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EFFECT OF SALT ON THE CONFORMATION OF GEL-FORMING β-1,3-D-GLUCAN IN ALKALINE SOLUTION

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Effect of sodium chloride on the conformation of a gel-forming β-1,3-D-glucan in sodium hydroxide solution was studied. The increase of the salt concentration causes the conformational transition of the glucan from a random coil to an ordered structure when the alkaline concentration is below 0.3 N.

In our previous paper[1)], a conformational transition of a gel-forming β-1,3-D-glucan with alkaline concentration was studied, and this transition was attributed to the ionization of the hydroxyl groups of the glucan. In the present paper, we wish to report the effect of salt on the glucan conformation in alkaline solution.

The sample studied was polysaccharide 13140, one of the curdlan type polysaccharides[2)], supplied by Takeda Chemical Industries Ltd. This glucan was reported to be a linear polymer of D-glucose linked by β-1,3-glucosidic linkage[2)]. $\overline{DP}n$ of the glucan was determined to be 400 by the modified Somogyi-Nelson method[3)] using laminaribiose as a standard sample.

Measurements of the ORD (230-450 nm) and the rotation angle at a fixed wavelength (439 nm) were carried out with a Yanagimoto Model ORD-185 Recording Spectropolarimeter and a Union Giken Model PM-70 High Sensitivity Polarimeter, respectively at 30°C. The reduced viscosity was determined with a Ubbelohde-type dilution viscometer (capillary diameter, 0.5 mm) at 30°C. To avoid undesirable effects of atmospheric CO_2, viscosity measurements were carried out in a nitrogen atmosphere. The extinction angle of flow birefringence was measured by using a Flow Birefringence Viscometer Model No. B-23 (Rao Instrument Co.) at 30°C. The visible absorption spectrum was obtained by a Hitachi Model 323 Recording Spectrophotometer at 25°C.

As shown in Fig. 1[1)], the rotation angle of the glucan changed sharply with sodium hydroxide concentration in the range of 0.19-0.24 N. The corresponding changes also could be observed in the viscosity and the extinction angle measurements; the latter could not be followed above 0.22 N NaOH. These changes were found to be reversible with respect to alkaline concentration. Our previous conclusion[1)] was that these changes result from a conformational transition of the glucan from an ordered structure (probably helix) to a random coil. In Fig. 1, the change of the absorption maximum (λ_{max}) of the glucan-congored system with the alkaline concentration[4)] is also presented. This change is closely related with the changes of the

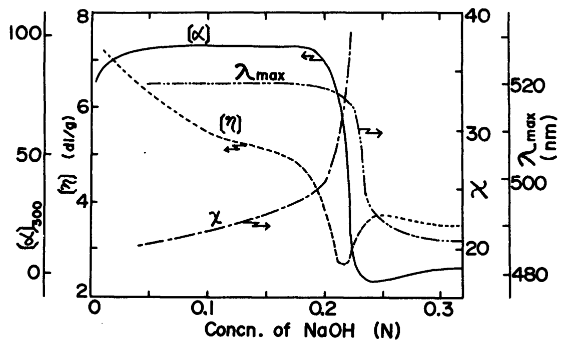

Fig. 1. The NaOH concentration dependence of the intrinsic viscosity ([η]), the extinction angle (χ), and the specific rotation angle at the wavelength of 300 nm, ($[\alpha]_{300}$) of the glucan, and also the absorption maximum (λ_{max}) of the glucan-congored system. The extinction angle was measured with a 0.5 g/dl glucan solution at the rate of shear of 6000 sec^{-1}. Values of the λ_{max} were obtained at the congored concentration, $D_0=2.3\times10^{-5}$M, the glucan concentration denoted in residue concentration, $R_0=3.1\times10^{-2}$M, and 25°C; the λ_{max} of free congored decreases monotonously with the alkaline concentration from 490 nm (at 0.05 N) to 485 nm (at 0.3 N).

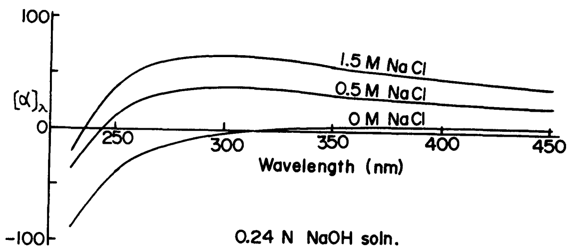

Fig. 2. ORD curves of the glucan-0.24 N NaOH solutions in the presence of various concentrations of NaCl at 30°C.

above three measurements, indicating that the glucan with an ordered conformation forms complex with congored[4].

Fig. 2 shows the effect of NaCl concentration on the ORD curve of the glucan in 0.24 N NaOH solution where in the absence of the salt, the value of rotation angle is minimum (Fig. 1) and the glucan exists as a random coil[1]. The rotation angle of ORD curve increases with the salt concentration. This change of ORD curve is similar to that observed with decreasing alkaline concentration from 0.24 to 0.19 N, in the absence of NaCl[1].

The NaCl concentration dependence of the specific rotation angle, the reduced viscosity, and the extinction angle of the glucan at various NaOH concentrations is shown in Figs. 3, 4, and 5, respectively. At low alkaline concentrations below 0.19 N, changes in the optical rotation, the viscosity, and the extinction angle with NaCl concentration, are small. Results obtained with the 0.21-0.30 N alkaline solutions are interesting in that at higher salt concentrations, measured values of each method approach those in dilute alkaline solutions below 0.19 N. At 0.5 N alkaline solution, however, no significant changes in the optical rotation and the viscosity were observed, and the extinction angle could not be observed at any salt concentrations below 2 M[5]. All the changes in rotation angle and the viscosity were reversible when NaCl concentration was varied.

From the present results (Figs. 2-5), the salt effect may be discussed as follows. In the alkaline solution below 0.22 N, slight changes in the optical rotation, the viscosity, and the extinction angle by the addition of small amount of NaCl will be caused by a little destruction of an ordered structure of the glucan resulting from the promotion of the ionization of the hydroxyl groups of the glucan.

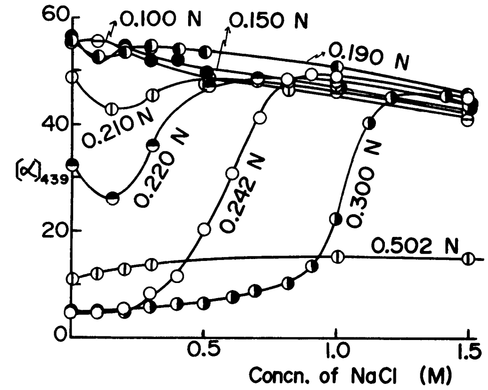

Fig. 3. The NaCl concentration dependence of the specific rotation angle at the wavelength of 439 nm, ($[\alpha]_{439}$) of the glucan at various NaOH concentrations (as indicated in the figure) and 30°C.

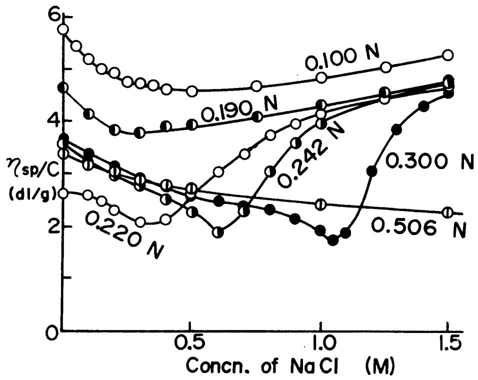

Fig. 4. The NaCl concentration dependence of the reduced viscosity (η_{sp}/C) of the glucan at various NaOH concentrations (as indicated in the figure) and 30°C. Measurements were carried out at a constant glucan concentration of 0.02 g/dl.

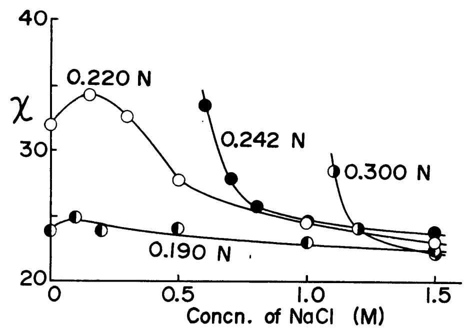

Fig. 5. The NaCl concentration dependence of the extinction angle (χ) of the glucan at various NaOH concentrations (as indicated in the figure) and 30°C. Measurements were carried out at a constant glucan concentration of 0.5 g/dl and a rate of shear of 6000 sec^{-1}.

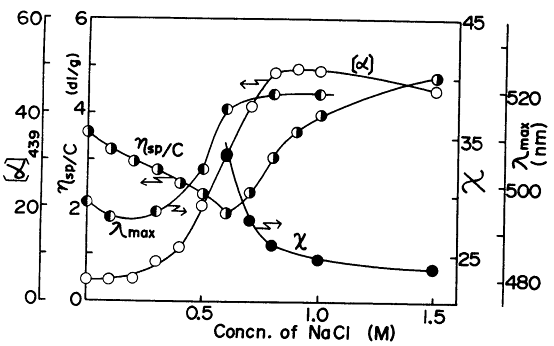

Fig. 6. The NaCl concentration dependence of η_{sp}/C, χ, and $[\alpha]_{439}$ of the glucan, and the λ_{max} of the glucan-congored system at 0.24 N NaOH concentration. The reduced viscosity was measured at the glucan concentration of 0.02 g/dl and the extinction angle at 0.5 g/dl. The λ_{max} was obtained at $D_0=1\times10^{-5}$M, $R_0=1.5\times10^{-2}$M and 25°C.

By the addition of larger amount of salt, however, the glucan restored its ordered structure. In 0.24 and 0.30 N NaOH solutions, the conformational transition from the random coil to the ordered structure occurs with increasing salt concentration. This transition is more clearly understood when Fig. 6 is compared with Fig. 1. A large red shift of the λ_{max} at higher salt concentration in Fig. 6 indicates the formation of the glucan-congored complex, and supports the conformational transition of the glucan in the studied media.

An interpretation of the present NaCl salt effect will be possible when we assume the glucan conformation as follows. The ordered structure of the glucan is formed by intermolecular association of several molecules, e.g. multistranded helix[6] or β-form, and this associated state of the glucan still remains at 0.30 N alkaline concentration, though the glucan is dissociated to individual molecules at 0.5 N NaOH. Addition of a small amount of NaCl results in the promotion of the ionization of the hydroxyl groups of the glucan, and consequently in partial destruction of the ordered form. In the presence of a large amount of salt, however, water molecules contained among glucan molecules which are in the associated state, are removed by the dehydration action of the salt; this salt effect forces the glucan to restore the ordered structure.

FOOTNOTES AND REFERENCES

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5) In the 0.5 N NaOH solution, the glucan was precipitated immediately after addition of 2.5 M NaCl; at low alkaline concentrations below 0.3 N, after addition of 1.7 M salt.
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