also supported by the results of Ogawa and Hatano[16] that, on the basis of circular dichroism measurements, show that the single-helix part of the D-glucan chain produces the complex with Congo Red. Thus, the gradual change in the shift of the absorption maximum of Congo Red with the concentration of sodium hydroxide, in contrast to the behavior of the linear (1 → 3)-β-D-glucans, may be satisfactorily explained by the view that the relative proportions of the helix and the random-coil conformations change with the concentration of sodium hydroxide between 0.1 and 0.2M. Bearing in mind that the difference of the chemical shift (Δ) between the helix and the random-coil conformations is 1.1 p.p.m. (see Fig.

2), the time needed for the interconversion, if it occurs, between the helix and the random-coil forms should be much less than 60 s, using the formula[17] $k < (\pi/\sqrt{2})\Delta$.

Fraction IV. — We also examined the sodium hydroxide-induced change in the ^{13}C-n.m.r. spectrum of lower-molecular-weight lentinan, namely, fraction IV, mol. wt. 16,200 (see Fig. 6). As with the original lentinan, no ^{13}C-signals due to β-D-(1 → 3)-linked D-glucosyl residues could be seen for the neutral, gel state (except for a low-intensity C-6 signal)[3]. Fig. 6A shows the ^{13}C-signals of the β-D-(1 → 3)-linked D-glucosyl residues, which appear in the presence of lower concentrations (0.06M) of alkali. It appears that the line widths of the C-1 and C-3 signals of the single-helix form are much smaller than those of the original lentinan (approximately half the width of those of the latter). As described previously[4], the line width for the helical, β-D-(1 → 3)-linked, D-glucosyl residues varies with the number average degree of polymerization of the primary molecules, suggesting that the line width is mainly

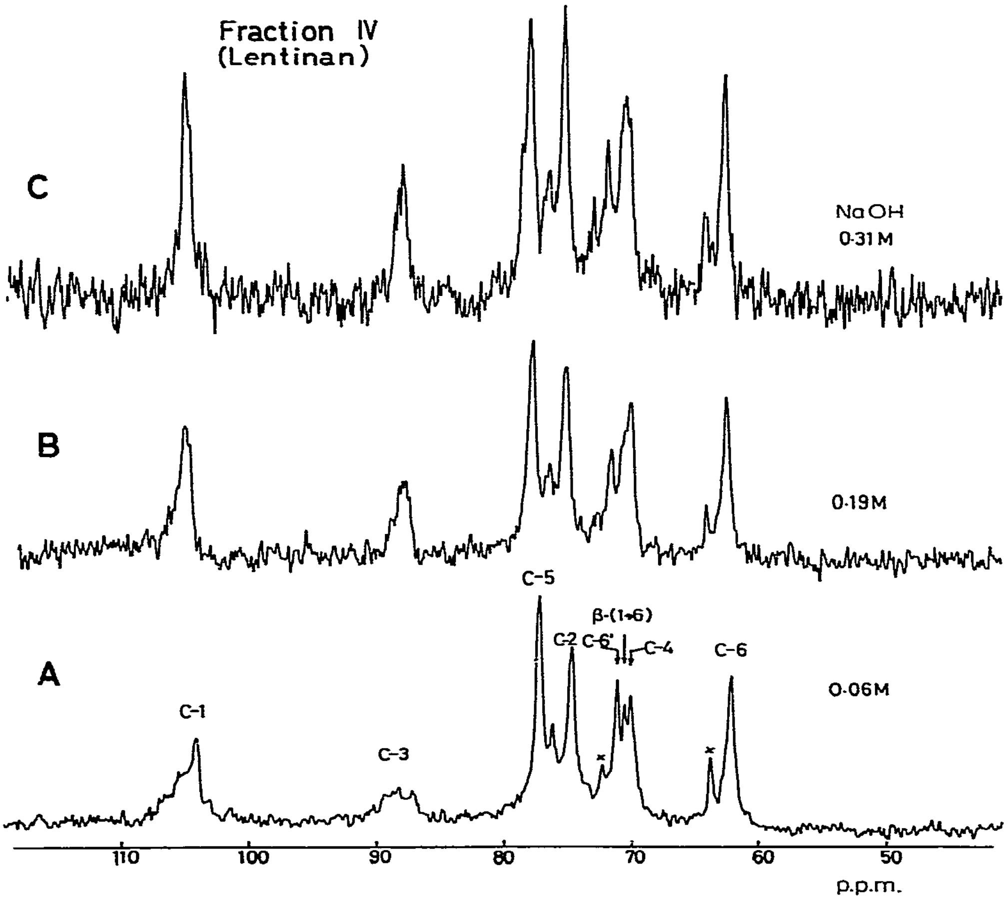

Fig. 6. ^{13}C-N.m.r. spectra of fraction IV in the presence of sodium hydroxide. [A, 0.06M NaOH (85,000 accumulations); B, 0.19M NaOH (18,000 accumulations); and C, 0.31M NaOH (15,500 accumulations).]

determined by the degree of cross-linking[6], by formation of double- or triple-helical junction-zones[2,4]. It may be expected that the branched structure is more favorable for the formation of the physically cross-linked structure, in view of the classical theory of gelation[18,19]. Accordingly, the line width for fraction IV results in a value larger than that expected from the molecular-weight-dependence of the linear D-glucans[4]. In agreement with expectations, the final helix–coil transition was found to occur at the same alkaline concentration (0.19M NaOH) as for the original lentinan

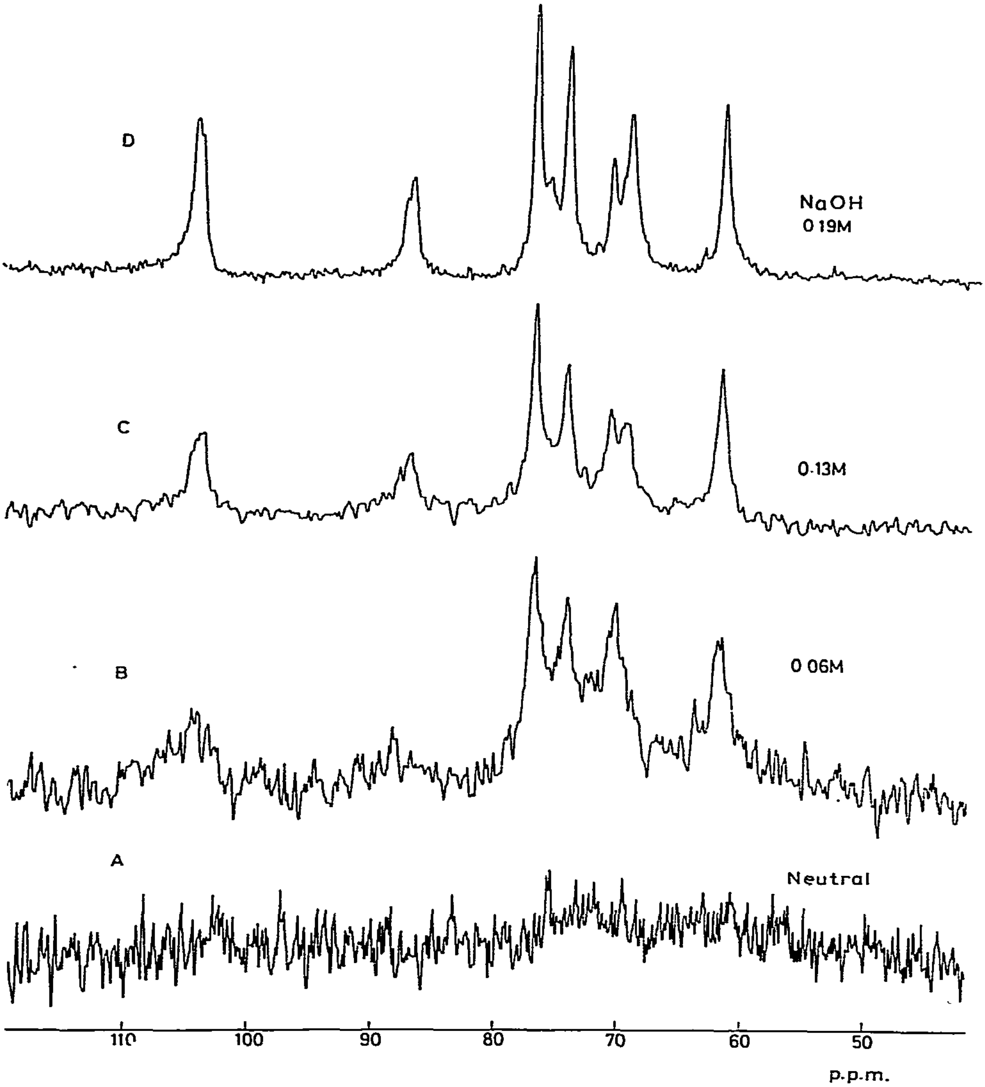

Fig. 7. ^{13}C-N.m.r. spectra of schizophyllan in the gel states and their changes with the concentration of sodium hydroxide. [A, Neutral (64,000 accumulations); B, 0.06M NaOH (125,199 accumulations); C, 0.13M NaOH (26,500 accumulations); and D, 0.19M NaOH (82.503 accumulations).]

(see Fig. 6). In Figs. 2 and 3, the chemical shifts (closed circle) and line width (X) of fraction IV are indicated.

B. *Conformational change of schizophyllan*

Schizophyllan is also a branched (1 → 3)-β-D-glucan; from chemical analysis, it has a single D-glucosyl group as a branch, the distance (three to five D-glucosyl residues) between the branch points being very similar to that in lentinan. This is consistent with the observation that very similar ^{13}C-spectra (having the same peak-positions and relative peak-intensities) are given by lentinan and schizophyllan in dimethyl sulfoxide, or in the more highly alkaline solutions. At neutral pH, however, all of the ^{13}C-n.m.r. signals disappear completely for the soft-gel state[3], as with lentinan and A_3 (ref. 1). Again, the ^{13}C-n.m.r. peaks of the β-D-(1 → 3)-linked D-glucosyl residues of schizophyllan may be seen on stepwise addition of sodium hydroxide, as illustrated in Fig. 7. Examination of the ^{13}C chemical-shifts of C-1 and C-3 also reveals that the backbone, β-D-(1 → 3)-linked D-glucosyl residues adopt the single-helix conformation at the lower concentrations of sodium hydroxide ($<$0.13M). It is also found that the changes induced by sodium hydroxide in the line width, peak intensity, and chemical-shift position observed in the spectrum of schizophyllan are very similar to those for lentinan and fraction IV. In addition, the relative peak-intensities of schizophyllan, with respect to those at 0.19M NaOH, increase gradually with the concentration of sodium hydroxide, in parallel with those of lentinan (see Fig. 5).

This result strongly supports the conclusion that organization of the gel network is very similar among the branched (1 → 3)-β-D-glucans studied herein. It is also emphasized here that the asymmetrical nature of the C-3 peak-profiles, due to the presence of both the helix and the random-coil forms (0.13M NaOH), also appears in the present instance, as an intermediate of the conformational change from the helix to the random-coil form.

C. *Gel networks, and dynamic aspects of branched* (1 → 3)-*β*-D-*glucans*

There are some obvious differences between the physicochemical properties of the linear and branched (1 → 3)-β-D-glucans**. In particular, branched D-glucans form soft gels without heating, whereas the linear glucan is capable of forming a firm, elastic, resilient gel[20,21] after heating at a temperature above 55°. Furthermore, turbidity develops in the elastic gel of the linear D-glucan either when the gels are prepared at higher temperatures[20,21] (high-set gel, > 80°), or when the gels are prepared at a lower temperature (60°, low-set gel) and kept for a long time[4]. In contrast, no elasticity was gained in the gels of the branched D-glucans. This distinction may be a reflection

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**Because ^{13}C-resonance peaks of β-D-(1 → 6)-linked D-glucosyl residues of lentinan are fully observed in the neutral gel-state, there appears to be no specific role for this linkage in gel formation. Thus, the gelation phenomenon may be discussed solely in terms of the association of β-D-(1 → 3)-linked D-glucosyl residues.

of a difference in the molecular organization of the gel networks. Nevertheless, it should be emphasized that, both for the linear and the branched D-glucans, the conformation of the molecular chain (other than the cross-links) is a single helix, and the cross-links for the gelation are composed of the multiple-stranded helix.

From chemical analysis, the primary structure of lentinan and schizophyllan was elucidated as that of (1 → 3)-β-D-glucans having two branches for every five D-glucosyl residues (lentinan)[13] or one for every three or four residues (schizophyllan)[14]. From chemical analysis alone, however, it cannot be definitely determined whether type (A) (tree-like) or (B) (linear) depicted in Fig. 8 represents, at least, a plausible model of the primary structure for lentinan and schizophyllan. It is conceivable that a structure of type (A) would be more readily capable of forming a physically cross-linked structure (by multiple-stranding), because it is highly branched, than one of type (B), or a linear D-glucan such as curdlan.

However, in type (A), many of the cross-links may be loosely formed, in view of possible steric hindrance due to the presence of branching for the formation of the multiple-stranded helices, although the number of cross-links may be increased considerably. The present ^{13}C-n.m.r.-spectral results are consistent with this view: no ^{13}C-n.m.r. signal of the single-helix portion of the branched D-glucans in the neutral gel-state could be observed; this is obviously due to the increased number of cross-links in the gels of the branched D-glucans compared with those of the linear D-glucans. The loosely formed cross-links (very short, double- or triple-stranded helices) may be readily attacked by sodium hydroxide, resulting in diminution of the cross-links to cause a broader conformational change (helix–coil transition) at lower concentrations (0.1–0.2M) of sodium hydroxide. Firmly held cross-links resembling those in the linear D-glucans may be broken at 0.2M sodium hydroxide, the same concentration as for the linear D-glucan.

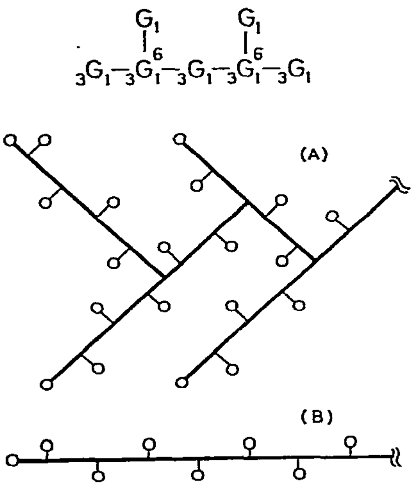
$$\begin{array}{ccccc} & G_1 & & G_1 & \\ & |_6 & & |_6 & \\ {}_3G_1- & {}_3G_1- & {}_3G_1- & {}_3G_1- & {}_3G_1 \end{array}$$

Fig. 8. Schematic representation of the primary structure of branched (1 → 3)-β-D-glucans (G = β-D-glucopyranosyl group or residue; A = tree-like; B = linear).

As already mentioned, for the linear D-glucan (curdlan)[4,20], a turbidity of the gels develops together with syneresis of the gels; this phenomenon was interpreted in terms of further aggregation of the multiple-stranded helices to form long microfibrils observable by electron microscopy[20,21]. For the gels of highly branched, (1 → 3)-β-D-glucans, as in the type (A) structure in Fig. 8, such an aggregation may be unfavorable, because of steric hindrance. Therefore, the absence of the syneresis phenomenon in the gels of the branched D-glucans also can be nicely accounted for on the basis of a type (A) structure.

The spin–lattice relaxation-times (T_1 values), nuclear Overhauser enhancements (nOe values), and line widths for lentinan are summarized in Table I. From these relaxation parameters, it may be possible to estimate the correlation times in order to describe the tumbling motion of the constituent β-D-(1 → 3)- and β-D-(1 → 6)-D-glucosyl residues. Intrinsically, the segmental motion of the backbone and side chains in the gel state may be highly heterogeneous, because of a possible distribution of segments in different physical environments. Therefore, it may be more appropriate to adopt the model of the log $-\chi^2$ distribution of the correlation times[22], as an isotropic reorientation. Although the segmental motion of the β-D-(1 → 3)-glucosidic residues cannot be obtained, because of the lack of relaxation parameters in Table I, it may be possible to infer the values from the proportion of the β-D-(1 → 6)-linked D-glucosyl residues as a limit of the fast motion. On the basis of the truncated, log $-\chi^2$ distribution[23] (at 1.000 μs), which was successful in the prediction of relaxation parameters for the elastic gel of the linear (1 → 3)-β-D-glucans[4], it was found that the average correlation-time ($\bar{\tau}$) 7 ns (width parameter $p = 14$) satisfactorily reproduces the relaxation times observed, as shown in Table II. Previously, we had shown[4] that the average correlation-time for the β-(1 → 3)-D-glucosidic residues of resilient gel is 40 ns, with $p = 8$. Thus, a correlation time of 7 ns may be reasonable as a proportion of the more

TABLE I

SPIN–LATTICE RELAXATION-TIMES (NT_1 VALUES IN ms), nOe VALUES, AND LINE WIDTHS (Hz) OF LENTINAN

Atom	*β-D-(1 → 6)-Linkages*			*β-D-(1 → 3)-Linkages*			
	Neutral gel-state[a]			*in Me$_2$SO solution*			*Alkaline (0.38 M NaOH)*
	T_1	*nOe*	*Line width*	T_1	*nOe*	*Line width*	T_1
C-1	64	1.3	28	81	1.5	20	79
C-2	70	1.1	50	81	1.6	20	73
C-3	70	1.5	50	68	1.4	44	58
C-4	46	1.4	55	64	1.5	22	57
C-5	70	1.5	55	93	1.2	28	76
C-6	92	1.4	55	86	1.5	20	115

[a] All ^{13}C-signals of the β-D-(1 → 3)-linked backbone disappear in the neutral gel-state (line width > 1.000 kHz).

TABLE II

CALCULATION OF THE CORRELATION TIMES OF MOLECULAR TUMBLING MOTION IN THE GEL AND IN SOLUTION OF LENTINAN

	Neutral gel [β-(1 → 6)]			*in* Me_2SO [β-(1 → 3)]		
	T_1	*nOe*	*Line width*	T_1	*nOe*	*Line width*
Obs.	64	1.4	48	78	1.4	27
Calc.	62	1.6	48	74	1.9	29
$p = 14$				$p = 10$		
$\bar{\tau} = 7$				$\bar{\tau} = 2$		

flexible site. It is also likely that the correlation time of the β-D-(1 → 3)-linked D-glucosyl residues of the single-helix portion is not far from 40 ns, as the line width is strongly influenced by p, which is a function of the degree of cross-linking. The relaxation parameters, and also the correlation times of the backbone, β-D-(1 → 3)-linked D-glucosyl residues (random-coil conformation) in dimethyl sulfoxide are very similar to those of curdlan[2] (see Tables I and II).

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