

## Initial Stage of Gelation Process for Sodium-Type Gellan Gums with Different Molecular Weights

Etsuyo Ogawa<sup>1</sup>, Rheo Takahashi<sup>2</sup>, Hirofumi Yajima<sup>3</sup>, and Katsuyoshi Nishinari<sup>2</sup>

<sup>1</sup>Showagakuin Jr. College, Higashisugano, Ichikawa, Chiba 272-0823, Japan.

Fax: 81-47-322-6800; e-mail [QYK04426@nifty.ne.jp](mailto:QYK04426@nifty.ne.jp).

<sup>2</sup>Graduate School of Human Life Science, Osaka City University, Sugimoto, Sumiyoshiku, Osaka 558-8585, Japan.

Fax: 81-6-6605-3086; e-mail [nisinari@life.osaka-cu.ac.jp](mailto:nisinari@life.osaka-cu.ac.jp)

<sup>3</sup>Department of Chemistry, Tokyo University of Science, Kagurazaka, Shinjyukuku, Tokyo, 162-8601, Japan.

Fax: 81-3-3235-2214; e-mail [yajima@rskagu.tus.ac.jp](mailto:yajima@rskagu.tus.ac.jp)

Using 6 kinds of well purified sodium-type gellan gums (Na-gellan samples of G1~G6,  $M_w=12 \times 10^4 \sim 1.7 \times 10^4$  at 40°C), we investigated the effects of molecular weight on the initial stage of gelation, such as coil to double-helix transition and the aggregation of double-helices, in aqueous solutions with 25 mM NaCl by measurements of light scattering (LS), intrinsic viscosity  $[\eta]$ , and circular dichroism (CD). At 40 °C, the molecular weight dependences of  $[\eta]$  were quantitatively described by the Yamakawa-Fujii-Yoshizaki theory, suggesting that the Na-gellan in aqueous NaCl solutions behaves as a single stranded chain. While, at 25 and 15 °C, except for G6 having the lowest  $M_w$ , the values of  $M_w$  were higher than those obtained at 40 °C and increased with decreasing temperature. The G6 did not form double-helix at 25 °C. The coil to helix transition and aggregation of double-helices were detected from the temperature dependences of  $[\eta]$ , and CD spectra for the Na-gellans solutions. The coil to double-helix transition for Na-gellans of  $M_w \geq 3.2 \times 10^4$  takes place at almost the same temperatures, and is followed by the aggregation of double-helices with lowering temperature. The van't Hoff's enthalpy  $\Delta H_{vH}$  depends on molecular weight. It is concluded that the initial stage of gelation such as the coil to double-helix transition is accelerated with increasing molecular weight.

Keywords: gellan gum, molecular weight, aqueous solution, coil-helix transition, sol-gel transition

### INTRODUCTION

Gellan gum, an anionic polysaccharide produced by *Psuedomonas elodea* has complex tetrasaccharide repeating sequences of  $\beta$ -D-glucose,  $\beta$ -D-glucuronic acid,  $\beta$ -D-glucose and  $\alpha$ -L-rhamnose, and a carboxyl side group [1,2]. This polysaccharide is widely used in the food industry and biomedical fields because it forms a transparent gel which is resistant to heat and acid when compared to other polysaccharide gels [3].

The gelation mechanism of gellan gum solutions has been the subject of controversy, but it is now accepted that gellan gum undergoes a thermally-reversible coil to double-helix transition, and that junction zones of gellan gum gels are formed by aggregation of double-helical gellan gum molecules[4]. Thus, the coil to double-helix transition is a prerequisite for gel formation. The gelation mechanisms of gellan

gum solutions are influenced strongly by the presence of cations and extensive studies have been recently carried out [5,6]. However, the effects of molecular weight of gellan gum on the gelation mechanism have not studied yet.

In this study, using 6 samples of well-purified sodium-type gellan gum (Na-gellan) having different molecular weights, we investigated the effects of molecular weight on the coil to double-helix transition and the aggregation of double-helices in aqueous solutions with 25 mM NaCl by light scattering (LS) and circular dichroism (CD) measurements, and viscometry.

#### EXPERIMENTAL

**Materials** Na-gellan samples (G1~G6) were prepared from the deacetylated gellan gum powder (San-Eigen F.F.I) by vibrational grinding using a HEIKO Sample Mill Model TI-300 [7].

**Measurements** LS and CD measurements and viscometry for Na-gellan were carried out in aqueous solutions with 25 mM NaCl. LS was measured at 40, 25, and 15 °C using Wyatt Technology DAWN DSP Light Scattering Spectrometer. Viscometry was carried out over the temperature range from 45 to 10°C using a low shear Ubbelohde-type viscometer. CD measurements were performed from 60 to 5 °C using a Jasco J-75 Spectropolarimeter (Japan).

#### RESULTS AND DISCUSSION

$M_w$  values for Na-gellan samples obtained at 40 °C by LS measurements are shown in Table 1 together with

the values of intrinsic viscosities  $[\eta]$ . The molecular weight range for 6 samples of Na-gellan were from  $120 \times 10^3$  to  $17 \times 10^3$ . The data obtained at 40 °C were analyzed according to the Yamakawa-Fujii-Yoshizaki (Y-F-Y) theory for unperturbed wormlike chain [8,9]. The plots of  $[\eta]$  vs  $M_w$  fit a single curve calculated from Y-F-Y theory using the values of persistence length  $q = 8.5$  nm, molar mass per unit contour length of the chain  $M_L = 360 \text{ nm}^{-1}$ , and chain diameter  $d_v = 1.0$  nm, suggesting that at 40 °C Na-gellan in solutions with 25 mM NaCl behaves as a single stranded chain. The ratio of  $M_w$  obtained at 25 and 40 °C ( $M_w^{25}/M_w^{40}$ ), and that at 15 and 40°C ( $M_w^{15}/M_w^{40}$ ) are also shown in Table 1. The values of  $M_w^{25}/M_w^{40}$  were ca.2.0 for G1 and G2 and those for G3~G5 were 1.7~1.3, suggesting that at 25°C double-helix formation are occurred for G1 and G2 and partially occurred for G3~G5. While, G6 did not form double-helix at 25°C.

Fig.1 shows the temperature dependence of  $[\eta]$  for the Na-gellan solutions in aqueous solutions with 25 mM NaCl. On lowering the temperature, the  $[\eta]$  values of Na-gellan of G1~G5 were almost constant at higher temperature regions but increased rapidly in the temperature region of 32~ca. 25°C, and below ca.25°C, increases of the  $[\eta]$  values become a gradual. It is noted that the slope of  $[\eta]$  vs temperature plots in the region of 32~ca.25°C increases with increasing  $M_w$ . While, the  $[\eta]$  value of G6 increases slightly from 45 to 10°C. The variation of  $[\eta]$  can be interpreted as follows. On lowering the temperature, the double-helix formation take place for G1~G5 below 32°C (the coil to

Table 1 Weight-average molecular weights and intrinsic viscosity of Na-gellan samples.

Sample	$10^{-3}M_w^{40}$	$M_w^{25}/M_w^{40}$	$M_w^{15}/M_w^{40}$	$[\eta]^{40}(\text{cm}^3/\text{g} \cdot \text{l})$
G1	120	2.0	2.2	2180
G2	71	2.1	3.3	1210
G3	62	1.7		841
G4	57	1.5	1.8	674
G5	32	1.3	1.7	289
G6	17	1.0	1.3	97.6

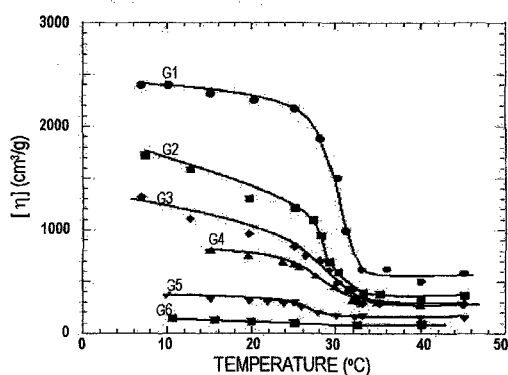


Fig.1 Temperature dependence of  $[\eta]$  for Na-gellan in aqueous solutions with 25 mM NaCl.

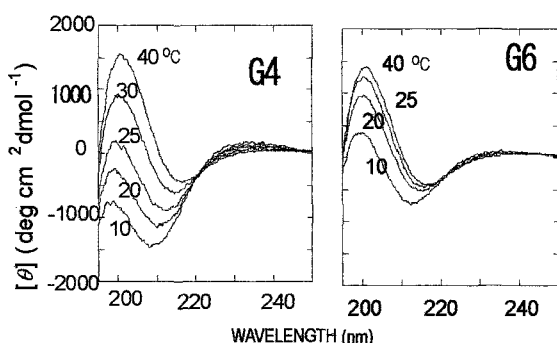


Fig.2 CD spectra of Na-gellans (G4 and G6) aqueous solutions with 25 mM NaCl.

double-helix transition temperature,  $T_{ch}$ ) and the aggregation of double-helices occurs below ca.25°C at least partly. In addition, the coil to double-helix transition is accelerated with increasing molecular weight.

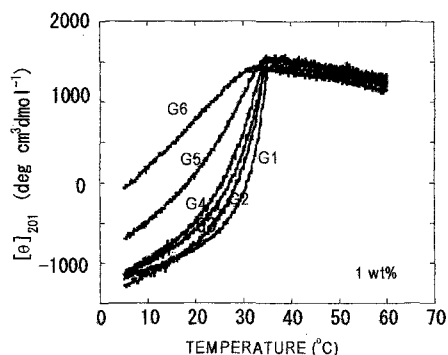


Fig.3 Temperature dependence of molar ellipticity at 201 nm for 1wt% Na-gellan aqueous solutions with 25 mM NaCl.

The temperature dependence of the CD spectra was measured for the 6 Na-gellan aqueous solutions with 25 mM NaCl (polymer concentration  $c_p=0.25, 0.5,$  and  $1.0$

wt%). Typical examples of the CD spectra are shown in Fig.2. The CD spectra for G1~G5 are similar, but G6 shows a somewhat dissimilar shape. The peak around 201 nm in the CD spectra reflects the optically active chemical structure of the glucuronic acid on gellan molecules in random coil form [6,10]. Therefore, the variation of molar ellipticity of the peak around 201 nm should correspond to a conformational change of gellan molecules. The molecular ellipticity at 201 nm,  $[\theta]_{201}$ , of Na-gellan is plotted against temperature. A typical example is shown in Fig.3. On lowering temperature, values of  $[\theta]_{201}$  for G1~G5 drastically decreased below  $T_{ch}$  (34-35°C), suggesting that the coil to double-helix transition and aggregation of double-helices occurred. While, values of  $[\theta]_{201}$  for G6 showed a gradual decrease below  $T_{ch}$  (30°C) which was lower than that for G1~G5.

The values of  $T_{ch}$  determined from temperature dependence of  $[\theta]_{201}$  are plotted against polymer concentration together with the values of  $T_{ch}$  obtained by viscometry in Fig.4. The values of  $T_{ch}$  increase slightly with increasing polymer concentration, but the values of  $T_{ch}$  for G1~G5 obtained by CD spectra (and also by viscometry) are almost the same. These results reveal that the values of  $T_{ch}$  do not depend on  $M_w$  at least  $M_w \geq 3.2 \times 10^4$ . While, the values of  $T_{ch}$  for G6 which could not be detected by viscometry are lower than those of G1~G5. Results for G6 may be interpreted as follows: on lowering temperature, only a small number of coils in

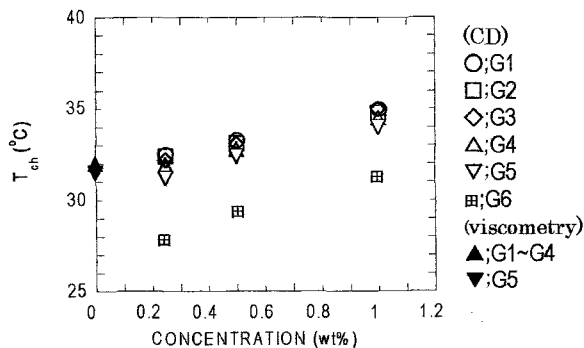


Fig.4 Polymer concentration dependence of the coil-helix transition temperature,  $T_{ch}$ , for Na-gellan aqueous solutions with 25 mM NaCl.

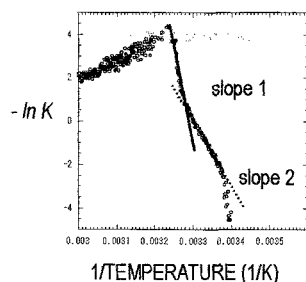


Fig.5 Van't Hoff plots of molar ellipticity data for Na-gellan (G4) aqueous solutions with 25 mM NaCl.

a molecular chain transformed into helices, and consequently, the double-helix formation could not occur due to insufficient number of helices needed to form a double-helix.

We analyzed the CD data using van't Hoff plots and calculated van't Hoff's enthalpy  $\Delta H_{\text{vH}}$ . It is assumed that the coil-helix transition is a two state equilibrium process and equilibrium constant obeys van't Hoff's relation:

$$\frac{d \ln K}{d(1/T)} = \frac{-\Delta H_{\text{vH}}}{R} \quad (1)$$

where  $K (= \theta / (1 - \theta))$ ,  $\theta$ ; the helix content) is the equilibrium constant for the transition and  $R$  the gas constant. The value of  $\theta(T)$  at different temperatures was calculated according to Eq. 2 [11].

$$\theta(T) = \frac{[\theta]_{201}(T) - [\theta]_{201c}(T)}{[\theta]_{201h}(T) - [\theta]_{201c}(T)} \quad (2)$$

Here  $[\theta]_{201}(T)$  is observed molecular ellipticity at 201 nm at a given temperature, and  $[\theta]_{201c}(T)$  and  $[\theta]_{201h}(T)$  are the molecular ellipticity assigned to the coil and the helix states, respectively. Assuming the value of  $[\theta]_{201c}(T) = 1550$  (deg cm<sup>2</sup>dmol<sup>-1</sup>) and  $[\theta]_{201h}(T) = -400$  (deg cm<sup>2</sup>dmol<sup>-1</sup>) which are the values of  $[\theta]_{201}$  for G1 obtained at 40 and 25°C, respectively, we calculate  $\theta(T)$  values. A typical example of van't Hoff plots is shown in Fig.5. Below  $T_{\text{ch}}$ , van't Hoff plots can be divided into two parts (slope 1 and 2). The problem arises which slope of the plot should be used for calculation of  $\Delta H_{\text{vH}}$ . From the results of  $M_w$  and  $[\eta]$ , on lowering temperature, the coil to double-helix transition occurs below  $T_{\text{ch}}$  and is followed by the aggregation of double-helices. Therefore, we thought

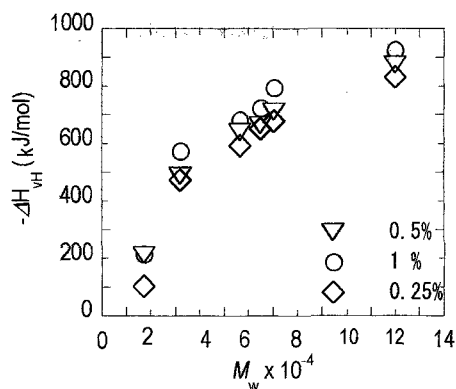


Fig.6 Dependence of van't Hoff enthalpy,  $\Delta H_{\text{vH}}$ , on molecular weight,  $M_w$  at 40°C of Na-gellans.

that  $\Delta H_{\text{vH}}$  for the coil to double-helix transition should be calculated from the  $[\theta]_{201}$  data obtained just below  $T_{\text{ch}}$ . The values of  $\Delta H_{\text{vH}}$  determined from slope 1 are plotted against  $M_w^{40}$  in Fig.6. Values of  $\Delta H_{\text{vH}}$  are slightly dependent on the polymer concentration and depend markedly on  $M_w^{40}$ . These results support the conclusion that the initial stage of gelation such as coil to double-helix transition is accelerated with increase of molecular weight.

#### ACKNOWLEDGEMENTS

This work is supported by a Grand-in-Aid for Nestle Science Promotion Committee.

#### REFERENCES

- 1) M.A.O'Neill, R.R.Selvenderan, and V. J. Morris, *Carbohydr. Res.*, **124**, 123-133 (1983).
- 2) P. Jansson, B. Lindberg, and P. A. Sandford, *Carbohydr. Res.*, **124**, 135-139 (1983).
- 3) G.R.Sanderson, Ed. by P. Harris, *Food Gels*. Elsevier Applied Science, London, 201-232 (1999).
- 4) G.Robinson, C.E.Manning and E.R.Morris, "Food Polymers, Gels and Collids", Ed. by E.Dickinson, Royal Society Chemistry, London, 22-33 (1991).
- 5) E. Miyoshi, T. Takaya and K. Nishinari, *Food Hydrocoll.*, **8**, 505-527 (1994).
- 6) E.Ogawa, H.Matsuzawa, and M.Iwahashi, *Food Hydrocoll.*, **16**, 1-9 (2002).
- 7) E.Ogawa and Y. Nozawa, *Rep. Prog. Polym. Phys.Japan*, **41**, 121-122 (1998).
- 8) H. Yamakawa and M.Fujii, *Macromolecules*, **7**, 128-135 (1974).
- 9) H. Yamakawa "Helical wormlike chains in polymer solutions" Springer, Berlin Heiderberg, New York, Chap.6 (1997).
- 10) Y. Nitta, S.Ikeda, T.Takaya and K.Nishinari, *Trans. Ma. Re. Soc.Jap.*, **26**, 621-624 (2001).
- 11) B. E. Christensen, K. D. Knudsen, O. Smidsrod, S.Kitamura and K.Takeo, *Biopolymers*, **33**, 151-161(1993).