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EFFECT OF 2-CHLOROETHANOL, DIOXANE, OR WATER ON THE CONFORMATION OF A GEL-FORMING β-1,3-D-GLUCAN IN DMSO

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The change of viscosity, optical rotation, and flow birefringence of a gel-forming β-1,3-D-glucan in DMSO with the addition of 2-chloroethanol, dioxane, or water was studied. The results indicate a conformational transition of the glucan from a flexible disordered form to a rigid ordered structure with increasing mole fraction of these nonsolvents.

We previously reported a conformational transition of a gel-forming β-1,3-D-glucan in aqueous alkaline solution with increasing alkaline concentration[1]. In our preliminary experiments, it was found that the glucan was soluble in dimethyl sulfoxide (DMSO), but it formed a gel by the addition of nonsolvents such as 2-chloroethanol, dioxane, and water[2]. It would be expected that certain informations about the conformational transition of the glucan in DMSO could be obtained with careful addition of the above nonsolvents. Therefore, we studied on the changes in viscosity, optical rotation (including ORD) and flow birefringence of the glucan in DMSO—2-chloroethanol, DMSO—dioxane, and DMSO—water systems.

The sample studied was polysaccharide 13140, one of the curdlan type polysaccharides[4], supplied by Takeda Chemical Industries Ltd. This glucan was reported[4] to be a linear polymer of D-glucose linked by β-1,3-glucosidic linkage, not to be soluble in aqueous media below pH 12, and to form a gel when its aqueous suspension was heated above 54°C. In our preliminary experiments, the gelation of the glucan was also observed when pH of the glucan-alkaline solution was lowered gradually below 12. $\overline{\mathrm{DP}}\mathrm{n}$ of the glucan was determined to be 462 by Nakanishi et al.[5]; they applied Manners´ enzymatic method[6] to the determination of $\overline{\mathrm{DP}}\mathrm{n}$ of the glucan. A low molecular weight β-1,3-D-glucan ($\overline{\mathrm{DP}}\mathrm{n}$=13.4) was separated from the partially degraded gel-forming glucan by fractional precipitation[7]. This fraction is soluble in neutral aqueous media and is named "soluble fraction"[7]. All the solvents used were of reagent grade and employed without further purification.

Measurements of the ORD (300-500 nm) and the rotation angle at 439 nm were carried out at 30°C with a Yanagimoto Model ORD-185 recording spectropolarimeter and a Union Giken Model PM-70 high sensitivity polarimeter, respectively. The reduced viscosity was determined with an Ubbelohde-type dilution viscometer (capillary diameter, 0.5 mm) at 30°C. The extinction angle of flow birefringence was measured by using a flow birefringence viscometer Model No. B-23 (Rao Instrument Co.) at 30°C.

As previously reported1)7), in the aqueous alkaline solution, the rotation angle, the viscosity, and the extinction angle of the original gel-forming glucan changed sharply with sodium hydroxide concentration in the range of 0.19-0.24 N, whereas the rotation angle of the soluble fractions ($\overline{\mathrm{DPn}}$=13.4-20.8) was little changed. We interpreted1)7) these phenomena as that the soluble fraction took a disordered form in both neutral and alkaline solutions, whereas the original glucan took an ordered conformation (probably helix) at dilute alkaline concentration below 0.19 N and a random coil above 0.24 N.

The ORD curve of the original glucan in DMSO was negatively normal, whereas in the presence of 0.346 mole fraction of 2-chloroethanol, rotation angle in the curve became positive (solid lines in Fig. 1). In both DMSO and DMSO—2-chloroethanol solutions, the curves of the soluble fraction were negatively normal (broken lines in Fig. 1). These changes were similar to those observed in aqueous alkaline media with decreasing alkaline concentration from 0.24 to 0.19 N1)7). By the addition of dioxane or water in the DMSO solutions, similar changes in ORD curve could be observed, although the responsible concentration range was differed for each nonsolvent.

Changes in optical rotation, viscosity, and extinction angle of the original glucan with mole fraction of 2-chloroethanol and dioxane are shown in Figs. 2 and 3, respectively. Abrupt changes in rotation angle and viscosity were observed in each specific mole fraction range of these nonsolvents, i.e. from 0.07 to 0.1 for 2-chloroethanol, and from 0.19 to 0.22 for dioxane, and above these mole fraction ranges, flow birefringence could be observed. All the changes were found to be reversible with respect to mole fraction of these nonsolvents. Above 0.41 mole fraction of 2-chloroethanol and 0.34 of dioxane (shaded parts in Figs. 2 and 3), gelation of the glucan was observed. These changes are similar to those observed in aqueous alkaline media1), and indicate that in the presence of large amount of the nonsolvents, the original glucan takes a rigid ordered structure similar to that in dilute alkaline solution. With the soluble fraction, change in rotation angle was monotonous with the concentration of these nonsolvents (Figs. 2 and 3). Therefore, this glucan is considered to take a disordered form in these DMSO—nonsolvent systems like in aqueous neutral and alkaline media7).

From the present results (Figs. 1-3), following description for the conformation of the original gel-forming glucan in DMSO may be allowed. The shape of both ORD curves of the original glucan and the soluble fraction in DMSO was negatively normal, and the changes in rotation angle of the both glucans were parallel with the concentration of the nonsolvents until it had reached the value that the abrupt change of the original glucan conformation could be observed. Therefore, the difference in rotation angle between both glucans in DMSO may be attributed solely to that of molecular weight; in other words, the contribution of terminal glucose units in the soluble fraction is appreciably large in the totally measured rotation angle, whereas it may be ignored for the original glucan8). These considerations indicate that the gel-forming glucan is in a flexible disordered structure9) similar to that of the soluble fraction in DMSO solution.

In DMSO—water system (Fig. 4)10), above 0.36 mole fraction of water, abrupt increase of rotation angle of the original glucan was observed and extinction angle

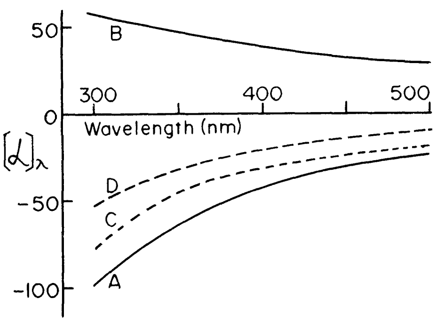

Fig. 1. ORD curves of the original glucan (solid lines) and the soluble fraction (broken lines) in DMSO (curves A and C) and DMSO–2-chloroethanol (curves B and D; mole fraction of 2-chloroethanol is 0.346) at 30°C. The glucan concentration was 0.200 g/dl.

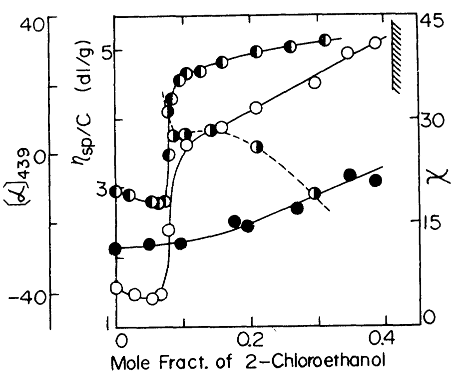

Fig. 2. The 2-chloroethanol concentration dependence of the reduced viscosity (η_{sp}/C:◐), the extinction angle at the rate of shear of 6000 sec^{-1} (χ:◑), and the specific rotation angle at the wavelength of 439 nm ($[\alpha]_{439}$) of the original glucan (○), and $[\alpha]_{439}$ of the soluble fraction (●) in DMSO solution at 30°C. The glucan concentrations were 0.200 g/dl for the rotation angle, 0.0500 g/dl for the viscosity, and 0.300 g/dl for the flow birefringence measurements, respectively. The shaded part shows 2-chloroethanol concentration where gelation of the original glucan is observed.

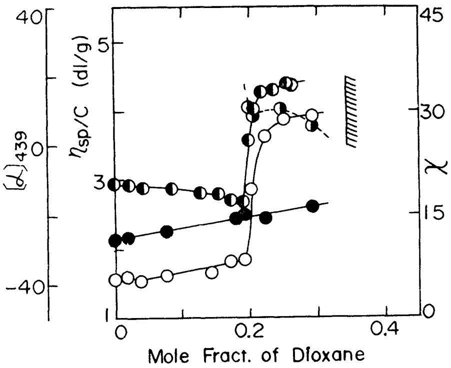

Fig. 3. The dioxane concentration dependence of the reduced viscosity (◐), the extinction angle at the rate of shear of 6000 sec^{-1} (◑), and the specific rotation angle at the wavelength of 439 nm of the original glucan (○), and $[\alpha]_{439}$ of the soluble fraction (●) in DMSO solution at 30°C. Other conditions were the same for Fig. 2.

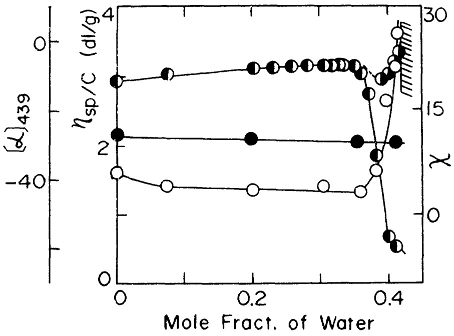

Fig. 4. The water concentration dependence of the reduced viscosity (◐), the extinction angle at the rate of shear of 6000 sec^{-1} (◑), and the specific rotation angle at the wavelength of 439 nm of the original glucan (○), and $[\alpha]_{439}$ of the soluble fraction (●) in DMSO solution at 30°C. Other conditions were the same for Fig. 2.

could be measured, but viscosity fell sharply. These changes were also reversible and the gelation of the glucan occurred above 0.42 mole fraction of water. These indicate that, above 0.36 mole fraction of water, both conformational transition and aggregation of the original glucan molecules simultaneously occur with increasing water concentration. The increase of the extinction angle with water concentration may be resulted from lowering of the axial ratio of the glucan particle caused by the aggregation of the glucan molecules.

DMSO is considered to be a good solvent for the gel-forming β-1,3-D-glucan. By addition of the nonsolvents, however, the polymer-solvent interaction is weakened, and the glucan takes an ordered structure similar to that in dilute alkaline solution1).

Recently, Suzuki and co-workers3) reported with the aid of X-ray analysis that the structure of the polysaccharide 13140 in DMSO was amorphous, whereas in the glucan gel prepared in both water and DMSO—water media, crystalline structure could be detected. These observations may support above our interpretations for the original glucan conformations in DMSO and in DMSO—nonsolvent systems.

FOOTNOTES AND REFERENCES

1) K. Ogawa, T. Watanabe, J. Tsurugi, and S. Ono, Carbohyd. Res., 23, 399 (1972).

2) S. Suzuki *et al*. also found3) the gel formation of the glucan in DMSO media by addition of various nonsolvents.

3) M. Takahashi, A. Aizawa, and S. Suzuki, presented at the 28th Annual Meeting of Chemical Society of Japan, Tokyo, April, 1973.

4) T. Harada, "Fermentation Technology Today" (Proceedings of the IVth International Fermentation Symposium, held in Kyoto, March, 1972) Ed. by G. Terui, Society of Fermentation Technology, Japan, 1972, p.603.

5) I. Nakanishi, S. Kusui, K. Kimura, T. Kanamaru, and K. Onishi, presented at the 278th Regional Meeting Kansai Branch, Agr. Chem. Soc. Japan, Osaka, 1972.

6) D. J. Manners, A. J. Masson, and R. J. Sturgeon, Carbohyd. Res., 17, 109 (1971).

7) K. Ogawa, J. Tsurugi, and T. Watanabe, *ibid.*, in press.

8) Similar difference in rotation angle between both glucans was also observed in aqueous alkaline media above 0.24 N NaOH concentration7).

9) However, Casu *et al*. have suggested in their NMR studies on laminaran(a low molecular weight β-1,3-D-glucan)-DMSO solution the presence of hydrogen bond between adjacent glucose residues of the glucan chain; B. Casu, M. Reggiani, G. G. Gallo, and A. Vigevani, Tetrahedron, 22, 3061 (1966). Hence, possibility of the presence of the partial ordered structure is not excluded.

10) While we were proceeding the present investigations, on the polysaccharide 13140 in organic solvents as above described, Suzuki *et al*. found similar behavior of the polysaccharide in DMSO—water system3). They interpreted that a sol-gel transition of the glucan occurred at 0.63 mole fraction of DMSO3). We carried out the same experiments as theirs and additional flow birefringence measurements.

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