A ^{13}C Nuclear Magnetic Resonance Study of Gel-Forming (1→3)-β-D-Glucans. Evidence of the Presence of Single-Helical Conformation in a Resilient Gel of a Curdlan-Type Polysaccharide 13140 from *Alcaligenes faecalis* var. *myxogenes* IFO 13140†

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ABSTRACT: A ^{13}C nuclear magnetic resonance (NMR) study of a resilient gel of a curdlan-type polysaccharide 13140, a (1→3)-β-D-glucan, from *Alcaligenes faecalis* var. *myxogenes* IFO 13140, was performed in an effort to understand the gel structure. It was found that very broad ^{13}C resonance peaks of line widths ca. 150 (C-1–C-5) and 50 Hz (C-6) are able to be seen in the gel state. Because of a fivefold increase of the peak intensities with respect to those in an aqueous suspension by gelation, these peaks are unequivocally ascribed to certain regions relevant to the gel structure. Those ^{13}C NMR peaks, however, account for only 20–30 (as viewed from C-1–C-5) and 60% (from C-6) of the total gel, the peak areas of the rest being lost. With respect to those of the disordered low molecular weight acid degraded fraction, fraction II, and laminaran, downfield displacements of C-1, C-3, and C-4 signals are found to take place by amounts of 2.8, 3.2, and 0.9 ppm, respectively, while the remaining peaks (C-2, C-5, and C-6) are unchanged. In view of similar differences of chemical shifts between cyclodextrins and linear (1→4)-α-D-glucans (Colson, P., Jennings, H. J., and Smith, I. C. P. (1974), *J. Am. Chem. Soc. 96,* 8081–8087), the observed downfield ^{13}C shifts of the glucosidic bonds in the gel are explained by the presence of the fixed conformation of the preferred dihedral angles, in which internal rotations around the glucosidic bonds are not allowed. Combined with the results on theoretical prediction and the downfield shift of C-4 signals, which is consistent with the presence of an O-4′···O-5 intramolecular hydrogen bond, the observed ^{13}C peaks are ascribed to a region of single helical conformation, whereas the peak-loss portion of ^{13}C NMR signals (70–80 and 40% from C-1–C-5 and C-6, respectively) is presumably ascribed to the multiple-helical junction zones for the gel structure and their vicinities. The variation of the line widths as well as the peak positions is also found to take place by stepwise addition of NaOH (>0.22 M). The onset of the conformational transition, helix to random coil, is in good agreement with the change of viscosity, specific rotation, optical rotatory dispersion, and absorption maximum shift by complex formation with Congo Red reported by Ogawa et al. (Ogawa, K., Watanabe, T., Tsurugi, J., and Ono, S. (1972), *Carbohydr. Res. 23,* 399–405).

Understanding of molecular assembly of various polysaccharide gels is of considerable importance with regard to their biological functions, as in the walls of plant cells, in animal fluids, and in connective tissues (Rees, 1968, 1972; Bryce et al., 1974). A picture of polysaccharide gelation has emerged in which solvent is trapped in the interstices of a three-dimensional network formed by the cooperative association of long regions of polymer chains in ordered conformation, so called junction zones (Rees, 1968, 1972). Hence, gel structure is closely related with conformation of constituting single or multiple polymer chains of polysaccharides in solution. The conformations of neutral polysaccharide, however, especially of (1→3)-β-D-glucan in solution, have not been fully explored, although double- or triple-helical (Atkins et al., 1969) conformations are present in the solid state and have been discussed in theoretical predictions (Rees and Scott, 1969; Sathyanarayana and Rao, 1971; Sundaralingam, 1968).

Recently, a thermally gelable (1→3)-β-D-glucan, curdlan-type polysaccharide 13140 (PS 13140),[1] which consists entirely of β-D-(1→3)-linked D-glucose residues and contains no other linkages as in pachyman and laminaran, was produced by cultivation of a mutant of a soil bacterium, *Alcaligenes faecalis* var. *myxogenes* IFO 13140 (Harada et al., 1968; Harada, 1972, 1974; Saito et al., 1968; Maeda et al., 1967; Nakanishi et al., 1974; Kimura et al., 1973). Apparently, for a study of conformation of (1→3)-β-D-glucan, it seems most appropriate to employ this polysaccharide, free from ambiguity due to other linkages. Physical properties of this compound in the resilient gel, formed by heating an aqueous suspension above 55 °C, as well as in dilute alkaline solution were reported (Maeda et al., 1967; Harada, 1972, 1974; Ogawa et al., 1972, 1973a,b; Nakanishi et al., 1974; Koreeda et al., 1974). It is noteworthy that PS 13140 and water-insoluble fractions of its acid-degraded products adopt an ordered conformation as manifested by studies of optical rotatary dispersion, intrinsic viscosity, flow birefringence, and shift of absorption maximum of the complex with Congo Red (Ogawa et al., 1972, 1973b) or Aniline Blue (Nakanishi et al., 1974).

In this paper, we attempted to elucidate the nature of the ordered conformation of (1→3)-β-D-glucan to provide insight

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[1] Abbreviations used are: PS 13140, Curdlan-type polysaccharide 13140; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser enhancement; T_1, spin-lattice relaxation time; T_2, spin-spin relaxation time.

into the gel structure of the resilient PS 13140 gel and conformational behavior in solution by ^{13}C nuclear magnetic resonance. Although the ^{13}C NMR method has proved to be a very powerful tool for characterization of polysaccharides, very few conformational studies have been done except for the observation (Colson et al., 1974) of substantial downfield ^{13}C shifts of the linked carbons, C-1 and C-4, of cyclodextrins with respect to those of linear (1→4)-α-D-glucans, suggesting the existence of conformation-dependent ^{13}C chemical shifts, and the conformational study of a branched (1→3)-β-D-glucan (Saitô et al., 1976). In this paper we first wish to demonstrate that very broad ^{13}C NMR signals are seen in the spectrum of the resilient gel of PS 13140 and that these peaks may be ascribed to the single-helical portion of the gel in view of the substantial downfield shifts of peaks, relating to the glucosidic bonds in comparison with those of the random-coil conformation, and extremely large line width (~150 Hz). It is then concluded that the resilient gel is composed of considerable amounts of the single helical regions in addition to the multiple-helical junction zones at which all of the ^{13}C peaks are lost because of the conformational rigidity. Furthermore, it is shown that a change of the line widths of ^{13}C signals by the stepwise addition of NaOH into an aqueous suspension of PS 13140 is characteristic of the conformational transition from helix to random coil, in good agreement with the change of absorption maximum of complex formation with the dyes.

Experimental Section

A linear (1→3)-β-D-glucan with dp_n 540, curdlan-type PS 13140, was kindly supplied by Takeda Chemical Industries, Ltd., Osaka, Japan. A partially degraded fraction, fraction II with dp_n 13, was prepared from PS 13140 with formic acid and fractionated with Amicon Diaflo ultrafiltration, followed by the stepwise addition of ethanol. Since PS 13140 was not soluble in D_2O at neutral pD, a viscous aqueous suspension was used (80 mg/mL). The resilient gel was prepared by heating the aqueous suspension over a steam bath at a temperature above 55 °C. All measurements were made at ambient temperature (28 °C). Samples were contained in 10-mm o.d. tubes at a concentration of 80 mg/mL.

^{13}C NMR spectra were obtained on a JEOL PFT-100/EC-100 pulsed Fourier transform spectrometer operating at 25.03 MHz. The 90° pulse requiring 22 μs was used to accumulate free induction decays with a repetition time of 0.6 s, since spin–lattice relaxation times were found to be less than 100 ms. 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 using 4K data points and a spectral width of 4K Hz. ^{13}C chemical shifts are expressed in parts per million downfield from external tetramethylsilane. All chemical shifts were measured digitally; the maxima of the broad lines in the gel were ensured by inspection of an expanded spectrum displayed on a Tektronix Monitor 400 scope in the EC-100 system. Inevitably by this procedure, estimated accuracy of the chemical shifts of the broad signals is ±0.3 ppm. The line width was taken as full width at half-height in an expanded spectrum, with an error of ±15–±20%. Spin–lattice relaxation times were obtained using the pulse sequence of 180°–t–90°, with an estimated error of ±10%. Nuclear Overhauser enhancements (NOE's) were obtained from the ratio of the intensity of fully decoupled spectra to the intensity of spectra in which the proton noise decoupler was gated off to remove the NOE (Freeman et al., 1972). The estimated error is ±15%.

Results and Discussion

(*A*) *Gel Structure Consisting of Single and Multiple Helical Region*

In Figure 1B, it is shown that ^{13}C NMR signals of the resilient gel of PS 13140 can be observed, although their line widths are considerably broadened in comparison with those of an alkaline solution (0.22 M NaOH) (Figure 2D) and also those of smaller molecular weight fraction (fraction II; see Figure 1A). First of all, we have checked the possibility that these signals arose from a smaller molecular weight polysaccharide dissolved in water, as often encountered in an unfractionated polysaccharide suspension or solution. However, exactly the same signal amplitude of the ^{13}C signals was observed, even after removing smaller molecular weight substances by ultrafiltration (mol wt <10 000). Moreover, it was confirmed that the ^{13}C signal amplitude is increased by fivefold by gelation from aqueous suspension compared with the signal amplitude of the suspension (see Figure 1B and Figure 2A). To our knowledge, this is the first observation of the ^{13}C NMR signals of the backbone constituting gels, in contrast to the cases of ι-carageenan (Bryce et al., 1974) and collagen (Chien and Wise, 1975) where restricted mobility of the double- and triple-helical backbone provides ^{13}C signals that are too wide to be observed by the high-resolution spectrometer. By comparing the integrated peak intensities (with NOE suppressed) of the ^{13}C signal of the gel with those of the random-coil conformation (0.22 M NaOH), as described later, it is possible to estimate the amount of the ^{13}C NMR visible regions in the gel. It is found that the peak areas of ^{13}C NMR account for approximately 20–30 and 60% for C-1–C-5 and C-6, respectively, of the total gel. It is not surprising to note that the peak area of C-6 is two to three times as high as that of C-1–C-5 in view of allowed internal rotation of the hydroxymethyl group (C-6). Hence, the remaining ^{13}C NMR invisible portion (70–80 and 40% from C-1–C-5 and C-6, respecitvely) should be ascribed to the junction zones of the gel structure and possibly to residues located closely to these junctions.

The line broadening of ^{13}C resonances in the gel might be interpreted in terms of immobilization of polymer chains in the presence of the junction zones. In fact, such broad resonance peaks were observed in cross-linked synthetic gels of poly(*N*-vinylpyrrolidone) and poly(hydroxyethyl methacrylate) under the condition of low water content (Yokota et al., 1977). In these cases, however, no chemical-shift displacements were noted between gel and polymer solution without cross-linking. In addition, we have reported that ^{13}C resonance peaks of α-(1→4) residues in the side chains of a branched (1→3)-β-D-glucan, A_3, in the gel state appear at the same positions as those of (1→4)-α-D-glucans in aqueous solution (Saitô et al., 1976). In view of these results, it is likely that ^{13}C resonance peaks due to a portion of disordered conformation in the gel would not be shifted by any special effect, for instance, of conformational distortion by the gel network. On the contrary, as described in detail below, it is clearly seen that C-1 and C-3 peaks of the gel are shifted downfield considerably with respect to those of fraction II (Figure 1). Together with the extensive studies in dilute alkaline solution by Ogawa et al. (1972, 1973b), this observation strongly suggests that the polymer chain of PS 13140 in the resilient gel adopts the ordered conformation. On the basis of the following arguments, the ^{13}C resonance peaks of the gel are ascribed to the region of single helical conformation.

(*1*) *Downfield Displacements of C-1 and C-3 Chemical*

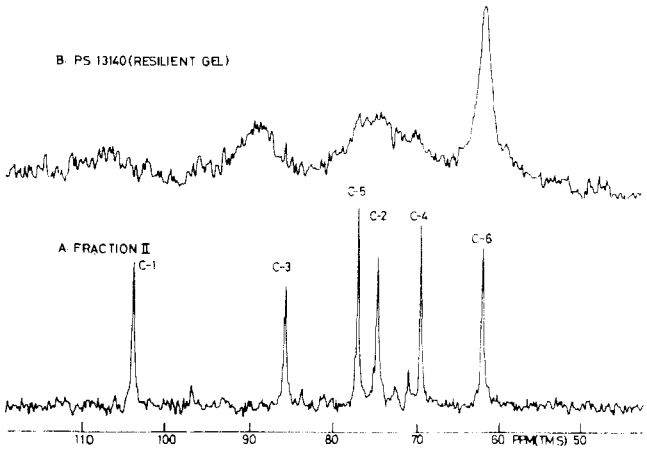

FIGURE 1: ^{13}C NMR spectra of the resilient gel of (A) small molecular weight fraction, fraction II (90° pulse, repetition time 1 s, 800 accumulations) and of (B) PS 13140 (90° pulse, repetition time 0.6 s, 88 000 accumulations).

Shifts. Figure 1 shows that ^{13}C peak positions of the resilient gel are shifted downfield by 2.8, 3.2, and 0.9 ppm for C-1, C-3, and C-4, respectively, whereas other signals (C-2, C-5, and C-6) remain unchanged (Table I). Interestingly, such substantial downfield shifts occur at carbons participating in the glucosidic linkages of (1→3)-β-D-glucan (C-1 and C-3). In a random-coil conformation[2] as in fraction II, a rapid internal rotation might be allowed around C-1–O and C-3–O in conjunction with segmental motions of the polymer chains. It is plausible, however, that in certain conformations, for instance, the right-handed or left-handed helix, such a rotation is not allowed, thus leaving only slight oscillation around the favored dihedral angle. Recently, Colson et al. (1974) showed that the chemical shifts of C-1 and C-4 of cyclodextrins differ substantially from those of the linear compound, amylose (1.6–2.0 and 2.9 ppm, respectively), and the shifts of other unlinked carbons are almost the same between cyclic and linear (1→4) α-D-glucans. From ab initio molecular orbital calculations of methanediol and methoxymethanol as models for the hemiacetal and acetal moieties of aldopyranose and methyl aldopyranosides, Jeffrey et al. (1972, 1974) predicted that the bond length of OCH_3 in methyl pyranosides varies with conformers around OCH_3 over a range of 0.01 Å. Apparently, this result implies that the electron densities at C-1 and C-3 of the glucosidic linkages vary depending on the rotamer population, and would be reflected in the conformation-dependent ^{13}C chemical-shift change described above.

(2) Energetically Preferred Conformations. The energetically preferred conformations of (1→3)-β-D-glucan were determined by calculation of the energy contour diagrams (Rees and Scott, 1969; Sathyanarayana and Rao, 1971) and consideration of the intramolecular hydrogen bonds between contiguous resides (Sundaralingam, 1968; Sathyanarayana and Rao, 1971). These results showed that wide and extended helical conformations are most probable for (1→3)-β-D-glucan. Therefore, it is reasonable to ascribe the ordered conformation of PS 13140 to the helix form. Recently, Takeda et al. (1977) showed, in the single-crystal x-ray analysis of laminaribiose, that the sole intramolecular hydrogen bond is formed between O-4′···O-5. This hydrogen bond would cause the ^{13}C signal of C-4, adjacent to the O-4′–H group, to shift downfield as depicted by the scheme $C\text{-}4^{+}\text{-}O\text{-}4'^{-}\text{-}H^{+}\cdots O\text{-}5^{-}$. Thus, the observed downfield shift of the C-4 signal (0.9 ppm) in the gel is consistent with the presence of an O-4′···O-5 hydrogen bond, which leads to the right-handed helical conformation.

TABLE I: ^{13}C Chemical Shifts of PS 13140 in the Resilient Gel and of the Fraction of Lower Molecular Weight (Fraction II).

	Resilient Gel	Fraction II	Difference
C-1	106.5	103.7 (103.8)[a]	2.8
C-2	74.2	74.5 (74.4)	−0.3
C-3	88.7	85.5 (85.5)	3.2
C-4	70.2	69.3 (69.3)	0.9
C-5	76.8	76.8 (76.8)	0
C-6	61.8	61.9 (61.9)	−0.1

[a] ^{13}C chemical shifts of laminaran (Colson et al., 1974).

(3) Line Width and Relaxation Time. Generally, ^{13}C relaxations of high polymers in solution are mainly determined by the segmental motions of chains instead of reorientational motions of the molecule as a whole. Even so, it was shown that ^{13}C peaks of molecules in the rigid double- or triple-helical conformation are completely lost (Chien and Wise, 1975; Bryce et al., 1974; Smith et al., 1975). In the case of single-helical polymers, however, rather narrow ^{13}C resonances (<40 Hz) have been reported (Saitô and Smith, 1973; Allerhand and Oldfield, 1973; Smith et al., 1975). Therefore, the ^{13}C NMR visible portion of the gel should be ascribed to the single-helical region. Nevertheless, the segments of the single-helical portion in the vicinity of the junction zone may be unlikely to gain enough mobility to produce ^{13}C resonance peaks. Thus, it is likely that the ^{13}C resonance peaks of the gel probably arise from free ends or rather flexible middle parts of the single-helical chains tied at the junction zones, in addition to helical chains trapped in interstices of the network.[3] This view is not inconsistent with the fact that considerably large spaces are made up in the gel, as described in section C. ^{13}C peaks of the junction zones, composed of double- or triple-helical chains, are naturally lost completely. The tendency of (1→3)-β-D-glucan to form a multiple helix was also proposed by Rees and Scott (1969).

The spin–lattice relaxation times (T_1's), nuclear Overhauser enhancements (NOE's), and line widths[4] (and spin–spin relaxation times, T_2's) of the resilient gel are summarized in Table II. It seems noteworthy that the T_1/T_2 ratio of the resilient gel is surprisingly large (45, 38, and 9.6 for C-1, C-3, and C-6, respectively). This is caused by the fact that T_2 tends to be more affected by the slow motion of long correlation time and T_1 is mainly determined by the fast motion of short correlation time. For this reason, as a first approximation to deduce the correlation times, a distribution of the correlation times is taken into account here following the Schaefer treatment of the log-χ^2 distribution (Schaefer, 1973; Lyerla and Torchia, 1975).

$$F^{(p)}(s) = (ps)^{p-1}e^{-ps}p/\Gamma(p) \qquad (1)$$

[2] It was noted by Ogawa et al. (1972) that the lower molecular weight fraction (dp_n <25) did not take ordered conformation.

[3] At the present it is very difficult to separate the relative contributions among them, because the extent of the cross-linking is not known in this experiment.

[4] Obviously, it is seen that the line width of C-1 is slightly larger than that of C-3. The difference may be partly due to anisotropic segmental motions. In our treatment, however, these effects are neglected for the sake of simplicity.

TABLE II: ^{13}C Spin-Lattice Relaxation Times, Line Widths, and Nuclear Overhauser Enhancements of PS 13140.

	Helix							Random Coil				
	Resilient Gel			0.06 M NaOH		0.19 M NaOH		0.22 M NaOH			Me_2SO	
	T_1[a]	Line Width (T_2)[b,c]	NOE	Line Width (T_2)	NOE	Line Width (T_2)	NOE	T_1	Line Width (T_2)	NOE	T_1	NOE
C-1	86	172 (1.9)	1.0	161 (2.0	1.2	167 (1.9)	1.3	76	14 (23)	1.5	77 (44)[d]	1.4
C-2	84		1.4		1.3		1.5	78	15 (21)	1.4	73 (48)	1.5
C-3	76	156 (2.0)	1.2	167 (1.9)	1.3	183 (1.7)	1.3	84	14 (23)	1.4	85 (46)	1.4
C-4								80	15 (21)	1.5	80 (52)	1.3
C-5	84		1.4		1.3		1.5	83	14 (23)	1.3	84 (50)	1.4
C-6	62	50 (6.4)	1.4	50 (6.4)	1.8	53 (6.0)	1.6	54	17 (19)	1.9	57 (40)	1.7

[a] In milliseconds. [b] In hertz. [c] In milliseconds calculated from $1/\pi$ (line width). [d] T_1's at 15 MHz.

TABLE III: Calculated Correlation Times of the Helix and Random-Coil Conformations (Nanoseconds).[a]

From	Helix Single Corr. Time (τ)	Distrib. of Corr. Times ($\bar{\tau}$)[b]	Random Coil Single Corr. Time (τ)
T_1	23, 0.63	30, 0.6	22, 0.66
NOE	7.3	39	4.3
T_2	120	38	6.4

[a] Calculated from the averages of C-1–C-5 carbons for observed T_1, NOE, and T_2. [b] $p = 26$, $b = 1000$.

$$s = \log b[1 + (b - 1)\tau_1/\bar{\tau}] \quad (2)$$

where the width of the distribution is characterized by p, the logarithmic time scale is determined by b, and the average and individual correlation times are given by $\bar{\tau}$ and τ_1, respectively. In the present case, it is found that the choice of $p = 26$ and $b = 1000$ produces a qualitatively consistent $\bar{\tau}$, 30–40 ns, which is much better in comparison with the approximation of a single correlation time (Doddrell et al., 1972), as shown in Table III.

(B) *Helix–Coil Transition*

In dilute alkaline solution up to 0.19 M NaOH concentration, the ^{13}C NMR spectra observed are essentially the same as those of the resilient gel. Therefore, it is concluded that the internal structures of the gels are apparently similar to each other. At 0.22 M NaOH concentration, the line widths of the ^{13}C signals decrease to less than one-tenth those of the helix (see Figure 2B and 2C), which is characteristic of the random-coil conformation. The transition behavior could be more clearly seen in a plot of the line width, C-1, C-3, and C-6, vs. NaOH concentration, as shown in Figure 3. This plot is in good agreement with that of the shift of absorption maximum of complex formation with Congo Red and with that of specific rotation, and the viscosity change of PS 13140 with respect to NaOH concentration presented by Ogawa et al. (1972), in which the transition occurred at a NaOH concentration between 0.19 and 0.24 M. Because NaOH would break up hydrogen bonds stabilizing the single- and multiple-helical conformation, this transition might be considered as the helix–coil transition of the polysaccharide chains accompanying the macroscopic gel to sol transition. There seems no obvious increase of the peak intensities of ^{13}C resonances on adding NaOH up to 0.19 M, in contrast to those of the branched

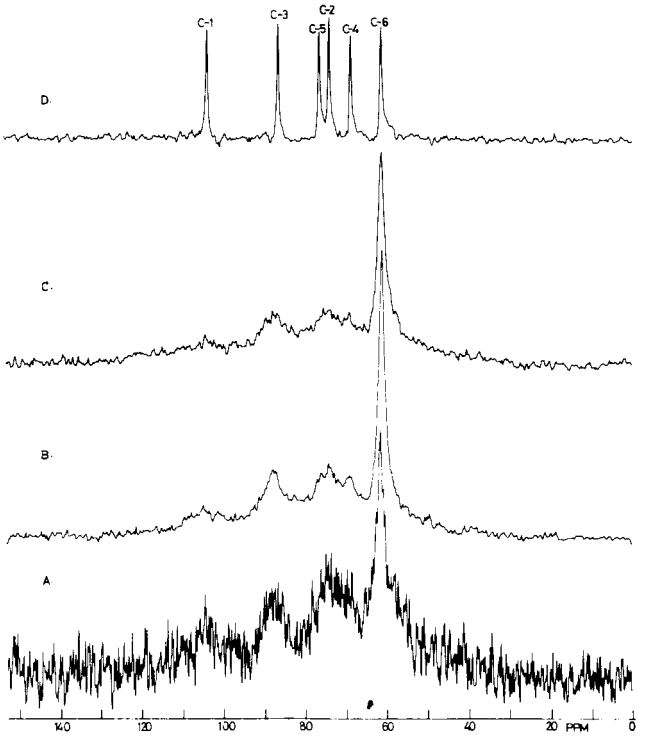

FIGURE 2: ^{13}C NMR spectra of PS 13140 of an aqueous suspension and alkaline solutions (90° pulse, repetition time 0.6 s): (A) aqueous suspension (neutral), 80 000 accumulations; (B) 0.06 M NaOH solution, 77 000 accumulations; (C) 0.19 M NaOH solution, 99 000 accumulations; (D) 0.22 M NaOH solution, 4500 accumulations.

(1→3)-β-D-glucans.[5] For this reason, the helix–coil transition seems to occur simultaneously from *both* the single and multiple helix to random coil as a cooperative process.

In contrast to the substantial change of the line widths, the changes of the T_1's and NOE's, however, were found to be not sensitive to the helix–coil transition (Table II). However, it should be noted that all of the peak intensities, most of which are lost in the gel state, are recovered in this transition. Thus, the most drastic change might be expected for the T_1's, NOE's, and T_2's of the junction zones, though it is not possible to obtain those in the present type of experiment. The correlation time of the segmental motion of the random-coil conformation was

[5] In these cases, an obvious increase of the peaks ascribable to the single-helical region is noted in the intermediate of the helix–coil transition (Saitô et al., 1976, 1977).

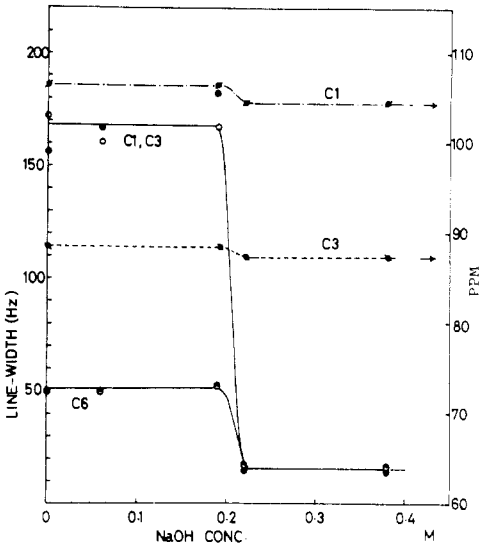

FIGURE 3: A plot of the line widths (C-1, C-3, and C-6), and ^{13}C chemical shifts of C-1 and C-3 (right) vs. NaOH concentration: line widths (left) (○) C-1, (●) C-3, (⊖) C-6; chemical shifts (right) (φ) C-1, (◊) C-3.

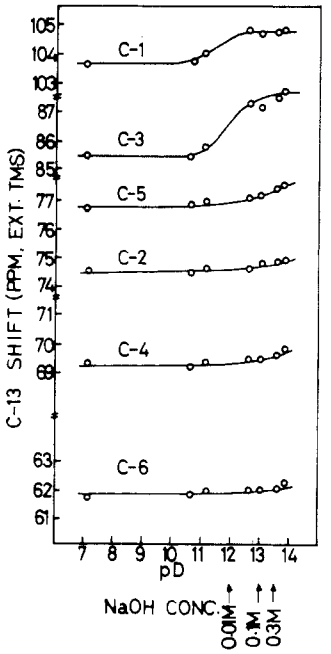

FIGURE 4: A plot of ^{13}C NMR signals of laminaran vs. pD.

TABLE IV: ^{13}C Spin–Lattice Relaxation Times (Milliseconds) and Nuclear Overhauser Enhancements of Fraction II in Solution and in Gel.

	Solution (pD 7)		Gel[a]	
	T_1	NOE	T_1	NOE
C-1	150	2.8	120	2.3
C-2	170	2.7	130	2.2
C-3	150	2.7	120	2.5
C-4	170	2.9	160	2.2
C-5	190	2.6	130	2.1
C-6	90	2.9	70	2.2

[a] The resilient gel consisting of PS 13140 (74%) and fraction II (26%).

calculated as 4.3–22 ns by a more satisfactory description of the single correlation time (Table III). The change of the correlation times, from the helix (30–40 ns) to coil (4.3–22 ns), seems to be unexpectedly small. The correlation time of the latter may be long in comparison with those of fraction II, 0.3–0.7 ns, obtained from the T_1 and NOE values (Table IV), and of amylose at pD 13.7, 0.5–1 ns (Saitô et al., unpublished results). These results suggest that the molecular mobility is still restricted in the random-coil conformation. In fact, it was shown in the computation of the energy contour map that the allowed conformations constitute only about 4% of the total conformations (Sathyanarayana and Rao, 1971). This conclusion is consistent with the finding by Casu et al. (1966) that considerable amounts of the intramolecular hydrogen bonds between contiguous residues of laminaran remain in dimethyl sulfoxide solution, leading to the suggestion that a helix or at least a coil of short helical segment is substantially retained in dimethyl sulfoxide. This is also true for alkaline solution (>0.22 M NaOH) in view of the very similar relaxation parameters taken in both solutions. Thus, the substantial decrease of the line widths in the random coil is interpreted in terms of disappearance of the cooperativity of longer segments which is characterized by the lower frequency end of the distribution of the correlation times determining larger line widths.

In accordance with the conformational transition, ^{13}C chemical shifts remain unchanged while taking the helical conformation even if the NaOH concentration is raised to 0.19 M (pD 13.2) (Table V and Figure 3). This trend is in contrast to the titration behavior of the ^{13}C shifts of laminaran (Figure 4),[6] in which downfield displacements of the linked C-1 and C-3 occur at pD between 10.5 and 12.9 the midpoint being at pD 11.8. Clearly, ^{13}C chemical shifts are influenced by both the conformational and ionization behaviors, both of which displace the ^{13}C chemical shifts of the glucosidic bonds (C-1 and C-3) downfield. However, the former contribution is evidently predominant in the helical (1→3)-β-D-glucan, PS 13140, because the C-1 and C-3 chemical shifts are constant up to pD 13.2. The interpretation is not clear at present; however, the rotamer population around the glucosidic bonds also seems to be related to the ionization-dependent ^{13}C chemical shifts.

(C) *Gels Involving Fraction II*

Clearly, there seems to exist a minimal chain length to form the multiple helix from a statistical point of view, since the possibility of forming the three-dimensional network should be abruptly diminished if the chain length is too short. Among acid-degraded low molecular weight fractions, Ogawa et al. (1972) showed that those fractions with dp_n higher than 25 are capable of forming the ordered conformation. As a clue to further understanding of the gel network, ^{13}C peak intensities of the single helical regions are compared with those of fraction II of a known concentration for a gel formed by a mixture of PS 13140 and fraction II. As shown in Figures 5B and 5C, very sharp ^{13}C signals rising from fraction II are seen in addition to the very broad peaks due to the gel structure. This result clearly shows that the fraction II molecule does not participate

[6] In the course of pD titration of laminaran, partial degradation of the polymer possibly by a peeling reaction was noted at higher pD regions, whereas no such degradation was observed for the PS 13140 sample. In this regard, the gel formation was proved to be reversible. ^{13}C NMR spectra of neutralized PS 13140 sample from pD 13.9 (0.37 M NaOH) exhibited the same pattern as those of Figures 2B and 2C.

TABLE V: ^{13}C Chemical-Shift Changes of the Helix and Random-Coil Conformation (in Parts per Million) from External Me_4Si).

	Helix		Random Coil		
	Resilient Gel	0.19 M NaOH (pD 13.2)	0.22 M NaOH (pD 13.4)	0.38 M NaOH (pD 13.6)	Laminaran pD 14[a]
C-1	106.5	106.4	104.7	104.8	104.7
C-2	74.2	74.8	74.9	74.9	74.9
C-3	88.7	88.5	87.4	87.4	88.0
C-4	70.2	69.7	69.8	69.6	69.9
C-5	76.8	77.0	77.4	77.4	77.8
C-6	61.8	62.1	62.3	62.1	62.5

[a] Taken from Colson et al. (1974).

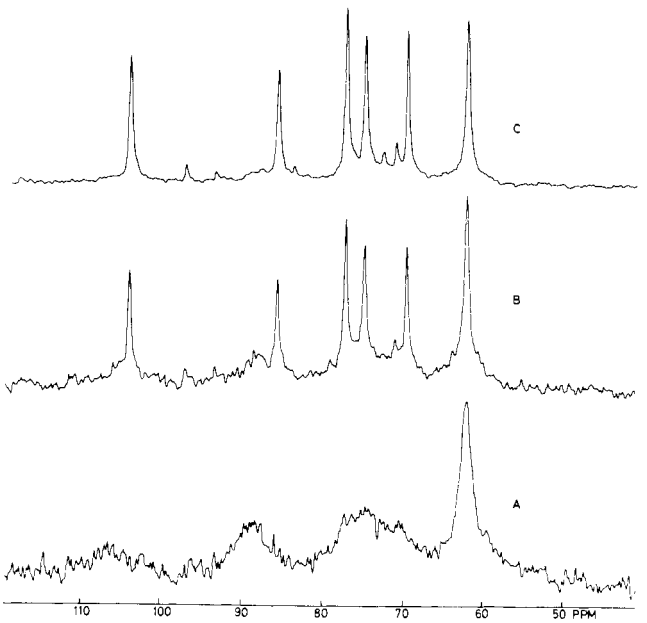

FIGURE 5: ^{13}C spectra of the resilient gel consisting of PS 13140 and fraction II: (A) PS 13140 only; (B) PS 13140, 87.5%; fraction II, 12.5%; (C) PS 13140, 74%; fraction II, 26%.

in the gel structure but dissolves in the interstices of the gel structure without perturbation from the network (see also Table IV). Accordingly, it may be concluded that there exists a large space that is enough to allow the fraction II molecule of dp_n 13 to move freely therein. Such a space achieved by the network seems to relate to the resiliency of this polysaccharide. In this regard, it is interesting to note that very small or almost no single-helical regions are seen in the branched (1→3)-β-D-glucans which do not exhibit any elastic property, A_3 from *Pleurotus ostreatus* (Saitô et al., 1976), lentinan from *Lentinus edodes*, and schizophyllan from *Schizophyllum commune* (Saitô et al., 1977), although in the intermediate of the helix-coil transition ^{13}C signals due to the single helix are clearly observed.

By measuring the ratio of the peak intensities of either C-1 or C-3 of PS 13140 (w_1) to those of the fraction II (w_2) together with the known quantity of the fraction II (f) and the NOE values (η_1 and η_2) (Table IV), the amount of the helical regions may be calculated from:

$$f(w_1/\eta_1)/(w_2/\eta_2)$$

The value 30–50% was obtained by this procedure which is in good agreement with that described in section A.

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Escherichia coli DNA-Directed β-Galactosidase Synthesis in Presence and Absence of Ca^{2+}†

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ABSTRACT: DNA-dependent synthesis of β-galactosidase was optimized in extracts made from cells lysed by a standard French pressure cell. Extracts made at 3200 psi synthesized up to 25-fold more β-galactosidase than extracts made at 7500 psi. β-Galactosidase synthesis was cyclic 3′,5′ AMP dependent, as expected, and in optimal conditions transcription and translation proceeded at 8.6 nucleotides and 2.7 amino acids per s, respectively. The high pressure extracts were stimulated 3- to 5-fold by Ca^{2+}, especially at low Mg^{2+} concentrations. In contrast, extracts prepared at low pressure were inhibited as much as 50-fold by Ca^{2+} ions. The inhibition by Ca^{2+} was analyzed further. Addition of kasugamycin, an antibiotic that acts on ribosomes, to reactions containing Ca^{2+} stimulated β-galactosidase synthesis to nearly control levels. Extracts from a kasugamycin resistant mutant were neither inhibited by Ca^{2+} nor stimulated by the addition of kasugamycin to in vitro reactions containing Ca^{2+}. The change in the mutant was ascribed to the ribosomes by testing combinations of soluble proteins, ribosome wash, and ribosomes from parental and mutant strains. These results suggest that Ca^{2+} ions inhibit translation by ribosomes, very likely at an initiation step; and that they enhance enzyme synthesis only in conditions where translation is inefficient (high-pressure extracts at low concentrations of Mg^{2+}, for example). This latter effect is probably a consequence of increased RNA stability in the presence of Ca^{2+} (Cremer, K., and Schlessinger, D. (1974), *J. Biol. Chem. 249,* 4730).

The DNA-coupled system of Zubay has provided a powerful way to reproduce in vitro many features of gene expression (Zubay, 1973). Major components required for DNA-directed synthesis of enzymes have been fractionated to a high state of purity (Kung et al., 1973, 1975a), and the systems have been used to analyze the regulation of transcription and translation of the *lac* operon (de Crombrugghe et al., 1971a; Kung et al., 1975a,b: Zubay et al., 1970), *gal* operon (Parks et al., 1971; Wetekam et al., 1972; Schumacher and Ehring, 1975), *ara* operon (Wilcox et al., 1974), *trp* operon (Pouwels and Van Rotterdam, 1972, 1975; Zalkin et al., 1974), the genes coding for the β and β′ subunits of *E. coli* RNA polymerase (Austin, 1974), the ribosomal proteins of *E. coli* (Kaltschmidt et al., 1974), and the N gene of bacteriophage λ (Dottin and Pearson, 1973; Greenblatt, 1973). Of singular importance to all these studies is an understanding of the components of the in vitro system which affect protein synthesis. For example, three features of the results have varied widely in different reports: the absolute levels of enzyme formed (Austin, 1974; Kung et al., 1973; Parks et al., 1971; Wetekam et al., 1971; Wilcox et al., 1974; Zubay et al., 1970); the dependence or lack of dependence on Ca^{2+} for enzyme synthesis (de Crombrugghe et al., 1970; Kung et al., 1973; Parks et al., 1971; Wetekam et al., 1972; Zubay et al., 1970); and the levels of Mg^{2+} required for efficient protein synthesis (de Crombrugghe et al., 1970; Parks et al., 1971; Pouwels and Van Rotterdam, 1972, 1975; Wetekam et al., 1972; Zalkin et al., 1974; Zubay et al., 1970).

We report here that these three parameters are interrelated and give conditions for their optimization for the case of β-galactosidase synthesis directed by DNA. This has required a further analysis of the effects of Ca^{2+}. The results indicate that, in addition to its inhibitory effect on nucleases (Cremer and Schlessinger, 1974), Ca^{2+} is an effective inhibitor of translation, probably at the initiation step. This inhibition is overcome by modifications of the ribosome which are induced either by certain antibiotics or by a specific ribosomal mutation.

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