CONFORMATIONAL BEHAVIOR OF A GEL-FORMING (1→3)-β-D-GLUCAN IN ALKALINE SOLUTION

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ABSTRACT

Conformational behavior of a gel-forming (1→3)-β-D-glucan in sodium hydroxide solution was studied by optical rotatory dispersion, viscosity, and flow birefringence measurements, the alkali concentration being changed from 5mM up to 4.8M. In the wavelength range studied (230–500nm), optical rotatory dispersion curves were anomalous, except the curve obtained with 4.8M sodium hydroxide. At longer wavelengths and low alkali concentration, the sign of rotation angle was positive, and at shorter wavelengths, it decreased sharply resulting in a broad maximum near 265nm. The angle of rotation at a given wavelength fell sharply with alkali concentration in the range of 0.19–0.24M. These changes were reversible when the sodium hydroxide concentration was varied. Intrinsic viscosity and flow birefringence also showed large changes corresponding to the rotation angle change. These observations suggest that, in solutions of low sodium hydroxide concentration, the (1→3)-β-D-glucan takes an ordered conformation, whereas it practically behaves as a random coil at high concentration, a conformational transition occurring in the region between 0.19 and 0.24M. When sodium hydroxide was replaced with lithium hydroxide, a similar dependence of the optical rotation and the viscosity on the alkali concentration was observed.

INTRODUCTION

It has been shown[1,2] that the bacterial gel-forming (1→3)-β-D-glucan, isolated by Harada *et al.*[3–5] is soluble in aqueous alkali but not in neutral or acid solution, and that it forms a gel when its aqueous suspension (at pH < 12) is heated above 54°. In the present paper, the (1→3)-β-D-glucan was studied in alkaline solution by optical rotatory dispersion, viscosity, and flow birefringence measurements.

EXPERIMENTAL

Material. — The gel-forming (1→3)-β-D-glucan, a linear polymer consisting of (1→3)-linked β-D-glucose residues, obtained from the culture filtrate of a mutant

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strain (NTK-u, IFO 13140) of *Alcaligenes faecalis* var. *myxogenes* strain 10C3K[1–5], was supplied by Takeda Chemical Industries Ltd. The number-average molecular weight of the glucan, as determined by the end-group assay of Takagi *et al.*[6], was 6.5×10^4, indicating a degree of polymerization of 400.

Methods. — Since the presence of oxygen may accelerate the degradation of the glucan in alkaline solution, the glucan was dissolved in a nitrogen atmosphere in 0.005–4.8M aqueous sodium hydroxide solutions with magnetic stirring. After the stirring had been continued for 1 h, the viscosity or the flow birefringence of the solution was measured. Even in a nitrogen atmosphere, the liberation of glucose residues from the reducing end through peeling could not be avoided. No attempt was made to eliminate this effect, since this degradation seemed slow, and, when the measurement was made within several hours after preparation of the solution, the degradation effect could be ignored.

O.r.d. measurements were performed with a Yanagimoto Model ORD-185 Recording Spectropolarimeter at 25° from 230 to 500 nm. The reduced mean residue rotation, $[R]_\lambda$, was calculated according to the equation:

$$[R]_\lambda = [3/(n_\lambda^2+2)](M_0[\alpha]_\lambda/100)$$

where $[\alpha]_\lambda$ is the specific rotation and n_λ the solvent refractive index at wavelength λ, and M_0 is the mean residue molecular weight. The value of n_λ was calculated by Sellmeier formula:

$$n_\lambda^2 = 1+a\lambda^2/(\lambda^2-\lambda_0^2)$$

where a and λ_0 are constants; these constants were calculated by use of the experimental values of n_λ which were determined by a differential refractometer type DR-1 (Shimazu Seisakusho Co. Ltd.) at 25°. In o.r.d. measurements, the influence of alkali degradation on the glucan could be ignored because the same o.r.d. curves were obtained with the solutions examined immediately after preparation and with the ones examined after storage for 3 days in a nitrogen atmosphere.

The intrinsic viscosity was determined with a Ubbelohde-type dilution viscometer (capillary diameter, 0.5 mm) at 30° in a nitrogen atmosphere. All measurements were performed within 2.5 h after preparation of the solution. Under these conditions, the influence of the alkali on the glucan was negligible; for example, the intrinsic viscosity of a glucan solution in M sodium hydroxide showed a slight decrease from 3.22 (immediately after preparation) to 3.05 (after a 24-h storage in a nitrogen atmosphere).

The flow birefringence was determined with a flow birefringence viscometer Model No. B-23 (Rao Instrument Co.) at 30°. At low rate of shear, the position of the arms of the cross of isoclyne could not be determined accurately. Therefore, the measurements were performed at the high rate of shear of 5000–6200 sec^{-1}, and finished within 10 min from the start of the revolution of the driving motor. Under such a high rate of shear, the extinction angle was observed to increase about 1 degree in the interval of 12 min, probably owing to the degradation of the glucan under shear.

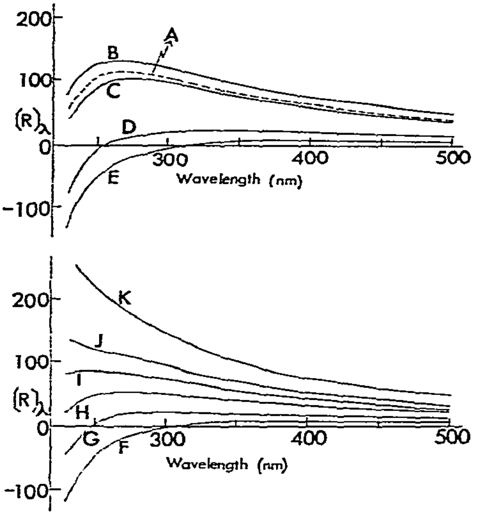

Fig. 1. O.r.d. curves of (1→3)-β-D-glucan solutions at various concentrations of sodium hydroxide and 25°. The concentrations of sodium hydroxide are (A) 0.005, (B) 0.19, (C) 0.21, (D) 0.22, (E) 0.24, (F) 0.26, (G) 0.58, (H) 1.0, (I) 1.5, (J) 2.0, and (K) 4.8M, respectively.

RESULTS

The o.r.d. curves of the glucan dissolved in various concentrations of aqueous sodium hydroxide are shown in Fig. 1. In the range of concentrations between 5mM and 0.21M*, the glucan is dextrorotatory at all the wavelengths studied and the o.r.d. curves are anomalous and show a maximum value near 265 nm. At higher sodium hydroxide concentration, a steep decrease in the optical rotation was observed with a minimum value at 0.24M sodium hydroxide concentration. From 0.24 to 4.8M, the optical rotation increases with the sodium hydroxide concentration. All o.r.d. curves, except the curve at 4.8M, are anomalous. The results shown in Fig. 1 were obtained with solutions of the glucan at a concentration of about 1 g/l. However, when the reduced mean residue rotation, $[R]_\lambda$, was plotted against the wavelength, all o.r.d. curves obtained for concentrations of the glucan in the range of 3–0.25 g/l were found to be independent of the concentration of the glucan. The values of $[R]_\lambda$, at wavelengths of 240, 265, 300, and 500 nm, respectively, as a function of sodium hydroxide concentration are shown in Fig. 2. An abrupt change in optical rotation was observed at sodium hydroxide concentrations between 0.19 and 0.24M, at any wavelength.

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*At a concentration lower than 5mM, the glucan did not give a clear solution.

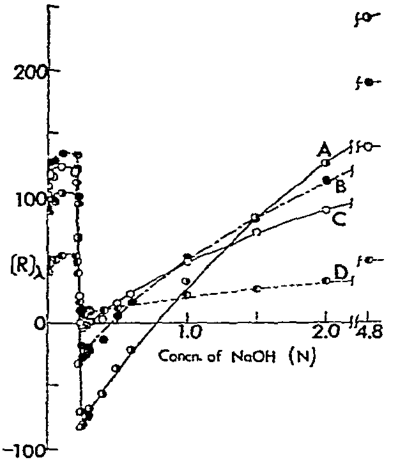

Fig. 2. Dependence of the reduced mean residue rotation ($[R]_\lambda$) of (1→3)-β-D-glucan on the concentration of sodium hydroxide at 25°. The wavelengths are (A) 240, (B) 265, (C) 300, and (D) 500 nm respectively.

In order to confirm the reversibility of the angle of rotation with the change of sodium hydroxide concentration, the values of $[R]_\lambda$ at the same alkali concentration were compared; one solution was prepared by dissolution of the glucan in an aqueous solution at a given concentration of alkali, the other solution was obtained by dilution of a more concentrated solution. The values obtained for both solutions were identical. The reversibility of the angle of rotation was shown for all the concentrations of alkali.

The results observed for the glucan solutions were also obtained with solutions of pachyman*, but not with solutions of amylose. In this case, the o.r.d. curves obtained for concentrations of alkali from 0.01 to 4.8M, where amylose molecules should be in the form of a random coil[7], were normal. Furthermore, the o.r.d. curve observed at pH 4.6, where the molecule takes the form of a deformed helix[7–12], was also normal.

The plot of reduced viscosity, (η_{sp}/c), against glucan concentration (c) was approximately linear in the range of glucan concentrations between 1–0.2 g/l at any concentrations of sodium hydroxide. When the intrinsic viscosity, {[η] (dl/g)}, is plotted against the sodium hydroxide concentration (Fig. 3), the value of [η] decreases with the increase of the alkali concentration from 0.005 to 0.19M. At higher concentrations, the viscosity falls abruptly between 0.19 and 0.22M, then rises sharply at 0.24M, and finally decreases gradually with the increase of the concentration.

Fig. 3 shows also the results of various determinations made on a 5 g/l solution of glucan at the rate of shear of 6000 sec^{-1}. The extinction angle (χ) of flow birefringence increases with the concentration of alkali under 0.19M; above this concentration, it showed a sharp increase, and at concentrations higher than 0.22M, it

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*Pachyman is also a gel-forming (1→3)-β-D-glucan which was extracted from powdered Bukuryo (*Poria cocos*, supplied by Takeda Chemical Industries Ltd.) according to the method described by Saito *et al.*[2].

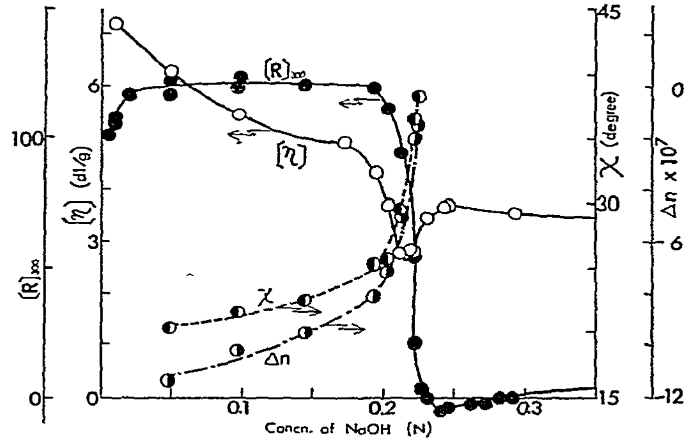

Fig. 3. Dependence of the intrinsic viscosity ([η]), the extinction angle (χ), the birefringence (Δn), and the reduced mean residue rotation at the wavelength of 300 nm, ([R]$_{300}$) of (1→3)-β-D-glucan solutions on the concentration of sodium hydroxide. The values of χ and Δn were obtained on a 5 g/l glucan solution at the rate of shear of 6000 sec^{-1} and 30°.

could not be observed. The values of birefringence (Δn) shows changes similar to those of the extinction angle. The similarity of the relationship between χ and Δn and sodium hydroxide concentration (Fig. 3) was observed at rates of shear other than 6000 sec^{-1} and also at different concentrations of the glucan.

When sodium hydroxide was replaced by lithium hydroxide, a similar dependency of the o.r.d. and viscosity curves on alkali concentration was observed. However, the abrupt changes were observed at a concentration between 0.17 and 0.21M (Fig. 4)*.

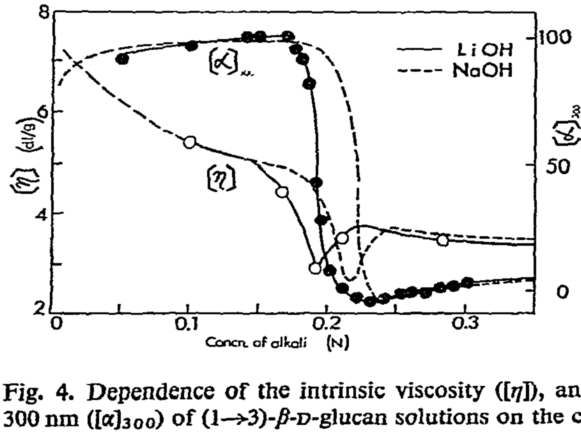

Fig. 4. Dependence of the intrinsic viscosity ([η]), and the specific rotation at the wavelength of 300 nm ([α]$_{300}$) of (1→3)-β-D-glucan solutions on the concentration of alkali.

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*A similar shift of the viscosity-alkali concentration curve of amylose solutions, when sodium ions are replaced by lithium ions, was assumed by Erlander and Purvinas[13] on the basis of their experience with sodium and potassium ions.

DISCUSSION

Changes in optical rotation and intrinsic viscosity, such as those reported in Fig. 3, are known to take place with poly-L-glutamic acid solution, in a pH range where the reversible helix–random coil transition occurs[14]. They take place also with amylose, at a pH of approximately 12, where the probably stiff, wormlike structure (deformed helix) existing in neutral solution changes to a flexible, expanded coil[7]. In the latter case this transition is a consequence of a conformation change caused by the interaction of the electrostatic charges resulting from the ionization of the hydroxyl groups[7].

On the basis of present and previous results, it can be suggested that the reversible conformational transition of the (1→3)-β-D-glucan from an ordered to a disordered form occurs in a range of sodium hydroxide concentration from 0.19 to 0.24M.

Sundaralingam[15], and Sathyanarayana and Rao[16] have suggested that the conformation of the chain of (1→3)-β-D-glucan is similar to that of amylose (helix) in the solid state. On this basis and on the similar dependences of the viscosity and optical rotation on the alkali concentration of solutions of both amylose[7] and gel-forming (1→3)-β-D-glucan, we can assume that the (1→3)-β-D-glucan takes a helical conformation at low concentration of alkali (below 0.19M for sodium hydroxide and 0.17M for lithium hydroxide). The axial ratio of rod-like particles was estimated with Kuhn's equation[17]. Based on Perrin's equation for a rod-like particle and on the values of the extinction angles, obtained by extrapolation of the glucan concentration to zero ($\chi_{c\rightarrow 0}$), the length of the glucan molecule in sodium hydroxide solution of low concentration (0.05–0.19M) was estimated to be 2800–2400 Å. The length, determined from the extinction angle measurement under such a high rate of shear, as a rule gives the weight average. However, it would be difficult to figure out a pertinent helical conformation (single or multistranded model) having this rod length, without any informations about the glucan conformation based on such direct observation methods as X-ray diffraction measurements.

The decrease in the value of $[\eta]$ with the increase of sodium hydroxide concentration from 0.01 to 0.19M (Fig. 3), may result from a decrease in the stiffness of the molecule without destruction of the ordered structure, owing to the gradual increase of the amount of ionization of the hydroxyl groups; the fact that, in the present experiment, a linear relationship between reduced viscosity and concentration of the glucan (an unusual relationship for a normal polyelectrolyte) was observed, was probably due to the stiff, rod-like structure of the glucan.

In the range of sodium hydroxide concentration of 0.19–0.24M, a conformation transition occurs because of the breakdown of the rod-like structure, which results in an increasing backbone flexibility. At sodium hydroxide concentrations from 0.19 up to 0.22M, where drastic changes in the rotation angle, the intrinsic viscosity, and the extinction angle are observed, the ordered structure of the glucan is destroyed by the ionization of hydroxyl groups resulting in the elimination of hydrogen bonds.

At concentrations of sodium hydroxide above 0.22M, a further breakdown of the ordered structure probably takes place. The increased degree of ionization results, however, in an increase of the hydrodynamic volume of the molecule owing to the electrostatic interaction between the ionized hydroxyl groups. Therefore, the rotation angle still decreases, the intrinsic viscosity passes through a minimum at 0.22M sodium hydroxide concentration, and the extinction angle cannot be observed because of the shortening of the axial length, probably below 1000 Å, the shortest length of the rod for the measurement of the extinction angle. The presence of a deep minimum in the intrinsic viscosity is the result of a conformational transition due to the presence of a large amount of sodium ions which sufficiently suppress the expansion of the glucan, as a result of the electrostatic interaction of the ionized hydroxyl groups.

At sodium hydroxide concentrations above 0.24M, the glucan exists practically as a random coil.

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REFERENCES

1 I. Maeda, H. Saito, M. Masada, A. Misaki, and T. Harada, *Agr. Biol. Chem. Tokyo*, 31 (1967) 1184.
2 H. Saito, A. Misaki, and T. Harada, *Agr. Biol. Chem. Tokyo*, 32 (1968) 1261.
3 T. Harada, M. Masada, K. Fujimori, and I. Maeda, *Agr. Biol. Chem. Tokyo*, 30 (1966) 196.
4 T. Harada, K. Fujimori, S. Hirose, and M. Masada, *Agr. Biol. Chem. Tokyo*, 30 (1966) 764.
5 T. Harada, A. Misaki, and H. Saito, *Arch. Biochem. Biophys.*, 124 (1968) 292.
6 M. Takagi, K. Tsuchiya, and S. Ono, *Abstr. 16th Polarographic Symposium Japan*, Kyoto, 1970, p. 24.
7 V. S. R. Rao and J. F. Foster, *Biopolymers*, 1 (1963) 527.
8 J. Holló and J. Szejtli, *Period. Polytech., Chem. Eng.*, (Budapest) 1 (1957) 223; *Stärke*, 10 (1958) 49.
9 T. Kuge and S. Ono, *Bull. Chem. Soc. Jap.*, 34 (1961) 1264.
10 S. Ono, T. Watanabe, K. Ogawa, and N. Okazaki, *Bull. Chem. Soc. Jap.*, 38 (1965) 643.
11 J. Szejtli, S. Augustat, and M. Richter, *Stärke*, 18 (1966) 38; *Biopolymers*, 5 (1967) 5, 17; *ibid.*, 6 (1968) 27.
12 T. Watanabe, K. Ogawa, and S. Ono, *Bull. Chem. Soc. Jap.*, 43 (1970) 950.
13 S. R. Erlander and R. M. Purvinas, *Stärke*, 20 (1968) 37.
14 P. Doty, A. Wada, J. T. Yang, and E. R. Blout, *J. Polymer Sci.*, 23 (1957) 851.
15 M. Sundaralingam, *Biopolymers*, 6 (1968) 189.
16 B. K. Sathyanarayana and V. S. R. Rao, *Carbohyd. Res.*, 15 (1970) 137.
17 W. Kuhn, *Kolloid-Z.*, 62 (1933) 269; *ibid.*, 68 (1934) 2.