ELSEVIER

PII: S0144-8617(96)00093-8

Carbohydrate Polymers 30 (1996) 109–119
Copyright © 1996 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0144-8617/96/$15.00 + .00

Rheological and thermal studies of gel–sol transition in gellan gum aqueous solutions

Emako Miyoshi, Tomohisa Takaya & Katsuyoshi Nishinari*

Department of Food and Nutrition, Faculty of Human Life Science, Osaka City University, Sumiyoshi 558, Osaka, Japan

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 G'' for gellan gum solutions of lower concentrations without salt showed 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 was attributed to the helix–coil transition, and was 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 was attributed to the sol–gel transition. Temperature dependence of G'' for gellan gum solutions of higher concentrations (>3.2%) showed a large hysteresis, moreover, the temperature at which G'' showed the second step decrease in the heating process 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 T_s and 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 with a concentration higher than 3.2%, two endothermic peaks were observed on heating while the cooling curve showed only one exothermic peak. The lower temperature endothermic peak corresponds to the first step decrease of G'' in the heating process, and the higher temperature endothermic peak corresponds to the second step decrease of G''. The viscoelastic behavior of gellan gum solutions was influenced much more strongly by divalent cations than by monovalent cations. DSC heating curves for a 1% gellan gum solution in the presence of sufficient monovalent cations showed multiple endothermic peaks, however, these multiple peaks were observed at significantly higher temperatures than those for a gellan gum solution of high concentration (3.2%) without salts. The endothermic peaks in the presence of sufficient divalent cations in the heating DSC curve were too broad to be resolved from the baseline, however, many small peaks were observed at higher temperatures and the largest endothermic peak was observed at a temperature higher than 100°C. Therefore, although divalent cations promote the formation of thermally stable junction zones much more strongly than monovalent cations, the gellan gum gels containing divalent cations may also consist of physical junction zones of hydrogen bonds because these junction zones can be unzipped on heating to 120°C. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The new microbial polysaccharides are of commercial importance and are produced on a large scale by industrial fermantation, and foremost amongst these are gellan gum and xanthan gum.

Gellan gum, an anionic polysaccharide produced by *Pseudomonas elodea* has a complex tetrasaccharide repeating sequence of β-D-glucose, β-D-glucuronic acid, β-D-glucose and α-L-rhamnose, and it has a carboxyl side group (Jansson *et al.*, 1983; O'Neill *et al.*, 1983). This polysaccharide is widely used in the food industry and biotechnology because it forms a transparent gel which is resistant to heat and acid when compared to other polysaccahride gels. The precise gelation properties depend crucially on the cation present. The gelation mechanism of gellan gum solutions has been the subject of some controversy, but it is now accepted that gellan

---

*Corresponding author.

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 (Grasdalen and Smidsrod, 1987; Crescenzi *et al.*, 1986, 1988; Dentini *et al.*, 1988; Chapman *et al.*, 1990; Milas *et al.*, 1990; Robinson *et al.*, 1991; Manning, 1992; Shi, 1990), in a way analogous to the gelation of carrageenans. Although helix aggregation and network formation of gellan gum are induced by the presence of cations, the gelation properties of gellan gum are related to the nature of the cations; divalent cations promote the gelation much more strongly than monovalent cations. Cation type, as well as valency is also important (Grasdalen and Smidsrod, 1987; Crescenzi *et al.*, 1986, 1988; Dentini *et al.*, 1988; Chapman *et al.*, 1990; Milas *et al.*, 1990; Robinson *et al.*, 1991; Manning, 1992; Shi, 1990). Previous studies (Miyoshi *et al.*, 1994a, b) have shown changes in the rheological and thermal properties of the sodium-form gellan gum solutions caused by the addition of metal ions. We have suggested that salts promote the aggregation of helices of gellan gum molecules at lower temperatures while they reduce coil dimensions of the gellan gum coil at higher temperatures. It was also found that in the presence of sufficient divalent cations, the junction zones formed on cooling below the setting temperature became more heat resistant and were different from those of thermoreversible gels formed by gellan gum alone or in the presence of monovalent cations.

In the present work, the samples of purified sodium-form gellan gum were used as in the previous studies and the rheological and thermal properties of gellan gum solutions with and without sodium chloride, potassium chloride, calcium chloride and magnesium chloride were studied subsequently by dynamic viscoelastic measurement and differential scanning calorimetry. The relation between thermal properties and rheological properties is also discussed in further detail.

EXPERIMENTAL

Materials

As described in previous studies (Miyoshi *et al.*, 1994a, b), 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^{2+} and Mg^{2+} were determined by Kelco as Na^+ 3.03%, K^+ 0.19%, Ca^{2+} 0.11% and Mg^{2+} 0.22%. Salts, NaCl, KCl, $CaCl_2$ and $MgCl_2$ used in this study were (Wako Pure Chemical Industries Ltd, Osaka, Japan), and were used without further purification.

The powdered sample was dispersed by stirring to swelling at 40°C overnight. The solutions for viscoelastic studies were prepared by stirring at 70°C for 2h and then heated at 90°C for 1h to attain complete transparency. The solutions were then kept at 70°C before each rheological or thermal measurement to prevent 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 100mM and that of $CaCl_2$ or $MgCl_2$ ranged from 0.43 to 6.8mM.

Rheological measurement

The rheological measurements were performed using a Dynamic Stress Rheometer DSR from Rheometrics Co. Ltd, NJ, USA equipped with parallel plate geometry of 50mm diameter, with radial grooves in order to avoid gel slippage (Miyoshi *et al.*, 1994a). The hot sample solution was poured directly onto the plate of the instrument. Temperature dependence of G' and G'' at a frequency of 0.1rad/s was observed by cooling the systems from 50 to 5°C and then reheating to 60°C, at a rate of 0.5°C/min. The frequency dependence of G' and G'' in the range from 10^{-2} to 10^1 rad/s was observed after attaining the plateau values of both moduli as a function of mixing ratio at various temperatures from 30 to 0°C.

DSC measurement

Diffrential scanning calorimetry (DSC) measurements were carried with a Setaram micro DSC-III calorimeter, Caluire, France (Miyoshi *et al.*, 1994b). Approximately 900mg 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 ±30μg of the weight of the sample pan. The two pans were then placed inside the calorimeter and heated to 110 or 120°C and kept at that temperature for 10min to eliminate the effect of thermal history. Then the temperature was lowered to 5°C at 0.1–0.5°C/min and raised again at the same rate up to 110 or 120°C.

RESULTS AND DISCUSSION

Rheological properties

Figure 1(A)–(C) shows the temperature dependence of G' and G'' during the cooling or heating process for 1, 2 and 3% gellan gum solutions without salt at 0.1rad/s at a cooling or heating rate of 0.5°C/min, and (D)–(F) shows the frequency dependence of G' and G'' for these solutions at various temperatures. For a 1.0% solution of gellan gum (Fig. 1(A)), G' was too small to be detected in both cooling and heating processes, however, G'' could be detected at temperatures lower than 50°C in both cooling and heating processes and showed a step-like change at 30°C. It was found that the temperature dependence of G'' for gellan gum solutions with a concentration of lower than 2.0% showed one step-like change at a certain temperature (not all the data are shown) corresponding to a transition. As illustrated by the frequency dependence of both moduli for a 1% gellan gum solution (Fig. 1(D)), at any temperature from 30 to 0°C, the viscoelastic behavior was typical of a dilute polymer solution (Morris *et al.*, 1980; Clark and Ross-Murphy, 1987; Nijenhuis, 1990; Almdal *et al.*, 1993; Graessley, 1974). As concluded from the frequency dependence of both moduli described in a previous study (Miyoshi *et al.*, 1994a), at any temperature from 30 to 0°C, the viscoelastic behaviour of gellan gum solutions with concentrations lower than 2.0% are characteristic of a dilute or concentrated polymer solution. Therefore, the transition observed in the temperature dependence of G'' at concentrations lower than 2.0% should not be attributed to the sol–gel transition, but may be induced by a coil–helix transition (Nishinari *et al.*, 1995). This temperature will be written as T_{ch}. At lower concentrations of gellan gum molecules, the helix formation and its partial aggregation may form an ordered structure, but does not lead to gel formation because the number of helical aggregates does not give a continuous network throughout the whole space. The difference between G'' at lower and higher temperatures provides the midpoint temperature of transition in the thermal scanning rheological measurements, this was called the relaxational strength in the previous study (Nishinari *et al.*, 1995). The relaxational strength at T_{hc} was shown to increase with increasing concentration of gellan gum in the present study. However, the thermal behavior of gellan gum solutions with concentrations higher than 2.0% showed two step-like changes in the present study. For a 2.0% gellan gum solution (Fig. 1(B)), G' could be detected at 34°C on cooling and then gradually increased, 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 and 10°C. For a 3.0% gellan gum solution (Fig. 1(C)), the cross-over of G' and G'' was observed at 28°C and this temperature was significantly higher than that for a 2.0% gellan gum solution (Fig. 1(B)), although the temperature at which G'' showed the first step increase in the cooling process

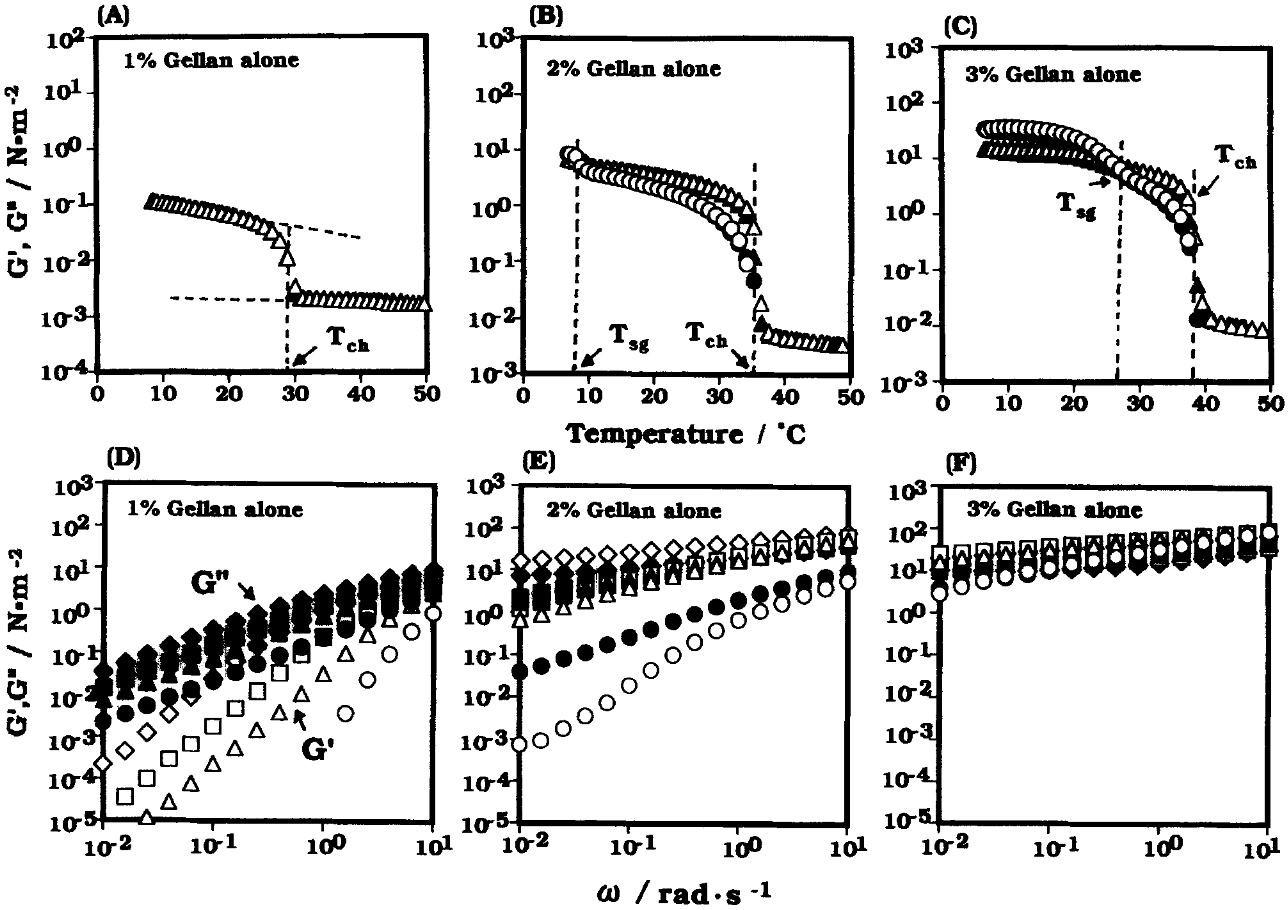

Fig. 1. Temperature dependence of the storage modulus G' and loss modulus G'' during a cooling or heating and frequency dependence of moduli for 1, 2 and 3% gellan gum solutions without salt. Temperature dependence: (○) G', (△) G'',cooling; (●) G', (▲) G'', heating; cooling and heating rate, 0.5°C/min; freq. $\omega = 0.1$ rad/s. Frequency dependence: (○) G', (●) G'', 30°C; (△) G', (▲) G'', 25°C; (□) G', (■) G'', 15°C; (◇) G', (◆) G'', 0°C. Polymer conc.: (A),(D) 1.0%; (B),(E) 2.0%; (C),(F) 3.0%.

was also slightly higher than that for 2.0% gellan gum solution. Such a behavior showing two step-like changes of G'' was observed for gellan gum solutions at concentrations 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 concentration 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 to 0°C (Miyoshi *et al.*, 1994a), the transition observed for these solutions cannot be attributed to a gel–sol transition, but to a helix–coil transition, as mentioned above. However, as shown in the frequency dependence of both moduli (Fig. 1(E)), 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 or 15°C, however the behavior changed to that of a weak gel at 0°C. For a 3.0% gellan gum solution (Fig. 1(F)), 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 the cross-over temperature of both moduli (27°C). Therefore, in concentrated solutions ($>2.0\%$), where the number of aggregates of helices exceeds a critical value on cooling, the rheological behaviour changes from sol-to-gel, and this appears as the second step increase in 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 (data not shown) showed the second step increase around 8°C in the cooling process, however G'' remained slightly larger than G' down to 5°C. For the case of the frequency dependence of both moduli (data not shown), the viscoelastic behavior for a 1.9% gellan gum solution was typical of a dilute solution at 10°C, and was typical of a concentrated polymer solution at 5°C, however, the behavior changed to that of a weak gel at 0°C. Therefore, the cross-over temperature of G' and G'' may be assigned to a sol–gel transition temperature, and the temperature will be written as T_{sg}. As shown in this figure, it was suggested that both T_{hc} corresponding to the helix–coil transition and T_{sg} corresponding to the sol–gel transition shifted to higher temperatures with increasing concentration of gellan gum.

Figure 2(A)–(C) shows the temperature dependence of G' and G'' during the cooling or heating process for 3.2, 3.3 and 3.5% gellan gum solutions without salt at 0.1 rad/s at a cooling or heating rate of 0.5°C/min. The thermal behavior for a 3.2% gellan gum solution (Fig. 2(A)) was evidently distinguished from that for gellan gum solutions with a concentration lower than 3.2% (Fig. 1); G'' showed a two-step increase at both 40 and 33.1°C in the cooling process, however it showed a two-step decrease at 39.1 and 48°C in the heating process, which indicated that the thermal hysteresis was greatly modified compared to that for gellan gum solutions with concentrations 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 the heating DSC curve, and the temperature at which G'' showed the second step decrease almost coincided with the higher temperature endothermic peak, as will be discussed later. For a 3.5% gellan gum solution (Fig. 2(C)), G'' showed a one-step increase at 40°C in the cooling process, in other words, T_{hc} corresponding to the helix–coil transition and T_{sg} corresponding to the sol–gel transition were observed at the same temperature. Therefore, this suggested that the coil–helix transition occurs concurrently with the sol–gel transition since the number of helices formed on cooling is enough to form a three-dimensional network throughout the whole space. 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 equal to that for a 3.2 or 3.3% gellan gum solution (Fig. 2(A) or (C)), 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 process decreased but that at the temperature of the second step decrease increased with increasing concentration of gellan gum.

As found in the frequency dependence of both moduli, the viscoelastic behavior for a 3.2, 3.3 or 3.5% gellan gum solution tended towards that of a weak gel at any temperature from 30 to 0°C (data not shown).

Figure 3(A)–(C) shows the temperature dependence of G' and G'' during the cooling or heating process for a 1.0% gellan gum solution containing KCl of various concentrations at 0.1 rad/s at a cooling or heating rate of 0.5°C/min, and (D)–(F) shows the frequency dependence of both moduli for these solutions at various temperatures. As shown in the temperature dependence of both moduli for a 1% gellan gum solution containing 5 mM KCl (Fig. 3(A)), G'' showed a step-like change around 32°C and this temperature was slightly higher than that for a gellan alone (Fig. 1(A)). The viscoelastic behavior was typical of a dilute polymer solution at any temperature for a 1% gellan gum solution containing 5 mM KCl as shown by the frequency dependence of

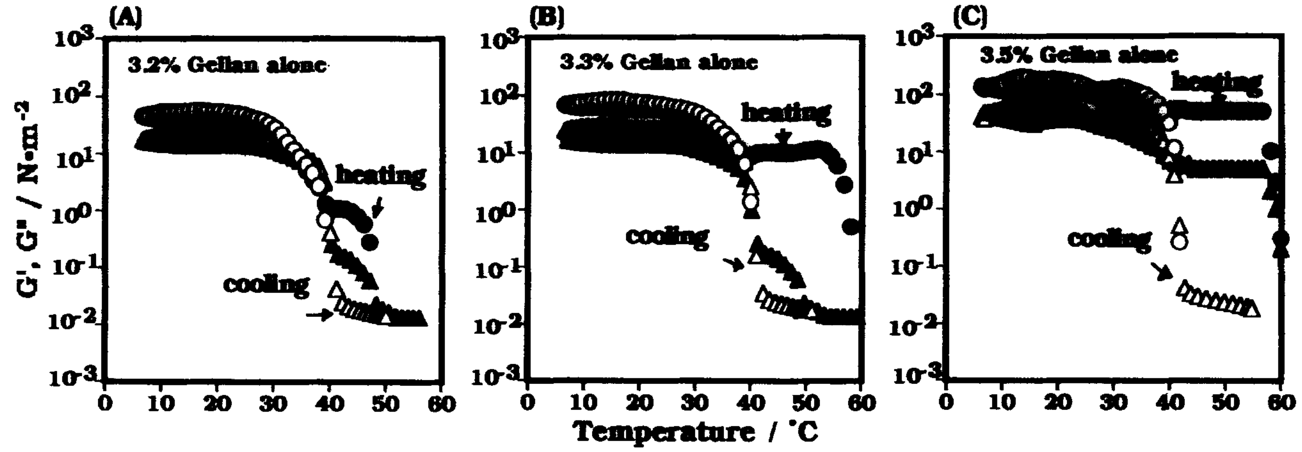

Fig. 2. Temperature dependence of the storage modulus G' and loss modulus G'' during a cooling or heating process for 3.2, 3.3 and 3.5% gellan gum solutions without salt. (○) G', (△) G'', cooling; (●) G', (▲) G'', heating; cooling and heating rate: 0.5°C/min; freq. $\omega = 0.1$ rad/s; polymer conc.: (A) 3.2%; (B) 3.3%; (C) 3.5%.

both moduli (Fig. 3(D)). On addition of 10mM KCl (Fig. 3(B)), G' began to be detected around 30°C during cooling and then increased gradually, moreover, the cross-over of G' and G'' was observed around 8°C and then G' became slightly larger than G'' at lower temperatures than the cross-over temperaure. G'' upon addition of 10mM KCl showed two step-like changes at both 33 and 22°C, indicating two thermal transitions, however, the lower transition temperature at which G'' drastically changes was significantly higher than the cross-over temperature. In the case of gellan gum solutions without salt, although G'' for gellan gum solutions whose concentrations are lower than 2.0% showed two step-like increases, the higher temperature process corresponded to the helix–coil transition (T_{hc}) and the lower temperature process almost coincided with that of the sol–gel transition (T_{sg}). The temperature at which G'' showed the first step increase on cooling, corresponded to the helix–coil transition (T_{hc}) for a gellan gum solution containing salts, however, the temperature at which it showed the second step increase should not be attributed to that of the sol–gel transition. As shown in the frequency dependence of both moduli for a 1% gellan gum solution in the presence of 10mM KCl (Fig. 3(D)), the viscoelastic behavior was typical of a dilute solution at 30 or 25°C and was typical of a concentated polymer solution at 15°C, however the behavior at 0°C changed to that of a weak gel. Thus, it seems that a certain ordered structure was formed by the addition of KCl 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. Therefore, the cross-over temperature of G' and G'' may be attributed to the sol–gel transition temperature (T_{sg}). It was found that the difference between the first and second step increase in G'' with temperature decreased and the cross-over temperature of both moduli shifted to higher temperatures with increasing concentration of KCl. In the case of the temperatrue dependence of both moduli upon addition of 30mM KCl (Fig. 3(F)), G' during the cooling process began to be detected at 37°C and then 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 43°C. This indicated that thermal hysteresis was significant. G'' in the presence of 30mM KCl showed a one step-like increase at 40°C in the cooling process, however, it showed a two-step decrease at both 40 and 43°C in the heating process. In the case of the frequency dependence of both moduli for a 1% gellan gum solution containing 30mM KCl, the viscoelastic behavior tended towards that of a weak gel even at a relatively higher temperature such as 30°C. Moreover, the values of G' and G'' in the presence of 30mM KCl at lower temperatures were significantly larger than those for 2 or 3% gellan gum solution without salt (Fig. 1(E) and (F)), which confirmed that the rheological properties of gellan gum solutions were influenced strongly by salt. The effect of NaCl on viscoelasticity of gellan gum solutions was almost similar to that of KCl, however KCl influences the viscoelastic behavior of gellan gum solutions more effectively than NaCl. This reason is interpreted on the basis that cations such as Na^+ are the structure ordering ions for water, and cations such as K^+ belong to the structure disordering ions (Hinton and Amis, 1971; Uedaira and Ohsaka, 1990) as described in the previous study (Miyoshi *et al.*, 1994a).

Figure 4(A)–(C) shows the temperature dependence of G' and G'' during the cooling or heating process for a 1.0% gellan gum solution containing $CaCl_2$ of various concentrations at 0.1 rad/s at a cooling or heating rate of 0.5°C/min, and (D)–(F) shows the frequency dependence of both moduli for these solutions at various temperatures. The viscoelastic behaviour of gellan gum solutions was influenced much more strongly by divalent cations than by monovalent cations. In the

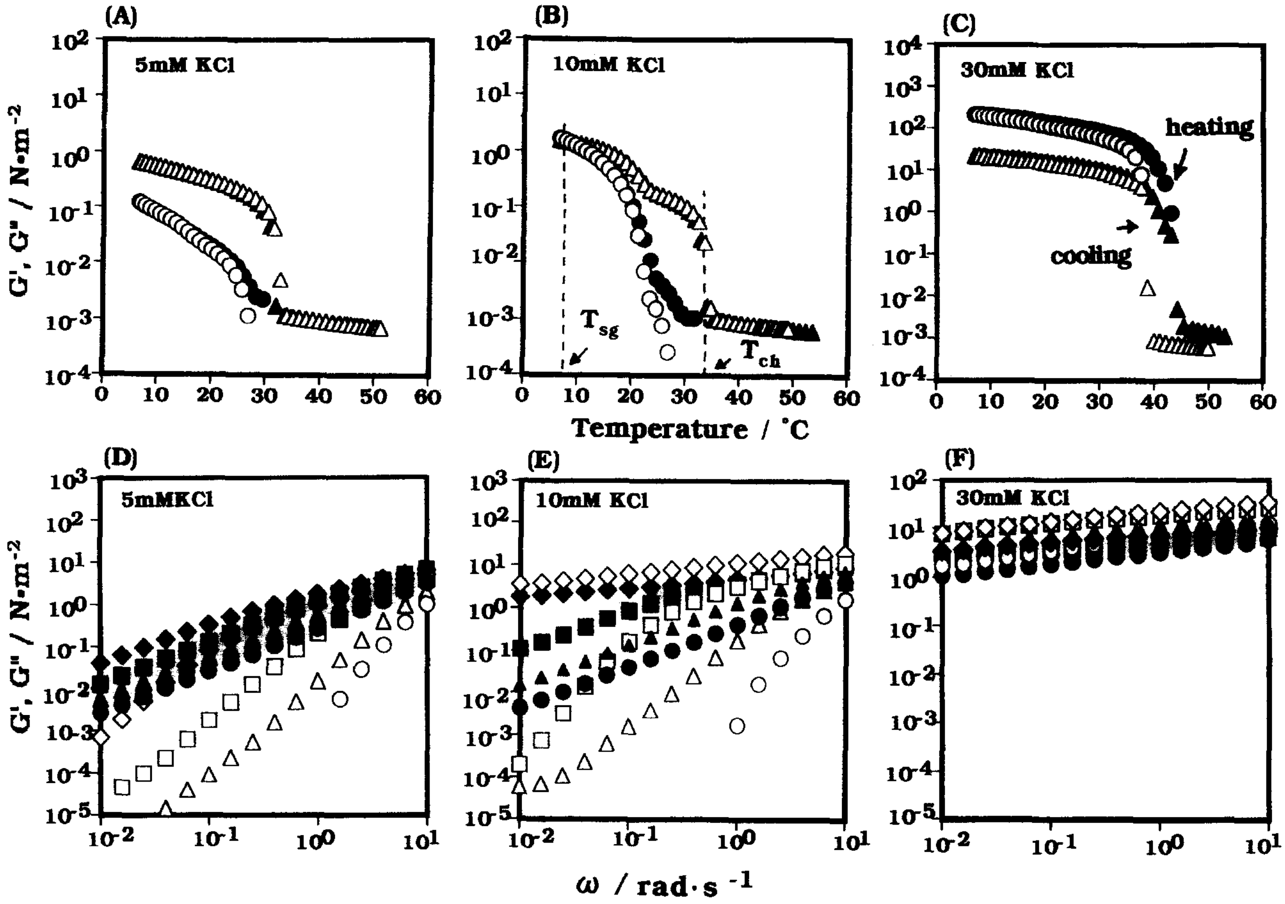

Fig. 3. Temperature dependence of the storage modulus G' and loss modulus G'' during a cooling or heating process for a 1.0% gellan gum solution containing NaCl of various concentrations. Temperature dependence: (○) G'(△) G'', cooling; (●) G', (▲) G'', heating; cooling and heating rate, 0.5°C/min; freq. $\omega = 0.1$ rad/s. Frequency dependence: (○) G', (●) G'', 30°C; (△) G', (▲) G'', 25°C; (□) G', (■) G'', 15°C; (◇) G', (◆) G'', 0°C. KCl conc.: (A),(D) 5mM; (B),(E) 10mM; (C),(F) 30mM.

temperature dependence of both moduli for a 1% gellan gum solution containing 0.85mM $CaCl_2$ (Fig. 4(A)). T_{hc} slightly shifted to higher temperatures compared to that for a gellan alone (Fig. 1(A)). The frequency dependence of both moduli for this solution (Fig. 4(D)) was typical of a dilute solution at any temperature from 30 to 0°C. In the temperature dependence of both moduli upon addition of 1.5mM $CaCl_2$ (Fig. 4(B)), T_{sg} was observed around 7°C, and thermal hysteresis was showed in this solution. In the frequency dependence of both moduli for this solution (Fig. 4(E)), the viscoelastic behavior remained typical of a dilute solution at any temperature higher than 7°C (T_{sg}), however, the behavior changed typical of a weak gel at 0°C, which corresponded well with that in the temperature dependence of both moduli. The temperature dependence of both moduli in the presence of 2.04mM $CaCl_2$ (Fig. 4(C)) was markedly different; G'' increased rapidly and G' began to be detected at 33°C in the cooling process, however, there was no evidence for a transition on heating up to 60°C. In the case of added sufficient monovalent cations (Fig. 3(C)), although the temperature at which G' and G'' increased rapidly was significantly higher than that in the presence of 2.04mM $MgCl_2$, 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 by the DSC measurement described later. In the case of the frequency dependence of both moduli upon addition of 2.04mM $CaCl_2$ (Fig. 4(F)), the viscoelastic behavior tended towards that of an elastic gel (Morris, 1982; Clark and Ross-Murphy, 1987; Nijenhuis, 1990; Almdal *et al.*, 1993) even at a relatively high tempeature such as 30°C.

The effect of $MgCl_2$ on the viscoelasticity of gellan gum solutions was almost similar to that of $CaCl_2$, however $CaCl_2$ can influence the viscoelastic behavior of gellan gum solutions more effectively than $MgCl_2$.

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 (Grasdalen and Smidsrod, 1987;

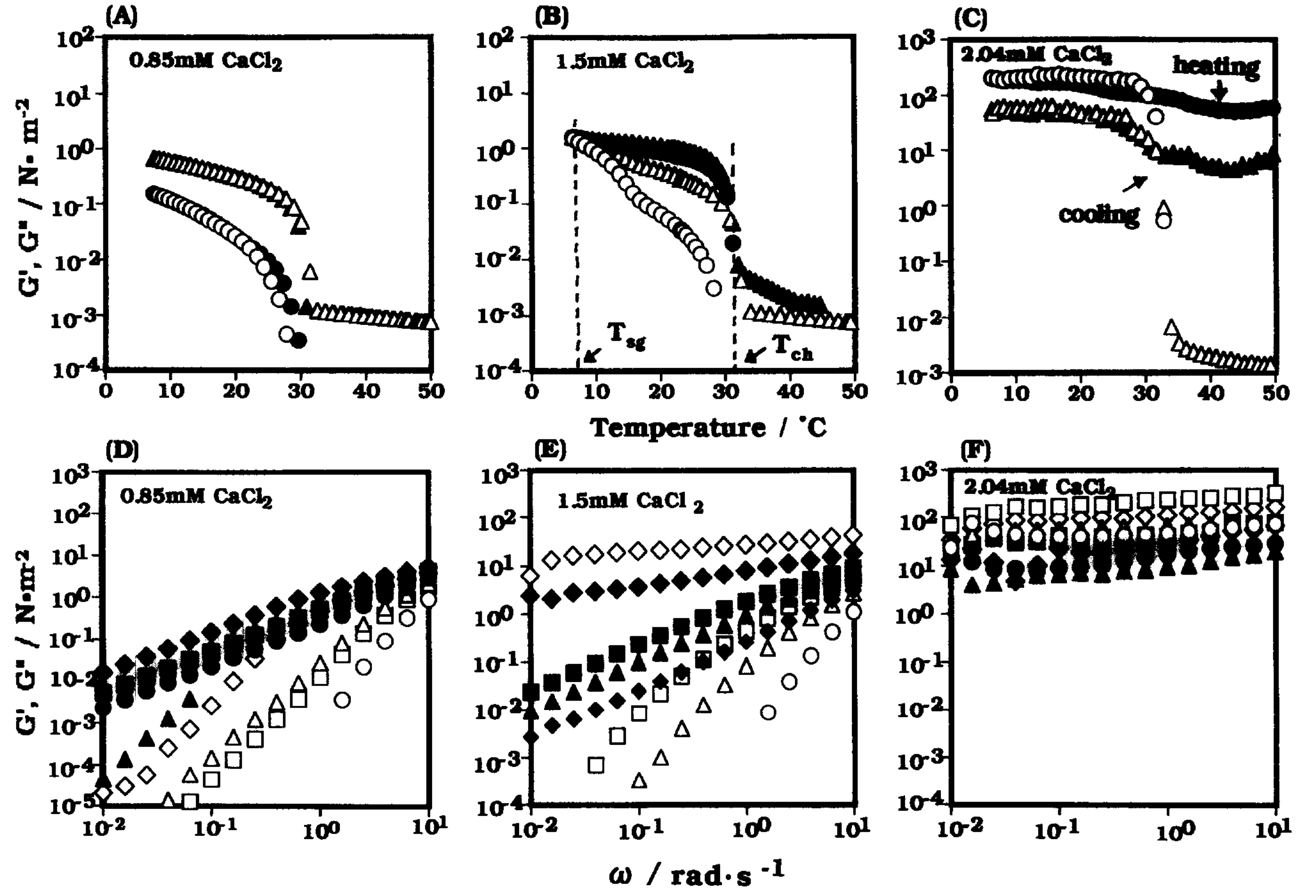

Fig. 4. Temperature dependence of the storage modulus G' and loss modulus G'' during cooling or heating process for a 0.8% gellan gum solution containing $MgCl_2$ of various concentrations. Temperature dependence: (○) G', (△) G'', cooling; (●) G', (▲) G'', heating; cooling and heating rate, 0.5°C/min; freq. $\omega = 0.1$ rad/s. Frequency dependence: (○) G', (●) G'', 30°C; (△) G', (▲) G'', 25°C; (□) G', (■) G'', 15°C; (◇) G', (◆) G'', 0°C. $CaCl_2$ conc.: (A),(D) 0.85mM; (B),(E) 1.7mM; (C),(F) 2.04mM.

Crescenzi *et al.*, 1986, 1988; Dentini *et al.*, 1988; Chapman *et al.*, 1990; Milas *et al.*, 1990; Robinson *et al.*, 1991; Manning, 1992; Shi, 1990), so that the introduction of cations can shield the electrostatic repulsion and thereby permit tight binding and aggregation of helices at lower temperatures or lead to the reduction of coil dimensions at higher temperatures (Morris *et al.*, 1980; Kobayashi *et al.*, 1994). 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 (Miyoshi *et al.*, 1994a) and divalent cations were more effective than monovalent cations as shown in Figs 3 and 4.

DSC

Figure 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.

The cooling curve for a 1% gellan gum solution without salt showed a single exothermic peak at 29.0°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 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 a 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 of these helices (Nishinari *et al.*, 1995). 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, which corresponds well with rheological results. Moreover, the temperature T_{hc} corresponding to the helix–coil transition in the 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 1–2 and 5). However, for solutions of concentrations higher than 3.2%, the heating curve showed two endothermic peaks, while the cooling curve showed a main exothermic peak and other smaller exothermic peaks at higher temperatures. It is clear that the lower temperature endothermic peak corresponds to the first step decrease in G'' observed on heating of rheological measurement, and the higher temperature endothermic peak corresponded with the second step decrease of G''. 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 the temperature dependence of elastic moduli shown above (Fig. 2(A)–(C)). It was thus considered that gellan gum solutions whose concentrations were higher than 3.2% formed the gel network containing thermally-stable junction zones. Manning (1992) 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.

We propose the following interpretation for the rheological and thermal results for gellan gum solutions without salts, as outlined schematically in Fig. 6. 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 decreasing temperature, at a lower concentration of gellan gum, the helix formation and its partial aggregation may form a certain ordered structure, but does not lead to gel formation, so that the temperature dependence of loss modulus for these solutions showed one step-like change at the coil–helix transition temperature T_{ch}. However, for concentrated gellan gum 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'' at the sol–gel transition temperature T_{sg}. In the case of concentrated gellan gum solutions at concentrations lower than 3.2%, the first step increase of G'' in the cooling process (a) almost coincided with the second step decrease of G'' in the heating process (d), and the second step increase of G'' in the cooling process (b) almost coincided with the first step decrease of G'' in the heating process (c). With a more progressive increase of concentrations of gellan gum, the individual helices were formed and immediately the aggregation of helices occurred in the cooling process, so that for 3.5% gellan gum solution, G'' showed a one-step increase in the cooling process, as shown in the rheological measurement (Fig. 2(C)). However, in the heating process, the temperature dependence of G'' for concentrated gellan gum solutions whose concentrations were higher than 3.2% showed a larger hysteresis, and these solutions showed two endothermic peaks in the heating DSC curve. Therefore, in the heating process, 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. It was concluded from both rheological and thermal results that for gellan gum solutions with a concentration higher than 3.2%, the conformational transition temperatures in the heating process did not coincide with those in the cooling process.

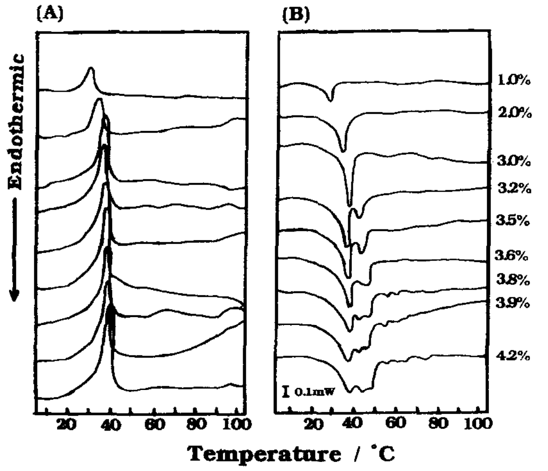

Fig. 5. Cooling (A) and heating (B) DSC curves for gellan gum solutions of various concentrations without salt. Figures beside each curve represent the concentration of gellan gum. Cooling and heating rate: 0.5°C/min.

Figure 7 shows cooling and heating DSC curves of a 3.2% gellan gum solution without salt and of a 1% gellan gum solution with 75mM NaCl and a cooling or heating rate of 0.5°C/min. In the previous study (Miyoshi *et al.*, 1994b), we found that in the presence of a small amount of monovalent cations, the DSC heating curves showed a single endothermic peak, however, on further addition of salt, they gradually developed bimodal character and eventually were split into multiple peaks, although the DSC cooling curves showed a single exothermic peak in all cases. In terms of a zipper model approach (Nishinari *et al.*, 1990), the appearance of two or more endothermic peaks in the heating DSC curves for gellan gum solutions in the presence of sufficient salt suggests that the zippers with different bonding energies 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 concentrations were higher than 3.2% showed two endothermic peaks in the heating DSC curve, therefore, it was suggested that the gellan

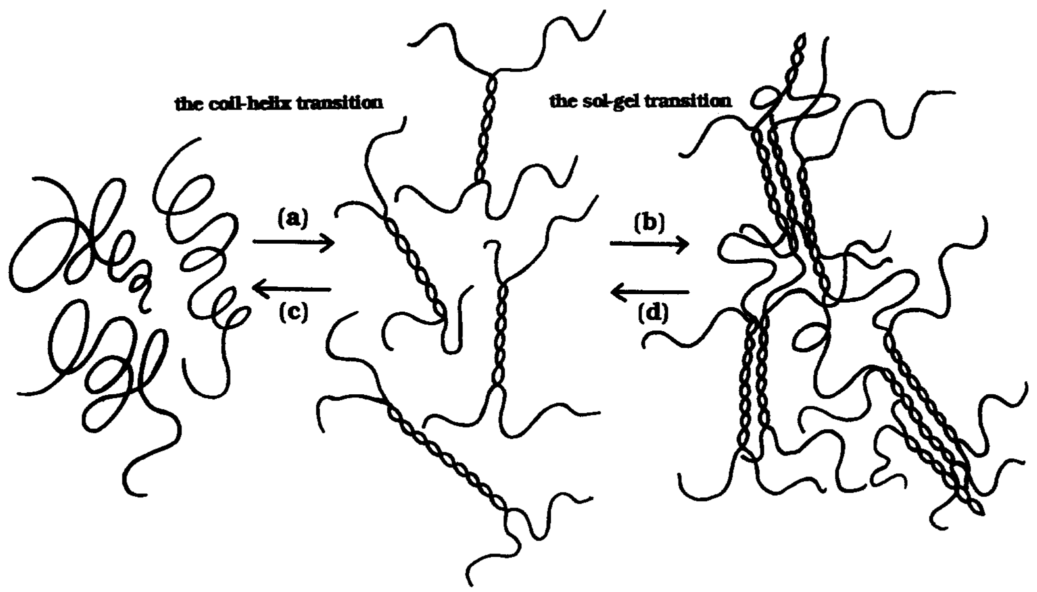

Fig. 6. Schematic representation of the structure of gellan gum, showing various processes during cooling or heating process. (a) The first step increase of G'' in the cooling process, (b) the second step increase of G'' in the cooling process, (c) the first step decrease of G'' in the heating process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

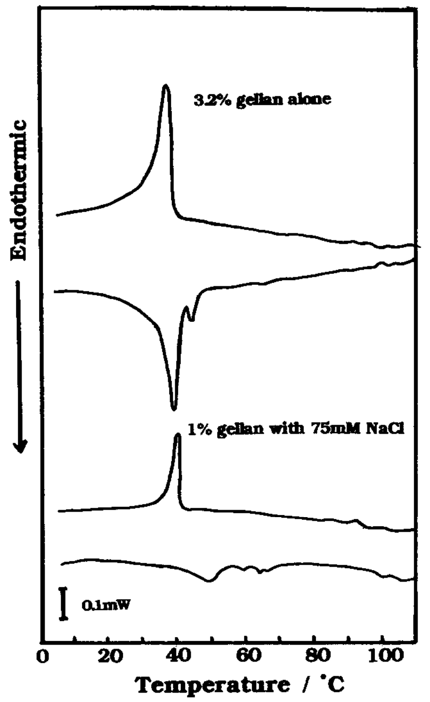

Fig. 7. Cooling and heating DSC curves for a 3.2% gellan gum solution without salt and of a 1% gellan gum solution with 75mM NaCl. Cooling and heating rate: 0.5°C/min.

gum solutions of high concentrations formed ordered stuructures involving thermally-stable junction zones as in the case of added salt. However, as shown in this figure, it is clearly demonstrated that these multiple endothermic peaks for a 1% gellan gum solution in the presence of suffifient monovalent cations were observed at significantly higher temperatures than those for a gellan gum solution with high concentration (3.2%) without salt. Moreover, the exothermic and endothermic enthalpies for a 1% solution with 75mM NaCl were estimated as 13.4 and 21.8J per gram of gellan gum, while those for a 3.2% gellan gum solution without salt were estimated as 12.4 and 11.9J per gram of gellan gum. 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 permit tight binding and aggregation of helices.

In the previous study (Miyoshi *et al.*, 1994b), we found that the thermal behavior of gellan gum solutions in the presence of divalent cations was markedly different from that in the presence of monovalent cations. In the presence of divalent 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 certain concentration of salt and then decreased. However, 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 gellan gum solutions in the presence of sufficient divalent cations 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.

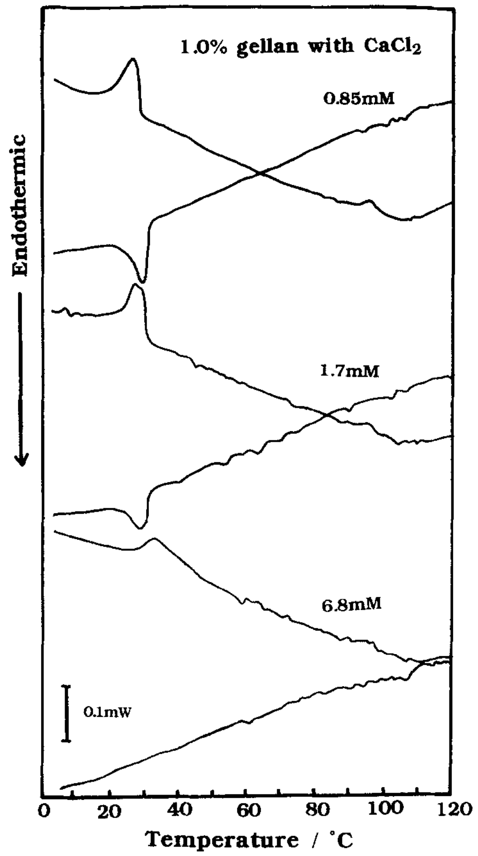

Fig. 8. Cooling and heating DSC curves for 1% gellan gum solutions containing CaCl$_2$ of various concentrations. Figures beside each curve represent the concentration of CaCl$_2$. Cooling and heating rate: 0.5°C/min.

The detailed experiments in the previous study (Miyoshi *et al.*, 1994b) suggested that gellan gum solutions with sufficient divalent cations form firm gels on cooling to below the setting temperature, and these gels became more heat resistant, i.e. a heating DSC curve did not show a clear endothermic peak. This behavior was very different from that of thermoreversible gels formed by gellan gum alone or in the presence of monovalent cations. Hence, although the cooling DSC curve in the presence of sufficient divalent cations showed a main exothermic peak around 35°C, the various junction zones did not seem to be unzipped completely on heating below 100°C. In the present study, DSC curves were obtained by cooling and heating samples between 120 and 5°C (Fig. 8). For a 1% gellan gum solution in the presence of 6.8mM CaCl$_2$, many small peaks were observed especially at higher temperatures in the heating DSC curve and the largest endothermic peak was observed at 105.2°C. Therefore, although both T_s and T_m shifted to higher temperatures with increasing concentration of divalent cations, it seemed that in the presence of sufficient divalent cations, the main endothermic peak shifted to a temperature higher than 100°C. It was, moreover, suggested that sufficient divalent cations induced unhomogenization of gellan gum solutions, so that the network of gellan gum gels containing divalent cations was formed by various junction zones with different thermal stabilities. However, although divalent cations promote the formation of thermally stable junction zones much more strongly than monovalent cations, the gellan gum gels containing divalent cations may also consist of the physical junction zones by hydrogen bonds because these junction zones can be unzipped on heating to 120°C. Some workers (Manning, 1992; Tutsumi *et al.*, 1993) have suggested 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 screening effects. 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 mentioned above. The bond energy for ionic bonds or covalent bonds is far higher than that for hydrogen bonds or for hydrophobic interactions. Indeed, more detailed studies based on other methods to describe the phenomena at the molecular level are required.

ACKNOWLEDGEMENTS

We thank Dr G. Sanderson of Kelco Division of Merck & Co. Inc., CA, USA for the gift of the sodium form of gellan gum.

REFERENCES

Almdal, K., Dyre, J., Hvidt, S. & Kramer, O. (1993). Towards a phenomenological definition of the term 'gel'. *Polym. Gels and Networks*, **1**, 5–17.

Chapman, H. D., Chilvers, G. R., Miles, M. J. and Morris, V. J. (1990) Studies on the gelation for xanthan-galactomannan. In *Gums and Stabilisers for the Food Industry*, Vol. 4, eds G. O. Phillips, D. J. Wedlock and P. A. Williams. Pergamon Press, Oxford, pp. 147–163.

Clark, A. H. & Ross-Murphy, S. B. (1987). Structural and mechanical properties of biopolymers gels. *Adv. Polym. Sci.*, **83**, 57–192.

Crescenzi, V., Dentini, M., Coviello, T. & Rizzo, R. (1986). Comparative analysis of the behaviour of gellan gum (S-60) and welan gum (S-130) in diluteaqueous solution. *Carbohydr. Res.*, **149**, 425–432.

Crescenzi, V., Dentini, M. & Dea, I. C. M. (1988). The influence of side-chains on the dilute-solution properties of three structurally related. *Carbohydr. Res.*, **21**, 283–302.

Dentini, M., Coviello, T., Burchard, W. & Crescenzi, V. (1988). Solution properties of exocellular microbial polysaccharides. 3. Light scattering from gellan and from the exocellular poly macromolecules. *Macromolecules*, **21**, 3312–3320.

Graessley, W. W. (1974). The entanglement concept in polymer rheology. *Adv. Polym. Sci.*, **16**, 1–179.

Grasdalen, H. & Smidsrod, O. (1987). Gelation of gellan gum. *Carbohydr. Polym.*, **7,** 371–393.

Hinton, J. F. & Amis, E. S. (1971). Solvention numbers of ions. *Chem. Phys.*, **71,** 627–674.

Jansson, P., Lindberg, B. & Sandford, P. A. (1983). Structural studies of gellan gum, an extracellular polysaccharide elaborated by *Pseudomonas elodea. Carbohydr. Res.*, **124,** 135–139.

Kobayashi, Y., Okamoto, A. & Nishinari, K. (1994). Viscoelasticity of hyaluronic acid with different molecular weights. *Biorheology*, **31,** 235–244.

Manning, C. E. (1992) Formation and melting of gellan polysaccharide gels, Thesis, Cranfield Institute of Technology, Silsoe College, Silsoe.

Milas, M., Shi, X. & Rinaudo, M. (1990). On the physicochemical properties of gellan gum. *Biopolymers*, **30,** 451–464.

Miyoshi, E., Takaya, T. and Nishinari, K. (1994a) Gel–sol transition in gellan gum solutions I. Rheological studies on the effects of salts. *Food Hydrocolloids*, in press.

Miyoshi, E., Takaya, T. and Nishinari, K. (1994b) Gel–sol transition in gellan gum solutions. *Food Hydrocolloids*, in press.

Morris, E. R. (1982) Rheology of hydrocolloids. In *Gums and Stabilisers for the Food Indurstry*, Vol. 2, eds G. O. Phillips, D. J. Wedlock and P. A. Williams. Pergamon Press, Oxford, 1982, pp. 57–78.

Morris, E. R., Rees, D. A. & Robinson, G. (1980). Cation-specific aggregation of carrageenan helices: domain model of polymer gel structure. *J. Mol. Biol.*, **138,** 349–362.

Nijenhuis, K. T. (1990) Viscoelastic properties of thermoreversible gels. In *Physical Networks: Polymers and Gels*, eds W. Burchard and S. B. Ross-Murphy. Elsevier Applied Science, London, pp. 15–33.

Nishinari, K., Watase, M., Kohyama, K., Nishinari, N., Oakenfull, D., Koide, S., Ogino, K., Williams, P. A. & Phillips, G. O. (1992). The effect of sucrose on the thermoreversible gel-sol transition in agarose and gelatin. *Polym. J.*, **24,** 871–877.

Nishinari, K., Miyoshi, E., Takaya, T. & Williams, P. A. (1995) Rheological and DSC studies on the interaction between gellan gum and konjac glucomannan. *Rheol. Acta*, in press.

Nishinari, K., Koide, S., Williams, P. A. & Phillips, G. O. (1990). A zipper model approach to the thermoreversible gel-sol transition. *J. Phys. France*, **51,** 1759–1768.

O'Neill, M. A., Selvenderan, R. R. & Morris, V. J. (1983). Structure of the acidic extracellular gelling polysaccharide produced by *Pseudomonas elodea. Carbohydr. Res.*, **124,** 123–133.

Robinson, G., Manning, C. E. & Morris, E. R. (1991) Conformation and physical properties of the bacterial polysaccharides gellan, welan, and rhamsan. In *Food Polymers, Gels and Colloids*, ed. Dickinson. Roy. Soc. Chem., UK, pp. 22–33.

Sanderson, G. R. (1990) Gellan gum. In *Food Gels*, ed. P. Harris. Elsevier Applied Science, London, 1990, pp. 201–232.

Shi, X. (1990) Relation entre la conformation et les proprietes d'un polysaccharide bacterien, le gellane, Thesis, Centre de Recherches sur les Macromolecules Vegetables-CNRS, l'Universite Joseph-Fourier.

Tutsumi, A., Ya, D., Hiraoki, T., Mochiku, H., Yamaguchi, R. & Takahashi, N. (1993). ESR studies of Mn(II) binding to gellan and carrageenan gels. *Food Hydrocolloids*, **7,** 427–434.

Uedaira, H. and Ohsaka, A. (1990) *Water in Biological Systems* (in Japanese). Kodansha, Tokyo, p. 30.

Watase, M., Nishinari, K., Clark, A. H. & Ross-Murphy, S. B. (1989). DSC, rheology, X-ray and NMR of very concentrated agarose gels. *Macromolecules*, **22,** 1196–1201.

Watase, M. & Nishinari, K. (1981). Effect of alkali metal ions on the rheological properties of kappa-carrageenan gels. *J. Texture Stud.*, **12,** 427–434.

Watase, M. & Nishinari, K. (1982). The rheological study of the interaction between alkali metal ions and kappa-carrageenan gels. *Colloid and Polym. Sci.*, **260,** 971–975.

Watase, M. & Nishinari, K. (1982). Effect of alkali metal ions on the viscoelasticity of concentrated kappa-carrageenan and aggarose gels. *Rheol. Acta*, **21,** 318–324.

Watase, M. and Nishinari, K. (1984) The rheological change of kappa-carrageenan gels containing alkali metal ions. In *Gums and Stabilisers for the Food Industry*, Vol. 2, eds G. O. Phillips, D. J. Wedlock and P. A. Williams. Pergamon Press, Oxford, pp. 541–550.

Watase, M. and Nishinari, K. (1986) Thermal and rheological properties of kappa-carrageenan gels containing alkali earth metal ions. In *Gums and Stabilisers for the Food Industry*, Vol. 3, eds G. O. Phillips, D. J. Wedlock and P. A. Williams. Elsevier Applied Science Publishers, London, pp. 185–194.

Watase, M. & Nishinari, K. (1989). The effect of monovalent cations and anions on the rheological properties of kappa-carrageenan gels. *J. Texture Stud.*, **19,** 259–273.