

SOL-GEL TRANSITION OF SONICATED GELLAN GUMS IN AQUEOUS SOLUTIONS

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Gellan gum undergoes gelation by forming domains composed of associated double-helices. In this study, we investigated the coil to double-helix transition and the sol to gel transition of sonicated sodium-type gellan gums (Na-gellans) in aqueous solutions by rheological measurements. Three well-purified Na-gellan samples with different molar masses were prepared by sonic irradiation and purification. Temperature dependence of storage modulus (G') and loss modulus (G'') of these three samples were measured from 60 to 5 °C in aqueous solutions. It was found that the coil to double-helix transition temperatures (T_{ch}) for the three sonicated Na-gellans were almost the same, however, the sol-gel transition temperature (T_{sg}) depended markedly on molar mass and T_{sg} of Na-gellans decreased with decreasing molar mass of the samples.

Key words: gellan gum, sol-gel transition, coil-helix transition, aqueous solution, rheology

Introduction

Gellan gum is a microbial polysaccharide produced by *Sphingomonas elodea*. The polymer is a linear heteropolysaccharide composed of tetrasaccharide (β -D-glucose, β -D-glucuronic acid, β -D-glucose and α -L-rhamnose) repeating units, and it has a carboxyl side group [1, 2]. This polysaccharide is widely used in the food industry and biotechnology since it forms a transparent gel which is resistant to heat and acid in comparison to other polysaccharide gels. The gelation mechanism of various polymer solutions and thermoreversible sol-gel transitions have been studied [3]. It has been accepted that gellan gum undergoes a thermally reversible helix-coil transition, and that the junction zones of gellan gum gels are formed by

aggregation of double-helical gellan molecules. The gelation of gellan gum solutions is influenced strongly by the kinds and amount of cations in the solutions. Extensive studies of the effects of cations on gelation of gellan gum solutions have been carried out by many researchers [4-8]. However, gelation is influenced not only by the cations, but also by the molar mass of gellan gums. Only a small number of studies [9-11] concerning molar mass effects on the initial stage of gelation, such as coil to helix transition, have been carried out due to the difficulty of sample preparation. Recently, it has been found that the coil to double-helix transitions of sodium-type gellan gums are all-or-none type and the coil to double-helix transition is promoted with increasing molar mass [10,11]. Additionally the lowest molar mass

below which no double-helix is formed at 25°C in aqueous solutions with 25 mmol NaCl lies between $M_w=32 \times 10^3$ and $M_w=17 \times 10^3$ [11]. However, the molar mass dependence of gel-formation such as sol-gel transition remains unclear.

In this study, using 3 samples of well-purified sonicated sodium-type gellan gum (Na-gellan) with different molar masses, we investigated the effects of molar mass on the coil to double-helix transition and the sol to gel transition by rheological and CD measurements.

EXPERIMENTAL

Materials Na-gellan samples (GF1–GF3) were prepared from the deacetylated gellan gum powder (San-Ei Gen F.F.I., Osaka, Japan, Lot 4F1191A) by sonication using a sonicator (Nihonseiki Ltd., US -300T). The characteristics of the samples are listed in Table 1.

Measurements The rheological measurements were performed using a RS 600 (Thermo HAAKE) with parallel plate geometry of 60 mm diameter. Temperature dependence of storage modulus, G' , and loss modulus, G'' , was observed by cooling the systems from 60 to 5 °C and then reheating to 60 °C, at the rate of 0.5 °C/min, at the constant frequency of 0.3 Hz. CD measurements for Na-gellan were carried out in aqueous solution using a spectropolarimeter (Jasco J-75, Japan)[10,11]. Light scattering and intrinsic viscosities [9] were measured in 25 mmol NaCl aqueous solutions using a purpose-built spectrometer with 488 nm line of Ar ion laser and Ubbelohde low-shear capillary viscometer, respectively.

Molar mass of the Na-gellan samples and viscosities obtained in aqueous 25 mmol NaCl at 40 °C are shown in Table 1. Takahashi et al. [9] reported that the molar mass dependence of intrinsic viscosity of Na-gellan in aqueous solutions with 25 mmol NaCl at 40 °C was described by the Yamakawa-Fujii-Yoshizaki (YFY) theory [12, 13] for an unperturbed wormlike chain using the values of persistence length of the chain $q=9.4$ nm, molar mass per unit contour length of the chain $M_L=355$ nm⁻¹, and chain diameter $d_v=1.0$ nm. The molar mass dependence of the intrinsic viscosity of the GF1, GF2, and GF3 samples obtained at 40 °C was also described by the YFY theory using the same parameters.

The CD spectra for the Na-gellan (GF1, GF2, and GF3) aqueous solutions (polymer concentration $c_p=1.0$ %) were measured at 10, 15, 20, 30, 40, and 60 °C over the wavelength range of 195–250 nm (data not shown). The CD spectra for GF1– GF3 are similar and a peak was observed at around 201 nm which corresponds to coil to double-helix conformational change of Na-gellan molecules [9, 10]. The molar ellipticity at 201 nm $[\theta]_{201}$ of Na-gellans is plotted against temperature (Fig.1). On lowering temperature, values of $[\theta]_{201}$ for GF1– GF3 drastically decreased below T_{ch} , suggesting that a coil to double-helix transition took place. As shown in Figure 1, values of T_{ch} for GF1, GF2, and GF3 were almost the same (around 30 °C).

We measured the temperature dependences of the storage modulus, G' , and the loss modulus, G'' for Na-gellan (GF1, GF2, and GF3) solutions of various polymer concentrations ($c_p=0.5$ – 5.0 %). In addition, in

Table 1 Characteristics of the Na-gellan samples

Sample	$M_w \times 10^{-3}$ a)	$[\eta]$ a) (cm ³ /g)	Metal contents (μ g / g)				
			Na	K	Ca	Mg	Fe
GF1	125	556	28200	105	ND	ND	ND
GF2	95.3	506	25500	52	ND	ND	ND
GF3	73.8	400	29000	76	ND	ND	ND
4F1197A			4940	18100	1750	ND	ND

a) Values are determined by LS measurements at 40°C in aqueous solutions with 25 mmol NaCl. ND; not detected

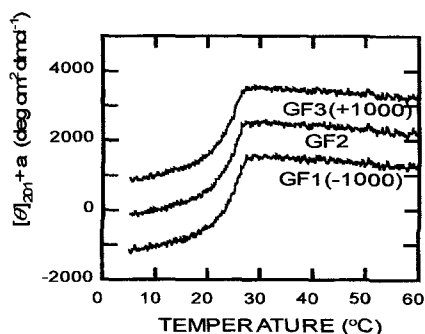


Fig.1 Temperature dependence of molar ellipticity during the cooling process for Na-gellan (GF1, GF2, GF3) aqueous solutions. Cooling rate; 0.5 °C/min. The data of GF1 and GF3 are shifted along the vertical axis by shift factor $a=1000$ to avoid overlapping.

order to know the rheological character, we made torque vs angle Lissajous plots (data not shown). Typical examples for the temperature dependence of G' and G'' are shown in Fig.2. It was reported [4,5] that in the cooling process, the steepest change in G'' in the

thermally scanning rheology was attributed to the coil to double-helix transition, T_{ch} , of Na-gellan molecules, and the crossover temperature of G' and G'' was attributed to the sol-gel transition T_{sg} . The crossover temperature was determined by data management software. The values of T_{ch} and T_{sg} determined from temperature dependence of G' and G'' for GF1–GF3 are plotted against the polymer concentrations together with the T_{ch} obtained from the temperature dependence of $[\theta]_{201}$ in Fig.3. The T_{ch} for 1% Na-gellan (GF1–GF3) solutions obtained from rheometric data are coincident with the values obtained from CD spectra, and T_{ch} shift to higher temperature with increasing polymer concentration. Reid et al. reported [14] that the coil to helix transition temperature of ι -carrageenan in aqueous solutions

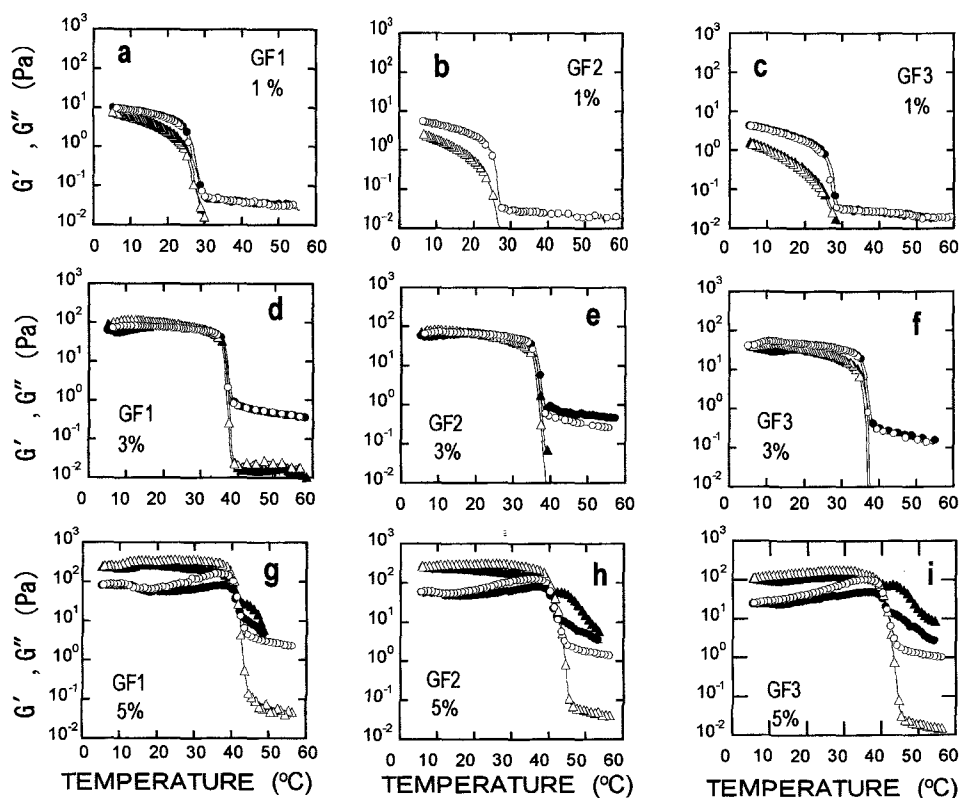


Fig.2 Temperature dependence of the storage modulus, G' , and the loss modulus, G'' , during the cooling and heating processes for the Na-gellan (GF1, GF2, and GF3) aqueous solutions. (Δ) G' , (\circ) G'' , cooling; (\blacktriangle) G' , (\bullet) G'' , heating. (a) GF1,1%; (b)GF2,1%; (c)GF3,1%; (d)GF1,3%; (e)GF2,3%; (f)GF3,3%; (g)GF1,5%; (h)GF2,5%; (i)GF3,5%.

increased with increasing polymer concentration. As shown in Fig 2, T_{ch} for GF1, GF2, and GF3 are almost the same, suggesting that the temperature at which the coil to double-helix transition takes place does not depend on M_w . Previously [10, 11] using 4 Na-gellan samples ($M_w = 32 \times 10^3 - M_w = 120 \times 10^3$), we investigated the polymer concentration dependence of T_{ch} from the measurements of $[\theta]_{201}$, $[\eta]$ and DSC curve in a concentration range (0–1.2%). It was concluded that values of T_{ch} for Na-gellan solutions were independent of M_w . In this study, we carried out the rheological measurements over a wide polymer concentration range (0.5–5.0%) and obtained the same conclusion. On the other hand T_{sg} corresponding to the sol-gel transition of Na-gellan aqueous solution, depends markedly on M_w . Below $c_p = 5\%$, T_{sg} decreased with decreasing M_w ; GF1 > GF2 > GF3. At low concentrations of Na-gellan (below $c_p = 1.5\%$, 2%, and 3% for GF1, GF2, and GF3, respectively) T_{sg} could not be observed. The coil to double-helix transition and the sol-gel transition occurred separately below $c_p = 5\%$. As shown in Fig.3, however, T_{sg} for the three Na-gellan solutions rapidly increased with increasing polymer concentration and the differences between T_{ch} and T_{sg} for each sample gradually decreased with increasing polymer concentration. At $c_p = 5\%$ solution, ultimately, T_{sg} for the GF1, GF2, and GF3 almost overlap with T_{ch} , indicating that the double-helix formation and gel formation occurred concurrently. The thermal scanning rheological behavior for 5% Na-gellan solutions (Fig.2) showed a thermal hysteresis, which often accompanies with gel formation. The double-helical molecules aggregate and form junction zones in gels [3]. Maxima of G' and G'' were observed near the transition temperature in Figs.2g-i. From results of Lissajous pattern, slippage between sample and measuring geometry did not occur. It is considered that these maxima could be attributed to change in microstructure of the gel network.

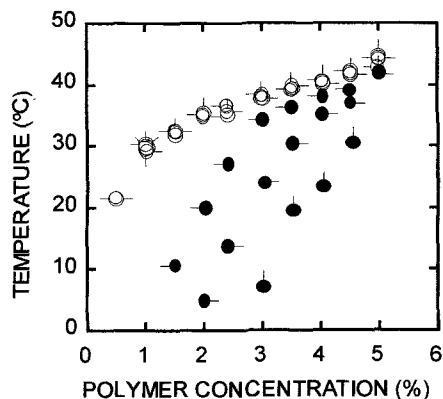


Fig.3 Polymer concentration dependence of T_{ch} and T_{sg} for Na-gellan (GF1, GF2, and GF3) aqueous solutions. T_{ch} obtained from rheological data. (○)GF1, (◻)GF2, (◻)GF3; T_{ch} obtained from CD data. (▽)GF1, (◻)GF2, (◻)GF3; T_{sg} : (●)GF1, (◻)GF2, (◻)GF3.

Below $c_p = 5\%$, T_{sg} depend on M_w suggesting that the gel formation of Na-gellan aqueous solutions is influenced strongly by the length of double-helices together with the number of double-helices.

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