

Effects of Metal Ions on the Conformational Change of Gellan Gum in Aqueous Solutions

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The effects of cationic ions on the conformational properties of gellan gum in aqueous solutions with and without salts are studied using highly purified Li, Na, and K-type gellan samples by measuring osmotic pressure, viscosity, circular dichroism, and electron microscope. The temperature dependence of M_n , θ_{201} and η_{sp}/c_p for the gellan solutions with and without salts showed drastic change at the coil-helix transition temperatures(T). In aqueous solutions without salt, T for the gellan solutions increased with increasing the polymer concentration but almost independent of the chemical species of cations. In aqueous solutions with salts, T depends on the polymer and salt concentration and the chemical species. The electron microphotograph of gellan solutions show straight fibers and their diameter depends on the chemical species. Key words: sodium-type gellan gum, lithium-type gellan gum, potassium-type gellan gum, coil to helix transition, aqueous solution

INTRODUCTION

Gellan gum, an anionic polysaccharide, is used in food and biotechnological industries because it forms transparent, heat-, and acid resistant gels. The gellan gum is composed of tetrasaccharide(- β -D-glucose, β -D-glucuronic acid, β -D-glucose, and α -L-rhamnose) repeating unit containing a carboxyl side group[1,2]. Many studies on the sol-gel transition of gellan gum molecules in aqueous solutions have been carried out [3-5]. The gelation mechanism of gellan gum solutions is now regarded as that the molecular conformation of gellan gum in solution changes from a disordered state(single coil) to an ordered state(double-helix) on lowering the temperature, and on further decreasing the temperature or increasing the concentration of solutions, aggregation of helices occurs, leading to gel formation. Therefore, helix formation is a prerequisite for the gel formation[3d]. The temperature that the coil to helix transition and subsequent aggregation of helices of gellan gum occurs in aqueous solutions is influenced strongly not only by the polymer concentration but also by the presence of cations. Moreover, these temperatures are also affected by the cationic species and their concentrations.

Previously, we studied by osmometry and viscometry the effect of sodium cation on the

conformational properties of gellan gum in aqueous solutions using sodium-type gellan gums(Na-gellans)[6a-c]. In the present study, the effects of cationic ions on the conformational properties of gellan gum in aqueous solutions are studied using highly purified lithium-type gellan gum (Li-gellan), Na-gellan, and potassium-type gellan gum(K-gellan) samples by measuring the osmotic pressure, viscosity, circular dichroism(CD), and electron microscope.

To obviate any confusion, we will use the superscripts, for example (K), (KCl), and (aq), to designate the K-gellan sample, the aqueous solution with added KCl, and the aqueous solution without salt, respectively, and the subscripts, CD and η to denote the measurements of CD and viscometry, respectively; e.g. $M_n(K/KCl)$ designates the number average molecular weight of K-gellan obtained in aqueous solution with added KCl, $T_{CD}(K/KCl)$ the transition temperature of K-gellan aqueous solution with added KCl obtained by CD measurement and $T_\eta(K/aq)$ the transition temperature for K-gellan aqueous solution without added salt obtained by viscometry.

EXPERIMENTAL

Materials The samples of Li, Na, and K-gellan were prepared from deacetylated gellan gum (San-Ei Gen F.F.I., Inc.Osaka, Japan, Lot 62058A)[6d]. Table I

Table I. Meatal contents in the gellan gum samples.

sample	Li	Na	K	Ca	Mg (%)
gellan(lot62058)		0.19	2.08	0.512	0.145
Li-gellan	0.80	0.09	0.06	0.007	0.0008
Na-gellan		3.00	0.07	0.005	0.0006
K-gellan		0.03	5.00	0.005	0.0008

shows the ionic contents of the samples.

Measurements Osmometry were carried out using a Hewlett-Packard high-speed membrane osmometer, Model 503[6d]. Viscometry was made using an Ubbelohde-type viscometer[6c]. CD measurements were carried out with a JASCO-720 spectropolarimeter. The electron microscopic analysis was made using JEOL, 2000FX₂. Preparation for electron microscopy was carried out by negative staining method using phosphotungstic acid solution.

RESULTS AND DISCUSSION

Osmotic pressure measurements were carried out at 45 and 28°C for the Li, Na, and K-gellan aqueous solutions with added salts of LiCl, NaCl, and KCl, respectively. The concentration c_s of the added salts such as LiCl, NaCl, and KCl is 50 mmol/10³cm³. The polymer concentration c_p was ranged from 0.5 to 0.1g/10²cm³. During the measurements at 28°C, we noticed small increases in the solution viscosities of Li and Na-gellan aqueous salt solutions at higher concentrations above about $c_p=0.3$ (g/10²cm³). In case of the solution of K-gellan, we could not make osmometry at 28°C due to its high solution viscosity. M_n values (Table II) were determined by osmometry employing the usual method [6a-c].

Previously we studied the conformational properties of Na-type gellan in aqueous solutions by osmometry [6a-c]. The M_n values of Na-gellan were measured in NaCl solutions at 25, 28, 32, 36, 40, and 45°C by osmometry using the same sample as the one used in this study[6a,b]. The M_n values obtained above 36°C in NaCl solutions were almost coincident and the average M_n value(4.6x10⁴) obtained above 36°C was just half the average value of M_n (9.4x10⁴) obtained below 28°C suggesting that two molecules associate and form a dimer at 28 and 25°C.

Table II. Number average molecular weights of Li-gellan, Na-gellan, and K-gellan in salts solutions at 45 and 28°C.

sample	$M_n \times 10^{-4}$	
	45°C	28°C
Na-gellan	4.8	9.2
Li-gellan	4.3	10.9
K-gellan	4.9	—

As shown in Table II, the value of M_n (Na/NaCl) (4.8x10⁴) obtained at 45°C and M_n (Na/NaCl)(9.2x10⁴) at 28°C were coincident with our previous value of M_n (Na/NaCl)(4.3₉x10⁴) at 45°C and M_n (Na/NaCl) (9.4₄x10⁴) at 28°C[6a,b], respectively. In this work, the M_n (Na/NaCl) obtained at 45°C was almost half the value of M_n (Na/NaCl) (9.2x10⁴) obtained at 28°C, and the M_n (Li/LiCl) (4.3x10⁴) obtained at 45°C was slightly less than half the value of M_n (Li/LiCl) (10.9x10⁴) obtained at 28°C. These results suggest that the coil to double-helix conformational transition occurs in the Li, Na, and K-gellan molecules, and aggregation of helices of the Li and K-gellan may also occurs at least partly at 28°C.

The temperature dependence of CD spectrum was measured for the Li, Na, and K-gellan aqueous solutions without salt ($c_p=0.25, 0.5, \text{ and } 1.0\text{wt}\%$). The CD spectra for the Li, Na, and K-gellan solutions are very similar. The peak around 201nm in the CD spectrum reflects the optically active chemical structure of glucuronic acid of gellan molecule in random coil form[5,7-9]. Therefore, the variation of ellipticity of the peak around 201 nm should correspond to the conformational change of gellan molecules. The ellipticity at 201 nm, θ_{201} , of the Li, Na, and K-gellan aqueous solutions with three polymer concentrations are plotted against temperature. A typical example is shown in Fig.1. The temperature dependence of θ_{201} of the Li, Na, and K-gellan aqueous solutions shows very similar behavior. The transition temperature T_{CD} was defined as the intersection point of the extrapolated lines and plotted against polymer concentration in Fig.2.

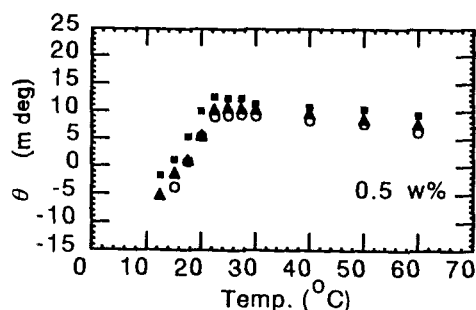


Fig.1. Temperature dependence of ellipticity at 201 nm for Li(●), Na(○), and K(▲)-gellan solutions without salts. $c_p=0.5\text{wt}\%$.

Viscosity measurements were carried out over the temperature range from 50 to 5°C in aqueous solutions with and without salts. Fig. 3 shows the viscosity number of solution η_{sp}/c_p versus temperature plots for the Li, Na, and K-gellan aqueous solutions without salts. On lowering temperature, the η_{sp}/c_p values of the Li, Na, and K-gellan aqueous solutions showed almost constant or only a slight increase at higher temperature regions but increased rapidly below the transition temperatures (T_η). The variation of η_{sp}/c_p is a reflection of conformational change of the Li, Na, and K-gellan molecules and can be interpreted as follows. In aqueous solutions, on lowering temperature, the coil to double-helix transition takes place below the transition temperature T_η and the solution with higher polymer concentration has higher tendency to form double-helix than that of lower polymer concentration. The concentration dependence of transition temperature T_η of the gellan solutions is also shown in Fig.2. The transition temperatures for the Li, Na, and K-gellans determined by CD and viscometry are almost coincident, which increase with increasing polymer concentration. The transition temperatures for the Li, Na, and K-gellan are not affected by the species of counterions suggesting that the electrostatic repulsive interaction between carboxylanions of gellan molecules seems to be similar among the Li, Na, and K-gellans within these experimental conditions.

Fig. 4 shows the temperature dependence of (η_{sp}/c_p) of the Li, Na, and K-gellan aqueous solutions with

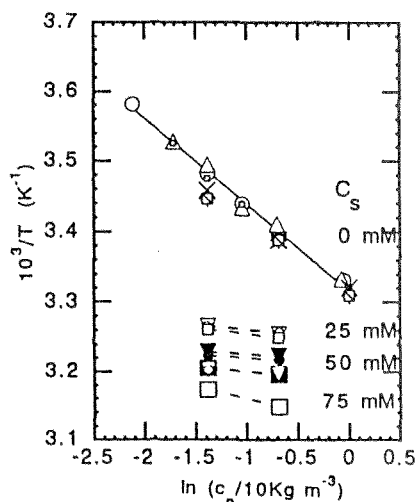


Fig.2. Plots of the transition temperature against gellan concentration for Li,Na, and K-gellan aqueous solutions without and with salts. The solid and dashed lines denote experimental fit to the data. T_η (Li/aq) (Δ), T_η (Na/aq) (\circ), T_η (K/aq) (\square), T_η (Li/25LiCl) (∇), T_η (Na/25NaCl) (\diamond), T_η (K/25KCl) (\square), T_η (Li/50LiCl) (\blacktriangledown), T_η (Na/50NaCl) (\bullet), T_η (K/50KCl) (\blacksquare), T_η (Li/75LiCl) (∇), T_η (Na/75NaCl) (\circ), T_η (K/75KCl) (\square), T_{CD} (Li/aq) (\times), T_{CD} (Na/aq) ($+$), T_{CD} (K/aq) (\boxtimes).

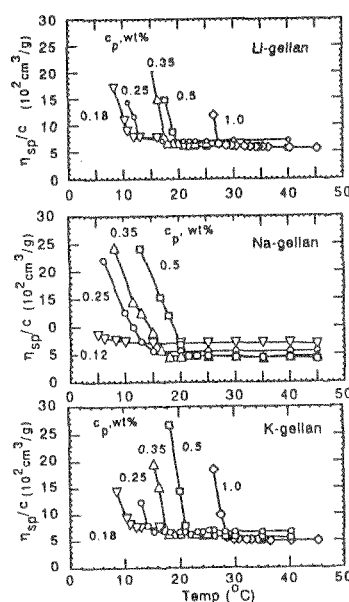


Fig.3. Temperature dependence of (η_{sp}/c_p) for Li, Na, and K-gellan aqueous solutions without salts.

LiCl, NaCl, and KCl respectively. The transition temperatures T_η are plotted against the polymer concentrations in Fig. 2. As shown in Fig. 4, on lowering temperature, the (η_{sp}/c_p) of these solutions keep almost constant values down to the transition temperatures, but the (η_{sp}/c_p) values increase steeply below the transition temperatures. As shown in Fig. 2, the T_η (Li/LiCl), T_η (Na/NaCl), and T_η (K/KCl) are slightly increase with increasing polymer concentration but greatly increase with increasing concentration of added salts, which reveals that counterions play an important role to the coil-double helix conformational transition of gellan molecules in aqueous solutions with added salts. It is considered that the counterions can shield the electrostatic repulsion between ionized carboxyl groups in the gellan molecule due to counterion condensation and thereby lead to the reduction of the coil-dimension, and hence can promote the double-helix formation and the association of two molecules. Moreover,

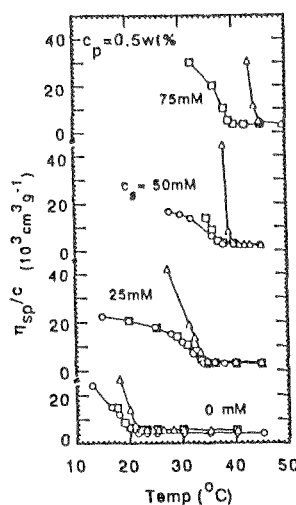


Fig.4. Temperature dependence of (η_{sp}/c_p) for Li(\circ), Na(\square), and K(\triangle)-gellan aqueous solutions ($c_p = 0.5wt\%$) with and without salts.

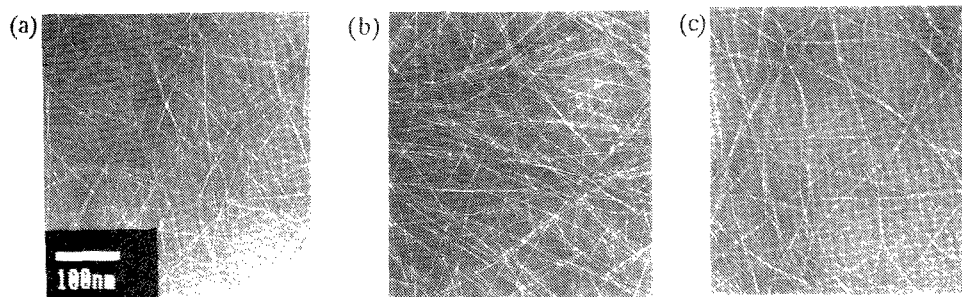
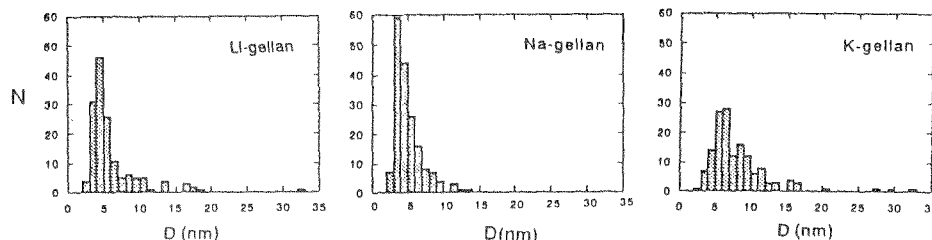


Fig.5. The electron micrographs of Li(a), Na(b), and K(c)-gellan aqueous solutions without salts.

Fig.6. Distribution of the fiber diameter for Li, Na, and K-gellan fibers in Fig.5.



counterions bind to the surface of individual helices, resulting in lowering the charge density of the helices and reducing their electrostatic barrier to association.

In the solutions of $c_g \approx 50$ and $75 \text{ mmol}/10^3 \text{ cm}^3$, the T_η (K/KCl) is higher than T_η (Li/LiCl) and/or T_η (Na/NaCl) (Fig.4). In the added salt solutions, the slopes of (η_{sp}/c_p) versus temperature plots below T_η increased in the order of K-gellan \gg Li-gellan \geq Na-gellan. These results suggest that below the transition temperatures, a partial aggregation of double-helices occurs and increases with decreasing temperature, and the order of effectiveness of the cationic species in promoting the partial aggregation is $\text{K}^+ \gg \text{Li}^+ \geq \text{Na}^+$. The reason why KCl can influence the coil to double-helix transition and aggregation of helices of gellan molecules more effectively than NaCl and LiCl could be interpreted as follows. The cations such as Na^+ and Li^+ are the structure ordering ions for water, and the cation such as K^+ belong to the structure disordering ions. Therefore, the latter group can shield the electrostatic repulsion of carboxyl groups in the gellan molecules more directly than the former groups. The hydrogen bonds between gellan chains enhance the formation of double-helix and the aggregation of the double-helices. Matsukawa et al. [8a] reported that the hydrogen bond structure formed at low temperature is stable in K-gellan compared with that in Li-gellan or Na-gellan solutions due to higher shielding-effect of K^+ .

The electron micrographs of the Li, Na, and K-gellan are shown in Fig. 5. The polymer concentrations (0.002wt%) of the gellan solutions used for the preparation of electron microscopy was too low to permit the coil-double helix transition and aggregation of helices at room temperature, therefore the gellan will exist in the coil form. During air-drying in the preparation of electron microscopy, however, the coil to double-helix transition will

occur and helices will associate and/or superimpose upon each other due to the increase of polymer concentration. As shown in Fig.5, the Li, Na, and K-gellan have a similar network pattern which has a straight fiber, not entangled. We measured the diameter of these fibers and analyzed statistically (Fig.6). The diameter of the fibers increased as the following order; K-gellan \gg Li-gellan \geq Na-gellan. These results are corresponding to the conclusions obtained from the viscometry, CD, and osmometry.

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