# Structural Change in Polymer Chains of Sodium Gellan Gums Studied by Circular Dichroism

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Gellan gum undergoes gelation by forming domains composed of associated double-helices. In this study, using five well purified sodium type gellan gums with different molecular weights (Na-gellan samples of G1-G5,  $M_w$ =12x10<sup>4</sup>-3.2x10<sup>4</sup> at 40°C), we investigated the molecular weight effect on the coil to double-helix transition of Na-gellans in aqueous solutions by circular dichroism (CD) measurements and viscometry. The coil to double-helix transitions of Na-gellans were investigated from the temperature dependences of viscosity number  $\eta_{sp}/c_p$  and molar ellipticity obtained at 201 nm  $[\theta]_{201}$ . The coil to double-helix transition temperatures  $T_{ch}$  for G1-G5 Na-gellans determined from  $\eta_{sp}/c_p$  and  $[\theta]_{201}$  data are coincident.  $T_{ch}$  values for G1-G5 Na-gellans are almost the same, irrespective of  $M_w$ , which increase with increasing polymer concentrations. CD data were analyzed according to the van't Hoff's equation. The van't Hoff's transition enthalpy  $\Delta H_{vH}$  depends markedly on  $M_w$ , suggesting that the initial stage of gelation such as the coil to double-helix transitions is accelerated with increasing molecular weights. Keywords: gellan gum, molecular weight, aqueous solution, coil-helix transition, sol-gel transition

## INTRODUCTION

Gellan gum is an anionic microbial heteropolysaccharide produced by Pseudomonas elodea. The chemical structure of the polymer is based on the tetrasaccharide repeating units,  $[(3)-\beta-D-glucose-$ (1-4)-, $\beta$ -D-glucuronic acid-(1-4),  $\beta$ -D-glucose and  $\alpha$ -L-rhamnose], and it has a carboxyl side group[1,2]. It is now accepted that the gelation mechanism of gellan gum is as follows; gellan gum molecules undergo a thermally reversible (coil to double-helix) transition, and junction zones of gellan gels are formed by aggregation of double-helical gellan gum molecules. Thus, helix formation is a prerequisite for gel formation. The coil to helix transition in aqueous solutions is influenced strongly by the presence of metal ions [3,4] and also by the polymer characteristics such as polymer molecular weight [5,6]. Previously, using five sodium-type gellan gums (Na-gellans, G1–G5) with different weight average molecular weights ( $M_w$ ), we studied the effects of molecular weight on the conformational transition of Na-gellans in aqueous solution with 25 mM NaCl from the measurements of circular dichroism (CD), intrinsic viscosity and light scattering (LS)[6]. It was found that the coil to double-helix transition temperature for Na-gellans of G1–G5 in aqueous solutions with 25mM NaCl were the same, irrespective of  $M_{w}$ , and the coil to double-helix transitions accelerated with increasing  $M_{w}$ .

In this study, we investigate the effects of  $M_w$  on the conformational transition of Na-gellans in aqueous solutions without NaCl using the Na-gellan samples which were used in the previous study [6].

#### EXPERIMENTAL

*Materials* Na-gellan samples (G1–G5) were prepared from the deacetylated gellan gum powder (San-EiGen F.F.I) by vibrational grinding using a HEIKO Sample Mill Model TI-300 [6,7]. The characteristics of the 5 Na-gellan samples are listed in Table 1.

*Measurements* CD measurements and viscometry for Na-gellan were carried out in aqueous solutions without salt. CD measurements were performed from 60 to 5  $^{\circ}$ C using a Jasco J-75 Spectropolarimeter (Japan).

Viscometry was carried out over the temperature range from 45 to 10  $^{\circ}$ C using an Ubbelohde-type viscometer.

#### **RESULTS AND DISCUSSION**

The temperature dependence of viscosity number  $\eta_{sp}/c_p$ (=  $(\eta_s \cdot \eta_o)/\eta_o c_p$ ) for the Na-gellans was determined in aqueous solutions (polymer concentration  $c_p$ = 0.25, 0.5 and 1.0 wt%). Here,  $\eta_s$  and  $\eta_o$  are the viscosities of the solution and the solvent, respectively. Typical examples of the  $\eta_{sp}/c_p$  versus temperature plots for the Na-gellans in aqueous solutions are shown in Fig.1. On lowering the temperature, the  $\eta_{sp}/c_p$  of G1 and G4 sample



Fig.1 Temperature dependence of  $\eta_{sp}/c_p$  for Na-gellans in aqueous solutions.

solutions with various polymer concentrations showed an almost constant or only a slight increase at higher temperature regions, but increased rapidly below the coil to double-helix transition temperatures ( $T_{ch}$ ). The transition temperatures of the Na-gellan solutions increased with increasing polymer concentrations.

The variation of  $\eta_{sp}/c_p$  is a reflection of the conformational change of Na-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  $T_{ch}$  and the solutions of higher polymer concentrations have a greater tendency to form double-helices than those of lower polymer concentrations. At the temperatures, above  $T_{ch}$ , where polymer chains take a coil form, the values of  $\eta_{sp}/c_p$  increase with decreasing polymer

Table 1 Characteristics of Na-gellan Samples

Samples	$M_{\rm w}  {\rm x10^{-3}}^{\rm a}$	Metal contents $(10^{-2} \text{ g/g})$					
		Na	K	Ca	Mg	Fe	_
G1	120	3.00	0.07	0.005	0.0006		
G2	71	3.33	0.00345	0.00251	0.00019	0.00176	
G3	62	3.10	0.00251	0.00101	0.00021	0.00243	
G4	57	3.20	0.00322	0.00245	0.00017	0.00385	
G5	32	3.29	0.00849	0.00154	0.00019	0.00213	

a) Values are determined by LS measurements at 40°C in aqueous solutions with 25 mM NaCl [6].



Fig.2 CD spectra of Na-gellans (G1 and G4) aqueous solutions.



Fig.3 Temperature dependence of molar ellipticity at 201nm for 1wt% Na-gellan aqueous solutions.



Fig.4 Polymer concentration dependence of the coil to double-helix transition temperature  $T_{ch}$  for Na-gellan aqueous solutions. Lines  $(\dots, -)$  are experimental fit of the data.

concentrations, which are commonly observed for a flexible polyelectrolyte in aqueous solutions in the absence of added salt. This effect should be attributed to the fact that, with decreasing the polyelectrolyte concentration, the condensed counteractions escape into the surrounding solution and hence the ionic strength increases, so that the polymer chain is expanded by the electrostatic repulsive interactions.

The temperature dependence of the CD spectra for the 5 Na-gellan aqueous solutions ( $c_p$ = 0.25, 0.5 and 1.0 wt%) was measured. Typical examples are shown in Fig.2. The CD spectra for G1–G5 are similar and a peak was observed at around 201 nm which corresponds to the coil to double-helix conformational change of Na-gellan molecules [4,8]. The molecular ellipticity at 201 nm [ $\theta$ ]<sub>201</sub> of Na-gellans is plotted against temperature. A typical example is shown in Fig.3. On lowering temperature, values of [ $\theta$ ]<sub>201</sub> for G1–G5 drastically decreased below  $T_{ch}$ , suggesting that a coil to double-helix transition took place.

In Fig.4, the values of  $T_{ch}$  for G1–G5 determined from the temperature dependence of  $[\theta]_{201}$  are plotted against polymer concentration together with the values of  $T_{ch}$  obtained by viscometry in Fig.1. In aqueous solutions without NaCl,  $T_{ch}$  obtained from  $[\theta]_{201}$  and  $\eta_{sp}/c_p$  data were coincident, and these  $T_{ch}$  values increase with increasing polymer concentration. It is noted that the  $T_{ch}$  values for G1–G5 are the same, suggesting that these values do not depend on  $M_w$  when  $M_w$  is higher than  $32 \times 10^3$ . Previously, we investigated the molecular weight dependence of  $T_{ch}$ for G1–G5 in aqueous solutions with 25 mM NaCl [5]. The previous results are shown in Fig.4. In aqueous



Fig.5 van't Hoff plots of molar ellipticity data for Na-gellan (G1, $c_p=0.5$ wt%) aqueous solutions.



Fig.6 Dependence of van't Hoff enthalpy,  $\triangle H_{VH}$ , on molecular weight  $M_w$  at 40°C of Na-gellans.  $\bigcirc$ ; 1wt%, $\triangle$ ; 0.5 wt%, $\bigtriangledown$ ; 0.25wt%

solutions with NaCl,  $T_{ch}$  for G1–G5 did not depend on  $M_w$  in the same way as  $T_{ch}$  in aqueous solutions without NaCl.  $T_{ch}$  values for G1–G5 in aqueous solutions without NaCl are much lower than those in solutions with NaCl, and  $T_{ch}$  values in aqueous solutions without NaCl increase rapidly with increasing polymer concentration. It is considered that these results should be attributed to the fact that the polymer chains are expanded by the electrostatic repulsive interactions in dilute aqueous solutions without salt.

We analyzed the CD data using van't Hoff plots and calculated the van't Hoff's transition enthalpy  $\Delta H_{VH}$ . Previously we concluded that the coil to double-helix transition for Na-gellan in aqueous solutions with 25 mM NaCl is an all-or-none type transition [9]. Assuming that coils and double-helices coexist in aqueous solutions, double-helix contents in a chain *h* can be calculated from Eq.1 [5,10], and the equilibrium constant *K* obeys van't Hoff's relation (Eq.2).

$$[\theta]_{201} = h[\theta]_{201h} + (1-h)[\theta]_{201c}$$
(1)

$$K = h / (1-h)^2$$
 (2)

$$\frac{\mathrm{d}\ln K}{\mathrm{d}(1/T)} = \frac{-\Delta H_{\mathrm{VH}}}{R} \tag{3}$$

Here  $[\theta]_{201}$  is observed molecular ellipticity at 201 nm at a given temperature, and  $[\theta]_{201c}$  and  $[\theta]_{201h}$  are the molecular ellipticity assigned to the coil and the double-helix state, respectively. *R* is the gas constant. Using the values of  $[\theta]_{201c}$  and  $[\theta]_{201h}$  which were obtained previously [5,9], we calculated K values, and plotted them against 1/T. A typical example of the van't Hoff plots is shown in Fig.5. For G1-G5, ln K in the van't Hoff plots showed a steep decrease at the transition temperatures.  $\triangle H_{VH}$  values for G1-G5 are determined from the slopes just below  $T_{ch}$  and are plotted against  $M_w$  in Fig.6.  $\triangle H_{VH}$  values for G1-G5, which are nearly the same as the values obtained in aqueous NaCl solutions, are slightly dependent on the polymer concentration but depend markedly on  $M_w$ . These results suggest that, in the same way as the results obtained in aqueous solutions with salt, the coil to double-helix transitions in aqueous solutions without salt are accelerated with increasing  $M_w$ .

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