(12) Colson, P.; Jennings, H. J.; Smith, I. C. P. *J. Am. Chem. Soc.* **1974,** *96,* 8081. Beragon, R. J.; Channing, M. A.; Gibeily, G. J.; Pillor, D. M. *J. Am. Chem. Soc.* **1977,** *99,* 5146.

(13) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976,** *64,* 2229.

(14) Mildvan, A. S.; Gupta, R. K. *Methods Enzymol.* **1978,** *49G,* 322-359. Swift, T. J.; Connick, R. E. *J. Chem. Phys.* **1962,** *37,* 307. Bertini, I.; Luchinat, C. *NMR of Paramagnetic Molecules in Biological Systems*; Benjamin Cummings: Menlo Park, 1986.

(15) MacLennan, J. M.; Stezowski, J. J. *Biochem. Biophys. Res. Commun.* **1980,** *92,* 929-932. Chacko, K. K.; Saenger, W. *J. Am. Chem. Soc.* **1981,** *103,* 1708-1715. Zabel, V.; Saenger, W.; Mason, S. A. *J. Am. Chem. Soc.* **1986,** *108,* 3664-3673.

Effect of Hydration on Conformational Change or Stabilization of (1→3)-β-D-Glucans of Various Chain Lengths in the Solid State As Studied by High-Resolution Solid-State ^{13}C NMR Spectroscopy

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ABSTRACT: ^{13}C NMR spectra of hydrated (1→3)-β-D-glucans from various sources were recorded after equilibrating samples in an atmosphere of 96% relative humidity in a desiccator over 8 h to gain insight into hydration-induced conformational change or stabilization. It was found that the effect of hydration depends strongly on the initial conformation of the glucans studied. The ^{13}C NMR peaks of curdlan hydrate ($\overline{DP}_n$ 540) were substantially displaced (up to 2.5 ppm) from those of curdlan powder (form II) owing to hydration-induced conformational change as an intermediate step to form the triple helix. The resultant conformation (form II′) turned out to be the same as the single-helical portion of the elastic gel because the ^{13}C chemical shifts of both states were identical within an error of ±0.1 ppm. Hydration, however, did not induce any significant displacement of peaks for either glucans of low molecular weight ($DP_n \leq 38$) or annealed curdlan, adopting form I (oligomer type) or form III (triple helix), respectively, but narrowed all spectral peaks except for those of laminariheptaose. The peak narrowing is explained by distorted or interrupted conformations of the anhydrous state readjusting on hydration. It is now clear that the presence of a hydration-induced single-helical conformation is an essential condition for gel-forming ability in linear (1→3)-β-D-glucans. Finally, the hydration-induced spectral change of paramylon is discussed.

Introduction

It is well recognized that a variety of physical properties of (1→3)-β-D-glucans such as density, crystallinity, solubility, gel-forming ability, etc., vary with the degree of polymerization and extent of branching, which depend on the source and history of the samples.[1-3] A linear (1→3)-β-D-glucan of high molecular weight, curdlan ($\overline{DP}_n$ 540), is known to form an elastic gel when its aqueous suspension is heated above 54 °C.[1] From a study of acid-degraded glucans, linear glucans with number-average degree of polymerization (DP_n) > 20 were found to be insoluble in aqueous media,[4] whereas branched glucans are soluble irrespective of their chain lengths.[4,5] These physical properties are related to secondary or tertiary structures of individual chains, molecular assembly, or aggregation and consequently to their biological function as structural components in cell walls or as reserve polysaccharides in bacteria, algae, fungi, or plants. In addition, it has been proposed that such physical properties are closely related to their specific activity as biological response modifiers (BRM), including antitumor activity.[6-9]

Previous solid-state ^{13}C NMR studies[2,3,11-13] showed that the existence of three types of conformations,[14] form I (lower molecular weight oligomers), form II (single chain), and form III (triple helix), are distinguishable in linear and branched (1→3)-β-D-glucans by examination of the conformation-dependent displacements of the C-3 ^{13}C NMR peak[17-19] at the glycosidic linkages as well as a peak profile of C-2, C-4, and C-5 carbons among several types of samples used (Table I). Conformational characterization by X-ray diffraction[20-22] is feasible only for form III samples, as the others are noncrystalline. Further, we found that any attempt to achieve better crystallinity resulted in conformational changes, as judged by the ^{13}C NMR pattern.[2,3] In this connection, conformational characterization by ^{13}C NMR is indispensable for interpretation of the physical properties of (1→3)-β-D-glucans as a complementary means of X-ray diffraction study.

Hydration has been recognized as a very important process in conformational stabilization of a variety of biological macromolecules. Previous ^{13}C NMR studies[23-25] have demonstrated that hydration of amylose, starches, or silk fibroins caused either substantial narrowing or displacements of ^{13}C NMR peaks as a result of conformational stabilization or change, respectively, depending on the types of molecular structures. Hydration/dehydration processes will induce conformational changes if water molecules are involved at chain positions of crucial importance in maintaining a secondary structure. As for (1→3)-β-D-glucans, it has been shown that hydration induces substantial spectral changes in curdlan and paramylon,[26,27] although the resulting conformations have not yet been clarified. In particular, Stipanovic and Giammatteo demonstrated that the three conformations "anhydrous", "hydrate", and "swollen" are distinguishable by solid-state ^{13}C NMR spectra.[27,28]

In this connection, it seems to be very important to reveal which types of initial conformations are susceptible

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Table I
Characterization of Three Forms of Anhydrous Conformations in Linear (1→3)-β-D-Glucans in the Solid State[a]

form	characteristic C-3 peaks,[b] ppm	sources and history		example	
		$DP_n \le 38$	$\overline{DP}_n > 250$	$DP_n \le 38$	$\overline{DP}_n > 250$
I	91.2	lyophilized from aqueous or DMSO soln		laminariheptaose	
II	89.5		lyophilized from aqueous or DMSO soln	laminaran	curdlan, pachyman
III	85.6	lyophilized from aqueous soln	annealed at 180 °C in the presence of water	laminaran	annealed curdlan

[a] Based on the findings in ref 2, 3, and 13. [b] Estimated error ±0.3 ppm.

Table II
^{13}C Chemical Shifts of Curdlan, Pachyman, and Paramylon in Anhydrous, Hydrate, Annealed, and Gel State (ppm from TMS)

	curdlan				pachyman		paramylon	
	anhydrous[a]	hydrate	annealed[a]	gel[b]	anhydrous[b]	hydrate	anhydrous[a]	hydrate
C-1	104.5	104.3	103.5	104.0	103.6	104.2	105.3, 103.2	103.3
C-2	73.5	73.8	74.3	73.5	73.0	73.2	75.8	74.0
C-3	89.8	87.3	86.5	87.5	89.3	88.5	88.9–83.2	86.7
C-4	68.8	69.1	68.3	69.1	69.6	69.0	68.6	67.9
C-5	75.5	75.8	77.5	75.9	75.5	75.8	75.8	78.7/77.4
C-6	61.8	61.2	61.6	61.4	61.6	61.5	61.7	61.5

[a] Taken from ref 3. [b] Taken from ref 2.

to hydration-induced conformational change or stabilization among a variety of (1→3)-β-D-glucans:[2,3,13] (a) lower molecular weight oligomers adopting form I, (b) laminaran (DP_n 38) adopting either form I or III, (c) curdlan ($\overline{DP}_n$ 540) adopting form II or III, and (d) highly crystalline paramylon.

Experimental Section

Spray-dried curdlan powder from *Alcaligenes faecalis* ($\overline{DP}_n$ 540) was provided by Takeda Chemical Industries, Osaka, Japan. Laminaran from *Laminaria* species was purchased from Nutritional Biochemistry Corp., Ohio. Laminaran ($\overline{DP}_n$ 38) was purified as a gelatinous precipitate by centrifugation (8000 rpm) after dissolving the commercial sample in 20% NaCl solution saturated with thymol, in order to remove readily soluble fractions containing higher proportions of branching points.[29] The precipitate was washed repeatedly with ethanol, dissolved in DMSO, and then dialyzed against deionized water followed by lyophilization. High-resolution ^{13}C NMR spectrum of thus purified laminaran dissolved in DMSO-d_6 solution showed that no peaks from either mannitol or branching points were visible. Therefore, the number of branching points should be at most one per molecule, if any.[29] Preparation of acid-degraded curdlan, $\overline{DP}_n$ 14, was described previously.[4] Laminariheptaose was purchased from Seikagaku Kogyo, Tokyo (Lot. No. 407191). Preparation of HA-β-glucan was previously reported.[30] Pachyman[2] was provided by Dr. G. Chihara of the National Cancer Center Research Institute. Paramylon was a generous gift from Professor B. A. Stone of La Trobe University, Australia.

The annealed curdlan samples from curdlan powder used in this study were prepared as described in the previous paper:[3] sample 1, annealed sample at 180 °C followed by slow cooling; sample 2, annealed sample at 150 °C followed by rapid cooling; sample 3, annealed sample at 80 °C followed by slow cooling. Anhydrous samples were prepared by lyophilization either from aqueous or DMSO solution. All of the hydrated samples were prepared by placing one of the above-mentioned samples spread on a glass dish in a desiccator of 96% or 75% relative humidity for over 8 h.

^{13}C NMR spectra (75.46 MHz) in the solid state were recorded on a Bruker CXP-300 spectrometer equipped with an accessory for cross polarization-magic angle spinning (CP-MAS). Pulse width, contact time, and repetition time were 3.5 μs, 1 ms, and 4 s, respectively. ^{13}C spin–lattice relaxation times in the laboratory frame were measured by the Torchia pulse sequence.[31] All samples were contained in an alumina rotor with a double air-bearing. No special care was taken to maintain the desired relative humidity of hydrated samples during data acquisition. ^{13}C chemical shifts were measured relative to carbon chemical shift of glycine carboxyl group (176.03 ppm) and converted to the chemical shift referenced to tetramethylsilane.

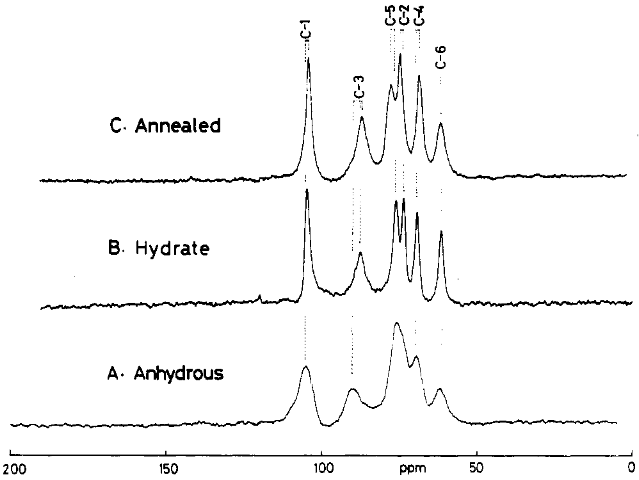

Figure 1. 75.46-MHz ^{13}C NMR spectra of curdlan in the solid state: (A) anhydrous, (B) hydrate, (C) annealed (at 180 °C followed by slow cooling).

Results

Figure 1 illustrates ^{13}C NMR spectra of a hydrate sample of curdlan as compared with those of anhydrous and annealed curdlans previously reported.[3] It is noteworthy that the ^{13}C NMR line widths of hydrate sample are much narrowed as compared with those of anhydrous sample (parts A and B of Figure 1) and are comparable to those of the annealed sample (Figure 1C). The C-3 ^{13}C chemical shift of curdlan hydrate is displaced upfield by 2.5 ppm with respect to that of anhydrous state but seems to be very similar, at first glance, to that of annealed curdlan (see Table II). However, distinction of spectra between the hydrated and annealed curdlan is straightforward by the C-5 chemical shifts (75.8 and 77.5 ppm for the former and latter, respectively) and peak separation between the C-5 and C-2 carbons (2.0 and 3.2 ppm, respectively). Similar hydration-induced spectral change was noted by going from anhydrous curdlan lyophilized from DMSO

Table III
^{13}C Chemical Shifts of Laminaran, Laminariheptaose, and Acid-Degraded Curdlan ($\overline{DP}_n$ 14) in Anhydrous and Hydrate State (ppm from TMS)

	laminaran						
	lyophilized from DMSO soln		lyophilized from aqueous soln		laminariheptaose		acid-degraded curdlan ($\overline{DP}_n$ 14):
	anhydrous	hydrate	anhydrous[a]	hydrate	anhydrous[a]	hydrate	hydrate
C-1	105.5	104.8	103.2	103.4	104.0	104.3	104.2
C-2	71.4	73.0	74.2	74.0	72.9	73.0	72.9
C-3	90.6	91.0	85.4	85.6	91.3	91.0	91.0
C-4	69.7	69.6	68.4	68.2	69.7	69.6	69.9
C-5	75.4	75.9	77.4	77.3	76.4	75.6	76.5
C-6	61.5	61.7	61.9	61.5	61.5	61.3	61.8

[a] Data taken from ref 3 and 13.

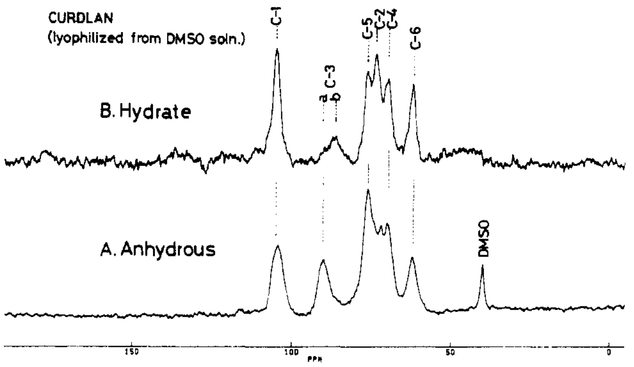

Figure 2. 75.46-MHz ^{13}C NMR spectra of anhydrous curdlan lyophilized from DMSO solution: (A) anhydrous, (B) hydrate.

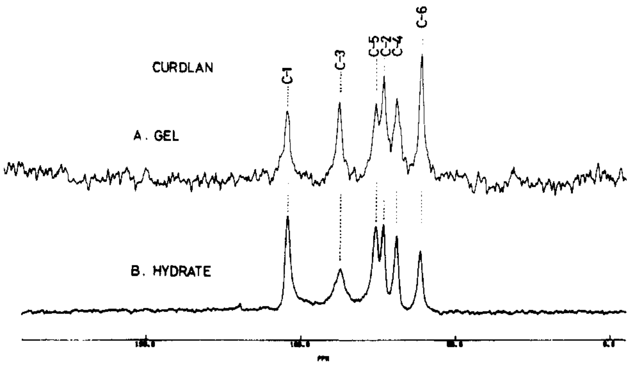

Figure 3. 75.46-MHz ^{13}C NMR spectra of curdlan gel (A) and curdlan hydrate in the solid state (B).

solution (A) to its hydrate form (B) (Figure 2). Pachyman also exhibits similar hydration-induced spectral change as summarized in Table II. The methyl ^{13}C signal from bound DMSO at 39.5 ppm (Figure 2A) disappeared in the hydrated sample (Figure 2B). Figure 3 illustrates ^{13}C NMR spectra of hydrate and elastic gel of curdlan, the latter of which was previously recorded under condition of standard high-resolution spectroscopy.[2] Surprisingly, the ^{13}C chemical shifts from both states are in good agreement (within ±0.1 ppm), as summarized in Table II.

Figure 4 demonstrates hydration-induced spectral changes in three kinds of anhydrous annealed curdlan samples: sample 1, density 1.49, high crystallinity (A); sample 2, density 1.49, low crystallinity (C); sample 3, density 1.46, very low crystallinity (E).[3] Resulting spectral change varies depending on the variety of conformations of samples used. The ^{13}C NMR profile of sample 1 is virtually unchanged between the anhydrous and hydrated states, although the line widths of the latter were substantially narrowed (parts A and B of Figure 4). It appears that the spectral pattern of hydrated sample 2 is very close to that of anhydrous sample 1. On the other hand, hydrated sample 3 exhibits a rather broader spectral pattern than that of curdlan hydrate (Figure 1B).

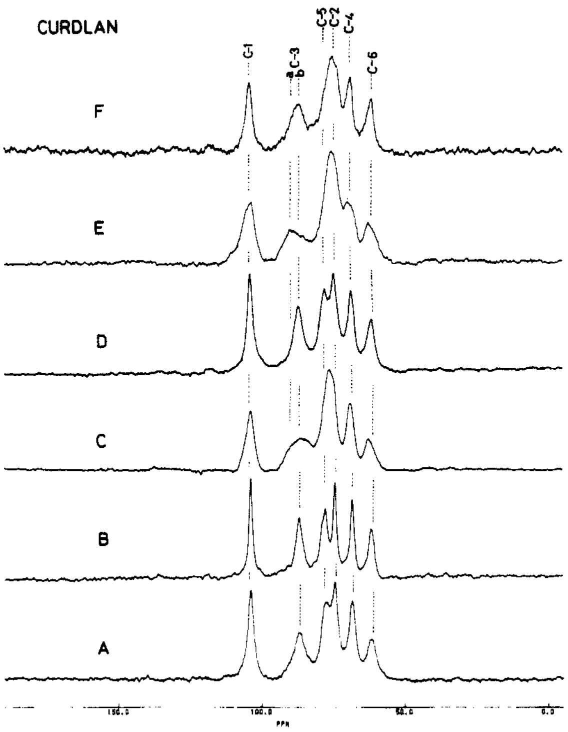

Figure 4. 75.46-MHz ^{13}C NMR of annealed curdlan samples at various conditions and their hydrates: (A) sample 1, annealed at 180 °C followed by slow cooling; (B) hydrated sample 1; (C) sample 2, annealed at 150 °C followed by rapid cooling; (D) hydrated sample 2; (E) sample 3, annealed at 80 °C followed by slow cooling; (F) hydrated sample 3.

Figures 5 and 6 demonstrate ^{13}C NMR spectra of anhydrous and hydrated (1→3)-β-D-glucans of low molecular weight. No spectral changes were observed between the anhydrous and hydrated states of laminariheptaose (parts A and B of Figure 5). The hydrated form of acid-degraded curdlan ($\overline{DP}_n$ 14) exhibits the same spectral pattern as that of laminariheptaose (Figure 5C and Table III). In addition, hydration did not induce any spectral change (parts B and D of Figure 6) in either anhydrous laminaran having a triple-helix form (Figure 6A) or single chain form (Figure 6C). However, a conformational change of laminaran from single chain to triple helix was induced by dissolving in aqueous solution (Figure 6E), although the ^{13}C NMR signals are broader than those of sample 1 (Figure 6A). These broad signals were converted to narrow peaks on hydration (Figure 6F). Figure 7 illustrates hydration-induced spectral change of paramylon: the ^{13}C NMR spectrum of the hydrated form of paramylon is very close to that of hydrated annealed curdlan, although the spectral resolution of the

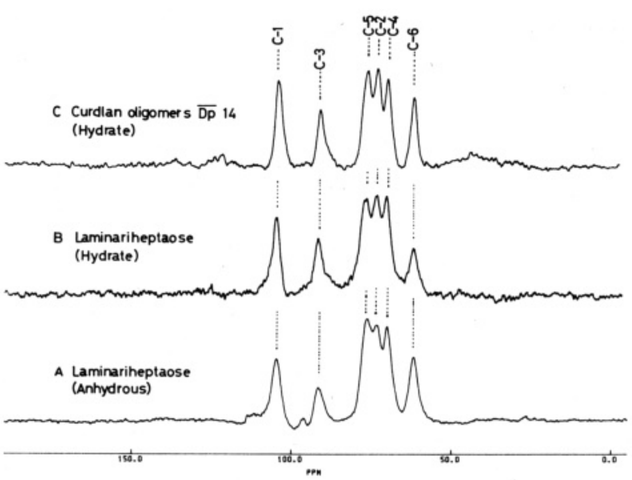

Figure 5. 75.46-MHz ^{13}C NMR spectra of laminariheptaose and acid-degraded curdlan $\overline{DP}_n$ 14: (A) anhydrous laminariheptaose, (B) hydrated laminariheptaose, (C) hydrated acid-degraded curdlan, $\overline{DP}_n$ 14.

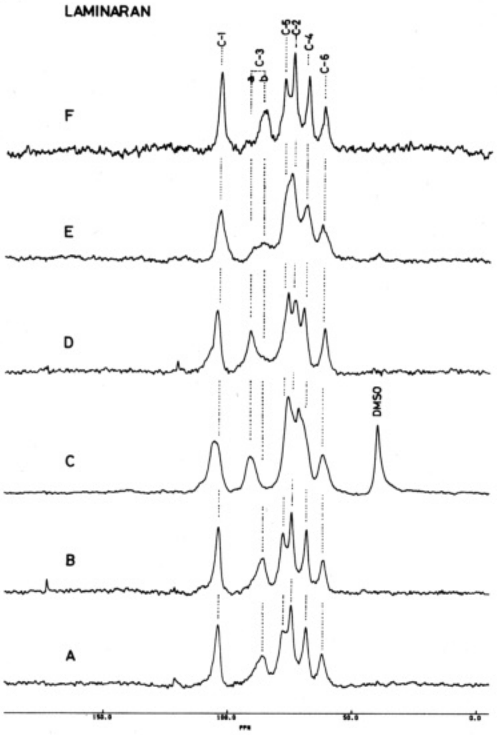

Figure 6. 75.46-MHz ^{13}C NMR spectra of water-soluble fraction of laminaran (DP$_n$ 38) from *laminaria* species adopting various conformations and their hydrates: (A) anhydrous laminaran lyophilized from aqueous solution, (B) its hydrate, (C) anhydrous laminaran lyophilized from DMSO solution, (D) its hydrate, (E) anhydrous laminaran lyophilized from aqueous solution after dissolving the laminaran sample in D, (F) its hydrate.

former is much better. It is noteworthy that the C-5 signal of both samples appears as doublet. ^{13}C spin–lattice relaxation times of paramylon hydrate are summarized in Table IV, together with those of anhydrous paramylon and annealed curdlan previously reported.[3,13]

Discussion

Classification of Hydration-Induced Spectral Change. Figure 8 summarizes how hydration-induced conformational change (as judged by displacement of peaks) or stabilization (as judged by peak narrowing) of linear (1→3)-β-D-glucan occurs depending on the initial conformations, which are determined by the degree of polymerization and sample history. The following three types of anhydrous conformations (see Table I) are distinguishable by differential response to hydration as well as by their characteristic C-3 peak positions[17–19] among a variety of linear (1→3)-β-D-glucans in the solid state: form I, hydration-indifferent; form II, hydration-induced conformational change; form III, hydration-induced conformational stabilization. In addition, the hydrated form (form II′) is readily distinguishable from the others.

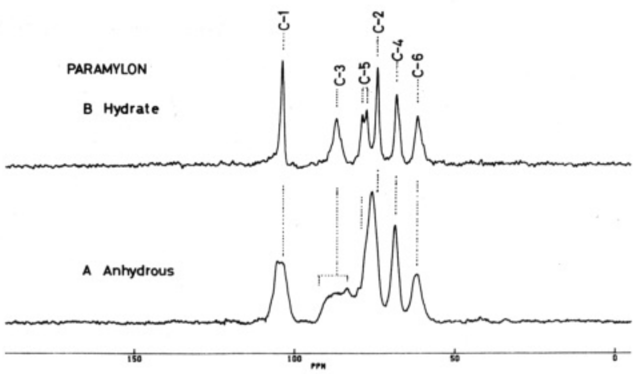

Figure 7. 75.46-MHz ^{13}C NMR spectra of anhydrous (A) and hydrate (96% R.H.) (B) sample of paramylon.

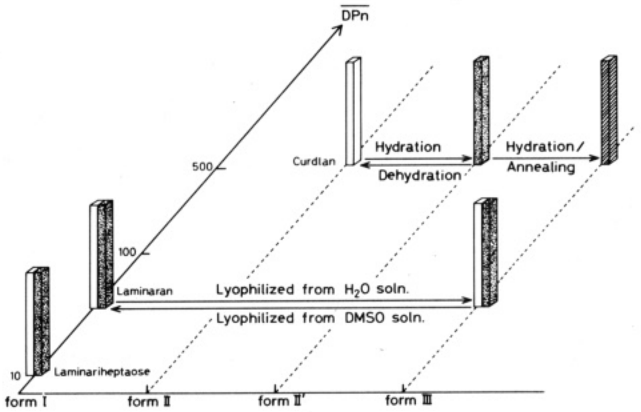

Figure 8. Schematic representation of hydration-induced conformational stabilization or change among a variety of (1→3)-β-D-glucans adopting different initial conformation: □, anhydrous; ▩, hydrate; ▨, hydrate/annealing.

Table IV
^{13}C Spin–Lattice Relaxation Times of Laboratory Frame of Anhydrous and Hydrated Paramylon in the Solid State (s)

	paramylon		annealed curdlan[b]
	hydrate	anhydrous[a]	anhydrous
C-1	13	30	16
C-2	13	22	18
C-3	13	30	16
C-4	5.7	12	8.2
C-5	6.8	22	9.3
C-6	0.61	6.3	1.2

[a] Averaged value from two or three peaks. Data taken from ref 3. [b] Taken from ref 3.

The present data demonstrate that the presence of form II′ is characteristic of (1→3)-β-D-glucans of high molecular weight ($\overline{DP}_n > 250$) (Figure 8) and can be considered as an intermediate form between single chain and triple-helix conformation. The hydrated form was rather stable (at least 1 week) even at low relative humidity (spectra not shown). However, this form was reversibly returned to the anhydrous form by removing hydrated water molecules in vacuo. The reversibility of the spectral change provides evidence that form II′ is not a triple helix as expected but a single chain stabilized by hydration.[32] The glycosidic torsion angles may vary to some extent on going from the anhydrous to the hydrated state, as manifested by significant displacement of the C-3 ^{13}C chemical shifts (2.5 ppm), which are sensitive to changes of the torsion angles.[18,19] This finding provides additional evidence about the existence of a single-helical conformation in both the hydrate and swollen gel, as will be discussed later.

In other words, this form II′ conformation could be inevitably distorted if hydrated water molecules were lost (anhydrous form), as judged by broadened line widths. Nevertheless, this sort of conformational instabilization could be partially prevented when solvent molecules remained in the vicinity of polymer chains in anhydrous solids: in fact, DMSO molecules are bound to the single chain portion of anhydrous curdlan lyophilized from DMSO solution,[2,13] and resulting ^{13}C NMR peaks are usually narrower than those of the anhydrous sample lyophilized from aqueous solution.[13] However, such DMSO molecules were readily replaced by water molecules by hydration (Figure 2). These forms are not always present as a single species. Instead, molecular organization of annealed curdlan is heterogeneous, reflecting complexity in the gel network of the starting materials: they consist of two or three domains such as forms II, II′, and III, depending on anhydrous or hydrated condition.

Hydration, however, did not result in any spectral change in laminariheptaose (Figure 5). This compound adopts a single chain conformation slightly different from that of form II, because the C-3 ^{13}C chemical shift (91.2 ppm) is not the same but close to that of form II (89.5 ppm). This observation can be explained by the fact that the chain length of laminariheptaose is too short to form either 6/1[21,22] or 7/1[35] triple helix.

Hydration-Induced Conformational Change. Anhydrous Single Chain and Hydrate Single Helix. The conformation of hydrated samples turned out to be a single-helix based on the similarity of ^{13}C chemical shifts with those of gel sample[36] and reversibility of hydration-induced conformational change (Table II). It is interesting to note that such single-helix conformation is achieved at ambient temperature simply by hydration without raising the temperature to 54 °C (gelation temperature). This means that no further conformational change is associated in gelation of curdlan powder, once hydration is achieved. This is in contrast to the well-known gelation mechanism of agarose or carrageenan in which conformational change from random coil to double helix is a major step for gelation.[37,38] Besides, it is expected that the glycosidic torsion angles of form II′ (single helix) are not far from those of triple helix, because the displacements of the C-1 and C-3 signals at the glycosidic linkage are less than 0.8 ppm. This view seems to account well for the fact that triple-helix conformation is achieved by twining three stranded chains in the presence of water molecules. The presence of these water molecules, however, is not always essential in maintaining the triple helix, once it is achieved: water molecules are located at inter-triple-helix spaces.[22]

The dynamics of single-helical chains in curdlan hydrate differs substantially from that of the elastic gel in view of substantial differences under the condition of spectral observations. Molecular reorientation in the elastic gel occurs in a time shorter than 10^{-9} s so as to permit one to observe isotropic ^{13}C NMR signals by conventional high-resolution spectrometer,[2,4,17,36] whereas the manner of molecular fluctuation of chain, if any, in the hydrated sample is no longer isotropic and its rate should be much longer than 10^{-8} s and ^{13}C NMR signals are subsequently made visible by use of cross polarization magic angle spinning NMR method.

Hydration-Induced Conformational Stabilization. Anhydrous and Hydrate Triple Helices. The anhydrous sample does not necessarily assume the single chain of form II: anhydrous laminaran lyophilized from aqueous solution adopts the triple-helix (form III) conformation.[3] Further, many branched (1→3)-β-D-glucans lyophilized from aqueous solution adopt the triple-helix conformation,[12] although spray-dried lentinan adopts a single chain conformation.[2,12] It is interesting to note that only linear (1→3)-β-D-glucans of intermediate chain length, laminaran ($\overline{\mathrm{DP}}_n$ 38),[39] assume a triple-helix conformation on lyophilization from aqueous solution, which is theoretically predicted to be the most stable conformation.[33,34] The triple-helix conformation of high molecular weight, however, can be formed by annealing the sample in the presence of water at 150–180 °C, followed by slow cooling.[3] This means that glucans neither of shorter chains ($\overline{\mathrm{DP}}_n < 14$) nor longer chains ($\overline{\mathrm{DP}}_n > 250$) are able to form the triple-helix conformation under the condition of ambient temperature.

The presence of such longer single-chain or single-helical segments in high molecular weight glucans is considered to be a consequence of failure to form the longer triple-helix conformation. This may be mainly ascribed to the limited solubility of (1→3)-β-D-glucans of larger $\overline{\mathrm{DP}}_n$,[1,4] due to formation of aggregates arising from either single-helical or triple-helical chains in aqueous media. To support this view, it was found that the proportion of triple-helix conformation was reduced to about 10% for samples of $\overline{\mathrm{DP}}_n > 250$, whereas it was roughly estimated as 60% for samples with DP_n between 14 and 130.[2] Nevertheless, triple-helical chains are more easily formed in branched (1→3)-β-D-glucans,[12] because steric hindrance due to branched residues at C-6 position would prevent closer packing of helical chains, resulting in increased solubility in aqueous media.

Previous X-ray diffraction studies[20-22] showed the presence of two polymorphs in annealed curdlan, dry (0% RH; where RH = relative humidity) and hydrated (75% RH) forms, depending on relative humidity. However, the ^{13}C NMR patterns of both forms are very similar (parts A and B of Figure 4) in spite of differences in the space group between anhydrous ($P6_3$) and hydrated ($P1$ or $P3$) forms. This finding suggests that conformations of the triple-helical chains are not significantly different between the two forms, because the C-1 and C-3 chemical shifts are closely related to the glycosidic torsion angles. Consistent with this, X-ray diffraction data showed that glycosidic torsion angles, ϕ and ψ, are very similar between anhydrous and hydrated samples of triple-helical conformation, with differences of only 6° and 2° between the respective angles.[22] In fact, water molecules in the hydrated form of annealed curdlan are located at inter-triple-helix spaces and half of those in the unit cell are clustered near the O-4 and O-5 atoms of the glucose residue, with about one water for every residue.[22]

The ^{13}C NMR spectrum of paramylon hydrate is in good agreement with that of annealed curdlan (at 150–180 °C) followed by slow cooling (sample 1; Figures 4B and 7B), consistent with the data of X-ray diffraction.[22] In agreement with this observation, the ^{13}C spin–lattice relaxation times of paramylon hydrate are very similar to those of annealed curdlan (Table IV).[3] As pointed out previously,[3] the ^{13}C T_1 values of annealed curdlan are mainly influenced by dipolar interaction with hydroxymethyl protons undergoing rapid reorientation. Under anhydrous conditions (<75% RH), however, the ^{13}C NMR spectrum of paramylon cannot be accounted for by triple-helix conformation alone (Figure 7A), because the ^{13}C NMR peaks are split into several sets of peaks due to the presence of more than two major conformations. Further, the C-6 ^{13}C spin–lattice relaxation time of the anhydrous paramylon sample becomes 1 order of magnitude longer than that of the hydrated sample (Table IV), probably because the hydroxymethyl group ceases to rotate.[21] Such a drastic conformational change under dehydration did not occur in the case of annealed curdlan, however. This behavior clearly arises from differences in tertiary structures between annealed curdlan and paramylon. In fact, the spectral resolution of hydrated paramylon, especially with regard to peak splitting in the C-5 signal, is much better than that of hydrated annealed curdlan, reflecting the higher density of paramylon (1.53) compared to annealed curdlan (1.49).[3]

There appears a discrepancy in the changeover point of anhydrous and hydrate forms of annealed curdlan or paramylon between X-ray diffraction (20% RH)[20] and ^{13}C NMR (ca. 75% RH). Such an obvious discrepancy might be explained by differential response of hydrated water molecules between NMR and X-ray diffraction. X-ray diffraction is mainly influenced by water molecules involved in the crystalline lattice because water molecules located in other regions, including amorphous regions, are indifferent to Bragg reflections. By contrast, the former and latter contribute equally to hydration- or dehydration-induced spectral change of ^{13}C NMR pattern. This would be more pronounced when conformational change is induced in a portions other than crystalline regions as a result of dehydration, as in the case of paramylon. The presence of such portions is responsible for hydration-induced conformational readjustment or change of molecular chains. This view may well explain why hydration at higher relative humidity is required for (1→3)-β-D-glucans as far as the effect of hydration is examined by ^{13}C NMR spectroscopy. In this connection, it is worthwhile to examine hydration of various types of molecular systems by NMR and X-ray diffraction.

Concluding Remarks

It is demonstrated that hydration of a variety of (1→3)-β-D-glucans results in either conformational change or stabilization, depending on the type of initial conformations, as examined by high-resolution solid-state ^{13}C NMR spectroscopy. The hydrated form of high molecular weight glucans such as curdlan and pachyman adopts a single-helix conformation that is identical with that of the elastic gel. This finding provides additional evidence concerning the existence of single-helical conformation both in the hydrated and gel states of (1→3)-β-D-glucan. Hydration of annealed curdlan, however, did not significantly change the ^{13}C NMR profile. On the other hand, the ^{13}C NMR signals of paramylon triple helix are substantially narrowed by hydration in spite of similarity in secondary structures as examined by X-ray diffraction. Undoubtedly, the ^{13}C NMR approach will be a very powerful means to study hydration-induced conformational change or stabilization.

Registry No. Curdlan, 54724-00-4; laminaran, 9008-22-4; HA-β-glucan, 9051-97-2; pachyman, 9037-88-1; paramylon, 51052-65-4; laminariheptaose, 72627-90-8.

References and Notes

(1) Harada, T. *ACS Symp. Ser.* **1977**, *45*, 265.
(2) Saitô, H.; Tabeta, R.; Sasaki, T.; Yoshioka, Y. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 2093.
(3) Saitô, H.; Tabeta, R.; Yokoi, M.; Erata, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 4259.
(4) Saitô, H.; Miyata, E.; Sasaki, T. *Macromolecules* **1978**, *11*, 1244.
(5) Saitô, H.; Ohki, N.; Takasuka, T.; Sasaki, T. *Carbohydr. Res.* **1977**, *58*, 293.
(6) Whistler, R.; Bushway, A. A.; Singh, P. M.; Nakahara, W.; Tokuzen, R. *Adv. Carbohydr. Chem. Biochem.* **1976**, *32*, 235.
(7) Sasaki, T.; Abiko, N.; Sugino, Y.; Nitta, K. *Cancer Res.* **1978**, *38*, 379.
(8) Maeda, Y. Y.; Watanabe, S. T.; Chihara, G.; Rokutanda, M. *Cancer Res.* **1988**, *48*, 671.
(9) Hamuro, J.; Chihara, G. In *Immune Modulation Agents and Their Mechanism*; Fenichel, R. L., Chirigos, M. A., Eds.; Dekker: New York, 1984; pp 409–434.
(10) Saitô, H.; Ohki, T.; Sasaki, T. *Carbohydr. Res.* **1979**, *74*, 227.
(11) Saitô, H.; Tabeta, R.; Harada, T. *Chem. Lett.* **1981**, 713.
(12) Saitô, H.; Tabeta, R.; Yoshioka, Y.; Hara, C.; Kiho, C.; Ukai, S. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 4267.
(13) Saitô, H.; Yokoi, M. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 392.
(14) In our previous papers, conformation of curdlan and pachyman has been ascribed to the single-helix conformation.[2–5,10–12,15] This assignment, however, was revised to a single chain form in our previous paper,[13] because the ^{13}C chemical shifts of the hydrated form are much closer to those of curdlan gel (see Table II). The single-helix conformation of curdlan gel was based on the following findings: (1) The absorption maximum of Congo Red in the visible region was largely shifted to a longer wavelength by addition of (1→3)-β-D-glucan of high molecular weight.[15] (2) The annealed film of curdlan was considerably less stained with Congo Red than original glucan.[16] (3) ^{13}C NMR signals are visible from elastic gels whose C-1 and C-3 peaks are substantially displaced from those of randomly coiled low molecular weight fraction.[4,5,10,17]
(15) Ogawa, K.; Tsurugi, J.; Watanabe, T. *Chem. Lett.* **1972**, 689.
(16) Ogawa, K.; Hatano, M. *Carbohydr. Res.* **1978**, *67*, 527.
(17) Saitô, H.; Ohki, T.; Sasaki, T. *Biochemistry* **1977**, *16*, 908.
(18) Saitô, H. *Magn. Reson. Chem.* **1986**, *24*, 835.
(19) Saitô, H.; Ando, I. *Annu. Rep. NMR Spectrosc.* **1989**, *21*, 209–290.
(20) Marchessault, R. H.; Deslandes, Y.; Ogawa, K.; Sundararajan, P. R. *Can. J. Chem.* **1977**, *55*, 300.
(21) Deslandes, Y.; Marchessault, R. H.; Sarko, A. *Macromolecules* **1980**, *13*, 1466.
(22) Chuah, C. T.; Sarko, A.; Deslandes, Y.; Marchessault, R. H. *Macromolecules* **1983**, *16*, 1375.
(23) Marchessault, R. H.; Taylor, M. G.; Fyfe, C. A.; Veregin, R. P. *Carbohydr. Res.* **1985**, *144*, C1.
(24) Horii, F.; Hirai, A.; Kitamaru, R. *Macromolecules* **1986**, *19*, 930.
(25) Ishida, M.; Asakura, T.; Yokoi, M.; Saitô, H. *Macromolecules*, in press.
(26) Fyfe, C. A.; Stephenson, P. J.; Taylor, M. G.; Bluhm, T. L.; Deslandes, Y.; Marchessault, R. H. *Macromolecules* **1984**, *17*, 501.
(27) Stipanovic, A. J.; Giammatteo, P. J. In *Industrial Polysaccharides: Genetic Engineering, Structure/Property Relations and Applications*; Yalpani, M., Ed.; Elsevier, New York, 1987; pp 281–292.
(28) Note that their "hydrate" and "swollen" forms differ from ours and correspond with "annealed" and "hydrated" forms, respectively. As pointed out by Marchessault et al.,[20] there exist polymorphs, dry and hydrated in annealed curdlan. Therefore, it is more appropriate to use hydrated and annealed as in the present paper.
(29) It is well-known that structural variation of laminaran occurs among species and with season and habitat. These variations occur mainly with respect to the proportion of mannitol end groups, the degree of branching points, and the proportion of units that are linked through positions 1 and 6 only. Typically, brown algal laminaran is a (1→3)-β-D-glucan with a number average of DP of 20 to 25, and up to three units per molecule are branched through position 6: Painter, T. J. In *The Polysaccharides*; Aspinall, G. O., Ed.; Academic Press: New York, 1983; Vol. 2, pp 195–285. Thus, we attempted to partially purify the commerical laminaran samples as described in the text. Here, it is pointed out that laminaran sample used in ref 2–4 was also purified in a similar manner described in the text.
(30) Yoshioka, Y.; Tabeta, R.; Saitô, H.; Uehara, N.; Fukuoka, F. *Carbohydr. Res.* **1985**, *140*, 93.
(31) Torchia, D. A. *J. Magn. Reson.* **1978**, *30*, 613.
(32) Bluhm and Sarko showed that on the basis of calculation of conformation single helix is less stable than double or triple helix in (1→3)-β-D-glucan.[33,34] This conclusion is true for

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prediction of conformational features in the anhydrous state. In the hydrated state, however, it is not unexpected that such a single-helix conformation can be stabilized by hydrogen-bonding interaction with water molecules.
(33) Bluhm, T. L.; Sarko, A. *Can. J. Chem.* **1977**, *55*, 293.
(34) Bluhm, T. L.; Sarko, A. *Carbohydr. Res.* **1977**, *54*, 125.
(35) Fulton, S.; Atkins, E. D. T. *ACS Symp. Ser.* **1980**, *141*, 385.
(36) Saitô, H. *ACS Symp. Ser.* **1981**, *150*, 125.
(37) Arnott, S.; Fulmer, A.; Scott, W. E.; Dea, I. C. M.; Moorhouse, R.; Rees, D. A. *J. Mol. Biol.* **1974**, *90*, 269.
(38) Arnott, S.; Scott, W. E.; Rees, D. A.; McNab, C. G. A. *J. Mol. Biol.* **1974**, *90*, 253.
(39) It is reasonable, on the basis of our finding as to the extent of branching points, to treat the laminaran sample used in this study as a linear glucan as far as the local secondary structure as deduced by ^{13}C NMR spectroscopy is concerned.

Reactant Preordering in Solid Polymer Matrices: Photo-Cross-Linking in Blends of Donor- and Acceptor-Substituted Poly(vinyl cinnamates)

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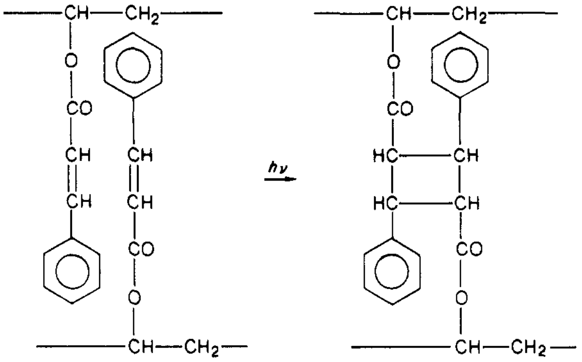
ABSTRACT: In blends of electron-donor- and electron-acceptor-substituted poly(vinyl cinnamates) a significant enhancement of photosensitivity is observed, compared to the sensitivity of the individual component polymers. The increase in cross-linking efficiency, which is the basis of the effect, is caused by reactant preordering in the solid polymer matrix. Donor–acceptor interactions between the functional cinnamoyl groups lead to a change from intramolecular to intermolecular pair formation. The magnitude of the effect is a function of the donor–acceptor interaction energy of the functional groups as measured by the difference in their Hammett substituent constants.

The performance of radiation-sensitive polymers depends not only on the inherent photoreactivity of their functional groups but also on the spatial arrangement of these groups in the solid matrix.[1] In conventional photopolymers the mutual geometries of reactants are a matter of chance and in these conditions the fraction of reactive configurations is low. It has been suggested that the number of reactive configurations or sites could be increased by reactant preordering. This is borne out by the solid-state chemistry of diacetylenes[2–4] and by the behavior of liquid-crystalline polymers.[5–8] We recall also the recent studies of Weiss et al.[9,10] on the effects of liquid-crystalline solvents on a number of photochemical probes. This paper is concerned with the partial preordering of photoreactive groups in amorphous polymer matrices. The idea of group preordering in solid polymers is of general interest since the ability of organizing the components of a polymeric system opens up a host of applications: Loosely ordered chromophore arrays may serve as pathways for the transport of photonic excitation, for the conduction of charge, and for the progression of chemical reactions.[11–13]

We have made an attempt at preordering photoreactive groups by means of electron-donor–acceptor interactions. The general aim was to attach electron donor or electron acceptor groups to the photoreactive moieties of a polymer and blend an electron-donor polymer and an electron-acceptor polymer together in a fluid medium (melt or solution), so that reactant pairs may be formed by electron-donor–acceptor interactions. The reactive configurations are then preserved in the solid state by cooling the melt or evaporating the solvent.

Poly(vinyl cinnamate) was chosen as a model system. It is a classical negative working photoresist where cross-links are formed by photocycloaddition between polymer-bound cinnamoyl groups.



Batches of poly(vinyl cinnamate) substituted with either electron-donor or electron-acceptor groups were prepared and it was hoped that blends of the donor and acceptor polymers coated from solution in the form of thin films would have a higher photosensitivity than either of the polymeric components. Watanabe and Ichimura[14] have reported interesting preliminary experiments on these lines and have indeed found increased photosensitivity. In the present paper preordering was to be detected not only by an increase in the photosensitivity of the films but also by the change in the number and in the nature of the reactive configurations.

The electron-donor or electron-acceptor groups substituted in the para position of the phenyl ring of the cinnamic acid moiety were, respectively, OCH_3, CH_3, Cl, $OCOCH_3$, and CN. In order to compare polymers of identical degree of polymerization and molecular weight distribution, the samples were prepared by polymer modification from the same batch of poly(vinyl alcohol).

The photosensitivity of the polymers was measured by determining their gel dose (E_G), that is, the quantum ex-

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