

Effects of Sodium Chloride and Calcium Chloride on the Interaction between Gellan Gum and Konjac Glucomannan

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Interaction between gellan gum (GELL) and konjac glucomannan (KGM) with and without sodium chloride and calcium chloride has been studied using dynamic viscoelastic measurements and differential scanning calorimetry (DSC). With progressive addition of NaCl, storage shear modulus G' and loss shear modulus G'' for mixtures increased, and not only the helix-coil transition temperature of GELL molecules in mixtures but also the sol-gel transition temperature for mixtures shifted to higher temperatures with increasing concentration of salts. In the presence of CaCl₂, G' increased up to a certain concentration and then decreased with increasing concentration of salts, although the helix-coil transition temperature of GELL molecules in mixtures monotonically increased with increasing concentration of salts. The exothermic and endothermic peak temperatures for mixtures in the cooling and heating DSC curves shifted to higher temperatures with increasing concentration of both NaCl and CaCl₂. In the presence of CaCl₂, the exothermic enthalpies for mixtures showed a maximum at a certain concentration and then decreased with increasing concentration of CaCl₂. KGM is attached to the surface of large aggregates of GELL helices, and since cations promote GELL self-aggregation by a screening effect, the synergistic interaction between GELL and KGM was promoted with increasing concentration of salts. However, sufficient CaCl₂ formed various aggregates of GELL helices with different thermal stabilities, so that the phase separation in GELL/KGM mixtures was promoted in the presence of sufficient CaCl₂.

Keywords: *Gellan gum; konjac glucomannan; rheology; differential scanning calorimetry; helix-coil transition; sol-gel transition; salts*

INTRODUCTION

The synergistic effects between galactomannan or glucomannan and certain polysaccharides such as xanthan, agarose, or κ -carrageenan have been extensively investigated (Dea and Morrison, 1975; Dea, 1979; Morris, 1986). Several models for these mixed gels have been proposed (Cairns et al., 1986, 1987; Brownsey et al., 1988; Tako and Nakamura, 1986; Fernandes et al., 1991, 1992; Rochas et al., 1990; Williams et al., 1991, 1993; Cheetham and Mashimba, 1988, 1991; Shatwell et al., 1991; Turquois et al., 1992; Kohyama et al., 1993), and there is still a vigorous debate as to whether the mixed gels consist of interpenetrating networks, phase-separated networks, or coupled networks. Dea et al. (1979) suggested that a specific interaction occurred between the ordered xanthan and galactomannan or glucomannan chains. However, Brownsey et al. (1988) suggested from X-ray diffraction studies that the xanthan molecules interacted with galactomannan or glucomannan in the disordered form since gelation occurred only when the solutions were mixed at a temperature above the order-disorder transition temperature of xanthan. Williams et al. (1991) proposed that the interaction of konjac mannan and xanthan occurred only after the xanthan chains had undergone ordering (Annable et al., 1994). Kohyama et al. (1993) carried out rheological and differential scanning calorimetry (DSC) studies on the gelation of konjac glucomannan/carrag-

enan mixtures using three konjac glucomannan fractions with different molecular weights. They proposed another new model where a main network of the mixture is formed by carrageenan molecules and carrageenan and konjac glucomannan form weak junction zones which contribute to the elasticity but not so much to thermal stability.

Gellan gum, an anionic polysaccharide produced by *Pseudomonas elodea* has a complex tetrasaccharide repeat unit (Jansson et al., 1983; O'Neill et al., 1983) and forms a transparent gel in the presence of cations (Grasdalen and Smidsrod, 1987; Crescenzi et al., 1986, 1987; 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 carrageenan. It has been suggested that at lower temperatures gellan gum molecules are in an ordered, elongated conformation while at higher temperatures gellan gum molecules are in a relatively disordered state (Crescenzi et al., 1988; Tanaka et al., 1993). The conformational change temperature was around 30 °C; however, this temperature was affected by the presence of cations (Grasdalen and Smidsrod, 1987; Crescenzi et al., 1986, 1987; Dentini et al., 1988; Chapman et al., 1990; Milas et al., 1990; Robinson et al., 1991; Manning, 1992; Shi, 1990). A previous study (Miyoshi et al., 1994a) has suggested that salts promote the aggregation of helices of gellan gum molecules at lower temperatures while they reduce the coil dimension of gellan gum molecules at higher temperatures. Many workers have reported that the formation and aggregation of helices in gellan gum molecules were much more promoted by divalent cations than by monovalent cations (Grasdalen and Smidsrod, 1987; Crescenzi et al., 1986, 1987; Dentini

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et al., 1988; Chapman et al., 1990; Milas et al., 1990; Robinson et al., 1991; Manning, 1992; Shi, 1990). It was concluded in previous studies (Miyoshi et al., 1994b, 1995a) that, in the presence of sufficient divalent cations, those junction zones formed on cooling below a setting temperature became more heat resistant than those formed in the presence of monovalent cations.

Extensive studies on the role of cations in the conformation of thermoreversible gels at the helix-coil transition have been carried out; however, the effects of cations on the properties of several mixed gels as mentioned above have not been studied in detail, except some work on xanthan/glucomannan mixtures by Annable et al. (1994a). In a previous paper (Nishinari et al., 1995), the interaction between gellan gum (GELL) and konjac glucomannan (KGM) with different molecular weights was studied by mechanical spectra and DSC. We suggested that the steplike change of elastic moduli in the temperature dependence as well as the exothermic and endothermic peaks in DSC cooling and heating curves were attributed to helix-coil transition of gellan molecules and the subsequent aggregation of these helices. It was also found that the viscoelastic behavior of GELL/KGM changed, depending on molecular weight of konjac glucomannan, and that the elasticity of mixtures mainly originates from aggregated helices of gellan gum molecules for systems of GELL/KGM with the lowest molecular weight KGM while the contribution from KGM is more important for systems of GELL/KGM with the medium or the highest molecular weight KGM. In the present work, the effects of NaCl and CaCl₂ on the interaction between GELL and KGM were studied using dynamic viscoelastic measurement and differential scanning calorimetry.

MATERIALS AND METHODS

Materials. Purified sodium-form gellan samples (in powder form) were kindly supplied by Kelco Division of Merck & Co. Inc., Los Angeles, as described in the previous studies (Miyoshi et al., 1994a,b, 1995a,b; Nishinari et al., 1995). The contents of the inorganic ions Na, K, Ca, and Mg were determined by Kelco using an inductively coupled argon plasma emission spectrophotometer (ICP) as Na 3.03%, K 0.19%, Ca 0.11%, and Mg 0.02%, respectively.

In the present work, a fraction of KGM with medium molecular weight (LM-1), prepared by enzymatic degradation, was chosen from three KGM samples used in the previous work (Nishinari et al., 1995). This fraction of KGM was kindly supplied by Shimizu Chemical Co., Hiroshima, Japan, as described previously (Nishinari et al., 1995). The molecular weight of the sample fraction was 9.5×10^5 (LM-1). This sample fraction will be called KGM hereafter.

The salts, NaCl and CaCl₂, used in this study were of the extrafine grade reagents (Wako Pure Chemical Industries Ltd., Osaka, Japan) and were used without further purification.

As described in the previous study (Nishinari et al., 1995), the solutions of GELL/KGM were prepared by mixing the appropriate amounts of each powder and dispersing by stirring to swell at 40 °C overnight. The solutions of mixtures for viscoelastic and DSC studies were prepared by stirring at 70 °C for 2 h and then heating at 90 °C for 1 h until completely transparent. The solutions of mixtures were then kept at 70 °C to prevent gelation before each solution was poured onto the plate of the rheological instrument or sealed into the DSC pan.

The total polysaccharide concentration was fixed at 0.8% (w/w) and the mixing ratio of GELL/KGM = 0.3/0.5 was chosen in the present work because a synergistic interaction was found at this ratio (Nishinari et al., 1995). For samples containing salts, the concentration of NaCl in solutions varied from 10 to 75 mM and that of CaCl₂ ranged from 0.85 to 6.4 mM. Each salt was added to solutions of GELL/KGM during stirring to swell before heating.

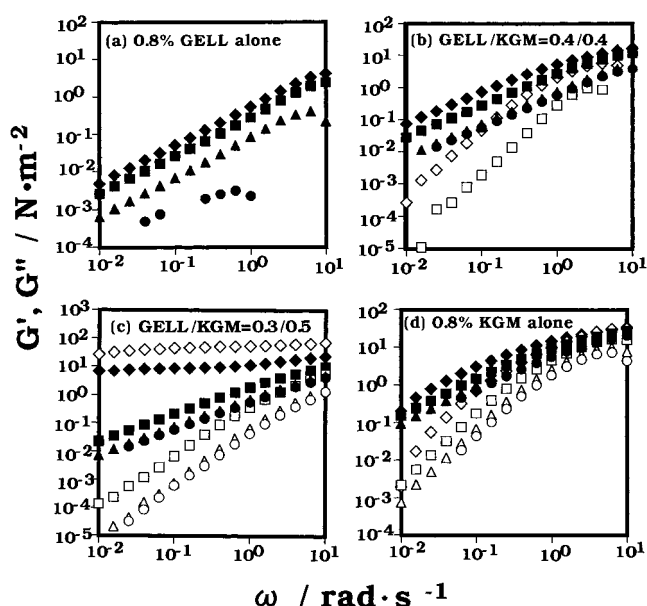


Figure 1. Frequency dependence of storage modulus G' and G'' for GELL/KGM mixtures (total polysaccharide 0.8%) with various mixing ratios in the absence of cations at various temperatures. (○) G' , (●) G'' , 30 °C; (△) G' , (▲) G'' , 25 °C; (□) G' , (■) G'' , 15 °C; (◇) G' , (◆) G'' , 0 °C.

Rheological Measurements. The mechanical spectra measurements were performed with a Dynamic Stress Rheometer DSR from Rheometrics Co. Ltd., Bayonne, NJ, at various temperatures. The details of the rheological measurements were described previously (Miyoshi et al., 1994a).

Frequency dependence of the storage shear modulus G' and loss shear modulus G'' at a frequency range from 10^{-2} to 10 rad/s was observed after the accomplishment of the plateau value of both moduli as a function of time at various temperatures from 30 to 0 °C. The temperature dependence of G' and G'' was observed by cooling the systems from 50 °C to 5 °C and then reheating to 50 °C, at the rate of 0.5 °C/min, at the constant frequency of 0.1 rad/s, as described in the previous study (Nishinari et al., 1995).

DSC Measurements. DSC measurements were carried out using a Setaram micro DSC-III calorimeter, Caluire, France. The details of DSC measurements were described previously (Miyoshi et al., 1994b). The sample and reference pans were placed inside the calorimeter and heated to 110 °C and kept for 10 min to ensure the mixture was in the form of a homogeneous solution. Then the temperature was lowered to 5 °C at 0.5 °C/min and raised again at the same rate to 110 °C. Finally, the temperature was scanned up and down at a preset rate, and the enthalpy profiles were recorded.

RESULTS

Rheological Properties. Figure 1 shows the frequency dependence of G' and G'' for GELL/KGM mixtures (total polysaccharide concentration 0.8%) with various mixing ratios in the absence of cations at various temperatures. At any temperature from 30 to 0 °C, G' for a solution of 0.8% GELL alone (Figure 1a) was strongly frequency dependent; however, G'' was too small to be detected. Thus, this indicated that GELL would not gel at this concentration. For a mixture with GELL/KGM = 0.4/0.4 (Figure 1b), although G' could be detected at 15 and 0 °C, the viscoelastic behavior was typical of that for dilute polymer solutions (Morris et al., 1982; Clark and Ross-Murphy, 1987; Te Nijenhuis et al., 1990) with $G' < G''$ throughout the accessible frequency range, and both moduli were strongly frequency dependent. For a mixture with GELL/KGM = 0.3/0.5 (Figure 1c), the viscoelastic behavior at 30, 25,

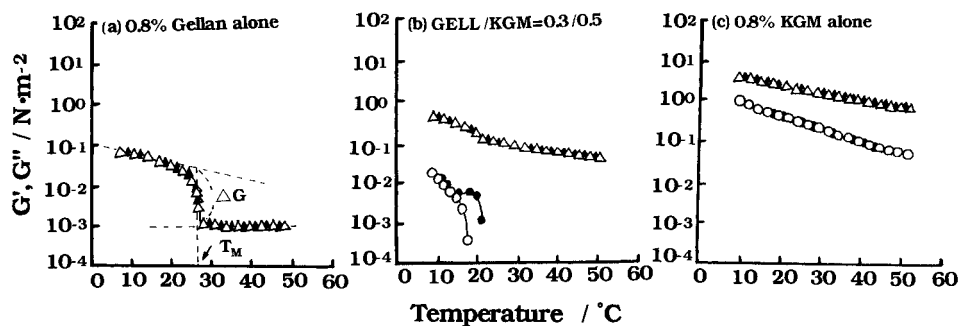


Figure 2. Temperature dependence of storage modulus G' and loss modulus G'' during the cooling and heating process for a solution of 0.8% GELL alone (a), a mixture with GELL/KGM = 0.3/0.5, (b), or a solution of 0.8% KGM alone (c) in the absence of cations. (○) G' , (△) G'' , cooling; (●) G' , (▲) G'' , heating; cooling and heating rate, 0.5 °C/min; frequency $\omega = 0.1$ rad/s.

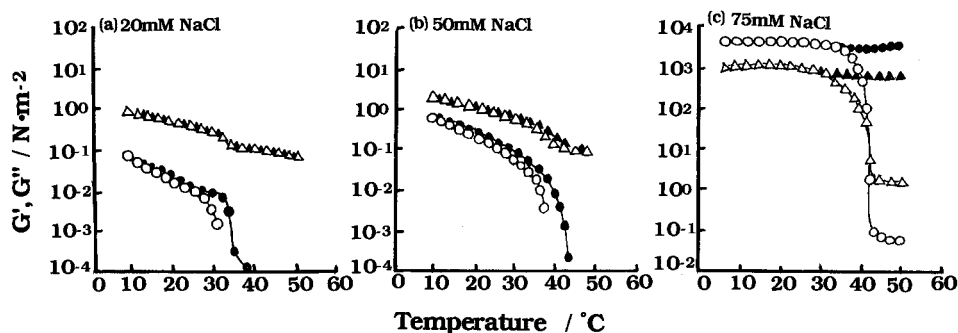


Figure 3. Temperature dependence of storage modulus G' and loss modulus G'' during the cooling and heating process for a mixture with GELL/KGM = 0.3/0.5 (total polysaccharide concentration 0.8%) containing NaCl of various concentrations. (○) G' , (△) G'' , cooling; (●) G' , (▲) G'' , heating; cooling and heating rate, 0.5 °C/min; frequency $\omega = 0.1$ rad/s.

or 15 °C remained typical of that for a dilute polymer solution; however, the behavior at 0 °C was quite different; G' was larger than G'' and both moduli were only slightly frequency dependent throughout the accessible frequency range. This behavior is classified rheologically as that of a weak gel (Morris et al., 1982; Clark and Ross-Murphy, 1987; Te Nijenhuis et al., 1990). However, for mixtures containing a higher content of KGM, the viscoelastic behavior at any temperature from 30 to 0 °C was typical of a dilute solution, and a solution of 0.8% KGM alone (Figure 1d) also indicated dilute polymer solution behavior at any temperature. Therefore, the gel-like behavior was observed at a mixing ratio of GELL/KGM = 0.3/0.5 only at 0 °C; however, all other solutions could not form a gel at any temperature. Since a mixing ratio of GELL/KGM = 0.3/0.5 corresponds to the maximum of synergistic interaction for mixtures, the following experiments to study the effects of various cations were performed using mixtures of this mixing ratio.

Figure 2a–c shows the temperature dependence of G' and G'' at 0.1 rad/s during cooling or heating at a scan rate of 0.5 °C/min for solutions of 0.8% GELL alone, a mixture with GELL/KGM = 0.3/0.5, or with 0.8% KGM alone in the absence of cation, respectively. For both the solution of 0.8% GELL alone and the mixture, the loss shear modulus G'' showed a steplike change at a certain temperature. As described in the previous study (Nishinari et al., 1995), this transition was attributed to the helix–coil transition in GELL molecules. The midpoint temperature of the transition T_M is defined as the temperature at which G'' changes most steeply, and the relaxational strength ΔG is defined as the difference between G'' at lower and higher temperature sides at T_M measured by the ordinate unit (Figure 2a). As was found previously (Nishinari et al., 1995), T_M shifted to lower temperatures and ΔG decreased monotonically with increasing content of KGM.

Figure 3a–c shows the temperature dependence of G' and G'' at 0.1 rad/s during cooling or heating at a scan rate of 0.5 °C/min for mixtures with GELL/KGM = 0.3/0.5 (total polysaccharide concentration 0.8%) containing NaCl of various concentrations. Upon addition of 20 mM NaCl (Figure 3a), T_M markedly shifted to higher temperatures, although ΔG for a mixture with 20 mM NaCl did not change so much. Upon addition of 50 mM NaCl (Figure 3b), T_M shifted to higher temperatures, moreover, $\tan \delta = G''/G'$ became small. Upon addition of 75 mM NaCl (Figure 3c), the thermal behavior of G' and G'' was quite different; both moduli could be detected at 50 °C in the cooling process and began to increase rapidly at ~40 °C, and then the values of G' were markedly larger than those of G'' below the crossover temperature. However, both moduli showed no drastic change up to 50 °C in the heating process. The frequency dependence of both moduli for a mixture with 75 mM NaCl showed a behavior of elastic gel at a relatively higher temperature, such as 30 °C (data not shown). Upon more progressive addition of NaCl, firm gels were formed. For a solution of 0.3% GELL alone (in the absence of 0.5% KGM) upon addition of 20, 50, or 75 mM NaCl, in all cases, G' was too small to be detected, which indicates that a solution of 0.3% GELL alone even in the presence of sufficient NaCl could not form gels (data not shown). In other words, at lower concentrations of gellan gum, the number of helices formed on cooling is not sufficient to cause the whole space and to form a three-dimensional network. Therefore, although the addition of KGM was necessary for a 0.3% GELL solution to reach a gel state, sufficient NaCl seems to promote not only the formation and aggregation of helices in GELL molecules but also the interaction between GELL and KGM, so that the mixtures in the presence of 75 mM NaCl form an elastic gel with a larger thermal hysteresis.

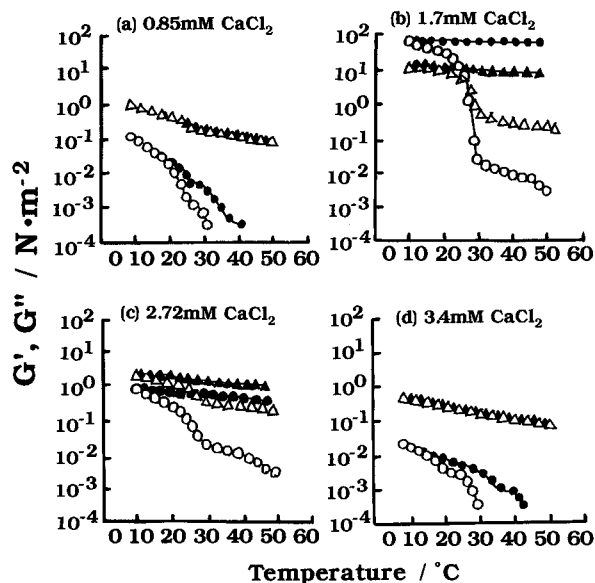


Figure 4. Temperature dependence of storage modulus G' and loss modulus G'' during the cooling and heating process for a mixture with GELL/KGM = 0.3/0.5 (total polysaccharide concentration 0.8%) containing CaCl_2 of various concentrations. (○) G' , (△) G'' , cooling; (●) G' , (▲) G'' , heating; cooling and heating rate, $0.5\text{ }^\circ\text{C}/\text{min}$; frequency $\omega = 0.1\text{ rad/s}$.

Figure 4a–d shows the temperature dependence of G' and G'' at 0.1 rad/s during cooling or heating at a scan rate of $0.5\text{ }^\circ\text{C}/\text{min}$ for mixtures with GELL/KGM = 0.3/0.5 (total polysaccharide concentration 0.8%) containing CaCl_2 of various concentrations. With progressive addition of salts, the sequence of thermal behavior for mixtures with CaCl_2 was quite different from that with NaCl, as shown above. Upon addition of 0.85 mM CaCl_2 (Figure 4a), the helix–coil transition temperature of GELL molecules in a mixture shifted to higher temperatures compared to those for a mixture without salt. Upon addition of 1.7 mM CaCl_2 (Figure 4b), the mixture showed a large thermal hysteresis, which was similar to that in the presence of 75 mM NaCl. However, with progressive addition of CaCl_2 , both moduli significantly decreased (Figure 4c); moreover, for mixtures containing $>3.06\text{ mM}$ CaCl_2 (Figure 4d), G'' became significantly larger than G' . However, both moduli upon addition of $>3.06\text{ mM}$ CaCl_2 became less temperature dependent, and the steplike change in G' in mixtures was not clearly observed in both cooling and heating process. The frequency dependence of both moduli for a mixture with 1.7 mM CaCl_2 at $<25\text{ }^\circ\text{C}$ showed a gel-like behavior; however, the mixtures upon addition of sufficient CaCl_2 ($>3.06\text{ mM}$), behaved as a dilute polymer solution at any temperature from 30 to $0\text{ }^\circ\text{C}$ (data not shown). As shown in this figure, G' and G'' for mixtures markedly increased with the addition of 1.7 mM CaCl_2 ; however, both moduli significantly decreased with the addition of excessive divalent cations. In the previous study (Miyoshi et al., 1994a), 1% GELL solutions in the presence of divalent cations formed an elastic gel at a relatively higher temperature such as $30\text{ }^\circ\text{C}$, which indicated that the formation and aggregation of GELL molecules were promoted by the addition of sufficient divalent cation. However, in the presence of CaCl_2 , 0.3% GELL solutions could not form a gel as in the presence of NaCl. Although the addition of KGM was necessary for a 0.3% gellan gum solution to reach gel state, it was suggested that in the presence of sufficient CaCl_2 , the synergistic interaction between GELL and KGM may decrease, so

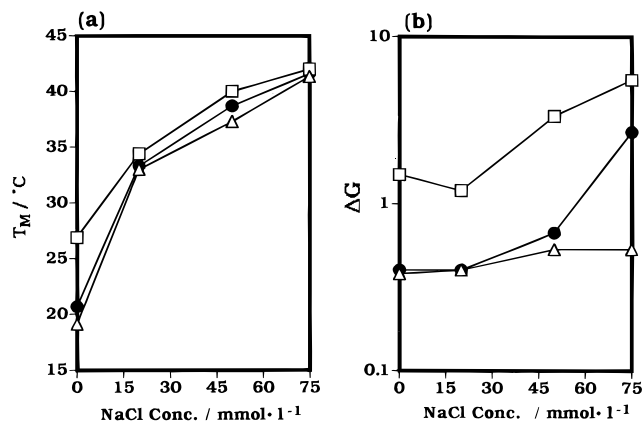


Figure 5. Dependence of midpoint temperature of transition T_M (a) and the relaxation strength ΔG (b) for mixtures with GELL/KGM = 0.3/0.5 (total polysaccharide concentration 0.8%) or 0.3% or 0.8% GELL solutions on the concentration of NaCl: (●) mixture; (△) 0.3% GELL; (□) 0.8% GELL.

that G' and G'' for mixtures with sufficient CaCl_2 markedly decreased.

Figure 5 shows the dependence of the midpoint temperature T_M (a) and the relaxational strength ΔG (b) for mixtures with GELL/KGM = 0.3/0.5 (total polysaccharide concentration 0.8%) on the concentration of NaCl, compared to those for solutions of 0.8 or 0.3% GELL alone. In all three cases, T_M shifted to higher temperatures with increasing concentration of NaCl. T_M for mixtures was significantly higher than that for solutions of 0.3% GELL alone in the presence of the same concentration of NaCl. T_M for mixtures was lower than that for solutions of 0.8% GELL alone in all cases; however, the difference between T_M for 0.8% GELL solutions and T_M for mixtures in the presence of the same concentration of NaCl significantly decreased with increasing concentration of NaCl. Values of ΔG for mixtures and a solution of 0.8% GELL alone were significantly increased with increasing concentrations of NaCl; however, ΔG for a solution of 0.3% GELL alone only slightly increased up to 50 mM NaCl and then leveled off. ΔG for mixtures was larger than that for 0.3% GELL alone at all added concentrations of NaCl; the difference between ΔG for mixtures and ΔG for 0.3% GELL alone markedly increased in the presence of 75 mM NaCl. We have recently found that the main contribution to the magnitude of ΔG is by the formation and the subsequent aggregation of helices of GELL molecules and suggested that this process was inhibited by the presence of KGM because ΔG for gellan alone was larger than that for mixtures at all mixing ratios (Nishinari et al., 1995). However, in the presence of sufficient monovalent cations, the formation and aggregation of helices in GELL molecules seemed to be promoted by the presence of KGM, because in the presence of 75 mM NaCl, ΔG for a mixture became significantly larger than that for a solution of 0.3% GELL alone in the presence of the same concentration of NaCl.

Figure 6 shows the dependence of the midpoint temperature T_M (a) and the relaxation strength ΔG (b) for mixtures with GELL/KGM = 0.3/0.5 (total polysaccharide concentration 0.8%) on the concentration of CaCl_2 , compared to those for solutions of 0.8 or 0.3% GELL alone. In all three cases, T_M shifted to higher temperatures with increasing concentration of CaCl_2 . Although T_M for solutions of 0.8% GELL alone was significantly larger than that for solutions of mixtures or 0.3% GELL alone at the same concentration of CaCl_2 ,

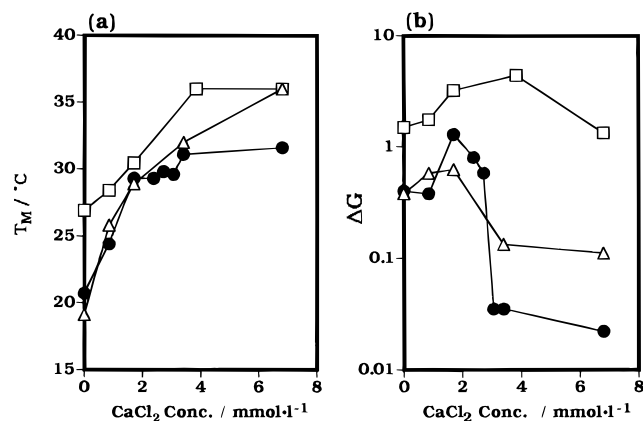


Figure 6. Dependence of midpoint temperature of transition T_M (a) and the relaxation strength ΔG (b) for mixtures with GELL/KGM = 0.3/0.5 (total polysaccharide concentration 0.8%) or 0.3% or 0.8% GELL solutions on the concentration of CaCl_2 : (●) mixture; (△) 0.3% GELL; (□) 0.8% GELL.

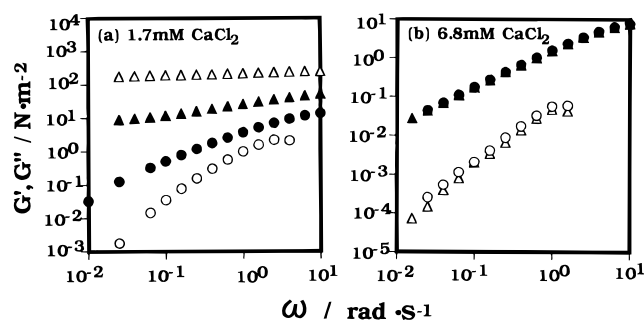


Figure 7. Frequency dependence of storage modulus G' and loss modulus G'' mixtures with GELL/KGM = 0.3/0.5 (total polysaccharide concentration 0.8%) containing 1.7 or 6.8 mM CaCl_2 at 30 °C, and 30 °C after cooling and heating cycles between 30 and 0 °C: (○) G' , (●) G'' , 30 °C; (△) G' , (▲) G'' , 30 °C after cooling and heating cycles between 30 and 0 °C; cooling and heating rate, 0.5 °C/min.

in the presence of 6.8 mM CaCl_2 , T_M for a solution of 0.3% GELL was similar to that for a solution of 0.8% GELL. Although T_M for mixtures almost coincided with that for solutions of 0.3% GELL alone at lower concentrations of CaCl_2 , T_M for mixtures leveled off around >1.7 mM CaCl_2 , so that the difference between T_M for a solution of 0.3% GELL alone and T_M for a mixture in the presence of the same concentration of CaCl_2 increased with increasing concentration of CaCl_2 . ΔG for a solution of 0.8% GELL alone gradually increased up to 4 mM CaCl_2 and then decreased with increasing concentration of CaCl_2 . For both mixtures and solutions of 0.3% GELL alone; ΔG passed through a maximum at 1.7 mM CaCl_2 and then decreased with increasing concentration of CaCl_2 . The maximum value of ΔG for a mixture was significantly larger than that for solutions of 0.3% GELL alone, however, in the presence of sufficient CaCl_2 , ΔG for mixtures was significantly smaller than that for solutions of 0.3% GELL alone.

The frequency dependence of both moduli for mixtures containing 1.7 or 6.8 mM CaCl_2 at 30 °C (the temperature was lowered from 60 to 30 °C at a cooling rate of 0.5 °C/min) and 30 °C after a cooling and heating cycle between 30 and 0 °C is shown in panels a and b of Figure 7, respectively. These solutions were cooled from 30 to 0 °C and reheated to 30 °C at a scanning rate of 0.5 °C/min, and then the frequency dependence of both moduli was observed after both moduli as a function time at 30 °C attained a plateau. In the presence of 1.7 mM CaCl_2 (Figure 7a), the viscoelastic behavior at 30 °C was typical of a dilute polymer solution; however,

it changed to that of a weak gel at 30 °C after a cooling and heating cycle between 30 and 0 °C. It was suggested that the mixture with 1.7 mM CaCl_2 that were cooled to below the setting temperature formed thermally stable junction zones, which was in good agreement with the result in the thermal scanning rheological measurements. However, in the presence of 6.8 mM CaCl_2 (Figure 7b), both behaviors at 30 °C with and without the cooling and heating cycle between 30 and 0 °C showed dilute polymer solutions. Moreover, both moduli at 30 °C after the cooling and heating cycles between 30 and 0 °C became slightly lower than those at 30 °C.

DSC. Figure 8 shows cooling and heating DSC curves for GELL/KGM mixtures (total polysaccharide concentration 0.8%) with various mixing ratios in the absence of cations. The cooling curve for a 0.8% GELL alone showed a single exothermic peak at 26.5 °C, and the heating curve showed a single endothermic peak at 28.0 °C. However, the cooling curves for mixtures with more than a KGM content of 0.2 showed a main exothermic peak at 15–25 °C and another small peak around 95 °C, while the heating curve showed a single endothermic peak. Both the main exothermic peak temperature T_s and the endothermic peak temperature T_m shifted to lower temperatures and these exothermic and endothermic enthalpies for mixtures decreased with increasing content of KGM in mixtures. Moreover, these main peaks became very broad for mixtures with excessive KGM. For 0.8% KGM alone, a cooling or heating DSC curve did not show any exothermic or endothermic peak, indicating that KGM alone does not show any conformational change.

Figure 9 shows cooling and heating DSC curves for solutions of 0.3% GELL alone or mixtures with GELL/KGM = 0.3/0.5 containing various concentrations of NaCl. As is seen in this figure, in all cases, the DSC cooling curves for both 0.3% GELL solutions and mixtures showed a main exothermic peak with T_s shifting to progressively higher temperatures with increasing concentration of added NaCl, although many other peaks were observed at both higher and lower temperatures with increasing concentration of NaCl. In the cooling DSC curves, T_s for mixtures was significantly higher than that for 0.3% GELL alone in the presence of the same concentration of NaCl. However in the absence of salts, T_s for a mixture was slightly lower than that for solutions of 0.3% GELL alone, as will be shown later (Figure 11a). This indicated that monovalent cations promote the formation and aggregation of helices of GELL molecules rather than interaction between GELL and KGM. Although the exothermic enthalpies in cooling DSC curves for both gellan gum alone and mixtures increased with increasing concentration of NaCl, the exothermic peak for a mixture with 75 mM NaCl became much sharper and this enthalpy significantly increased compared to that for a 0.3% GELL alone with 75 mM NaCl. This result was in good agreement with the rheological result; both moduli for a mixture with 75 mM NaCl significantly increased and this solution behaved as an elastic gel even at 30 °C, although a solution of 0.3% GELL alone with 75 mM NaCl could not form a gel at any temperature from 30 to 0 °C. For solutions of 0.3% GELL, T_s in the cooling DSC curves shifted to higher temperatures with increasing concentration of NaCl. However, upon addition of 50 or 75 mM NaCl, the heating DSC curve split into multiple peaks around 40–60 °C, which was similar to the results in the previous study (Miyoshi et al., 1994b, 1995a). We found in the previous studies

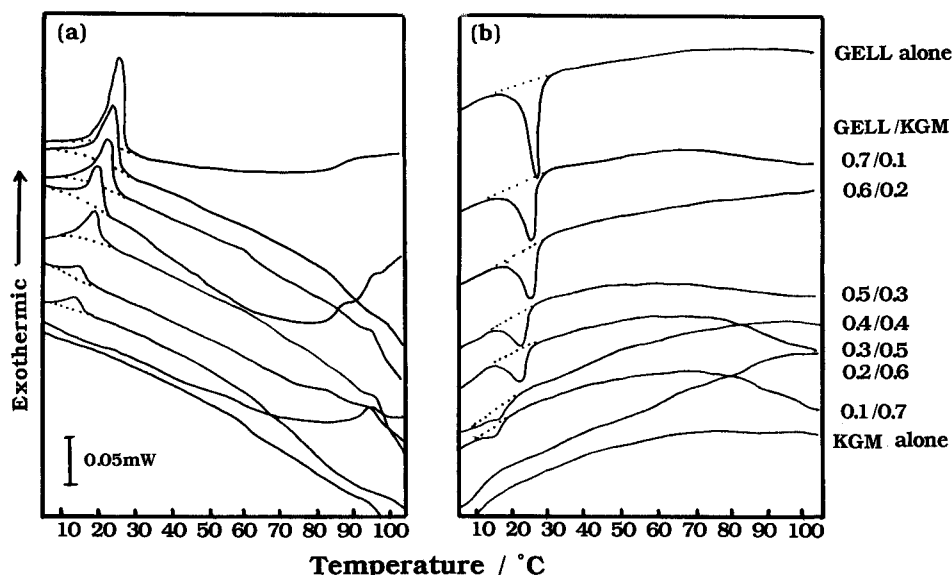


Figure 8. Cooling (a) and heating (b) DSC curves for GELL/KGM mixtures (total polysaccharide concentration 0.8%) with various mixing ratios. Cooling and heating rate, 0.5 °C/min.

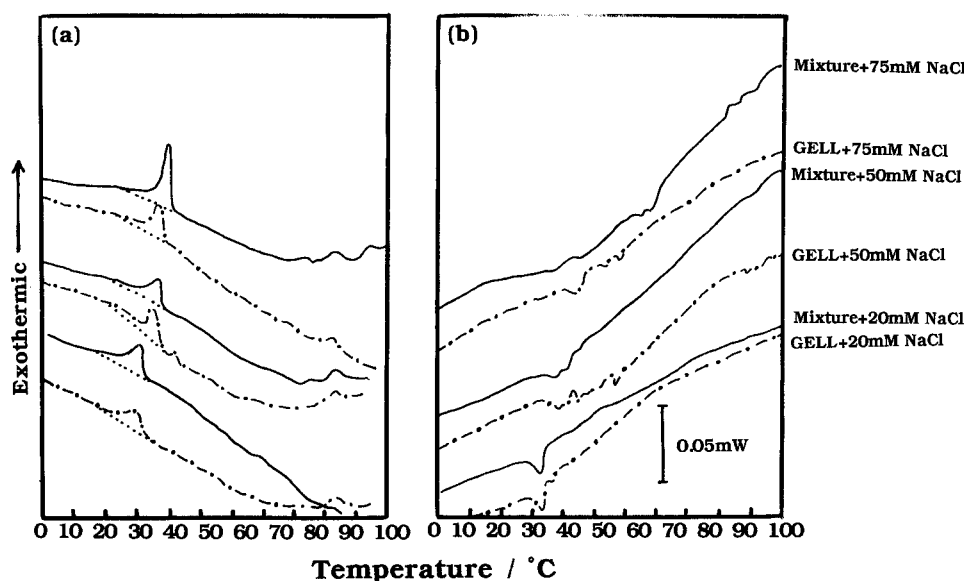


Figure 9. Cooling (a) and heating (b) DSC curves for mixtures with GELL/KGM = 0.3/0.5 (—) or 0.3% GELL solutions (---) containing NaCl of various concentrations.

(Miyoshi et al., 1994a,b) that the occurrence of detectable splitting in the heating DSC curves was observed for 1% gellan gum solutions with sufficient monovalent cations and coincided with the occurrence of elastic gel formation at relatively higher temperatures such as 30 °C. However, in the present study, the concentration of GELL investigated (0.3%) was too low to form an elastic gel even in the presence of sufficient monovalent cations, although solutions of 0.3% GELL with sufficient monovalent cations showed multiple endothermic peaks. T_m for mixtures, as well as T_s , was significantly higher than that for solutions of 0.3% GELL alone in the presence of the same concentration of NaCl. T_m for mixtures shifted to higher temperatures and the heating DSC curves for mixtures became broader with increasing concentration of NaCl. However, upon addition of 75 mM NaCl, the heating DSC curve for a mixture showed a main endothermic peak around 65 °C, and this temperature was significantly higher than that for 0.3% GELL solution with 75 mM NaCl. The fact that the main endothermic peak for a mixture with 75 mM NaCl was observed around 65 °C could explain the rheological result that elastic moduli for a mixture with 75 mM

NaCl did not show any drastic change during heating to 50 °C (Figure 3c). This result indicated that KGM could shift the helix-coil transition temperature in GELL molecules to higher temperatures, and in the presence of cations, KGM could contribute to the formation of thermally stable gels.

Figure 10 shows cooling and heating DSC curves for 0.3% solutions of GELL alone or of mixtures with GELL/KGM = 0.3/0.5 containing of various concentrations of CaCl₂. In the presence of CaCl₂, the cooling DSC curves for both GELL alone and mixtures showed a main exothermic peak around 15–35 °C and another small peak around 95 °C. Although T_s for both solutions of 0.3% GELL alone and mixtures shifted to higher temperatures with increasing concentration of CaCl₂, T_s for mixtures almost coincided with that for a 0.3% GELL solution in the presence of the same concentration of CaCl₂. The endothermic enthalpies for both GELL alone and mixtures showed a maximum at 1.7 mM CaCl₂ and then decreased with increasing concentration of CaCl₂. Upon addition of 6.8 mM CaCl₂, the cooling DSC curve for a mixture became much broader and this exothermic enthalpy significantly decreased compared

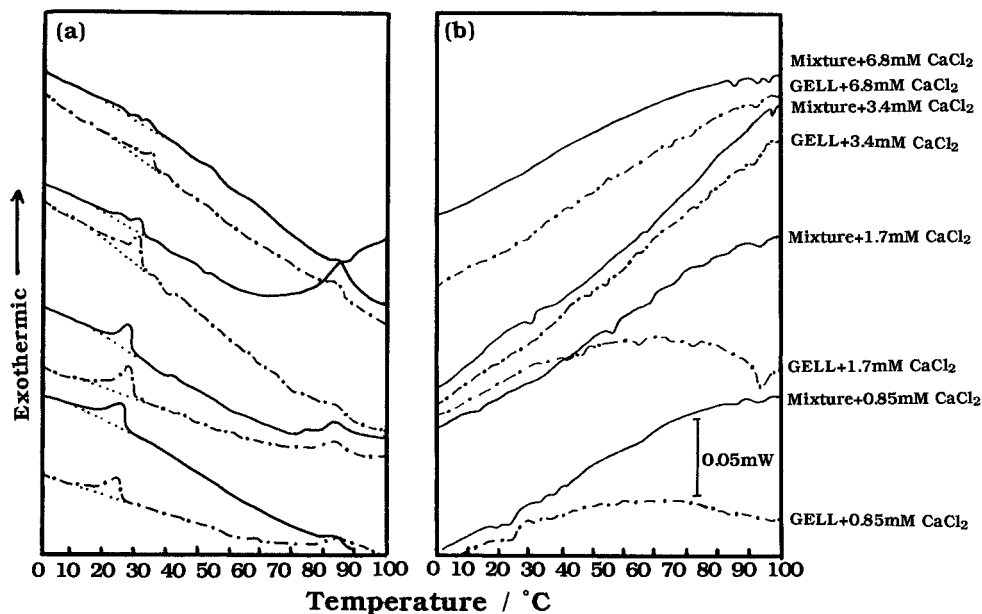


Figure 10. Cooling (a) and heating (b) DSC curves for mixtures with GELL/KGM = 0.3/0.5 (—) or 0.3% GELL solutions (---) containing CaCl_2 of various concentrations.

to that for a 0.3% GELL alone with 6.8 mM CaCl_2 . This corresponds well to the rheological result. Upon addition of 0.85 mM CaCl_2 , the heating DSC curve for both gellan gum alone and a mixture showed a main endothermic peak around 25 °C. However, the endothermic enthalpy for this mixture was significantly smaller than that for a 0.3% GELL solution with 0.85 mM CaCl_2 and other endothermic peaks were observed at much higher temperatures. For both 0.3% GELL solution and mixtures with 1.36 mM CaCl_2 , many small peaks were observed at both lower and higher temperatures and the largest peak was observed around 102 °C. With progressive addition of CaCl_2 , the heating DSC curves for both 0.3% GELL solution and mixtures became broader and many small peaks shifted to higher temperatures. As illustrated in the previous study (Miyoshi et al., 1994b, 1995a,b), although divalent cations promoted the formation of thermally stable junction zones much more strongly than monovalent cations, GELL solutions with sufficient divalent cations formed various junction zones with different thermal stabilities. Therefore, it was considered that sufficient divalent cations also induced inhomogeneous mixtures of GELL and KGM, so that the synergistic interaction between GELL and KGM molecules may be inhibited by the addition of sufficient divalent cations.

Figure 11 shows the dependence of the exothermic peak temperature T_s (a) and the main exothermic enthalpy ΔH_s (b) for mixtures with GELL/KGM = 0.3/0.5 on the concentration of NaCl, compared to those for solutions of 0.8 or 0.3% GELL alone. In all three cases, T_s shifted to higher temperatures with increasing concentration of NaCl. Although in the absence of NaCl, T_s for 0.8% GELL alone was significantly higher than that for 0.3% GELL alone or mixtures, T_s for mixtures drastically shifted to higher temperatures with increasing concentration of NaCl. Eventually, T_s for a mixture with 75 mM NaCl became higher rather than that for 0.8% GELL alone with 75 mM NaCl. This tendency coincided with the results of T_M in the rheological measurement. Although in the absence of salts, ΔH_s for a mixture was significantly smaller than that for a 0.8 or 0.3% GELL alone, ΔH_s for both 0.8 and 0.3% GELL alone did not change so much with increasing concentration of NaCl. In contrast, ΔH_s for mixtures

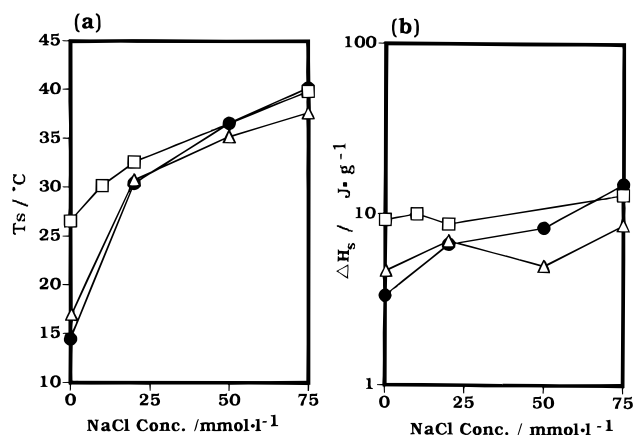


Figure 11. Dependence of the exothermic peak temperature T_s (a) and the exothermic enthalpy ΔH_s (b) for mixtures with GELL/KGM = 0.3/0.5 (total polysaccharide concentration 0.8%) or 0.3 or 0.8% GELL solutions on the concentration of NaCl: (●) mixture; (Δ) 0.3% GELL; (□) 0.8% GELL.

markedly increased with increasing concentration of NaCl. Eventually, upon addition of 75 mM NaCl, ΔH_s for mixture became larger than that for not only a 0.3% GELL alone but also a 0.8% GELL alone. This behavior of T_s and ΔH_s induced by the concentration change of NaCl coincided with those of T_M and ΔG in the rheological measurement.

Figure 12 shows the dependence of the exothermic peak temperature T_s (a) and the main exothermic enthalpy ΔH_s (b) for mixtures with GELL/KGM = 0.3/0.5 on the concentration of CaCl_2 , compared to those for solutions of 0.8 or 0.3% GELL alone. In all three cases, ΔH_s increased with increasing concentration of CaCl_2 , as well as T_s . In the absence of salt, T_s for a 0.8% GELL solution was significantly higher than that for a mixture or a 0.3% GELL solution. However, T_s for both mixtures and 0.3% GELL alone drastically increased with increasing concentration of CaCl_2 , and upon addition of 6.8 mM CaCl_2 , these values almost coincided with T_s for a 0.8% GELL alone. Although T_s for a mixture without salt was significantly lower than that for a 0.3% GELL alone without salt, in the presence of CaCl_2 , T_s for mixtures became similar to that for solutions of 0.3% GELL alone. ΔH_s for 0.8% GELL

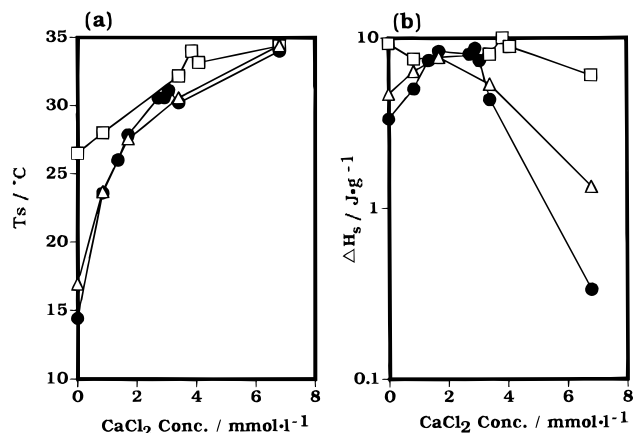


Figure 12. Dependence of the exothermic peak temperature T_s (a) and the exothermic enthalpy ΔH (b) for mixtures with GELL/KGM = 0.3/0.5 (total polysaccharide concentration 0.8%) or 0.3 or 0.8% GELL solutions on the concentration of CaCl_2 : (●) mixture; (Δ) 0.3% GELL; (\square) 0.8% GELL.

alone did not change so much with increasing concentration of CaCl_2 ; however, it slightly decreased by the addition of >4 mM CaCl_2 . ΔH_s for both mixtures and 0.3% GELL alone showed a maximum at 1.7 mM CaCl_2 and then significantly decreased with increasing concentration of CaCl_2 , which was in good agreement with the result of ΔG in the rheological measurement. The maximum values of ΔH_s for a mixture was slightly larger than that for a 0.3% GELL alone; however, in the presence of sufficient CaCl_2 , ΔH_s for mixtures was significantly smaller than that for 0.3% GELL alone.

DISCUSSION

In many hydrocolloid mixed systems containing two polymers in a common solvent, the polymers may or may not associate, as described in the Introduction. Association is usually described to explain the synergistic effects observed in some of mixtures, e.g., whey proteins and carboxymethyl cellulose (Hidalgo and Hansen, 1971) or xanthan and konjac glucomannan (Morris et al., 1991; Williams et al., 1991), whereas segregation is thought of as a microphase separation, driven by incompatibility between the two polymers (Annable et al., 1994b; Piculell et al., 1994).

The reason for the increase of G' by the addition of KGM may be ascribed to the following three possibilities: (1) an increase of effective concentration of gellan gum due to volume exclusion; (2) segregation due to thermodynamic incompatibility; (3) association of gellan and KGM. If molecular association occurs between GELL and KGM, the increase in G' should be more prominent in a mixture with higher molecular weight KGM. However, as illustrated in the previous study using three KGM samples (Nishinari et al., 1995), no synergistic interaction was observed in mixtures of GELL and KGM with the highest molecular weight, while mixtures of GELL and KGM with the lowest molecular weight showed a synergistic interaction over a wider temperature range as well as over a wider mixing ratio. Therefore, in the case of mixtures of GELL/KGM with the highest molecular weight, the first or second possibility seems to be more plausible. However, in the case of GELL/KGM with intermediate molecular weight (the same sample in the present study), the frequency dependence changed a gel-like behavior below 15 °C at a mixing ratio of GELL/KGM = 0.6/1.0 (total polysaccharide concentration 1.6%). As shown in the present study, the synergistic interaction

occurs only at 0 °C at a same mixing ratio of GELL/KGM = 0.3/0.5 (total polysaccharide concentration 0.8%) (Figure 1c). In the present study, solutions of 0.3% GELL alone could not form gels even if monovalent or divalent cations were present; however, a gel was formed on addition of 0.5% KGM at 0 °C (Figure 1c). This fact indicated that gel-like behavior was observed when the polymers are mixed at fairly low concentrations, where exclusion effects are likely to be negligible. We suggested that mixed aggregates of GELL/KGM consist of a core of aggregates of GELL helices with KGM chains attached to the surface, as discussed later. A similar conclusion was reached in recent studies on gels of κ -carrageenan mixed with galactomannan or glucomannan (Williams et al., 1993; Morris, 1990; Piculell and Lindman, 1992; Piculell et al., 1994). In this work, it seems that KGM chains attach to self-aggregates of GELL helices, rather than individual GELL helices. This reason could be explained as follows. In the cooling process, the individual helices of GELL molecules were formed at a certain temperature, and then these helices gradually aggregated with decreasing temperature, and as result, a certain ordered structure was formed. As shown in the both previous and present studies, the synergism of GELL and KGM occurred at much lower temperatures than the coil-helix transition temperature. Therefore, it was suggested that the synergism should occur at low temperatures, where individual helices in GELL molecules are sufficiently aggregated. This is similar to the results of Piculell et al. (1994) that the synergistic interaction disappeared in mixtures of KGM and κ -carrageenan in the presence of iodide ions, which inhibit the self-aggregation of κ -carrageenan helices. Since in the absence of cations, ΔG and ΔH_s for a mixture were slightly smaller than that for a 0.3% GELL alone (Figure 5b or Figure 6b and Figure 11b or Figure 12b), KGM molecules may hinder further aggregation of GELL helices. Upon addition of KGM, the interaction between GELL and KGM occurred so that the topological entanglement may act as temporary cross-linking point. In other words, the main ordered structure for mixtures was formed by aggregates of GELL helices and KGM attached to aggregates and forming weak junction zones. It seems that the degree of mixed aggregates is governed by the degree of self-aggregated GELL helices and by the mixing ratio of GELL/KGM. Upon addition of salt to mixtures, unequal partitioning of salt in mixtures occurred; hence, GELL-rich phase kept a higher ionic strength so that the coil-helix transition of GELL molecules in mixtures shifted to further high temperatures. Thus, in the presence of cations, both T_M in the rheological measurement and T_s in the DSC measurement slightly shifted to higher temperatures than those for 0.3% GELL alone (Figure 5a, Figure 6a, Figure 11a, and Figure 12a). Since cations can promote the aggregation of helices in gellan gum molecules by a screening effect, the introduction of cations to mixtures should permit KGM chains attached to aggregates of GELL helices at higher temperatures. The mixed aggregates of GELL and KGM became larger with increasing concentration of cations, because the self-aggregates of GELL helices became gradually larger with increasing concentration of cations (Grasdalen and Smidsrod, 1987; Manning, 1992; Miyoshi et al., 1994a,b, 1995a,b). Thus, elastic moduli for mixtures with 75 mM NaCl or 1.7 mM CaCl_2 (Figure 3c and Figure 4b) showed larger values and formed a gel at relatively higher temperatures, compared to a mixture in the absence of cations (Figure 2c). ΔG and ΔH_s for mixtures with 75

mM NaCl or 1.7 mM CaCl₂ also became larger than those for a 0.3% GELL alone with the same concentration of cations (Figure 5b, Figure 6b, Figure 11b, and Figure 12b). Moreover, since in the heating process, KGM molecules that are attached to large aggregates of GELL helices could retard the helix-coil transition in GELL molecules, the melting temperature shifted to higher temperatures; hence, a larger thermal hysteresis was observed for these solutions (Figure 3c and Figure 4b).

In the previous studies (Miyoshi et al., 1994b, 1995a,b), we reported that, in the presence of sufficient divalent cations, GELL could form various junction zones with different thermal stabilities. In the present study, many self-aggregates of GELL helices in mixtures were formed by the addition of sufficient divalent cations and then KGM chains could attach to each surface of its aggregate. However, since the size of each aggregate of GELL helices became smaller by the addition of sufficient divalent cations, the size of mixed aggregates also became smaller. This may explain the fact that the cooling DSC curve for mixtures with sufficient divalent cations became much broader compared to that for 0.3% GELL alone with the same concentration of divalent cations or mixtures with lower concentration of divalent cations. Thus, the phase separation in GELL/KGM mixtures was promoted by addition of sufficient divalent cations, which should lead to weaker ordered structures. This explanation is favored by the possibility that the intermolecular binding complexes between GELL and KGM molecules could not form.

It was noteworthy that mixtures with a low concentration of divalent or monovalent cations could not form gels even at 0 °C (data not shown), although a mixture without salt could form a weak gel only at 0 °C (Figure 1c). If KGM interacts with GELL in the coil conformation, since the electrostatic shield leads to the reduction of coil dimensions (Robinson et al., 1991; Miyoshi et al., 1994a), the overlap between GELL coils and KGM chains should decrease in the presence of salts, and hence, synergistic interaction between GELL and KGM would decrease. However, since with more progressive addition of salt, the elastic moduli for mixtures significantly increased and gel formation was observed, this possibility should be excluded. Although KGM was uncharged, cations changed water structures around KGM molecules or GELL molecules, so that the chance that KGM chains attach to the self-aggregated GELL helices may decrease. This simply may relate to the low total polysaccharide concentration; however, it seems to be difficult to explain this phenomenon by a simple factor.

Annable et al. (1994b) studied mixed potato maltodextrin (PMD)/hydrocolloid systems and reported that, in the case of mixed PMD/carboxymethyl cellulose (CMC), phase separation could not be observed at all in the absence of electrolyte for the compositions investigated but was observed when electrolyte was present. They also found that, in the case of mixed PMD/gum arabic phase separation was observed without added electrolyte but only at very high polymer concentration. Both high polymer concentration and addition of salt to such mixtures (one of polymers in mixture carried an ionic charge) should generally result in an increase of segregation. If the concentration of gellan gum increases, where gellan gum itself could form a gel whether cations are present or not, segregation due to thermodynamic incompatibility was promoted, so that no synergistic interaction may be observed in such high

polymer concentration mixtures. This should be investigated in further work.

We conclude that KGM is adsorbed onto the surface of large aggregates of GELL helices, and not only the gellan gum self-aggregation but also these interactions between GELL and KGM were promoted with increasing concentration of cations. Since sufficient divalent cations formed various aggregates of GELL helices with different thermal stabilities, the phase separation in GELL/KGM mixtures would be promoted in the presence of sufficient divalent cations. However, the present data were obtained only from rheological and thermal measurements. Indeed, more detailed studies based on other methods to describe the phenomena at the molecular level are required.

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