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Effects of salts on the gel-sol transition of gellan gum by differential scanning calorimetry and thermal scanning rheology[1]

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Abstract

The rheological and thermal properties of sodium form gellan gum solutions with and without sodium chloride, potassium chloride, calcium chloride and magnesium chloride were studied by dynamic viscoelastic measurement and differential scanning calorimetry. Temperature dependence of the loss modulus for gellan gum solutions of lower concentrations without salt showed a one step-like change at a certain temperature, however that for concentrated gellan gum solutions (>2.0%) showed two step-like changes. The higher temperature process T_{hc} may be attributed to the helix-coil transition and found in between the exothermic and endothermic peak temperatures T_s and T_m observed in cooling and heating DSC curves, while the lower temperature process T_{sg} may be attributed to the sol-gel transition. Temperature dependence for gellan gum solutions of higher concentrations (>3.2%) showed a large hysteresis, moreover, the temperature at which the loss shear modulus G'' showed the second step decrease shifted to higher temperatures with increasing concentration of gellan gum. The cooling or heating DSC curves for gellan gum solutions of lower concentrations showed a single exothermic or endothermic peak, and both exothermic peak temperature T_s and endothermic peak temperature T_m shifted to higher temperatures, and both exothermic and endothermic enthalpies increased with increasing concentration of gellan gum. However, for a gellan gum solution of a concentration higher than 3.2%, the endothermic peak in the heating DSC curve split into two peaks, while the cooling curve showed only one endothermic peak. The lower temperature endothermic peak in the heating DSC curve corresponds with the first step decrease of G'' in the heating process of rheological measurement, and the higher temperature endothermic peak corresponds with the second step decrease of G''. The viscoelastic change of gellan gum solutions was more remarkable by the addition of K^+ than Na^+, and by Ca^{2+} than by

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Mg^{2+}. The viscoelastic behavior of gellan gum solutions was influenced much more strongly by divalent cations than by monovalent cations. DSC cooling curves of gellan gum solutions showed a single exothermic peak shifting to progressively higher temperatures with increasing concentration of monovalent cations. At low concentration of monovalent cations, the DSC heating curves showed a single endothermic peak, however, with more progressive addition of salt, the endothermic peak gradually developed bimodal character and eventually split into multiple peaks. With increasing concentration of divalent cations, the exothermic and endothermic enthalpies estimated for a main peak increased up to a certain concentration and then decreased. Moreover, the endothermic peaks in the presence of sufficient salts were too broad to be resolved from the baseline, and many other peaks were observed at higher temperatures. Gellan gum solutions with sufficient divalent cations form firm gels on cooling to below the setting temperature, and then it was difficult to remelt them, which was quite different from the behavior of thermoreversible gels formed in the presence of monovalent cations.

Keywords: Gel-sol transition; Gellan gum; DSC; Thermal scanning rheology; Salt

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1. Introduction

Gellan gum is a microbial polysaccharide produced by *Pseudomonas elodea*. The polymer is a linear anionic heteropolysaccharide composed of tetrasaccharide (β-D-glucose, β-D-glucuronic acid, β-D-glucose and α-L-rhamnose) repeat units, and it has a carboxyl side group [1,2]. This polysaccharide is widely used in the food industry and biotechnology because it forms a transparent gel which is resistant to heat and acid in comparison to other polysaccharide gels, and its gel strength is known to increase by the addition of metal ions [3]. The gelation mechanism of various polymer solutions and thermoreversible gel-sol transitions have been studied [4–9], and it has been suggested that gellan gum may undergo a thermally reversible ordered helix-coil transition, and the junction zones of gellan gum gels are formed by aggregation of double helical gellan molecules. The conformational change temperature of gellan gum was around 30°C, however this temperature is controlled by the thermodynamic properties of the systems (temperature, cations, pH) [10–18], therefore, the rheological and thermal properties of gellan gum solutions have not been clarified sufficiently.

In the present work, a purified sodium form gellan gum was used and the rheological and thermal properties of gellan gum solutions with and without sodium chloride, potassium chloride, calcium chloride and magnesium chloride were studied by dynamic viscoelastic measurement and differential scanning calorimetry.

2. Experimental

2.1. Materials

As described in previous studies [19,20], gellan samples (in powder form) were kindly given by Kelco Division of Merck & Co., Inc., CA, USA. The contents of the inorganic ions Na, K, Ca and Mg were determined by Kelco using ICP as Na 3.03%, K 0.19%, Ca 0.11% and Mg 0.22% respectively. Salts, NaCl, KCl, $CaCl_2$ and $MgCl_2$ used in this study were of extra fine grade reagents (Wako Pure Chemical Industries Ltd., Osaka, Japan), and were used without further purification.

The powdered sample was dispersed by stirring to swell at 40°C overnight. The solutions for viscoelastic studies were prepared by stirring at 70°C for 2 h and then heated at 90°C for 1 h to attain complete transparency. The solutions were then kept at 70°C before each rheological or thermal measurement to prevent the gelation.

The concentration of gellan solutions changed from 0.2% to 4.2% (w/w). For samples containing salts, the concentration of NaCl or KCl in solutions varied from 5 to 100 mM and that of $CaCl_2$ or $MgCl_2$ ranged from 0.43 to 6.8 mM.

2.2. *Rheological measurement*

The rheological measurements were performed using a Dynamic Stress Rheometer DSR from Rheometrics Co. Ltd., NJ, USA at various temperatures [19].

The hot sample solution was poured directly onto the plate of the instrument, using a parallel plate geometry of 50 mm diameter, with radial grooves in order to avoid gel slippage. Temperature dependence of G' and G'' was observed by cooling the systems from 50°C to 5°C and then reheating to 60°C, at the rate of 0.5°C min^{-1}, at the constant frequency of 0.1 rad s^{-1}.

2.3. *DSC measurement*

Differential scanning calorimetry (DSC) measurements were carried out by a Setaram micro DSC-III calorimeter, Caluire, France [20]. Approximately 900 mg of the sample solution was sealed into the DSC pan hermetically, and then the pan was accurately weighed. For each sample, a reference pan was filled with distilled water, to within $\pm 30\,\mu g$ of the weight of the sample pan. The two pans were then placed inside the calorimeter and heated to 110°C and kept for 10 min to annihilate the thermal history. Then the temperature was lowered to 5°C at 0.1–0.5°C min^{-1} and raised again at the same rate up to 110°C.

3. Results and discussion

3.1. *Thermal scanning rheology*

Fig. 1A–I shows the temperature dependence of G' and G'' during the cooling or heating process for gellan gum solutions of various concentrations without salt at 0.1 rad s^{-1} at a cooling or heating rate of 0.5°C min^{-1}. G' for a 1.0% solution of gellan gum (Fig. 1A) was too small to be detected in both the cooling and heating processes, however, G'' could be detected at temperatures lower than 50°C in both the cooling and heating processes and showed a step-like change at 30°C. For a 1.2% gellan gum solution (Fig. 1B), G' began to be detected around 25°C during the cooling process and then increased gradually with decreasing temperature, while it decreased gradually during the heating process and then became too small to be detected around 28°C, however, G'' for a 1.2%

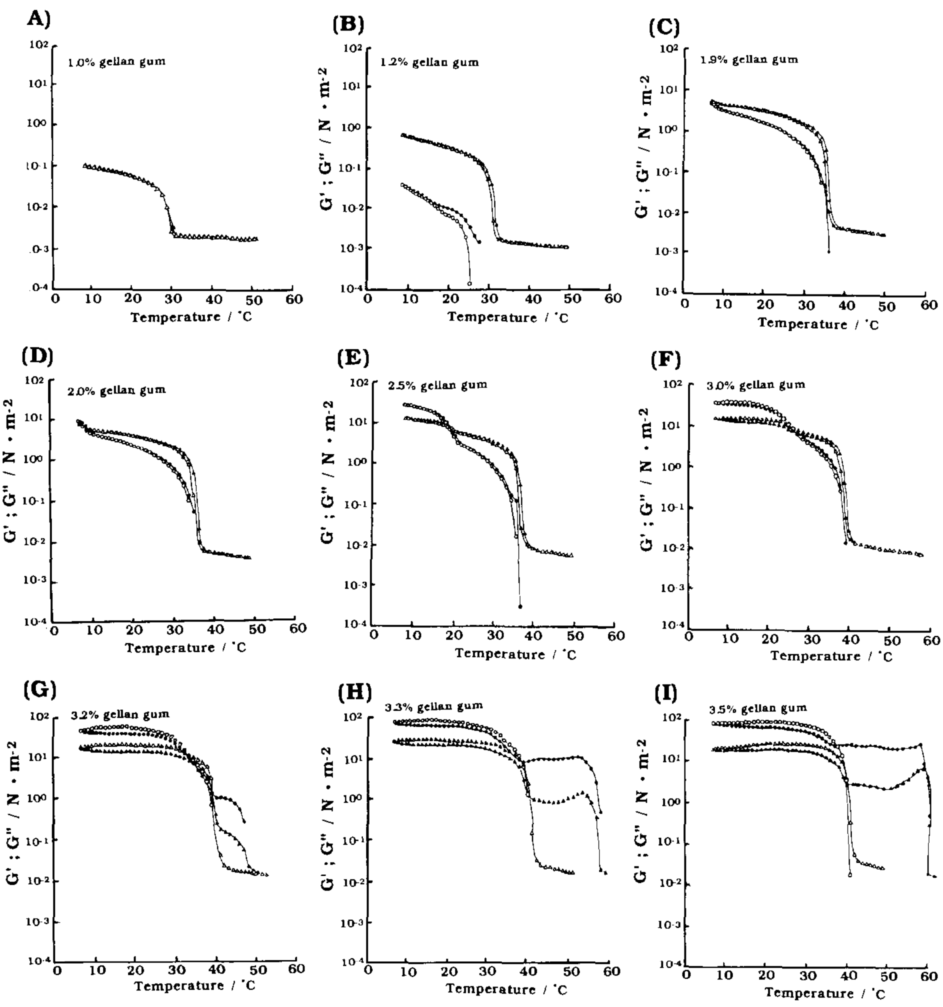

Fig. 1. Temperature dependence of storage modulus G' and loss modulus G'' during the cooling or heating process for gellan gum solutions of various concentrations without salt. (○) G', (△) G'', cooling; (●) G', (▲) G'', heating; cooling and heating rate, 0.5°C min^{-1}; frequency, $\omega = 0.1$ rad s^{-1}; polymer concentration, 1.0% (A), 1.2% (B), 1.9% (C), 2.0% (D), 2.5% (E), 3.0% (F), 3.2% (G), 3.3% (H), 3.5% (I).

gellan gum solution showed a step-like change at 31°C. The temperature dependence of G'' for gellan gum solutions at a concentration lower than 2.0% showed one step-like change at a certain temperature in the present study. The temperature at which G'' drastically changes should be a kind of transition temperature. As concluded from the frequency dependence of G' and G'' described in the previous study [19], at any temperature from 30°C to 0°C, the viscoelastic behavior of gellan gum solutions of a concentration lower than 2.0% are characteristic of dilute or concentrated polymer solution [4–7,22]. Therefore, the transition observed in the temperature dependence of G'' for a gellan gum solution of a concentration lower than 2.0% should not be attributed to the sol-gel transition, but may be induced by coil-helix transition [21], and the temperature is written as T_{ch} hereafter. At lower concentration of gellan gum molecules, the helix formation and its partial aggregation may form a certain ordered structure, but does not lead to gel formation because the number of helical aggregates is not sufficient to prevail the whole space. In the previous study [21], the difference between G'' at the lower and higher temperature side of the midpoint temperature of transition was called the relaxational strength. The relaxational strength at T_{hc} was shown to increase with increasing concentration of gellan gum in the present work. However, the thermal behavior of gellan gum solutions of a concentration higher than 2.0% was significantly different. For a 2.0% gellan gum solution (Fig. 1D), G' could be detected at 34°C and then gradually increased, and moreover it also increased rapidly around 10°C and G' became significantly larger than G' at temperatures lower than the cross-over temperature. G' for a 2.0% gellan gum solution showed two step-like changes at both 35°C and 10°C. For a 3.0% gellan gum solution (Fig. 1F), the cross-over of G' and G'' was observed at 28°C and this temperature was significantly higher than that for a 2.0% or 2.5% gellan gum solution (Fig. 1D,E), although the temperature at which G'' showed the first step increase in the cooling process was also slightly higher than that for a 2.0% gellan gum solution. Such a behavior showing two step-like changes of G'' was observed for gellan gum solutions at a concentration higher than 2.0% in the present work.

The elastic modulus of gellan gels will increase with increasing number of elastically active network chains or with increasing number of junction zones. The two step increase in elastic modulus should then be attributed to the two step increase in the number of junction zones. The higher temperature process may be induced by the helix-coil transition at T_{hc}, which corresponds to the temperature at which G'' for gellan gum solutions of low concentrations showed one step-like change, but the subsequent lower temperature process at which G' and G'' showed a cross-over may be attributed to the sol-gel transition. Since the gellan gum solutions whose concentrations are lower than 2.0% showed a typical mechanical spectrum for concentrated polymer solutions at any temperature from 30°C to 0°C [19], the transition observed in Fig. 1A–C cannot be attributed to a gel-sol transition but to a helix-coil transition. At lower concentrations, the number of helices formed on cooling is not sufficient to prevail the whole space and to form a three-dimensional network, as mentioned above. However, as shown in the previous study, the viscoelastic behavior for a 2.0% gellan gum solution is typical of a dilute solution at 30°C and is typical of a concentrated polymer solution at 25°C or 15°C, however the behavior tended towards that of a weak gel. For a 3.0% gellan gum solution (Fig. 1F), although the viscoelastic behavior remained typical of a concentrated solution at 30°C, the behavior tended towards that of a weak gel at temperatures lower than 25°C. Therefore, in concentrated solutions (>2.0%), where the number of aggregates of helices exceeds a critical value on cooling, the rheological behavior changes from sol to gel, and this appears as the second step increase of G'' shown in this figure. However, it is worthy of note that the temperature at which G' becomes larger than G'' was slightly lower than the temperature at which G'' shows the second step increase. Hence, G'' for a 1.9% gellan gum solution (Fig. 1C) showed the second step increase around 8°C in the cooling process, however G'' had slightly larger values than G' down to 5°C. Therefore, the cross-over temperature of G' and G'' may be defined as a sol-gel transition temperature, and the temperature will be written as T_{sg} hereafter. The thermal behavior for a 3.2% gellan gum solution (Fig. 1G) was evidently distinguished from that for gellan gum solutions of a concentration lower than 3.2%; G'' showed a two step increase at both 40°C and 33.1°C in the cooling process, however it showed a two step decrease at 39.1°C and 48°C in the heating process, which indicated that the thermal hysteresis was greatly modified compared to that for gellan gum solutions of a concentration lower than 3.2%. Moreover, the temperature at which G'' showed the first step decrease in the heating process almost coincided with the lower endothermic peak temperature in heating DSC curve, and the temperature at which G'' showed the second step decrease almost coincided with the higher temperature endothermic peak, as discussed later. For a 3.5% gellan gum solution (Fig. 1I), G'' showed one step increase at 40°C in the cooling process, thus, it was suggested that the coil-helix transition occurs concurrently with the sol-gel transition since the number of helices formed on cooling is enough to prevail the whole space and to form a three-dimensional net work. Although G'' for a 3.5% gellan gum solution showed a two step decrease in the heating process, the temperature where it showed the first step decrease was almost similar to that for a 3.2% or 3.3% gellan gum solution (Fig. 1G,H), but the temperature at which it showed the second step decrease significantly shifted to higher temperatures with increasing concentration of gellan gum. Moreover, the relaxational strength at the temperature of the first step decrease in the heating decreased but that at the temperature of the second step decrease increased with increasing concentration of gellan gum.

Fig. 2 shows the first step increase temperature of G'' in the cooling process (T_{hc}) and the second step increase temperature of G'' (T_{sg}) for gellan gum solutions as a function of concentration of gellan gum from 0 to 3.5%. As shown in this figure, it was suggested

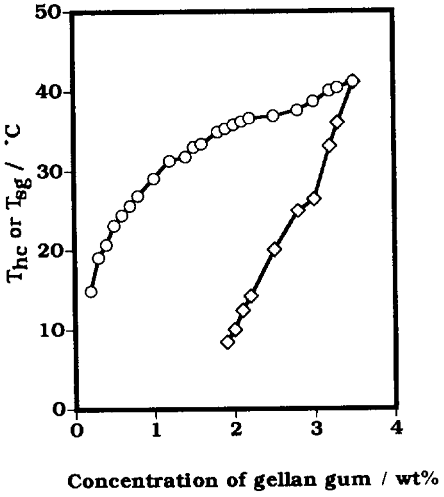

Fig. 2. First step increase temperature of G'', T_{hc} (○) and the second step increase temperature of G'', T_{sg} (◇) for gellan gum solutions in the cooling process as a function of concentration of gellan gum from 0 to 3.5%.

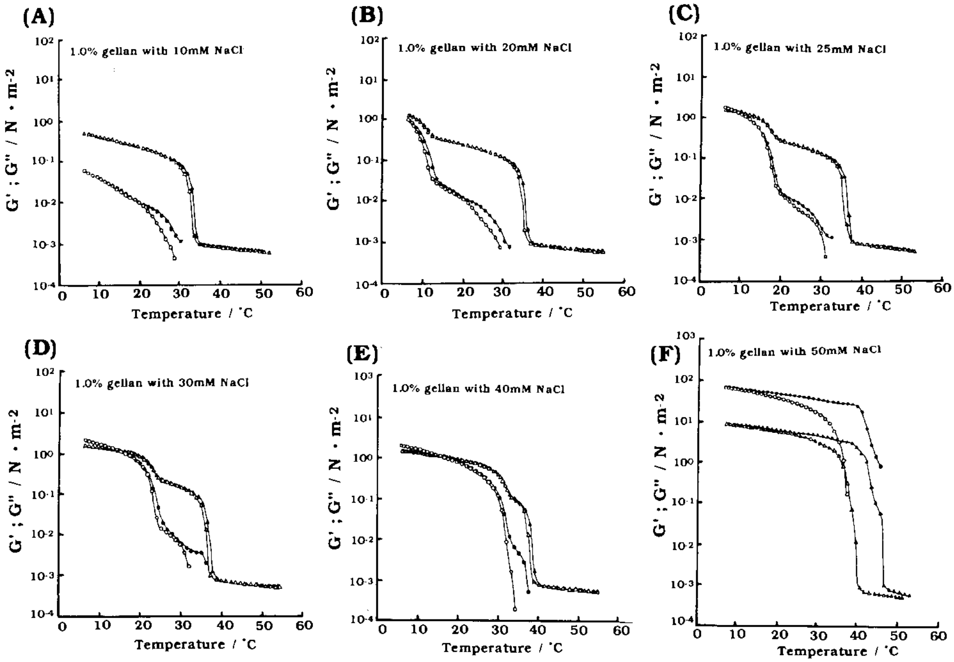

Fig. 3. Temperature dependence of storage modulus G' and loss modulus G'' during cooling or heating process for a 1.0% gellan gum solution containing NaCl of various concentrations. (○) G', (△) G'', cooling; (●) G', (▲) G'', heating; cooling and heating rate, 0.5°C min^{-1}; frequency $\omega = 0.1$ rad s^{-1}; NaCl concentration, 10 mM (A), 20 mM (B), 25 mM (C), 30 mM (D), 40 mM (E), 50 mM (F).

that both T_{hc} corresponding to the helix-coil transition and T_{sg} corresponding to the sol-gel transition significantly shifted to higher temperatures with increasing concentration of gellan gum. Moreover, the difference between T_{hc} and T_{sg} gradually decreased with increasing concentration of gellan gum, and eventually, for a 3.5% gellan gum solution, the helix-coil transition and the sol-gel transition occurred almost concurrently.

Fig. 3A–F shows the temperature dependence of G' and G'' during cooling or heating processes for a 1.0% gellan gum solution containing NaCl of various concentrations at 0.1 rad s^{-1} at a cooling or heating rate of 0.5°C min^{-1}. Both the storage modulus G' and loss modulus G'' of gellan gum solutions increased remarkably by the addition of NaCl as reported previously [19]. Upon addition of 10 mM NaCl (Fig. 3A), G' began to be detected around 29°C during the cooling process and became too small to be detected during the heating process around 30°C, while G'' showed a step-like change around 33°C. Upon addition of 20 mM NaCl (Fig. 3B), G' began to be detected around 30°C during the cooling process and then increased gradually, however, it showed another step-like increase around 10°C, while it decreased rapidly up to 10°C and then decreased gradually and eventually became too small to be detected around 32°C during the heating process. G'' upon addition of 20 mM NaCl showed two step-like changes at both 35°C and 10°C, indicating two thermal transitions, however G'' showed larger values than G'

throughout the accessible temperature range. In the case of gellan gum solutions without salt, although G'' for gellan gum solutions whose concentration is lower than 2.0% showed two step-like increase, the higher temperature process corresponded to the helix-coil transition (T_{hc}) and the lower temperature process almost coincided with that of sol-gel transition (T_{sg}). In a gellan gum solution containing salts, the temperature at which G'' showed the first step increase in the cooling corresponded to the helix-coil transition (T_{hc}), but the temperature at which it showed the second step increase should not be attributed to that of sol-gel transition. In the previous study, the viscoelastic behavior for a 1% gellan gum solution in the presence of 20 mM NaCl was typical of a dilute solution or concentrated polymer solution at any temperature from 30°C to 0°C. Thus, it seems that a certain ordered structure was formed by addition of NaCl at the temperature at which G'' showed the second step increase in the cooling process following the helix-coil transition, however, this transition does not produce enough aggregates to reach the gel state. Upon addition of 25 mM NaCl (Fig. 3C), G'' showed two step-like changes at both 37°C and 18°C, thus, both transition temperatures shifted significantly to higher temperatures than those in the presence of 20 mM NaCl (Fig. 3B), moreover, the cross-over of G' and G'' in the presence of 25 mM NaCl was observed around 10°C and then G' became slightly larger than G'' at lower temperatures than the cross-over temperature. This cross-over temperature of G' and G'' may be attributed to the sol-gel transition temperature (T_{sg}). Upon addition of 30 mM NaCl (Fig. 3D), the temperature at which G'' showed the second step increase in the cooling and the cross-over temperature (T_{sg}) shifted significantly to higher temperatures than those in the presence of 25 mM NaCl (Fig. 3C), although T_{hc} was not so different from that in the presence of 25 mM NaCl (Fig. 3C). Upon addition of 40 mM NaCl (Fig. 3E), G' during the cooling process began to be detected around 34°C and then increased monotonically with decreasing temperature, however, it showed two step-like changes during the heating process at both 35°C and 38°C. G'' in the presence of 40 mM NaCl showed two step-like changes at both 38°C and 32°C; during both the cooling and heating processes, the cross-over of both moduli was observed at 17°C. As shown in Fig. 3B–E, the difference between the first and second step increase temperatures of G'' decreased and the cross-over temperature of both moduli shifted to higher temperatures with increasing concentration of NaCl. Upon addition of 50 mM NaCl (Fig. 3F), G' during the cooling process began to increase rapidly at 37°C and then G' became significantly larger than G'', while it decreased monotonically with increasing temperature during the heating process, and eventually, it became too small to be detected at 47°C. This indicated that thermal hysteresis was significantly exhibited. G'' in the presence of 50 mM NaCl showed one step-like increase at 40°C in the cooling process, however, G'' showed two step decrease at both 43°C and 47°C in the heating process. Moreover, the values of G' and G'' in the presence of 50 mM NaCl at lower temperatures were significantly larger than those for 2% or 3% gellan gum solution without salt (Fig. 1B,C), which confirmed that the rheological properties of gellan gum solutions were influenced strongly by the presence of sufficient salt. The effect of KCl on the viscoelasticity of gellan gum solutions was almost similar to that of NaCl, however KCl influences the viscoelastic behavior of gellan gum solutions more effectively than NaCl. This is interpreted on the basis that cations such as Na^+ are the structure ordering ions for water, and the cations such as K^+ belong to the structure disordering ions [23,24]. There-

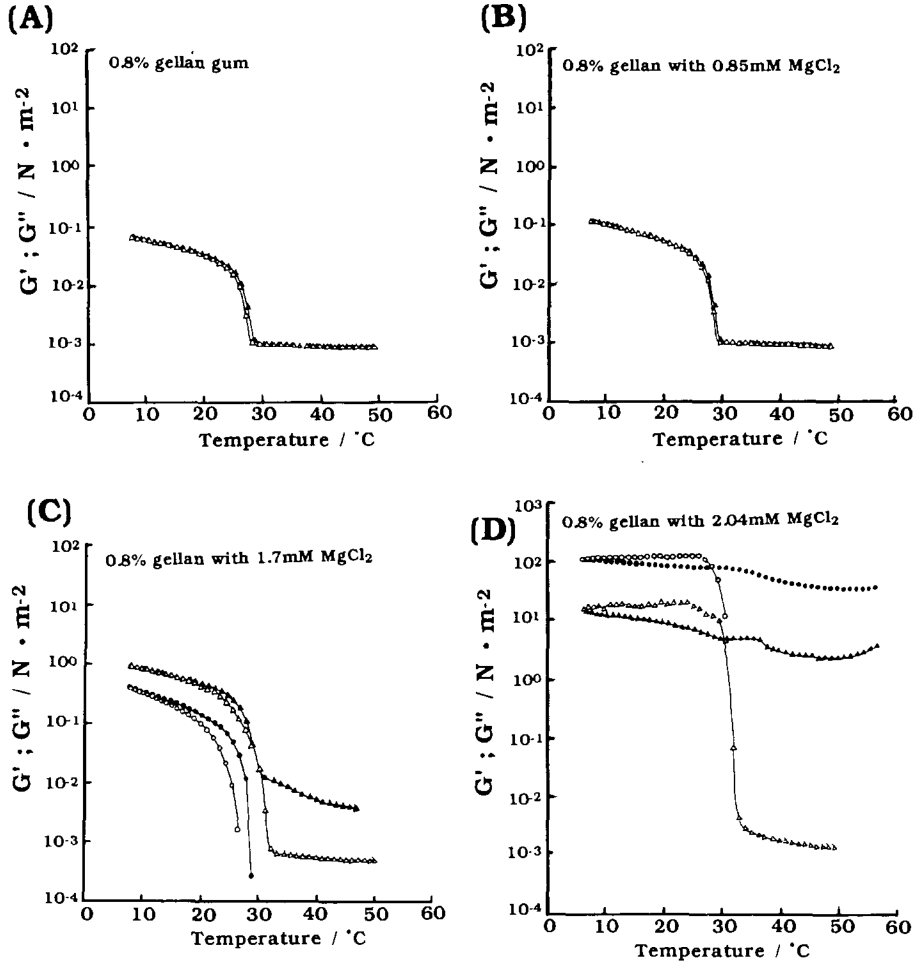

Fig. 4. Temperature dependence of storage modulus G' and loss modulus G'' during cooling or heating process for a 0.8% gellan gum solution containing $MgCl_2$ of various concentrations. (○) G'', (△) G'', cooling; (●) G', (▲) G'', heating; cooling and heating rate, 0.5°C min^{-1}; frequency $\omega = 0.1$ rad s^{-1}; $MgCl_2$ concentration, 0 mM (A), 0.85 mM (B), 1.7 mM (C), 2.04 mM (D).

fore, the latter group can shield the electrostatic repulsion of carboxyl side groups in the gellan gum molecules more directly than the former group, just as in the case of the addition of cations to κ-carrageenan; the structure disordering ions increased G' much more than the structure ordering ions by shielding the electrostatic repulsion of sulfate groups [25–30].

Fig. 4A–D shows the temperature dependence of G' and G'' during the cooling or heating process for a 0.8% gellan gum solution containing $MgCl_2$ of various concentrations at 0.1 rad s^{-1} at a cooling or heating rate of 0.5°C min^{-1}. The viscoelastic behavior of gellan gum solutions was influenced much more strongly by divalent cations than by monovalent cations. G' for an 0.8% gellan gum solution without salt (Fig. 4A) was too small to be detected in both the cooling and heating processes, and upon addition of 0.85 mM $MgCl_2$ (Fig. 4B), it was also too small to be detected in both the cooling and heating processes, while both solutions showed one step like change corresponding to the helix-coil transition (T_{hc}). However, T_{hc} in the presence of 0.85 mM $MgCl_2$ shifted to higher temperatures and the relaxational strength at T_{hc} increased slightly compared to that without salt. Upon addition of 1.7 mM $MgCl_2$, G' began to be detected at 27°C in the cooling process and became too small to be detected at 38°C in the heating process. G'' in the presence of 1.7 mM $MgCl_2$ (Fig. 4C) showed a step-like change at 30°C, however the difference between G'' in the cooling and heating process at higher temperature sides was significantly observed. The temperature dependence of both moduli in the presence of 2.04 mM $MgCl_2$ (Fig. 4D) was markedly different; G'' increased rapidly and G' began to be detected at 31°C in the cooling process, however, there was no evidence for remarkable change of both moduli up to 60°C. In the case of added sufficient monovalent cations (Fig. 3F), although the temperature at which G' and G'' increased rapidly was significantly higher than that in the presence of 2.04 mM $MgCl_2$, these junction zones formed in the presence of sufficient monovalent cations were completely melted on heating to 50°C. Therefore, the thermal behavior in the presence of divalent cations was obviously different from that without salts or in the presence of monovalent cations, as illustrated in the rheological measurement. The effect of $CaCl_2$ on the viscoelasticity of gellan gum solutions was almost similar to that of $MgCl_2$, however $CaCl_2$ can influence the viscoelastic behavior of gellan gum solutions more effectively than $MgCl_2$.

The carboxy side groups in gellan gum molecules repulse each other by electrostatic interaction, and this hinders the tight binding of helices and also the tight aggregation of helices [10–17], so that the introduction of cations can shield the electrostatic repulsion and thereby permits tight binding and aggregation of helices at lower temperatures or leads to the reduction of coil dimensions at higher temperatures [31,32]. It was suggested that the influence of cations on the viscoelastic behavior of gellan gum solutions was more remarkable for K^+ than Na^+, and for Ca^2 than Mg^{2+} as in the previous study [19], and divalent cations were more effective than monovalent cations shown in Figs. 3 and 4.

3.2. DSC

Fig. 5 shows cooling and heating DSC curves of gellan gum solutions of various concentrations without salt at a cooling or heating rate of 0.5°C min^{-1}. The cooling curve for a 1% gellan gum solution without salt showed a single exothermic peak at 29°C, and the heating curve showed a single endothermic peak at 30.5°C. The cooling curve for a 2.0% gellan gum solution showed a main exothermic peak at 33.9°C and another small peak at 95°C while the heating curve showed only a main endothermic peak at 35.4°C. As shown in the rheological measurement, G'' for a 2.0% gellan gum solution showed two step-like changes at both 35°C and 10°C. Although the subsequent lower temperature process at which G' and G'' showed a cross-over may be attributed to the sol-gel transition, this transition around 10°C was not observed in the DSC measurement. Therefore, at low concentration of gellan gum, these exothermic and endothermic peaks seem to be attributed to the helix-coil transition of gellan gum molecules and the subsequent aggregation

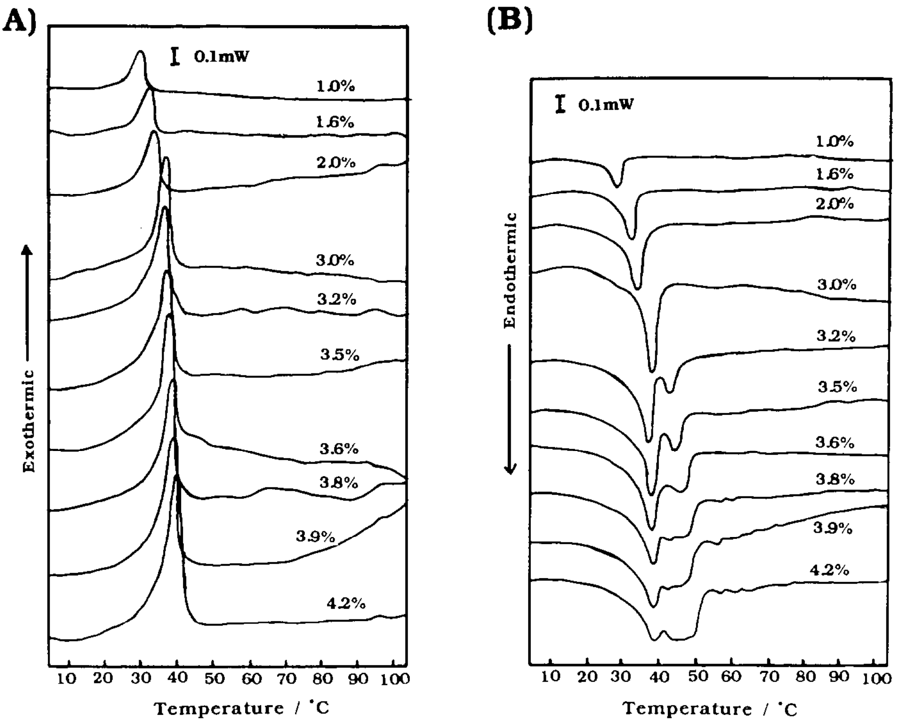

Fig. 5. Cooling (A) and heating (B) DSC curves for gellan gum solutions of various concentrations without salt. Figures beside each curve represent the concentration of gellan gum. Cooling and heating rate, 0.5°C min^{-1}.

of these helices [21]. Both exothermic peak temperature T_s and endothermic peak temperature T_m shifted to higher temperatures and both exothermic and endothermic enthalpies increased with increasing concentration of gellan gum up to 3.0%. However, for a gellan gum solution of a concentration higher than 3.2%, the endothermic peak in the heating curve split into two peaks, while the cooling curve showed a main exothermic peak and other small exothermic peaks at higher temperatures. As mentioned above, the detectable splitting in the heating DSC curve and the large hysteresis in elastic moduli during the heating process were observed for gellan gum solutions >3.2%. It is clear that the lower temperature endothermic peak corresponds with the first step decrease of G'' in the heating process of the rheological measurement, and the higher temperature endothermic peak corresponds with the second step decrease of G''. It was thus considered that gellan gum solutions whose concentration was higher than 3.2% formed a gel network containing thermally stable junction zones. Manning [18] suggested that the lower temperature peak may be caused by the melting of unaggregated helices while the higher temperature peak may be caused by the melting of aggregated helices. However, our interpretation is different from theirs.

In the cooling process, although the individual helices of gellan gum molecules were formed at a certain temperature and then these helices gradually aggregated with decreas-

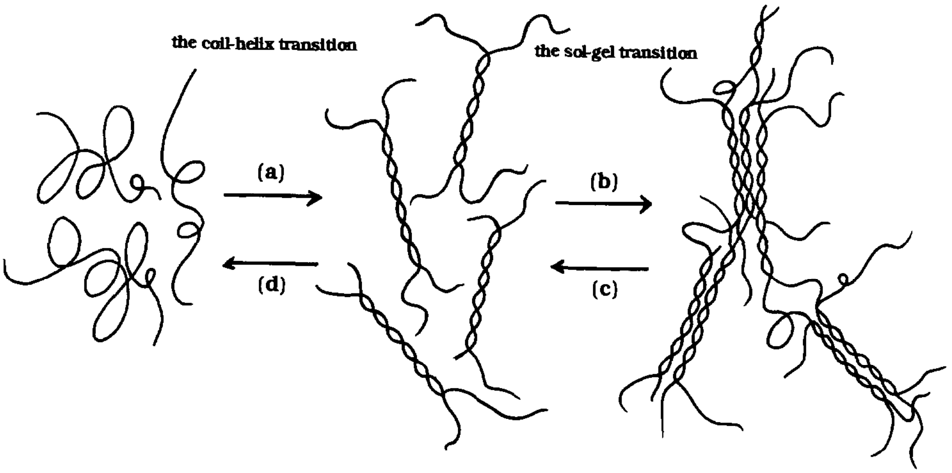

Fig. 6. Schematic representation of the structure of gellan gum, showing various processes during cooling or heating process. (a) the first step increase of G'' in the cooling process; (b) the second step increase of G'' in the cooling process; (c) the first step decrease of G'' in the heating process = the lower temperature process in the heating DSC curve; (d) the second step decrease of G'' in the heating process = the higher temperature process in the heating DSC curve.

ing temperature, at higher concentrations of gellan gum, the individual helices were formed and immediately the aggregation of helices occurred as shown in the rheological measurement (Fig. 1). However, the reason why the gellan gum solutions of a concentration higher than 3.2% showed two endothermic peaks in the heating DSC curve may be explained as follows. The lower temperature process may be caused by the separation of the aggregated helices into single helices, while the higher temperature process may be induced by the helix-coil transition (Fig. 6). Moreover, as illustrated in this figure, the enthalpy determined from the lower temperature endothermic peak decreased with increasing concentration of gellan gum, and in contrast, the enthalpy determined from the higher temperature endothermic peak increased with increasing concentration of gellan gum, which was in good agreement with that in the temperature dependence of elastic moduli shown above (Fig. 1G–I).

Fig. 7 shows the exothermic peak temperature T_s and endothermic peak temperature T_m for gellan gum solutions as a function of concentration of gellan gum from 0 to 4.2%. The experimental result that the endothermic peak temperature T_m in the heating DSC curve is higher than the exothermic peak temperature T_s in the cooling DSC curve is commonly observed for many thermoreversible systems [33]. As reported previously [20], however, extrapolation of the scan rate to zero will make this difference between T_m and T_s negligible for gellan gum solutions of lower concentrations. It is clearly demonstrated that both T_s and T_m shifted to higher temperatures with increasing concentration of gellan gum, which corresponds well with rheological results. Moreover, the temperature T_{hc} corresponding to the helix-coil transition in rheological measurement was found to be in between the exothermic and endothermic peak temperatures T_s and T_m observed in cooling and heating DSC curves (Figs. 2 and 7). However, the lower temperature en-

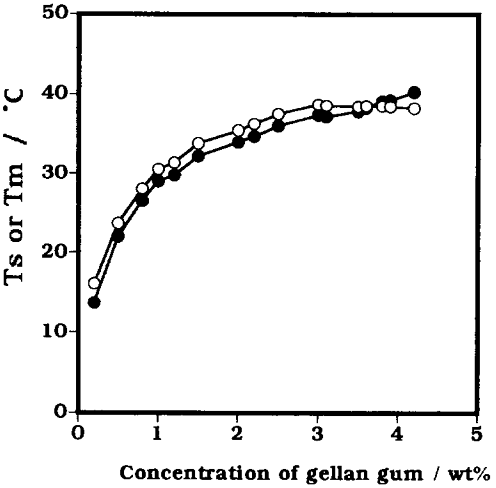

Fig. 7. Exothermic peak temperature T_s (●) and endothermic peak temperature T_m (○) for gellan gum solutions as a function of gellan gum concentration.

dothermic peak for gellan gum solutions of a concentration higher than 3.2% for these solutions maintained a plateau, and as a result, for gellan gum solutions of a concentration higher than 3.8%, the exothermic peak temperature became slightly higher than the lower endothermic peak temperature. Thus, it seems that the gelation mechanism for gellan gum solutions of higher concentrations becomes more complicated.

Fig. 8 shows cooling and heating DSC curves of 1% gellan gum solutions with and without KCl of various concentrations and at a cooling or heating rate of 0.5°C min^{-1}. In the case of added NaCl, the cooling and heating DSC curves for 1% gellan gum solutions showed a similar tendency to those with KCl, although the influence of KCl on the thermal behavior of gellan gum solutions is more effective than that of NaCl as in the rheological measurement. As is seen in this figure, the DSC cooling curves showed a single exothermic peak in all cases, and T_s shifted progressively to higher temperatures with increasing concentration of added KCl. At low concentration of NaCl or KCl, the DSC heating curves also showed a single endothermic peak, however, with more addition of salt, the endothermic peak gradually developed bimodal character and eventually split into multiple peaks. These results were in good agreement with those obtained by Manning [18]. Interestingly, the onset of detectable splitting occurred at a salt concentration of about 60 mM NaCl or 50 mM KCl and coincided with the onset of elastic gel-formation; it was found from viscoelastic measurements as stated above that an elastic gel network was formed even at as a high a temperature as 30°C in the presence of sufficient salt such as 60 mM NaCl or 50 mM KCl. In other words, upon the addition of sufficient salt to elastic gels, the gellan gum seemed to show multiple thermal transitions; the heating DSC curves of a 1% gellan gum solution containing sufficient salt showed more than two endothermic peaks. In terms of a zipper model approach [33], the appearance of two or more endothermic peaks in heating DSC curves for gellan gum solutions in the presence of sufficient salt suggests that the zippers with different bonding energies

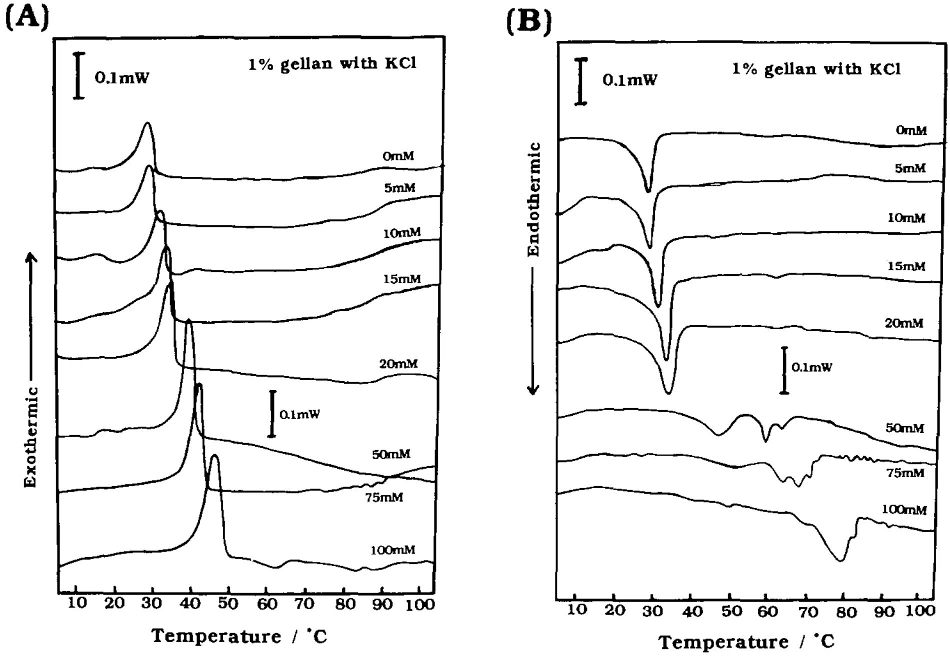

Fig. 8. Cooling (A) and heating (B) DSC curves for 1% gellan gum solutions containing KCl of various concentrations. Figures beside each curve represent the concentration of KCl. Cooling and heating rate, 0.5°C min^{-1}.

or different rotational freedoms are formed in the presence of sufficient metal ions. The lower temperature endothermic peak is attributed to the melting of the zippers with lower bonding energies or with higher rotational freedoms while the higher temperature endothermic peak corresponds to the melting of zippers with higher bonding energies or lower rotational freedoms. In the absence of salts, the gellan gum solutions whose concentration was higher than 3.2% showed two endothermic peaks in the heating DSC curve, therefore, it was suggested that the gellan gum solutions of high concentrations formed ordered structures involving thermally stable junction zones. However, these multiple endothermic peaks for a 1% gellan gum solution in the presence of sufficient monovalent cations were observed at significantly higher temperatures than those for gellan gum solutions of high concentration without salt (Fig. 5). Although the aggregation of gellan gum molecules was promoted with increasing concentration of both polymer and added salt, in the absence of salts, the carboxyl side groups in gellan gum molecules repulse each other by electrostatic interaction, and this hinders the tight binding of helices and also the tight aggregation of helices. However, the presence of cations can shield the electrostatic repulsion and thereby permits tight binding and aggregation of helices. Gellan gum solution in the presence of more sufficient monovalent cations (100 mM KCl) showed a single exothermic peak on cooling at 45°C, and a single endothermic peak on heating at 80°C; this thermal hysteresis was far more pronounced compared to that observed in gellan gum gels with less salt. Therefore, it was considered that with increasing

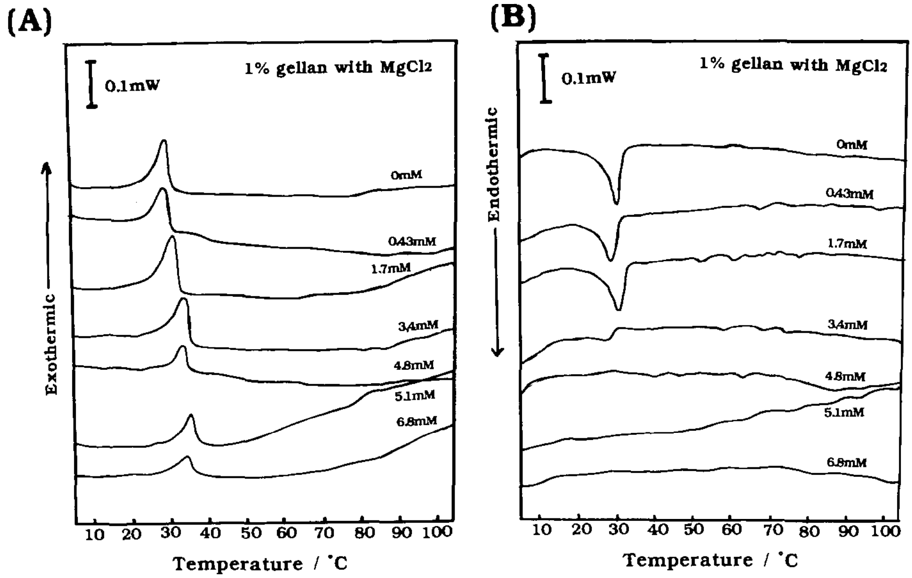

Fig. 9. Cooling (A) and heating (B) DSC curves for 1% gellan gum solutions containing $MgCl_2$ of various concentrations. Figures beside each curve represent the concentration of $MgCl_2$. Cooling and heating rate, 0.5°C min^{-1}.

concentration of added salt, the aggregation of helices was promoted, and the higher temperature peak originating from the aggregated helices formed by thermally stable junction zones with higher bonding energies was observed.

Fig. 9 shows cooling and heating DSC curves of 1% gellan gum solutions containing $MgCl_2$ of various concentrations. In the case of added $CaCl_2$, cooling and heating DSC curves for 1% gellan gum solutions showed a similar tendency to those with $MgCl_2$ [19]. However, the behavior of 1% gellan gum solutions with divalent cations was different from that with monovalent cations. T_s shifted to higher temperatures with increasing concentration of $CaCl_2$, and the exothermic and endothermic enthalpies estimated for a main peak increased up to a concentration of 1.7 mM and then decreased. In addition to a main peak, many other peaks were observed, especially at higher temperatures, with increasing concentration of salt. Moreover, the endothermic peaks in the heating DSC curve for gels containing more than 6.8 mM $CaCl_2$ were too broad to be resolved from baseline, however, in contrast, the main exothermic peak in the cooling DSC curve was much sharper and readily recognized.

Since the electric charges of divalent cations such as Ca^{2+} or Mg^{2+} are larger than those of monovalent cations such as Na^+ or K^+, divalent cations can shield the electrostatic repulsion of the carboxyl groups more effectively than can monovalent cations, as in the case of κ-carrageenan gels where the electrostatic repulsion of sulfate groups is shielded more effectively by divalent cations than by monovalent cations [34]. It is, moreover, suggested in the present work that the network of gellan gum gels containing divalent cations is formed by junction zones with different thermal stabilities.

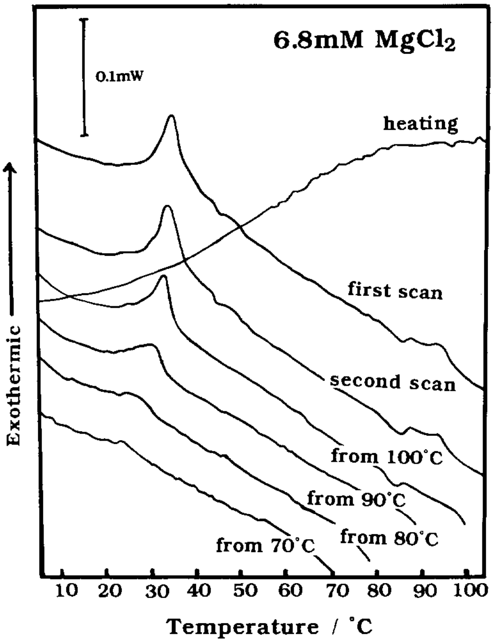

Fig. 10. Cooling and heating DSC curves in several cycles for 1% gellan gum solutions containing 6.8 mM $MgCl_2$. Figures beside each curve represent the initial heating temperature. Cooling or heating rate, 0.5°C min^{-1}.

Figs. 10 and 11 show cooling and heating DSC curves in several cycles for a 1% gellan gum solution with 6.8 mM $MgCl_2$ or 100 mM KCl. These experiments were carried out on the sample with sufficient divalent cations which showed broad exothermic peaks which cannot be resolved from baseline, or the sample with sufficient monovalent cations which showed multiple endothermic peaks in DSC measurements. In the presence of sufficient divalent cations (Fig. 10), the T_s at 34.2°C in the second scan from 110°C was in good agreement with that in the first scan, which indicates that the various junction zones in the presence of sufficient divalent cations were almost unzipped on heating to 110°C. However, in the case of cooling DSC curves from 100, 90, 80, 70°C, T_s significantly shifted to lower temperatures and the exothermic enthalpies estimated for a main peak around 30°C decreased with decreasing temperature of the initial heating, and eventually in the cooling DSC curve from 70°C, no main peak was observed around 30°C and many other peaks were observed at both high and low temperatures. This result was in good agreement with that in the case of sufficient $CaCl_2$, as described in the previous study [20]. Thus, gellan gum solutions with sufficient divalent cations from firm gels on cooling to below the setting temperature became more heat resistant. This behavior was very different from that of thermoreversible gels formed by gellan gum alone or in the presence of monovalent cations. It has been also considered that divalent cations may make cross-links by ionic bonds, so that those can form thermo-irreversible gels involving specific cation-polyanion interaction in addition to ion-specific charge screen-

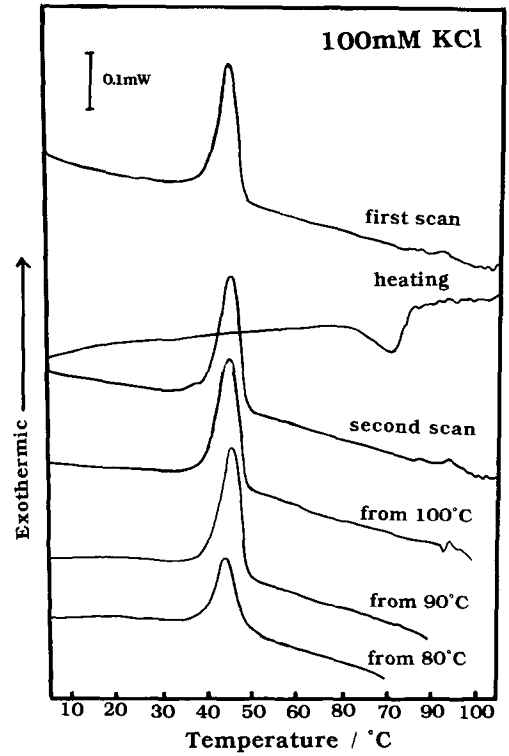

Fig. 11. Cooling and heating DSC curves in several cycles for 1% gellan gum solutions containing 100 mM KCl. Figures beside each curve represent the initial heating temperature. Cooling or heating rate, 0.5°C min^{-1}.

ing effects [18,29,34]. However, the possibility of the formation of ionic bonds by divalent cations in gellan gum molecules may be excluded because gellan gum gels in the presence of divalent ions melted completely on heating up to 110°C as described above. The bond energy for ionic bonds or covalent bonds is far higher than that for hydrogen bonds or for hydrophobic interactions.

In the presence of monovalent cations (Fig. 11), the T_s at 46.9°C in the second scan from 110°C was in good agreement with that in the first scan. T_s of 1% gellan gum solution in the presence of 100 mM KCl was significantly higher than that in the presence of 6.8 mM $MgCl_2$, however, the cooling DSC curve from 100°C or 90°C was also in close agreement with that from 110°C, which indicates that the ordered structures formed by the thermally stable junction zones were almost unzipped on heating to 90°C. It is explained by the fact that the endothermic peaks were observed at temperatures lower than 90°C. In the cooling DSC curve from 80°C, T_s significantly shifted to lower temperatures and the exothermic enthalpy decreased markedly compared to those in the curve from 90°C, 100°C or 110°C. Only aggregated helices formed by junction zones with low bonding energies or with high rotational freedom unzipped, and the other aggregated helices formed by junction zones with high bonding energies or with low rotational freedom are not unzipped by the heating up to 80°C. This result resembles that in the presence of sufficient NaCl, as described in the previous study [20]. Therefore, as illustrated in this work, the mechanism of gel formation in gellan gum with divalent cations is markedly different from that with monovalent cations. However, these phenomena are not completely clear yet; indeed, more detailed studies based on other methods are required to elucidate the mechanism of gel formation in gellan gum.

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