Investigation of the Gelation Mechanism in κ-Carrageenan/Konjac Mannan Mixtures Using Differential Scanning Calorimetry and Electron Spin Resonance Spectroscopy

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ABSTRACT: Differential scanning calorimetry (DSC) and electron spin resonance spectroscopy (ESR) have been used to probe the mechanism of gelation in gels formed by mixtures of konjac mannan and κ-carrageenan and have indicated that an association occurs between konjac mannan chains and (aggregated) κ-carrageenan helices. On cooling mixed systems at low ionic strength, a coil–helix transition of κ-carrageenan, as evidenced by a DSC exothermic peak (peak I), occurs at a temperature significantly higher than the "normal" transition temperature observed for κ-carrageenan alone. This transition is accompanied by a partial immobilization of the konjac mannan chains, as evidenced by ESR spectroscopy. When κ-carrageenan is in excess, a second transition (peak II) appears at the normal transition temperature. Both transitions are ion specific, and they shift in the same way with added salt, which strongly suggests that they both reflect the formation of normal κ-carrageenan helices. At sufficiently high ionic strength, only one transition is apparent in the DSC curve, and its effect on the mobility of the konjac mannan chains is considerably reduced. Both the peak I and peak II transitions show a marked thermal hysteresis, as evidenced by DSC heating experiments, which demonstrate that both transitions are accompanied by aggregation processes subsequent to the coil–helix transition. The transition enthalpy per mole of carrageenan is significantly less in the mixtures than for κ-carrageenan alone, indicating that the κ-carrageenan self-aggregation is affected by the presence of konjac mannan. The results are interpreted in terms of the formation of mixed aggregates of κ-carrageenan helices and konjac mannan, possibly involving bundles of self-aggregated κ-carrageenan helices covered with surface-adsorbed konjac mannan chains.

Introduction

Locust bean gum is a galactomannan obtained from the seeds of the carob tree (*Ceratonia siliqua*) and is commonly used in conjunction with κ-carrageenan in food products since it leads to gels with increased elasticity and strength and with a greater resistance towards syneresis.[1] Konjac mannan is a glucomannan obtained from the tubers of *Amorphophallus konjac*, and recently its potential for enhancing κ-carrageenan gel characteristics has been illustrated.[2]

The nature of the interaction between galactomannans and κ-carrageenan has been the subject of much interest since the early work of Rees[3,4] and Dea and co-workers[5–7] and is still a matter of controversy.[8,9] Rees[3,4] and Dea and co-workers[5–7] showed that gelation becomes more pronounced as the proportion of galactose residues along the mannan chain is reduced and proposed that a specific interaction occurs between unsubstituted regions along the ordered ribbon-like galactomannan backbone and the κ-carrageenan double helix. This conclusion was drawn essentially from optical rotation studies, and other observations including the facts that (i) galactomannans could induce segmented (very low molecular mass) nongelling carrageenan to gel, (ii) cuprammonium ions could not complex with galactomannans below the gel threshold temperature, and (iii) the liquor that leaked from the mixed gels after freeze–thaw treatment contained only galactomannan. X-ray diffraction studies by Morris and co-workers,[8–13] however, did not provide evidence for a specific interaction, and potassium and sulfur atom mapping of gels by monitoring X-ray emission found them to be evenly distributed throughout the gel matrix. This led them to conclude, therefore, that galactomannans and glucomannan were not incorporated into the carrageenan crystalline junction zones and hence did not contribute to the gel structure. This conclusion, however, does not explain the differences in gel properties observed between mixed gels formed with various galactomannans and glucomannan, and Morris and co-workers[11] later suggested that the model of Rees[3,4] and Dea and co-workers[5–7] could be modified to take into account the X-ray diffraction studies by considering that the gels contain large aggregates or microcrystalline regions linked by surface attachment of galacto- or glucomannan chains.

Tako and Nakamura[14] monitored the rheological properties of carrageenan/galactomannan mixtures and concluded that association occurred between galactomannan and single-stranded helical carrageenan molecules in contrast to the model of Rees and Dea and co-workers. At concentrations where the carrageenan was in excess, Tako and Nakamura noted that carrageenan self-association also took place. Rochas et al.[17] and Turquois et al.[18] provided further evidence for an association between carrageenan and galactomannans based on ^{13}C NMR and dialysis experiments although they did add the proviso that their observations could also be explained by autogelation of the galactomannan. This possibility was also suggested by Fernandes et al.[16] based on rheological measurements. Initial studies reported by us[19] using electron spin resonance spectroscopy (ESR) and differential scanning calorimetry (DSC) also indicated that association occurs in these systems, and this paper describes further work that has been carried out to elucidate the mechanism of interaction in konjac mannan/carrageenan mixtures.

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Experimental Section

Materials. Konjac mannan flour was obtained from FMC Marine Colloids Division and its characteristics have been described in detail elsewhere.[19] A portion of the konjac mannan was spin labeled through the hydroxyl groups along the polymer chain with 4-amino-Tempo according to the procedure previously reported.[20]

The κ-carrageenan sample was also obtained from FMC Marine Colloids Division and was converted to the sodium salt form by ion exchange using Amberlite IR 120 exchange resin. Once in the sodium form, the κ-carrageenan was dialyzed against distilled water for 1 week changing the water daily and freeze-dried.

Methods. Differential Scanning Calorimetry (DSC). Konjac mannan solutions were prepared by dissolving the flour in water at 85 °C for 1 h while mixing with a mechanical stirrer. The solutions were then centrifuged to remove insoluble material, and the quantity of polysaccharide in solution was accurately determined by dry weight analysis. Sodium κ-carrageenan solutions were prepared by dissolving the freeze-dried material in water at room temperature. Konjac mannan/sodium κ-carrageenan mixtures at various ratios, but containing 0.6% total polysaccharide, were prepared by mixing the appropriate amounts of each powder. The mixture was then heated to 85 °C for 30 min and various amounts of 2.5 mol dm^{-3} potassium chloride solution were added with continuous stirring to give final solution concentrations of 0.003–0.3 mol dm^{-3} KCl after accounting for evaporation losses.

Differential scanning calorimetry was carried out using a Setaram micro DSC "batch and flow" calorimeter. The calorimeter was fitted with 1-cm^3 batch sample and reference vessels, and the cooling and heating behavior of the various konjac mannan/κ-carrageenan mixtures was monitored between 10 and 85 °C. The samples were initially subjected to a heating/cooling cycle to ensure the solutions had the same thermal history.

Electron Spin Resonance (ESR) Spectroscopy. Mixtures of konjac mannan/κ-carrageenan solutions were prepared using the spin-labeled konjac mannan. Again a total polysaccharide concentration of 0.6% was maintained. The mixed solutions were poured into a flat quartz ESR cell while hot, and the ESR spectra were recorded at various temperatures on cooling from 75 to 25 °C at a cooling rate of 1 °C/min using a Bruker ESP300 ESR spectrometer.

Results

The DSC cooling curves for κ-carrageenan/konjac mannan mixtures in the presence of 0.05 mol dm^{-3} KCl are given in Figure 1. When the konjac mannan content is in excess (curves A and B), a single exothermic peak is observed with the midpoint temperature, T_{gel}, at 43 °C. This peak was shown to correspond to gelation as monitored rheologically as reported previously.[19] At approximately equivalent κ-carrageenan/konjac mannan ratios (curve C), the gelation peak appeared slightly broader. For mixtures with κ-carrageenan in excess (curves D–F), the cooling curves showed two exothermic peaks, one with T_{gel} at 43 °C (peak I) and the other with T_{gel} at 38 °C (peak II). κ-Carrageenan alone gave a single exothermic peak with T_{gel} at 38 °C (curve G), while konjac mannan alone did not gel under these conditions and there was no peak observed on cooling. The intensities (in terms of peak height) of peaks I and II are plotted as a function of the mixing ratio in Figure 2. Peak I increases as the κ-carrageenan content increases up to a mixing ratio of ~1:1 and then decreases to zero at 100% κ-carrageenan. Peak II is present only when the κ-carrageenan content is in excess and increases as the κ-carrageenan concentration increases. The enthalpy of gelation, ΔH_g, for the mixtures calculated by taking the total area under the curves is given in Figure 3 expressed in units of J g^{-1} of κ-carrageenan present in the mixture, ΔH_g values obtained for solutions of κ-carrageenan alone at various concentrations are also shown for comparison. In all cases ΔH_g for the mixed system was less than that for κ-carrageenan by itself.

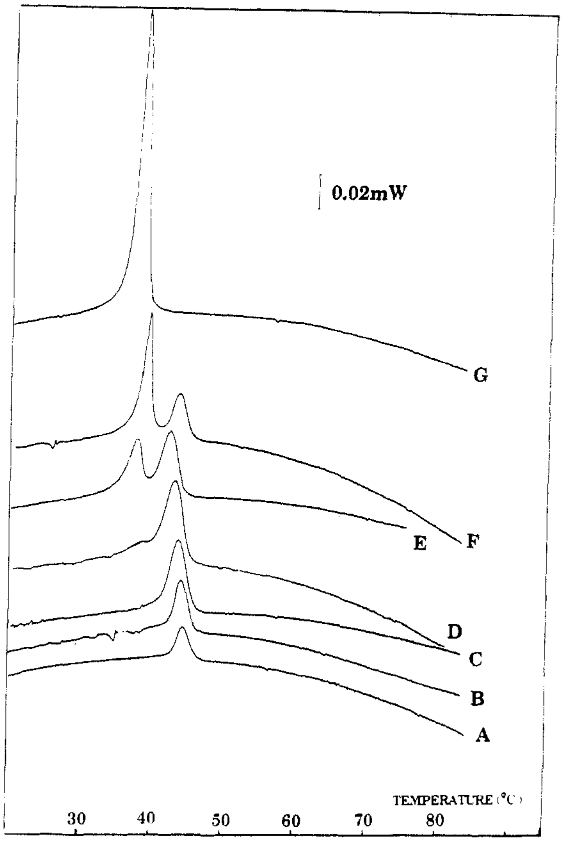

Figure 1. DSC cooling curves for various ratios of κ-carrageenan/konjac mannan mixtures in the presence of 50 mM KCl (0.6% total polymer concentration). Scanning rate was 0.1 °C/min. % κ-carrageenan/% konjac mannan: (A) 0.1/0.5; (B) 0.2/0.4; (C) 0.3/0.3; (D) 0.4/0.2; (E) 0.45/0.15; (F) 0.5/0.1; (G) 0.6/0.

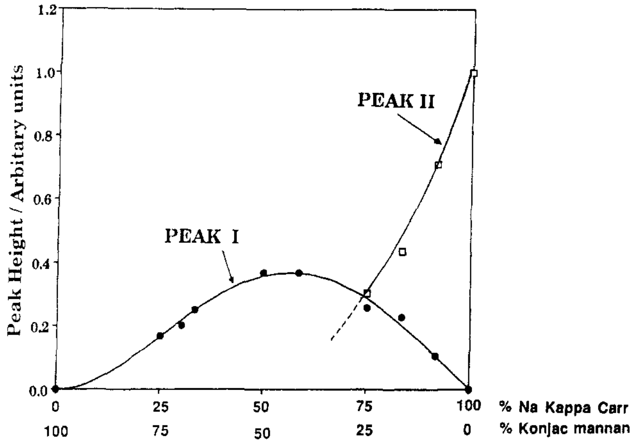

Figure 2. DSC peak heights of the higher (peak I) and lower (peak II) setting peaks as a function of the ratio of κ-carrageenan/konjac mannan in the mixture (0.6% total polymer concentration).

The DSC heating curves for κ-carrageenan/konjac mannan mixtures in the presence of 0.05 mol dm^{-3} KCl are given in Figure 4. When the konjac mannan content is in excess (curves A and B), a single endothermic peak is observed corresponding to gel melting with a midpoint temperature, T_m, at 63 °C. As the κ-carrageenan ratio increases (curves C–E), the peak broadens, and at higher κ-carrageenan ratios (curve F), a second melting peak appears with T_m at about 50 °C. This corresponds to the peak (curve G) obtained for carrageenan alone.

The effect of potassium ion concentration for a mixture showing two setting peaks (0.5% κ-carrageenan/0.1% konjac mannan) is illustrated in Figure 5 and raises several points of interest. The potassium causes the peaks to shift to higher temperatures, with both peaks I and II shifting by approximately the same degree. However, the

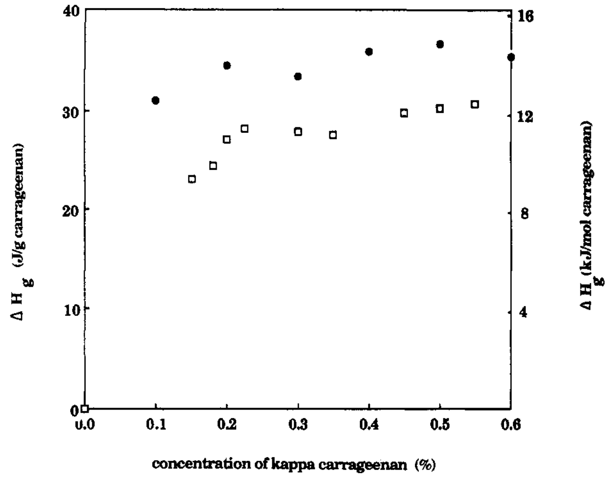

Figure 3. Enthalpy of gelation in J/g of κ-carrageenan for solutions of: (●) κ-carrageenan alone in 50 mM KCl and (□) κ-carrageenan/konjac mannan mixtures (0.6% total polysaccharide concentration) in 50 mM KCl.

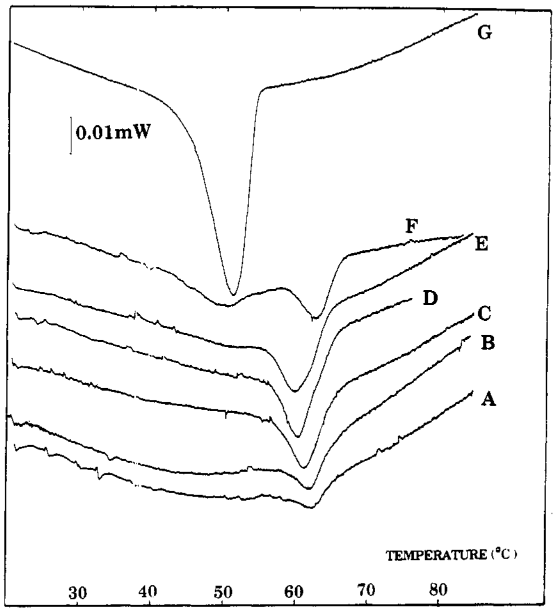

Figure 4. DSC heating curves for various ratios of κ-carrageenan/konjac mannan mixtures in the presence of 50 mM KCl (0.6% total polymer concentration). Scanning rate was 0.1 °C/min. % κ-carrageenan/% konjac mannan: (A) 0.1/0.5; (B) 0.2/0.4; (C) 0.3/0.3; (D) 0.4/0.2; (E) 0.45/0.15; (F) 0.5/0.1; (G) 0.6/0.

intensity of peak II increases at the expense of peak I with increasing potassium ion concentration until at 200 mmol dm^{-3} KCl where peak I appears to have disappeared completely. The plot of log(total cation concentration) (i.e., K$^+$ added and Na$^+$ counterion) against $1/T_{gel}$ is illustrated in Figure 6 both for peak II and peak I for the 0.5% κ-carrageenan/0.1% konjac mannan mixture. Both peaks give good linear plots as observed by other workers for carrageenan itself[21,22] with the slopes of the lines for the two peaks being the same at −6.69 × 10^3 K. Also shown in Figure 6 is the plot for κ-carrageenan in the absence of konjac mannan which has a marginally higher slope (−6.97 × 10^{-3} K) than those of the peaks in the mixtures.

In solutions containing 200 mmol dm^{-3} KCl the enthalpy associated with the gelation process for mixed κ-carrageenan/konjac mannan systems is less than that for κ-carrageenan alone. This is illustrated in Figure 7, which shows the DSC cooling curves for 0.3% κ-carrageenan/0.3% konjac mannan and 0.3% κ-carrageenan alone. The enthalpy for gelation of the mixed system (curve A) is 13.5 J g^{-1} of κ-carrageenan, while for κ-carrageenan alone it is 23.4 J g^{-1} of κ-carrageenan.

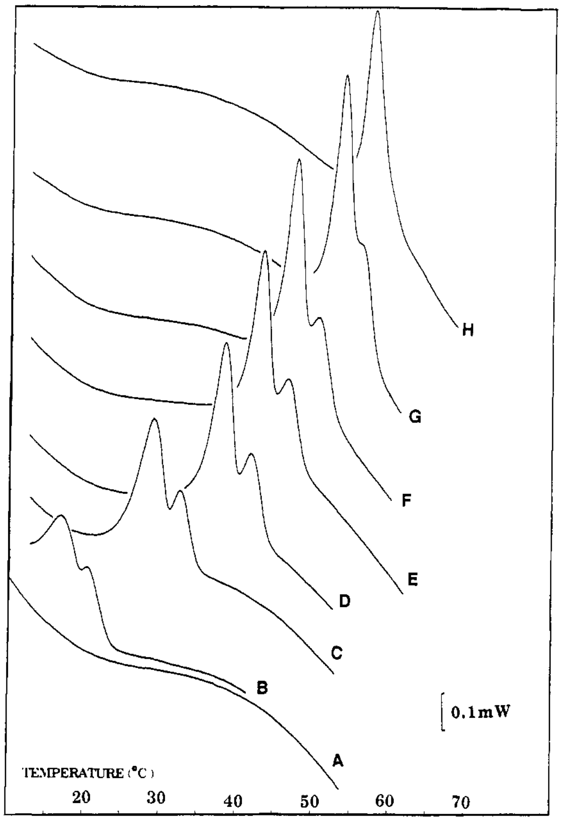

Figure 5. Effect of increasing potassium ion concentration on the DSC cooling curves for a 0.5% κ-carrageenan/0.1% konjac mannan mixture. Scanning rate was 1 °C/min. (A) No KCl; (B) 10 mM KCl; (C) 25 mM KCl; (D) 50 mM KCl; (E) 75 mM KCl; (F) 100 mM KCl; (G) 150 mM KCl; (H) 200 mM KCl.

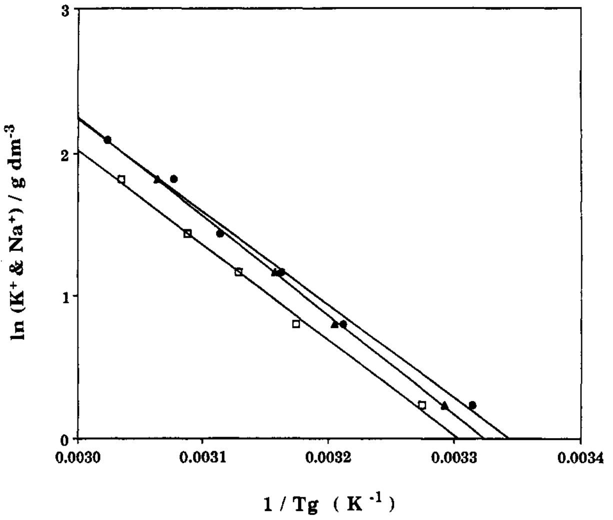

Figure 6. Plot of ln (total potassium and sodium concentration) against the reciprocal midpoint setting temperature (T_{gel}) for (●) 0.5% κ-carrageenan/0.1% konjac mannan, peak I; (□) 0.5% κ-carrageenan/0.1% konjac mannan, peak II; and (▲) 0.5% κ-carrageenan only.

The ESR spectra obtained for a 0.45% κ-carrageenan/0.15% konjac mannan mixture in the presence of 0.05 mol dm^{-3} KCl on cooling to temperatures just above the gelation temperature, at the gelation point, and just below the gelation temperature are given in Figure 8, and the ratio of the intensities of the low-field and center lines (h_{+1}/h_0), which can be used as an arbitrary measure of the mobility of the spin label, is given as a function of temperature in Figure 9. At high temperatures the spectra are essentially isotropic. On cooling, (h_{+1}/h_0) decreases as expected due to a reduction in the segmental motion of the polymer chains, and then at 43 °C, which corresponds to the temperature at which the DSC peak I appears, (h_{+1}/h_0)

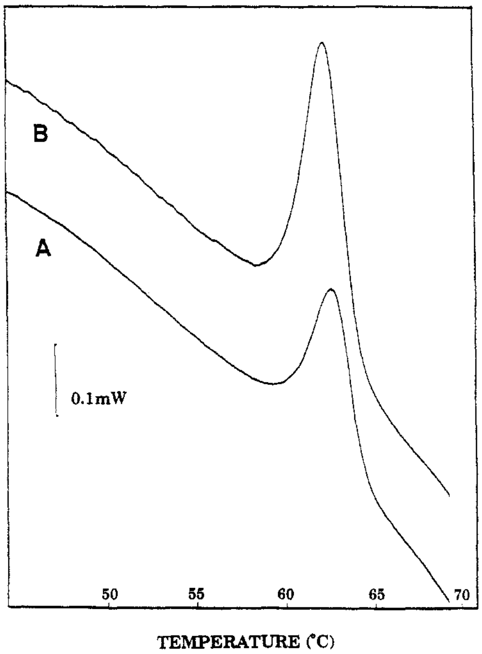

Figure 7. DSC cooling curves in 250 mM KCl for (A) 0.3% κ-carrageenan/0.3% konjac mannan and (B) 0.3% κ-carrageenan only. Scanning rate was 1 °C/min.

Figure 8. ESR spectra for 0.45% κ-carrageenan/0.15% konjac mannan mixture in 50 mM KCl illustrating the onset of line broadening close to the temperature corresponding to peak I in the DSC cooling curves: (A) above the gelation point; (B) at the gelation point; (C) below the gelation point.

shows an abrupt change and the spectra themselves show line broadening with the appearance of an anisotropic component. The spectra for a 0.2% solution of konjac mannan in isolation show no evidence of line broadening, and the plot of (h_{+1}/h_0) for this system, also given in Figure 9, shows no abrupt change in slope down to temperatures of 25 °C. The anisotropic component in the konjac mannan/κ-carrageenan mixture is believed to arise as a consequence of the aggregation of polymer segments as has been discussed previously,[20] and computer analysis reveals that it accounts for up to 30% of the total spectrum, indicating that 30% of the konjac mannan chain segments are involved in the aggregation process. The ESR spectra for a 0.5% κ-carrageenan/0.1% konjac mannan mixture in the presence of 200 mmol dm^{-3} KCl did not show any evidence of an anisotropic component following gelation. However, the plot of (h_{+1}/h_0) against temperature given in Figure 10 shows that the values decrease more rapidly than for konjac mannan alone as the temperature is reduced, signifying a greater reduction in segmental motion presumably brought about through interaction of konjac mannan and κ-carrageenan molecules.

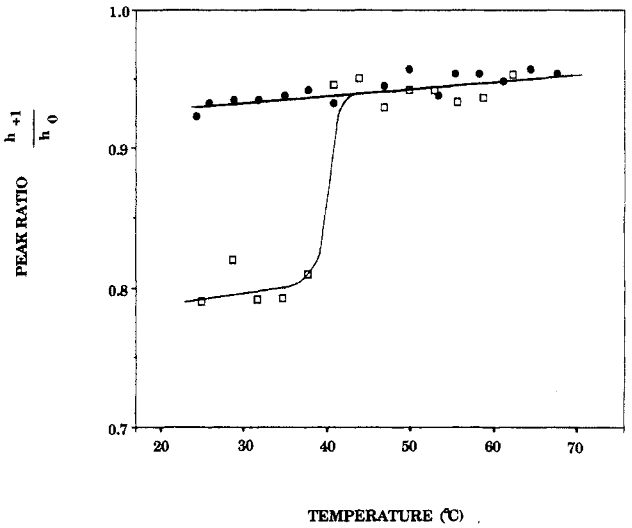

Figure 9. Plot of h_{+1}/h_0 for 0.45% κ-carrageenan/0.15% konjac mannan in 50 mM KCl (□) and 0.2% konjac mannan only (●) as a function of temperature on cooling the solutions.

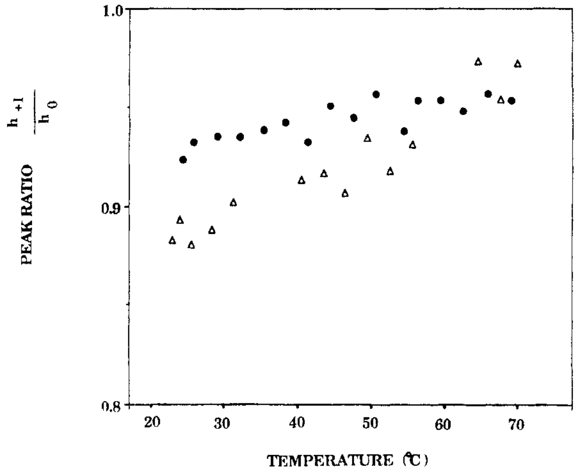

Figure 10. Plot of h_{+1}/h_0 for 0.5% κ-carrageenan/0.1% konjac mannan in 200 mM KCl (△) and 0.2% konjac mannan only (●) as a function of temperature on cooling the solutions.

Discussion

Before attempting an interpretation of the data presented here for the κ-carrageenan/konjac mannan mixtures, it is appropriate to review our present understanding of the gelation of κ-carrageenan alone. A necessary condition for gelation is that the carrageenan molecules form helices. However, helix formation is in itself not sufficient for gelation of κ-carrageenan, since certain salt conditions give rise to nongelling helical solutions.[23,24] Gelation only occurs under salt conditions when the helices aggregate further. The aggregation confers stability to the helices, and hence the gel melts at a temperature, T_m, which is higher than the gelation temperature, T_{gel}. Strong evidence suggests that T_{gel} is equal to the equilibrium temperature of helix formation, unaffected by subsequent aggregation. Thus, it is found that the variation of T_{gel} with salt under gelling conditions extrapolates into the salt dependence of the coil–helix transition temperature under nongelling conditions.[25] Both helix formation and aggregation of κ-carrageenan are strongly ion specific processes. For instance, certain monovalent cations, such as potassium, rubidium, and cesium, promote both helix formation and helix aggregation. Counterion NMR experiments have shown that helix formation is accompanied by changes in the chemical shifts, as well as dramatic increases in the line widths, of the gel-promoting cations, indicative of site binding of these ions.[23,26] A thermodynamic analysis has shown that both the cation specificity of the coil–helix transition and the ion-binding isotherms obtained from the NMR experiments can be consistently explained by the occurrence of binding sites, with an intrinsic ion-binding constant of the order of a few M^{-1}, on the κ-carrageenan helix.[27] It is emphasized that the sites are unique to the helical conformation of the low-sulfated carrageenans κ-carrageenan and furcellaran; no specific cation binding is found for disordered carrageenan chains, nor for the helical conformation of the more highly sulfated ι-carrageenan.[28] This implies that the cation binding is highly structure specific. No quantitative thermodynamic analysis of the effects of ions on helix aggregation has yet been carried out, but the indications are that the ion specificity of this process, found for κ-carrageenan, is a secondary effect, resulting from the decreased negative charge density, and hence reduced repulsion, caused by the site binding of the gel-promoting cations to the helices.

Turning now to the data on the κ-carrageenan/konjac mannan mixtures presented here, the two peaks (I and II) in the DSC cooling curves in 50 mmol dm^{-3} KCl under conditions of excess carrageenan demonstrate that two separate transitions occur in these mixtures. The correlation of the peak intensities with the κ-carrageenan content in the mixtures shows that both peaks may be ascribed to transitions involving κ-carrageenan. Moreover, all experimental features strongly suggest that the aggregates formed at both transitions (I and II) involve the same ordered conformation of κ-carrageenan, i.e., the κ-carrageenan helix, as is formed by κ-carrageenan alone, in the absence of konjac mannan. The transitions are promoted by the gel-inducing cations, and the salt-induced shift of T_{gel} is very similar, if not identical, for the three transitions (cf. Figure 6). This indicates that the ordered conformations formed at these different transitions all contain the same cation-binding sites and have the same charge density. This is strong evidence that they all correspond to the normal κ-carrageenan helix. This conclusion is further corroborated by preliminary experimental results from our laboratories combining DSC, NMR, and polarimetry on κ-carrageenan/konjac mannan mixtures in the prescence of CsCl. These experiments show that the high-temperature DSC transition (peak I) obtained on cooling is accompanied by significant cesium NMR shifts as well as an increase in optical rotation, both effects being very similar to those observed for κ-carrageenan alone. We may thus conclude that, under conditions of excess κ-carrageenan, the coil–helix transition of κ-carrageenan molecules in mixtures with konjac mannan occurs in two separate steps, each involving a fraction of the κ-carrageenan molecules, i.e., a high-temperature transition (corresponding to DSC peak I), which is affected by the presence of glucomannan (T_{gel} is shifted relative to T_{gel} of κ-carrageenan alone), and a transition at a slightly lower temperature (peak II), involving another part of the carrageenan, the position of which is unaffected by konjac mannan (T_{gel} is the same as for κ-carrageenan alone). The ESR data (Figure 9) indicate, furthermore, that the transition at peak I is accompanied by an association of the konjac mannan molecules and that this association process is completed over the temperature interval of peak I (no further association of konjac mannan occurs at lower temperatures). The simplest explanation of these findings and of the variations in peak intensities at different κ-carrageenan/glucomannan ratios (Figures 1 and 2) is that peak I corresponds to the formation of some kind of mixed aggregate, with a rather well-defined stoichiometry, between konjac mannan and κ-carrageenan helices. With excess κ-carrageenan, all the glucomannan chains (or possibly all the "reactive" parts of the chains) are consumed at the peak I transition before the conversion of κ-carrageenan chains to helices is complete; hence, the excess κ-carrageenan chains are unaffected, and form helices and gel at the normal, peak II temperature.

The various models discussed[11] for mixed polysaccharide gels prompt us to attempt to interpret the results in terms of a phase-separation event. In terms of such a model, peak II should be ascribed to the coil–helix transition of κ-carrageenan molecules in the separated phase, formed by a demixing which takes place as a result of the helix formation of some of the carrageenan molecules at peak I. Two cases may be distinguished for the phase separation. One is a segregation of κ-carrageenan helices and konjac mannan into two phases, each enriched in one of the polysaccharides, and the other is an association between κ-carrageenan helices and konjac mannan, resulting in the separation of a phase enriched in both polymers. An associative phase separation would be comparable with the formation of quasi-stoichiometric mixed aggregates, as proposed above, and peak II would then correspond to the transition of excess κ-carrageenan molecules in the remaining solution after the phase containing mixed aggregates has separated out. An interpretation of the data in terms of a segregative phase separation, on the other hand, would imply that the helical κ-carrageenan molecules self-aggregate (κ-carrageenan helices are known to aggregate under the salt conditions applied) and separate out in a phase of their own at peak I. To explain peak II by this mechanism, it has to be assumed that the remaining κ-carrageenan coils separate out with the helices (the position of peak II indicates that this transition takes place in an environment which does not contain konjac mannan), which does not seem very probable in view of the good miscibility between κ-carrageenan coils and konjac mannan. Furthermore, this interpretation would also imply that the konjac mannan molecules self-associate (cf. the ESR results, Figure 9) as a result of phase separation. The latter is considered to be unlikely, however, for the following reasons. First, the gelation process is reversible and konjac mannan self-association is well known for its reversibility. Second, we found that gelation did not occur for Na^+ carrageenan/konjac mannan mixtures in the absence of added potassium ions. Third, it is difficult to imagine a mechanism by which the helix formation and self-aggregation of κ-carrageenan molecules could induce a self-association of the konjac mannan molecules.

The increase in T_{gel} of transition I in the konjac mannan/κ-carrageenan mixtures, compared to T_{gel} of κ-carrageenan alone indicates that the coil–helix equilibrium in the presence of konjac mannan is shifted slightly toward an increased stability of the helix. It is tempting to ascribe this increased stability to an association of konjac mannan with the κ-carrageenan helix. This interpretation is complicated, however, by the occurrence of the hysteresis in the mixture, which clearly shows that additional aggregation occurs after the formation of the helices at peak I. In particular, this means that it is not clear at which stage the konjac mannan/κ-carrageenan association takes place. One possibility, as just noted, is that a primary mixed complex is formed involving a κ-carrageenan helix with one (or more) associated side chains. Such complexes would then have to further associate to account for the observed hysteresis. Another possibility is, however, that individual κ-carrageenan helices are first formed at peak I and that these self-aggregate, just as they would in the absence of konjac mannan. According to the latter view, the association of konjac mannan would occur at a later stage as an adsorption to the surfaces of the κ-carrageenan aggregates. The latter explanation is favored by the absence of evidence of mannan/κ-carrageenan complexation found in X-ray diffraction studies. Adsorption of konjac mannan onto the aggregated chains would inhibit further association, thus accounting for the lower enthalpy values for gelation of the mixture compared to carrageenan alone (Figure 3). Another reason not to uncritically ascribe the increase in T_{gel} to a favorable interaction between glucomannan chains and individual κ-carrageenan helices is that recent experiments on other mixtures indicate that an increased stability of the helical conformation by an added flexible polymer may be quite a general effect.[29,30] This may possibly be due to an increase in the configurational entropy of the added polymer if the helix-forming polymer is driven into its more compact helical form. More experiments and model calculations should clarify this point. At this stage, a comment on the apparent 1:1 stoichiometry of the κ-carrageenan/konjac mannan complex is appropriate. At first sight, this simple stoichiometry would seem to indicate the formation of a primary bimolecular complex involving one κ-carrageenan chain and one konjac mannan chain since both polymers are expected to have similar molecular mass values (~400 000). The stoichiometry is, however, also compatible with an aggregate consisting of a core of aggregated κ-carrageenan chains with adsorbed konjac mannan chains, provided that not all segments of the konjac mannan chains are adsorbed but that some parts extend in loops and tails, as implied by the ESR results presented here. (Note that an incomplete association of mannan chains is invoked in some models for synergistic galactan/mannan gelation: The nonbound fractions of the mannan chains serve to connect regions bound to different galactan aggregates, hence contributing to the network formation and the gel strength.) As shown in Figure 5, only one peak appears in the DSC cooling curves at sufficiently high concentrations of KCl. This means that as the salt concentration increases, either peak II grows at the expense of peak I or the transition temperatures of the two peaks merge at high salt. The experimental data do not allow a definite distinction between the two cases; indeed both processes may occur. A shift in the relative intensities of the two peaks would imply that the stoichiometry of the mixed aggregate changes with added salt or with increasing temperature, since T_{gel} increases with increasing salt concentration. This could be the case if the fraction of bound konjac mannan segments decreases with increasing temperature. Then less carrageenan chains would be required to "consume" a given amount of konjac mannan chains. This interpretation is supported by the diminished effect of carrageenan gelation on the ESR spectra of konjac mannan at high salt (cf. Figures 9 and 10). The alternative explanation, a merging of the two peaks, would imply that the difference in T_{gel} decreases with increasing temperature or with increasing salt. Since the origin of the difference in T_{gel} is unclear in the first place, it is difficult to speculate on the origin of such a decrease. It might be relevant, however, that the coil conformation of κ-carrageenan should be less expanded at high salt, owing to electrostatic screening; therefore any unfavorable overlap between κ-carrageenan coils and glucomannan chains should be less severe at high salt, and hence, the driving force to induce helix formation should be smaller. An indication that the two peaks actually merge at high temperature is that the enthalpy of the transition in the mixture is still significantly lower than in the pure κ-carrageenan gel (cf. Figure 7). This feature is difficult to explain without a significant effect of the konjac mannan on the κ-carrageenan self-aggregation.

In conclusion, this paper provides significant evidence for an association between κ-carrageenan helices and konjac mannan. The presence of konjac mannan also increases the transition temperature of κ-carrageenan relative to T_{gel} in solutions of κ-carrageenan alone, at least at low salt concentrations. The konjac mannan/κ-carrageenan association competes with κ-carrageenan self-association, so that the amount of κ-carrageenan self-association is less in the mixture. At high carrageenan/mannan ratios, all mannan chains are consumed by aggregation with carrageenan helices before the carrageenan coil → helix transition is complete. The remaining κ-carrageenan coils form helices at the normal transition temperature, unaffected by the aggregated mannan chains. The nature of the κ-carrageenan/konjac mannan aggregate is uncertain, but a core of aggregated carrageenan chains with mannan chains attached to the surface is clearly a possibility.

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