

Helix-Coil Transition in Gellan Gum Gels

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The temperature dependence of the loss Young's modulus E'' for a 1.6% potassium type gellan gum gel which was cylindrically moulded showed a step-like change at 30°C. The endothermic peak temperature in the heating DSC curve and the exothermic peak temperature in the cooling DSC curve appeared at the midpoint transition temperatures of the loss Young's modulus E'' and of the specific ellipticity at 202nm in circular dichroism. It is concluded that the helix-coil transition occurs at this temperature, while the cylindrical gel keeps the shape. On heating, some chain segments are released from helices into coils, and coils are reeled into helices on cooling, as has been proposed in a reel-chain model. This is the first clear experimental evidence for the helix-coil transition in an elastic gel, as far as the authors are aware.

Key words: gellan gum, helix-coil transition, dynamic viscoelasticity, DSC, CD

1. INTRODUCTION

Gellan gum is a microbial polysaccharide produced by *Sphingomonas elodea*. It is composed of a tetrasaccharide (β -D-glucose, β -D-glucuronic acid, β -D-glucose, and α -L-rhamnose) repeat unit, containing one carboxyl group (Fig.1) [1,2]. Gellan gum is of interest as food additives such as a gelling material, thickener, stabiliser or texture modifier in the food industry. A solution of gellan gum forms a transparent and thermoreversible gel in the presence of a sufficient amount of cations, when it is cooled below the gelation temperature [3]. It is accepted that, in a solution state, gellan gum molecules change from the disordered state (single chain) to the ordered state (double helix) with decreasing temperature and that, above a certain critical concentration, double helices form aggregates which play a role of junction zones [4]. It has been suggested that the gelation occurs when the helix content exceeds a threshold value above which the number of helical aggregates is sufficient to form a three dimensional network [5].

The conformational change of gellan gum in solutions and gelation of gellan gum solutions were discussed in the collaborative research group organised in Japan using a common sample. As is often the case with the biopolymers, the physicochemical properties of gellan solutions are influenced by a subtle difference in the position of the electrolytic groups or in molecular weight [6]. This indicates that the results obtained for different sample cannot be compared directly. Results of the collaborative research group can be compared and are useful to elucidate the conformation in solution and the gelation mechanism. The results of studies on the first, second and third common samples were published in special issues of *Food Hydrocolloids* 7:361-456 in 1993, *Carbohydrate*

Polymers 20:75-207 in 1996, and *Progress in Colloid and Polymer Science* 114:1-135 in 1999, respectively.

Previously, dynamic viscoelastic measurements and differential scanning calorimetry (DSC) were performed on the sol-gel transition of a solution of the first [7], the second [8,9], and the third common samples [10], respectively. Although the previous studies reported detailed and valuable results on the gelation of gellan gum solutions, the gelation mechanism and the gel structure as a thermoreversible gel are still unclarified. In this experiment, we focused on a gellan gel of which elastic moduli were large enough for the gel to maintain its shape. We used the first common sample which has a stronger gelling ability than other common samples. Since few studies about the sol-gel transition of gellan gels with large elastic modulus have been performed, combining results of rheological measurements of such gels with results of DSC and circular dichroism (CD) measurements might give useful information on the gelation mechanism and the structure of thermoreversible gels.

In this work, viscoelastic properties of gellan gum gels were studied by dynamic viscoelastic measurements and were compared with conformational changes of gellan gum studied by DSC and CD measurements.

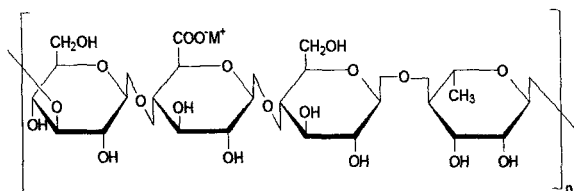


Fig.1 Chemical structure of deacylated gellan gum.

2. MATERIALS AND METHODS

Gellan gum was supplied by San-Ei-Gen FFI Ltd., Osaka, Japan. It is the first common sample in the collaborative research published in 1993 [11]. The metal contents in the sample are as follows: Na 1900 $\mu\text{g/g}$, K 20800 $\mu\text{g/g}$, Ca 5120 $\mu\text{g/g}$ and Mg 1460 $\mu\text{g/g}$ [11]. The sample was not influenced by storage as for its molecular weight [12]. Gellan gum powder was swollen in distilled water and stirred overnight at 40°C. Then the solution was heated for 2 hours at 90°C. The solution was poured into teflon moulds (30mm height and 20mm diameter) and cooled at room temperature for more than 2 hours to obtain the samples for rheological measurements. The cylindrical gellan gels thus obtained were then kept at 5°C for 12 hours.

The storage Young's modulus E' and the loss Young's modulus E'' were determined by the observation of longitudinal vibrations of cylindrically moulded gels. The apparatus used was a Rheograph Gel (Toyo Seiki Seisakusyo Ltd, Tokyo) [13]. The temperature was raised from 10 to 55°C and then lowered from 55 to 10°C with an interval of 5°C. E' and E'' were measured in the equilibrium state. The typical time interval that required for equilibrium was 15 min.

DSC measurements were carried out with a Setaram micro DSC- III calorimeter, Caluire, France. The temperature was raised from 5°C to 55°C at 0.5°C/min and then lowered from 55°C to 10°C at the same rate.

CD measurements were carried out with a JASCO-820A spectropolarimeter. The specific ellipticity at 202nm $[\Psi]_{202}$ was measured in the temperature range from 5°C to 55°C at 0.5°C/min.

3. RESULTS AND DISCUSSION

Fig.2 (a) shows the temperature dependence of the storage Young's modulus E' , the loss Young's modulus E'' , and mechanical loss $\tan\delta$ for a 1.6% gellan gum gel, which was cylindrically moulded, observed at each temperature after being kept 15 min on heating. Both E' and E'' decreased with increasing temperature. Since E'' decreased faster than E' , $\tan\delta$ decreased with increasing temperature. Gellan gels as well as other thermoreversible gels such as agarose, carrageenan and gelatin are believed to be formed by junction zones that are connected by chain molecules released out from junction zones. Junction zones are formed by aggregated helices. The widely accepted gelation mechanism of gellan is as follows: on cooling gellan aqueous solutions, the helices are formed from coiled molecules at a certain temperature. These helices aggregate on further cooling to form junction zones that play a role of knots of the three dimensional network. Gels are formed only if the polymer concentration is higher than the critical concentration, otherwise, even if helices are formed, they cannot percolate the whole space to form a gel. The gradual decrease in the storage Young's modulus E' and a steplike decrease in the loss Young's modulus E''

on heating shown in Fig.2 (a) are induced by the release of chain segments from junction zones as described in a reel-chain model [14].

Fig.2 (b) shows the temperature dependence of the specific ellipticity at 202 nm together with a heating DSC curve at 0.5°C/min. Both an endothermic peak in the heating DSC curve and the steep increase in the specific ellipticity suggest that helix to coil transition occurs at this temperature range.

Fig.3 (a) shows the temperature dependence of E' , E'' , and $\tan\delta$ observed at each temperature after being kept 15 min on cooling. Fig.3 (b) shows the temperature dependence of the specific ellipticity at 202 nm together with a cooling

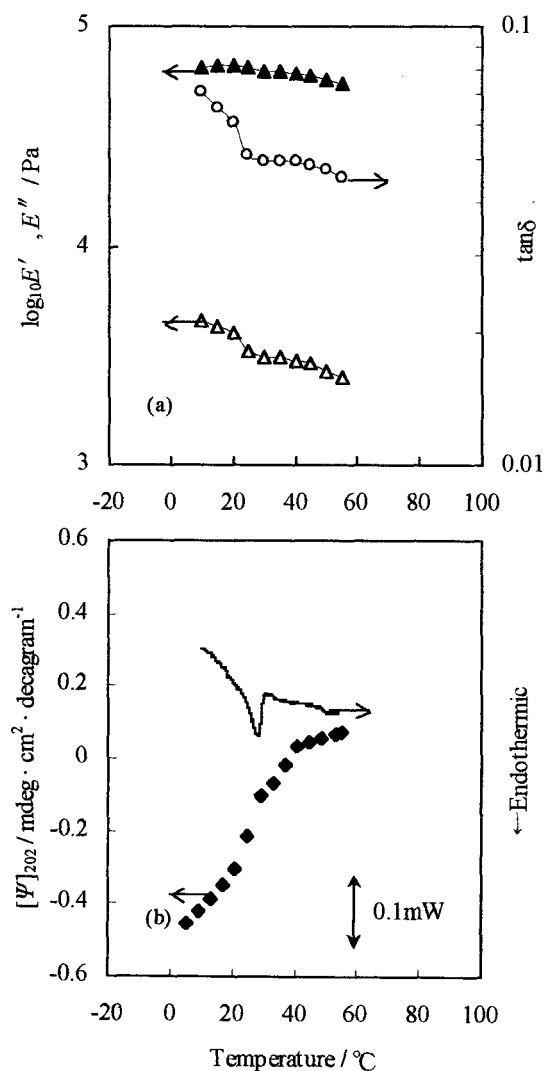


Fig.2 (a) Temperature dependence of the storage modulus E' (\blacktriangle), the loss modulus E'' (\triangle) and mechanical loss $\tan\delta$ (\circ) on heating and (b) the heating DSC curve (solid curve) and temperature dependence of the specific ellipticity at 202nm, $[\Psi]_{202}$, (\blacklozenge) on heating for 1.6% (w/w) gellan gum gels. E' and E'' measurements were done after 15min equilibration at each temperature. Heating rates of DSC and CD measurements; 0.5°C/min.

DSC curve at 0.5°C/min. The exothermic peak in a cooling DSC curve is far sharper than a corresponding endothermic peak in a heating DSC curve, which may be induced by a mechanism like a supercooling as has been discussed in relation with a zipper model approach to the thermoreversible gel-sol transition [15]. This is also true for the sharper change of elasticity and the specific ellipticity in the cooling process than in the heating process.

The loss Young's modulus E'' showed a step-like change at 30 °C. The endothermic peak temperature in the heating DSC curve and the exothermic peak temperature in the cooling DSC curve appeared at the midpoint transition temperature of the loss Young's modulus E'' and of the specific ellipticity.

It is generally believed in the gelation of polysaccharides such as agarose, carrageenan, gellan and also for gelatin that the helix formation is a prerequisite for the gel formation. It is generally accepted that helices are aggregated into a bundle which acts as a knot of the three dimensional network. Therefore, on cooling of a solution of these polymers, coil conformers are transformed into helices, and then a gel is formed. In this process, however, not all the coils are transformed into helices and some molecular chains remain isolated, or dangling chains which are released from junction zones or from aggregated helices remain not to be incorporated into the network structure. Then, on further cooling, these dangling chains are reeled into junction zones as described in the reel-chain model.

From simple extension of a theory of rubber-elasticity, the increase in the elastic modulus should be attributed to the increase in the number of elastically active network chains. This should be induced by the increase in the number of junction zones or cross-linking region. Whether coiled chains are transformed into helices and reeled into junction zones or the newly created helices are cross-linked by weak molecular forces such as hydrogen bonds still remain to be clarified.

There should be two coil-to-helix transition temperatures; one is lower than the sol-to-gel transition temperature, and the other is higher than that. Although helix-coil transitions in polymer solutions have been studied extensively, a helix-coil transition in a gel state has never been reported. The sharp change in the elasticity and in the specific ellipticity and the DSC peaks shown in Figs. 2 and 3 should be attributed to the lower temperature helix-coil transition. In this process, some elastically non-active coiled chains may change into elastically active helical chains incorporated in network structure.

A gel consists of liquid and a network. The majority of water in agarose gel has been reported to be in a state of the so-called free water [16]. Therefore, the mobility of long chain molecules is not so restricted, and long chain molecules can execute not only macrobrownian motion as in a

rubber state but also change their conformation such as in helix-coil transition.

Watase and Nishinari reported the increase in the elastic modulus of cylindrically moulded κ -carrageenan gels immersed in alkali salt solution with the lapse of time in 1982 [17]. As is widely accepted, the gelation mechanism of κ -carrageenan is very similar to that of gellan as described above. Cations are believed to shield the electrostatic repulsion of sulfate groups in κ -carrageenan molecules, and then they promote the formation and aggregation of helices, which leads to the increase in elastically active network chains via the increase in junction zones. They examined the concentration change in gels that are immersed in salt solutions. The concentration was found to

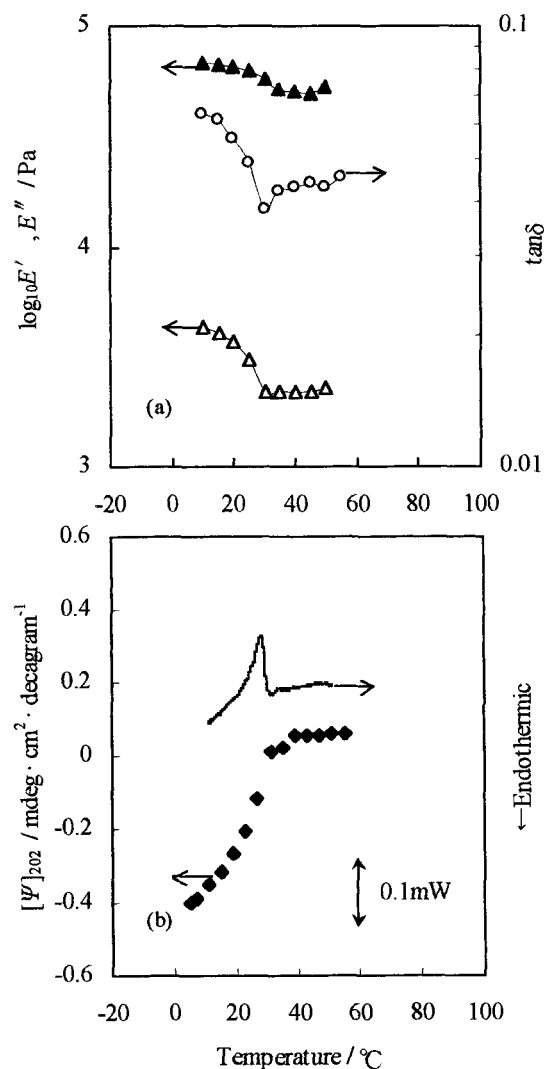


Fig.3 (a) Temperature dependence of the storage modulus E' (\blacktriangle), the loss modulus E'' (\triangle) and mechanical loss $\tan\delta$ (\circ) on cooling and (b) the cooling DSC curve (solid curve) and temperature dependence of the specific ellipticity at 202nm, $[\Psi]_{202}$, (\blacklozenge) on cooling for 1.6% (w/w) gellan gum gels. E' and E'' measurements were done after 15min equilibration at each temperature. Cooling rates of DSC and CD measurements; 0.5°C/min.

increase. Then, they estimated the elastic modulus taking into account of the concentration change based on the power law dependence of the elastic modulus on the concentration. Since the increase of the storage Young's modulus of gels was far larger than the calculated value by a method mentioned above, they concluded that the change in the elastic modulus should be rather attributed to the structural change of network in the gel and not to the concentration change of the gel. They suggested that the structural change such as the strengthening of the network structure in a gel can occur even after the gel is formed. In the light of the experimental results described in the present paper, the rheological change found in 1982 should have the same origin as described in the present work.

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REFERENCES

- [1] P. E. Jansson, B. Lindberg and P. A. Sandford, *Carbohydr. Res.*, **124**, 135-39 (1983).
- [2] M. A. O'Neill, R. R. Selvendran and V. J. Morris, *Carbohydr. Res.*, **124**, 123-33 (1983).
- [3] G. R. Sanderson, "Food Gels", Ed. by P. Harris, Elsevier Applied Science, London and New York (1990) pp. 201-32.
- [4] G. Robinson, C. E. Manning and E. R. Morris, "Food Polymers, Gels and Colloids", Ed. by E. Dickinson, Royal Society Chemistry, London (1991) pp. 22-33.
- [5] E. Miyoshi, T. Takaya and K. Nishinari, *Macromol. Symp.*, **99**, 83-91 (1995).
- [6] K. Nishinari, *Carbohydr. Polym.*, **30**, 75-76 (1996).
- [7] M. Watase and K. Nishinari, *Food Hydrocoll.*, **7**, 449-56 (1993).
- [8] E. Miyoshi, T. Takaya and K. Nishinari, *Thermochim. Acta.*, **267**, 269-87 (1995).
- [9] E. Miyoshi, T. Takaya and K. Nishinari, *Carbohydr. Polym.*, **30**, 109-20 (1996).
- [10] E. Miyoshi and K. Nishinari, *Progr. Colloid Polym. Sci.*, **114**, 68-82 (1999).
- [11] *Food Hydrocoll.*, **7**, 361-456 (1993).
- [12] E. Ogawa, *Carbohydr. Polym.*, **30**, 145-48 (1996).
- [13] K. Nishinari, H. Horiuchi, K. Ishida, K. Ikeda, M. Date and E. Fukada, *Nippon Shokuhin Kogyo Gakkaishi*, **27**, 227-33 (1980).
- [14] K. Nishinari, S. Koide and K. Ogino, *J. Phys. France*, **46**, 793-97 (1985).
- [15] K. Nishinari, S. Koide, P. A. Williams and G. O. Phillips, *J. Phys. France*, **51**, 1759-68 (1990).
- [16] S. Ablett, P. J. Lillford, A. M. A. Baghdadi and W. Derbyshire, *J. Colloid Interf. Sci.*, **67**, 355-77 (1978).
- [17] M. Watase and K. Nishinari, *Colloid & Polym. Sci.*, **260**, 971-75 (1982).

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