CHEMISTRY LETTERS, pp. 571-574, 1981.

HIGH RESOLUTION ^{13}C NMR STUDY OF (1→3)-β-D-GLUCANS BY CROSS POLARIZATION/MAGIC ANGLE SPINNING: EVIDENCE OF CONFORMATIONAL HETEROGENEITY

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Conformation of various (1→3)-β-D-glucans in solid state was examined by ^{13}C cross polarization/magic angle spinning NMR spectroscopy. It was found that high molecular-weight glucans adopt mainly helix form, whereas low molecular weight ones take considerable proportion of random-coil form in addition to the helix in solid.

(1→3)-β-D-glucans of high molecular-weight, such as curdlan[1] from Alcaligenes faecalis var. myxogenes and lentinan[2] from Lentinus edodes, have unique property to be able to form gels in aqueous media. The most important feature of these polysaccharides is that three-dimensional networks essential for gelation are formed by single helical chains held together by multiple-stranded helices and/or aggregation of helical chains, as manifested from high resolution ^{13}C NMR[3-8] and X-ray diffraction[9-11] studies. In particular, the presence of the single helical chains in curdlan of gel state was recently proved by the similarity of the ^{13}C chemical shifts of the C-1 and C-3 carbons at the glucosidic linkages, which are very sensitive to conformational change,[3-7] between gel and solid-state samples.[8]

Here we wish to report ^{13}C cross polarization/magic angle spinning (CP/MAS)[12] NMR spectra of various (1→3)-β-D-glucans with emphasis on revealing a relation between solid-state conformation and gel-forming ability. For this purpose, we used curdlan powder as a reference compound, since its conformation is known to adopt single helix at a temperature below 120°C.[9]

Native curdlan was obtained as the surface matter stripped from the colonies grown on solid culture on glucose yeast-extract for four days and washed with distilled water to remove the remained cells as possible. The other glucans were the same as those previously used.[3-6]

Figure 1 illustrates 75.46 MHz ^{13}C CP/MAS NMR spectra of various (1→3)-β-D-glucans. The reproducibility of the ^{13}C chemical-shift values taken at different frequencies (75.46 and 15.03 MHz) and different samples is generally good(<1 ppm) except for the C-1 and C-3 signals, which being sensitive to conformational change[3-7] (see Table 1). It appears that for unoriented powder samples the linewidths might be mainly determined by dispersion of chemical shifts arising from a

number of slightly different conformations.[12] In accordance with this expectation, the linewidths of the ^{13}C signals of curdlan powder (Fig. 1A) are found to be 4 and 7 ppm for the C-1 and C-3 signals, respectively, which are very close to those 4.5 and 6.5 ppm, observed at 15.03 MHz.[8] In some instances, such a dispersion of chemical shifts can be seen as splittings of peaks as in the C-1 and C-3 signals (see traces B, C, D and E). In particular, the substantial displacement of the ^{13}C chemical shifts (C-1a and C-3a), from those of solution, is characteristic of formation of the helix conformation as viewed from X-ray diffraction of curdlan fiber.[9,13]

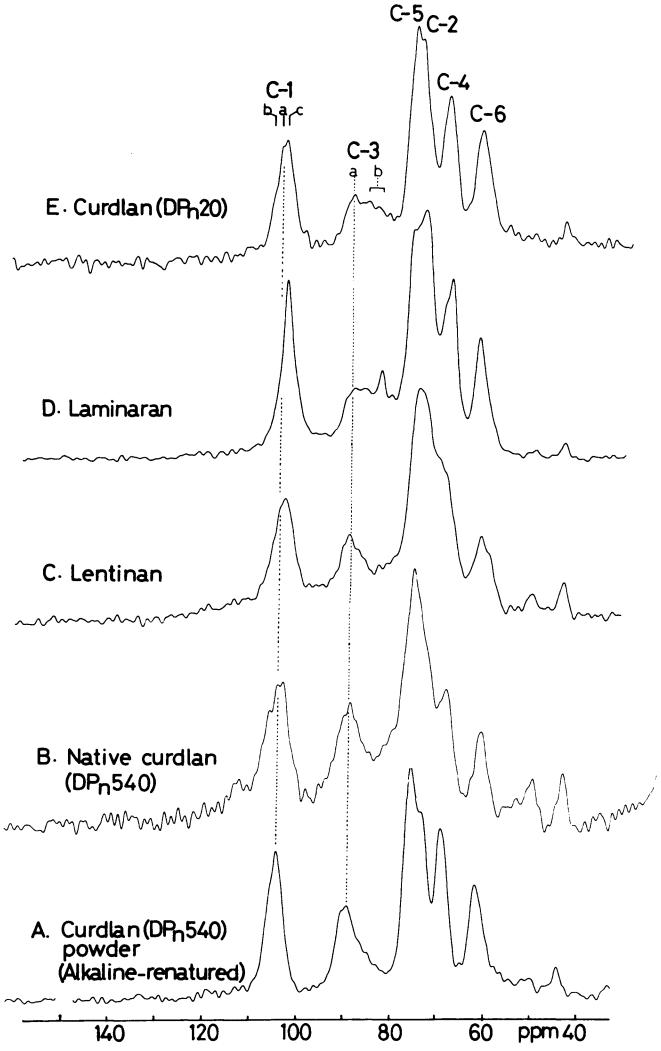

Figure 1. 75.46 MHz ^{13}C CP/MAS NMR spectra of various (1→3)-β-D-glucans, taken by a Bruker CXP-300 spectrometer. Samples were contained in a rotor machined from perdeuterated polymethylmethacrylate(~300 mg). ^{1}H H_1 field 14 gauss, spectral width 30 kHz, 4 K data points, repetition time 2 s, contact time 1 ms, and spinning rate 3.5 kHz. Peaks at 44.4 and 51.0 ppm are from the rotor.

Obviously, conformation of native curdlan is very similar to that of curdlan powder (alkaline-renatured) as manifested from the similarity of the C-1 and C-3 ^{13}C chemical shifts between the two samples. However, the more pronounced dispersion of the chemical shifts in the C-1 region of the former (more intense C-1b and C-1c peaks) might be caused by more heterogeneous nature of chain conformation. Such a conformational similarity between the native and renatured samples in (1→3)-β-D-glucans is in contrast to the similar system of (1→4)-β-D-glucans, cellulose, recently reported,[16,17] reflecting the differences in molecular conformation and assembly to tertiary structure between the two kinds of β-D-glucans.

The ^{13}C NMR spectrum of lentinan, a branched (1→3)-β-D-glucan of MW 1000,000, is again similar to that of curdlan powder, although the C-4, C-2 and C-5 signals were not well resolved. These unresolved signals arose from a superposition of an additional signal ascribable to the displaced C-6 signal due to the formation of β-(1→6) linkage at the branch point[2] (two branches for every five glucosidic residues). Thus, it is obvious that lentinan also adopts similar single helix conformation, at least as a major conformer. This finding is generally in agreement with the previous X-ray diffraction by Bluhm and Sarko[11], although tertiary

Table 1. ^{13}C Chemical Shifts of (1→3)-β-D-Glucans in Solid, Gel and Solution (ppm from TMS)

	CP/MAS NMR (solid)					High Resolution NMR	
	High Molecular Weight			Low Molecular Weight		††Gel	††Solution
	Curdlan Powder	Native Curdlan	Lentinan	Curdlan (DP_n20)	Laminaran	(DP_n540)	(DP_n13)
C-1a	104.7(105.1)†	105.1	104.3	104.8			
b		106.6	105.2	106.0		106.5	
c		103.7	103.3	103.7	103.4		103.7
C-2	73.9 (74.8)	*	*	74.7	73.6	74.2	74.5
C-3a	89.5 (90.1)	89.6	90.3	89.7	88.8	88.7	
b				86.4	83.0		85.3
C-4	69.5 (70.6)	69.5	69.5	69.0	68.0	70.2	69.3
C-5	76.2 (76.6)	76.9	76.0	76.1	76.5	76.8	76.8
C-6	62.2 (62.1)	62.0	62.2	62.2	62.2	61.8	61.9

*Signals in the shoulder; †Data observed at 15.03 MHz (from ref. 8); ††from ref.3.

structure may be more complicated than reported, because the branched structure was not taken into account in their analysis.

The most pronounced feature of the ^{13}C NMR spectra in the glucans without gel-forming ability (curdlan, DP_n=20 (and DP_n=125; spectrum not shown) and laminaran) is that an additional peak marked by b appears at the right hand of C-3a peak. No other signal than that of β-D-(1→3)-linkage was seen by high resolution ^{13}C NMR spectra recorded in DMSO and alkaline solution. Therefore, the C-3b peak should be ascribed to the presence of a conformation other than the helix form. Interestingly, the ^{13}C shift of C-3b is very close to that of aqueous solution of low molecular weight glucan (DP_n=13) adopting random-coil form (see Table 1). Accordingly, it is natural to assume that this signal comes from randomly-coiled or amorphous portion in solid state, in which the dihedral angle between C-3-O and C-1-O is deviated substantially from that of the helix. The increased peak height of the C-1c (traces D and E), the position of which being very close to the value observed in aqueous solution, is well explained by this view. In parallel with this finding, we previously noted that high resolution ^{13}C NMR spectra of these glucans in neutral and/or dilute alkaline (0.06 M NaOH) media afforded considerable amounts of peaks ascribable to the random-coil. For this reason, it is clear that low molecular weight (1→3)-β-D-glucans cannot afford sufficient number of helical conformers essential for formation of cross-linking.

In conclusion, it is demonstrated that ^{13}C CP/MAS NMR spectroscopy is a very useful tool to determine conformation of polysaccharide chains in non-crystalline region as well as in crystalline portion. So far, we ascribed the conformer giving rise to the major component of the ^{13}C chemical shifts to the single helix, because such a species can be visible in gels or dilute alkaline media by conventional high resolution ^{13}C NMR spectrometer. However, more elaborate assignment of peaks, including distinction between the single- and triple-helices, might be necessary by the CP/MAS NMR spectra with appropriate use of reference samples.

Such a work is in progress in our laboratory.

The authors thank Dr. G. Chihara of National Cancer Center Research Institute for generous gift of lentinan, and Takeda Chemical Industries for gift of curdlan powder and lower molecular-weight curdlan. This work was supported in part by a Grant-in-Aid for Scientific Research from Ministry of Education, Science and Culture of Japan.

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13) Curdlan powder contains only 30% crystalline portion.[14] Nevertheless, no separation of peaks was seen between the crystalline and non-crystalline portions like ^{13}C NMR spectra of synthetic polymers.[15] Obviously, this is caused by that the non-crystalline portion consists of helical conformation and that ^{13}C chemical shifts are rather insensitive to the intermolecular perturbation such as packing of helices to crystalline region.
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(Received February 16, 1981)