

Effects of Sugars on the Conformational Change of Gellan Gum in Aqueous Solutions

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The effects of sugars such as glucose, fructose, sucrose, and raffinose on the coil-helix transition of sodium-type gellan gum in aqueous solutions are studied by measuring viscosity and circular dichroism. The temperature dependence of η_{sp}/c_p and θ_{201} for the gellan solutions with sugars showed drastic change at the coil-helix transition temperature. The transition temperatures of gellan solutions with glucose, sucrose, and raffinose were slightly higher than the solutions without sugars, while the transition temperature of the solution with fructose was slightly lower. It seems that sugar indirectly influences the stabilizing of gellan gum due to the hydration and structural changes of water surrounding sugar.

Key words: sodium-type gellan gum, sugar, viscometry, coil to helix transition, aqueous solution

INTRODUCTION

Gellan gum is an anionic bacterial heteropolysaccharide whose structure [1,2] is based on the tetrasaccharide chemical repeat unit [-3)- β DGlc-(1-4)- β DGlcA-(1-4)- β DGlc- α Lrha-(1)]. The gelation mechanism of gellan gum which is now accepted is that gellan molecules undergo a thermally reversible coil-to-helix(double helix) conformational transition, and junction zones of gellan gels are formed by aggregation of double-helical gellan molecules [3]. Thus, helix formation is a prerequisite for gel formation [3].

Gellan gum forms transparent, heat-, and acid resistant gels which have already more than 10 years history of commercial application as a food additive, and now it has been applied to a very wide range of foods [4,5]. In the application of gellan gum to food, the effect of salt and sugar on gel formation of gellan gum is very important. Previously, we studied the effects of salt on the gel formation of gellan gum [6-9].

In the present study, the effects of sugars such as glucose, fructose, sucrose, and raffinose on the coil to double-helix conformational transition of sodium-type gellan gum (Na-gellan) aqueous solutions are studied by measuring viscometry and CD.

EXPERIMENTAL

The sample of Na-gellan was prepared from deacetylated gellan gum (San-Ei Gen F.F.I, Inc. Osaka Japan, Lot 62058A) by passing through a

column of cation exchange resin [10]. Its ion contents are Na 3.00%, K 0.07%, Ca 0.005%, and Mg 0.0008%. The number average molecular weight (M_n) obtained at 45 and 28°C are 4.8×10^4 and 9.2×10^4 , respectively. Viscometry was performed using an Ubbelohde-type viscometer and CD measurements were carried out with a JASCO-720 spectropolarimeter.

RESULTS AND DISCUSSION

Viscosity measurements were carried out over the temperature range from 10 to 40°C for the aqueous Na-gellan solutions with sugars, i.e. monosaccharide of glucose and fructose, disaccharide of sucrose, and trisaccharide of raffinose. Figs.1 and 2 show the viscosity number (η_{sp}/c_p) versus temperature plots for the Na-gellan aqueous solutions with and without sugar. Here, $\eta_{sp}/c_p (= \eta_s - \eta_o) / \eta_o c_p$ is the viscosity number of solution, η_s and η_o are the viscosities of solution and solvent, respectively, and c_p the polymer concentration. The temperature dependence of (η_{sp}/c_p) for the Na-gellan in sugar solutions seems to be similar to the behavior of the solution without sugar; on lowering temperature, the values of (η_{sp}/c_p) for these Na-gellan aqueous solutions with and without sugar keep almost constant down to the transition temperatures T_η , but below the transition temperatures the (η_{sp}/c_p) values increase steeply. The variation of (η_{sp}/c_p) is a reflection of con-

formational change of Na-gellan molecules[6,9], and can be interpreted as follows. In aqueous solutions with and without sugar, on lowering temperature, the coil to double-helix conformational transition and/or partial association of helices took place below the transition temperature.

As shown in Figs. 1 and 2, at the temperatures above the transition temperature, the (η_{sp}/c_p) value of the solution with and without sugar decreased with increasing polymer concentration. While, it was not or little influenced by the chemical species of sugar and its concentrations, indicating that the molecular dimension of polyelectrolyte gellan is extended due to repulsive interactions between ionized carboxylate groups which are not affected by the presence of sugar. Therefore, the interaction between sugar and gellan and that between water and gellan may have something common features. On the contrary, the (η_{sp}/c_p) value of a Na-gellan solution decreased remarkably by the addition of a little amounts of salt[6], which was essentially different in the presence of any sugar used in this study.

The transition temperature of a Na-gellan solution with any sugar could be detected as a temperature at which (η_{sp}/c_p) increased steeply. However, it must be concerned, an effective concentration should increase by the addition of a large amount of sugar due to volume exclusion. For example, the Na-gellan dissolved in sucrose solution of $1M/10^3 \text{ cm}^3$ which contain 34vol% sugar, and so the amount of water which is available for dissolution of the Na-gellan in this system may be very small compared to that in the absence of sugar. Therefore, the concentration was calibrated as a percentage of the Na-gellan in the system from which the amount of sugar was subtracted. Using our data of concentration dependence of T_η for the solution of Na-gellan alone shown in this issue, we calculated the transition

temperature $T_{\eta \text{ cal}}$ at the calibrated concentration. The $T_{\eta \text{ cal}}$ for the solutions of Na-gellan alone at the calibrated concentrations are shown in Fig.3, together with the experimental T_η for the solutions containing various sugars. As shown in Fig.3, there is little difference among the values of $T_{\eta \text{ cal}}$ of the Na-gellan solutions with monosaccharides (glucose and fructose), disaccharide (sucrose), and trisaccharide (raffinose), meaning that the calibrated concentrations of these solutions are almost the same. This suggests that these solutions contain almost the same amount of saccharide units. Therefore, the difference between T_η and $T_{\eta \text{ cal}}$ should reveal the contributions of the individual sugars to promote the coil-helix transition.

The T_η for the Na-gellan solutions containing raffinose, sucrose, and glucose shifted to higher temperatures with increasing concentration of added sugar, suggesting that the addition of these sugars promote the coil to helix transition of the Na-gellan molecules. In the solutions containing almost the same weight of sugar, trisaccharide (raffinose) was the most effective in promoting coil-helix transition, and disaccharide (sucrose) was more effective than monosaccharide (glucose and fructose). The T_η of the Na-gellan solutions containing fructose shifted to lower temperatures with increasing fructose concentration suggesting that the coil to helix transition is retarded by the addition of fructose. This behavior was significantly different from that in the presence of glucose, although both glucose and fructose are monosaccharide and have the same molecular weight. The effectiveness of sugars in promoting helix formation is the following order: raffinose > sucrose > glucose > fructose. This order is in good agreement with the dynamic hydration number (DHN) ($n\text{DHN}(\text{raffinose})=30.7$; $n\text{DHN}(\text{sucrose})=25.2$; $n\text{DHN}(\text{glucose})=18.6$; $n\text{DHN}(\text{fructose})=16.3$ and the

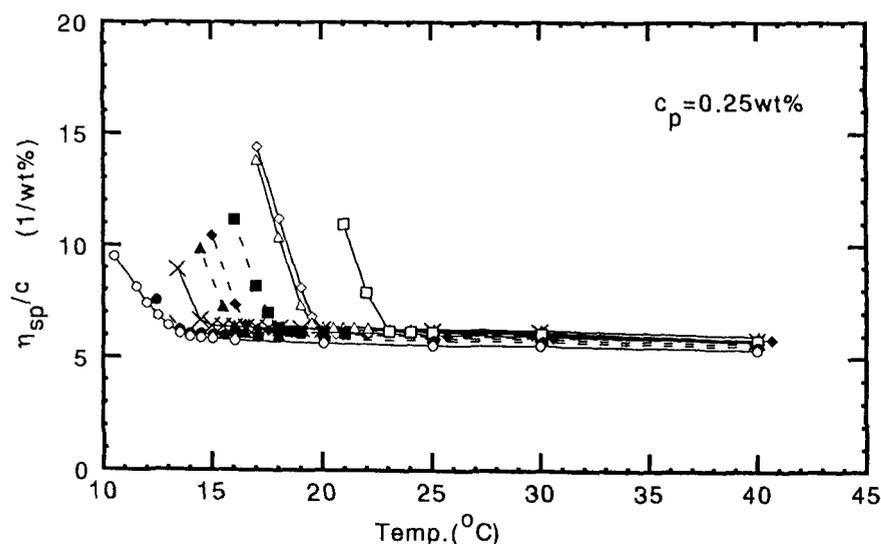


Fig.1. Plots of (η_{sp}/c_p) vs. temperature for aqueous solutions of the Na-gellan ($c_p=0.25\text{wt}\%$) with and without sugars. The sugar solution with 1M glucose(-♦-), 2M glucose(-◇-), 1M fructose(-●-), 2M fructose(-○-), 1/2M sucrose(-▲-), 1M sucrose(-△-), 1/3M raffinose(-■-), 2/3M raffinose(-□-), and Na-gellan alone(-×-).

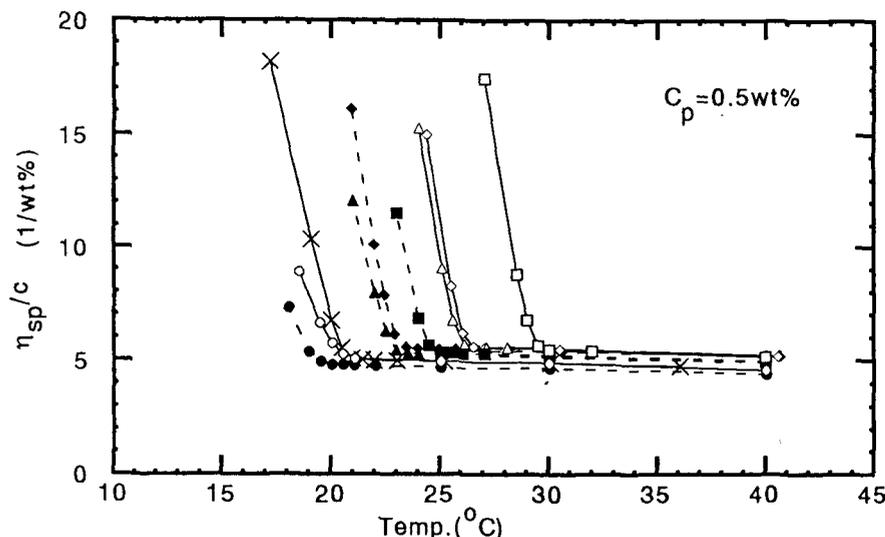


Fig.2. Plots of (η_{sp}/c_p) vs. temperature for aqueous solutions of the Na-gellan ($c_p=0.5wt\%$) with and without sugars. The sugar solution with 1M glucose(-♦-), 2M glucose(-○-), 1M fructose(-●-), 2M fructose(-○-), 1/2M sucrose(-▲-), 1M sucrose(-△-), 1/3M raffinose(-■-), 2/3M raffinose(-□-), and Na-gellan alone(-×-).

number of equatorially attached OH groups [$n(e-OH)(\text{raffinose})=8.3$; $n(e-OH)(\text{sucrose})=6.3$; $n(e-OH)(\text{glucose})=4.6$; $n(e-OH)(\text{fructose})=3$] [11-13]. It was reported that sugars with larger $n(e-OH)$ could stabilize the water structure much more, and there was a good correlation between the number of (e-OH) in saccharide and DHN[11-13].

The difference between T_η and $T_{\eta cal}$ increases with increasing the added sugar concentrations, which is similar to the results of added salt shown in this issue. In spite of a large amount of added sugar, however, the difference in a sugar solution is much smaller than that in NaCl solution; in a Na-gellan solution ($c_p=0.5wt\%$) with 1M sucrose and that with 25 mM NaCl(0.015wt% NaCl), the difference for the former is ca. 2°C, and the latter is ca. 20°C.

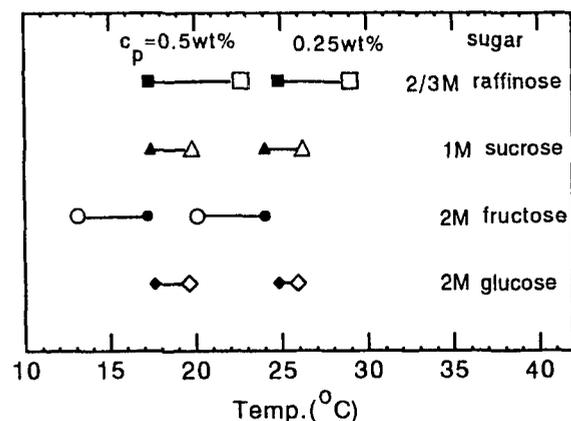
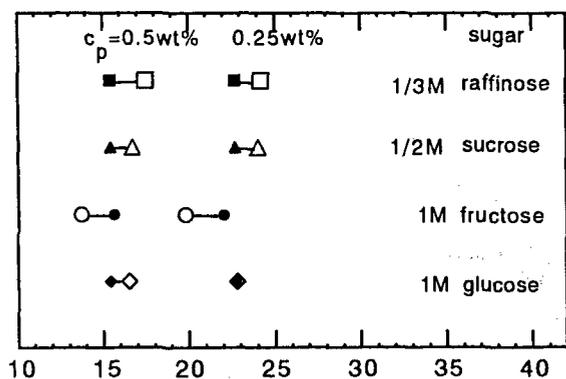


Fig.3. T_η and $T_{\eta cal}$ for the Na-gellan solutions with sugars. T_η (♦ ; ○ ; △ ; □) $T_{\eta cal}$ (● ; ● ; ▲ ; ■).

Miyoshi and Nishinari studied the effects of glucose, fructose and trehalose on the sol-gel transition in gellan gum aqueous solutions using rheological measurements and differential scanning calorimetry [14]. They observed that the added sugar influenced the coil-helix transition and its effectiveness was the following order: sucrose> trehalose> glucose> fructose. They also observed that the coil-helix transition was inhibited by the addition of sufficient fructose.

From these results obtained in this study, it is considered that the reason why sugar can influence the coil to double helix transition of gellan molecules should be essentially different from the reason of the cations. As discussed previously, cations could stabilize the double-helix formation of gellan by electrostatic interaction[6-9]. On the other hand, in the presence of sugar, the structural change of water surrounding the sugar, for example, the formation of large clusters, may occur, which decrease the degree of motional freedom of gellan molecules, and/or promote hydrogen bonding between hydroxyl groups in the polymers, resulting in the promotion of double-helix formation. Sugars may immobilize water and their order of the effectiveness is raffinose> sucrose>glucose> fructose based on the DHN. It is considered that in the presence of fructose, the water structures surrounding sugar may rather

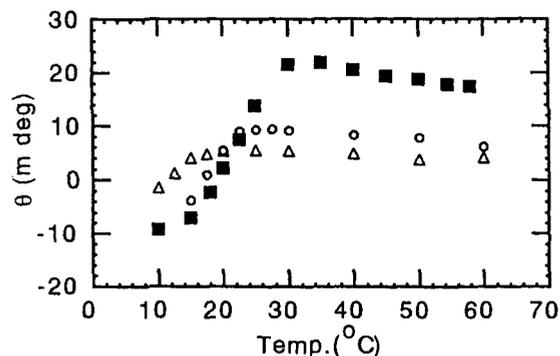


Fig.4. Temperature dependence of ellipticity at 201 nm for the Na-gellan aqueous solutions without sugars. $c_p = 1.0\text{wt}\%$ (■), $0.5\text{wt}\%$ (○), and $0.25\text{wt}\%$ (△)

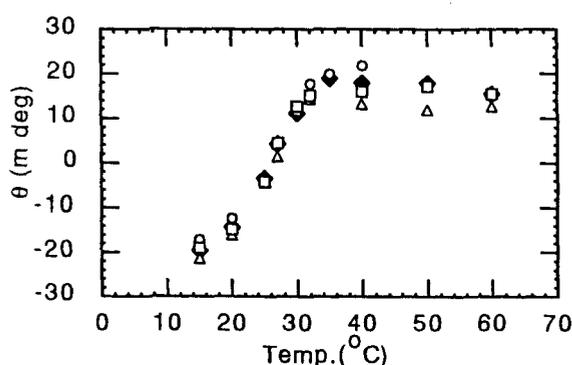


Fig.5. Temperature dependence of ellipticity at 201 nm for the Na-gellan aqueous solutions ($c_p = 1.0\text{wt}\%$) with sugars; 0.25M glucose (◆), 0.25M fructose (○), 0.125M sucrose (△), and 0.083M raffinose (□).

mobilize then, the helix formation was retarded. However, further study should be necessary.

In order to confirm the conformational transition of the Na-gellan in aqueous solutions, we measured CD spectra over the temperature range from 60 to 10°C. It was reported that the positive peak around 201 nm in the CD spectra relates to the optically active chemical structure of glucuronic acid unit in the random coil form of gellan and, therefore, the change of the ellipticity at 201 nm should correspond to a coil to double-helix conformational transition of gellan molecules [6,15-19]. The ellipticity at 201 nm for the Na-gellan solutions without sugar are plotted against temperature in Fig.4. The transition temperatures, which are defined as the intersection point of the extrapolated lines, for the Na-gellan solutions are almost coincident with those obtained from viscometry; $T_{CD} = 16.0^\circ\text{C}$ and $T_\eta = 14.5^\circ\text{C}$ for the $0.25\text{wt}\%$ Na-gellan solution, $T_{CD} = 22.0^\circ\text{C}$ and $T_\eta = 20.4^\circ\text{C}$ for the $0.5\text{wt}\%$ Na-gellan solution and $T_{CD} = 29.5^\circ\text{C}$ and $T_\eta = 29.0^\circ\text{C}$ for the $1.0\text{wt}\%$ Na-gellan solution. For the Na-gellan solution with

sugar, the peak at around 201 nm was overlapped with that of solvent (sugar solution), then we used sugar solutions with low sugar concentrations ($0.083 \sim 0.25\text{M}$) as solvents and subtracted the solvent peak from the total height. As shown in Fig. 5, temperature dependence of the ellipticity at 201 nm was very similar to that of the solution of Na-gellan alone. From these results, it was confirmed that the T_η determined from viscometry in this study corresponds to the coil-helix transition temperature of gellan molecule in sugar solutions.

REFERENCES

- [1] P.Jansson, B. Lindberg, and P.A.Sandford, *Carbohydr. Res.*, **124**, 135-139 (1983).
- [2] M.A.O'Neill, R.R. Selvendran, and V.J.Morris, *Carbohydr. Res.*, **124**, 123-133 (1983).
- [3] K. Nishinari, *Colloid Polym. Sci.*, **275**, 1093-1107 (1997).
Polym. Sci., **114**, 123-126 (1999)
- [5] N.A.Morrison, G.Sworn, R.C.Clark, Y.L.Che, and T. Talashek, *Prog. Colloid Polym. Sci.*, **114**, 127-131 (1999)
- [6] E.Ogawa, H.Matuzawa, M.Iwahashi, Y.Sagara, T. Shioya, and T.Kimura, this issue.
- [7] E. Ogawa, *Prog. Colloid Polym. Sci.*, **114**, 8-14 (1999)
- [8] E.Ogawa, *Recent Res. Dev. Macromol. Res.* **2**, 81-94 (1997)
- [9] E.Ogawa, *Macromolecules*, **29**, 5178-5182 (1996).
- [10] E.Ogawa, *Food Hydrocoll.* **7**, 397-405 (1993).
- [11] H.Uedaira, M.Ishimura, S.Tsuda, H.Uedaira, *Bull. Chem. Soc. Japan*, **62**, 574-575 (1990).
- [12] H.Uedaira, M.Ishimura, S.Tsuda, H.Uedaira, *Bull. Chem. Soc. Japan*, **63**, 3376-3379 (1990).
- [13] H.Uedaira and A.Ohsaka, *In: Water in biological systems*. Kodansha, Tokyo, pp30-62 (1990) (in Japanese)
- [14] E.Miyoshi and K.Nishinari, *Prog. Colloid Polym. Sci.*, **114**, 83-91 (1999).
- [15] K.te.Nijenhuis, Chapter 13 Gellan Gum, *Thermoreversible Networks*, **130 Advances Polym. Sci., Springer 219-235 (1997).**
- [16] V.Crescenzi, M. Dentini, T.Coviello, and R. Rizzo, *Carbohydr. Res.*, **149**, 425-432 (1986).
- [17] S.Matukawa, Z.Tang, and T. Watanabe, *Prog. Colloid Polym. Sci.*, **114**, 15-24 (1999)
- [18] S.Matsukawa, Z.Huang, and T.Watanabe, *Prog. Colloid Polym. Sci.*, **114**, 92-97 (1999).
- [19] Y. Tanaka, M. Sakurai, and K.Nakamura, *Food Hydrocoll.*, **10**, 133-136 (1996).