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The Effect of Sodium Thiocyanate on Thermal and Rheological Properties of kappa-Carrageenan and Agarose Gels

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ABSTRACT

In order to clarify the factors affecting the gelation mechanisms of agarose and kappa-carrageenan, the effects of sodium thiocyanate on the gel-sol transition temperature and on the dynamic elastic modulus of the gels were examined. Both the melting temperature T_m *and the setting temperature* T_s *determined by differential scanning calorimetry shifted to higher temperatures with increasing concentration of sodium thiocyanate for kappa-carrageenan, while the opposite tendency was observed for agarose. The elastic modulus of agarose gels decreased, while that of kappa-carrageenan gels increased with increasing concentration of sodium thiocyanate in the range from 0·2 mol/liter to 1 mol/liter.*

INTRODUCTION

Both carrageenan and agarose are polysaccharides which are extracted from red seaweeds Rhodophycophyta. Carrageenan is extracted from

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Eucheuma and *Chondrus* of the Gigartinales, while agarose is extracted from *Gelidium* of the Gelidiales. Both of these polysaccharides form thermo-reversible gels. Agarose is the component which plays a dominant role in determining the mechanical properties of agar–agar gels. Carrageenans are classified into kappa-, iota- and lambda-carrageenan according to the sulfate content and its position in the molecule.

Although kappa-carrageenan and agarose have similar structures, the gelling mechanism and the physico-chemical properties of gels seem quite different from each other. Since carrageenan molecules contain sulfate side groups, the rheological and thermal properties are strongly influenced by the addition of alkali metal or alkaline earth metal ions, while agarose is not influenced by these ions (Watase & Nishinari, 1981, 1982*a*, 1982*b*; Morris & Chilvers, 1981, 1982). The effects of glycerin and ethylene glycol on gels of agarose and kappa-carrageenan are different (Nishinari & Watase, 1987). Both urea and formamide weaken the gel-forming ability of agarose and kappa-carrageenan. Guanidine hydrochloride weakens the gel-forming ability of agarose, but strengthens that of kappa-carrageenan (Watase & Nishinari, 1986). Thiocyanate ions are known to break hydrogen bonds, and so denature proteins. However, sodium ions will strengthen the gelling ability of kappa-carrageenan. In order to clarify the factors governing the gelation mechanisms of kappa-carrageenan and agarose, the effect of sodium thiocyanate on the gel–sol transition temperature and on the dynamic viscoelasticity of the gels was examined by differential scanning calorimetry (DSC) and dynamic mechanical measurements.

EXPERIMENTAL

Materials

Agarose

Gelidium amansii, produced in 1987 in the Izu Suzaki region (Japan), was heated at 75°C in 6% NaOH for 1 h, then washed in water and dried. It was stirred at 130°C in an autoclave for 1 h and then extracted. Extracts were filtered by diatomaceous earth, and then acrynol powders were added to hot filtrates and heated for 0·5 h while stirring in order to prevent gelation. The solution was filtered again to remove agaropectin and insoluble ingredients. The temperature of the solution was lowered gradually to form a gel. The gel was cut into thin strips and then pressed to remove acrynol. Freeze-drying was repeated several times, and the strips were then immersed in ethyl alcohol for several days in order to remove acrynol completely.

kappa-Carrageenan

Eucheuma cottonii (120 g), produced in 1979 in Korea, was washed in water, and then dissolved in 2 liters of water in the temperature range from 60°C to 70°C. It was pretreated with 6% KOH for 1 h and then filtered. Two liters of 0·125 mol/liter KCl aqueous solution was gradually added to the solution, which was then stirred vigorously. After washing out the soluble part, the kappa-carrageenan precipitated by KCl was separated by centrifugation. The precipitate was washed with ethyl alcohol several times to remove the excess KCl and water and the kappa-carrageenan dried in vacuo. The weight-average molecular weight determined by gel filtration chromatography (HLC-8020, SC-8010 from Tohsoh Co. Ltd, using dextran as a standard material) was $6{\cdot}1 \times 10^6$. The potassium content in the specimen measured by atomic absorption spectrophotometry was 3·1 wt%.

The observed values of intrinsic viscosity of the specimens purified in this way in a 0·01 mol/liter NaSCN aqueous solution at 35°C were 3·3 (100 ml/g) for agarose, and 14·2 (100 ml/g) for kappa-carrageenan, respectively.

The test pieces for rheological measurements were prepared as follows. The dried powder of kappa-carrageenan and agarose were dispersed in distilled water, and then preheated at 70°C in a glycerin bath for 1 h. The temperature was raised to 100°C to attain a complete dissolution, which can be recognized by the attainment of a clear solution. The solution was then poured into Teflon molds, 20 mm diameter and 30 mm high. The temperature was lowered gradually to room temperature in a thermostated box. Annealed gels thus obtained were taken out from the molds and were kept at about 5°C in silicone oil for a week. Each gel sample was kept at the measured temperature for 1 h before measurements were taken.

Measurements

Dynamic viscoelasticity

The apparatus for measuring the complex Young's modulus was a Rheolograph CV-100. The measurement was made at 2·5 Hz in a silicone oil bath in the temperature range from 15°C to 85°C. The temperature was raised stepwise at intervals of 10°C, and was kept constant at each temperature for 20 min.

Differential scanning calorimetry

The apparatus used for DSC measurements was a high sensitivity DSC-10 from Seiko Electronic Co. Gelled samples (40 ± 0·1 mg) were sealed into a silver pan; distilled water of the same weight was used as a reference material. After the temperature was kept at 5°C for 10 min, it was raised at the rate of 2°C/min. Following melting, the gel was maintained at the higher temperature for 10 min. The temperature was then lowered at 2°C/min in order to observe the exothermic peak accompanying gelation.

RESULTS AND DISCUSSION

The temperature dependence of the dynamic Young's modulus E' and mechanical loss tangent tan δ of kappa-carrageenan and agarose gels of various concentrations are shown in Fig. 1. E' increased slightly up to a certain temperature T_c, decreased gradually at first and then rapidly with increasing temperature for gels whose concentration was higher than 4%. For kappa-carrageenan gels of concentration below 4%, E' decreased continuously with increasing temperature. E' increased and tan δ decreased with increasing concentration, implying that gels become more solid-like with increasing polymer concentration. T_c ranged from 25°C to 35°C, and was shifted to higher temperatures with increasing polymer concentration for kappa-carrageenan gels whose concentration was higher than 4%. For kappa-carrageenan gels of concentration below 4%, E' decreased uniformly (Fig. 1(a)), whilst T_c was about 35°C, and almost independent of agarose concentration, for agarose gels (Fig. 1(b)).

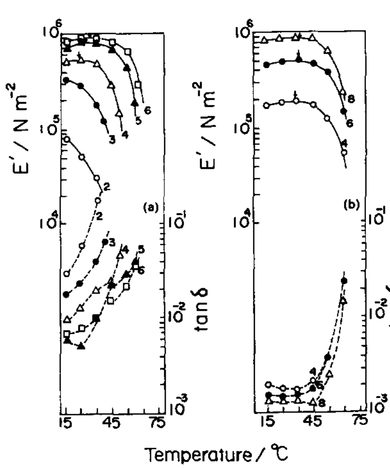

Fig. 1. Dynamic Young's modulus E' (——) and mechanical loss tangent tan δ (- - -) of (a) kappa-carrageenan and (b) agarose gels of various concentrations as a function of temperature. Figures beside each curve represent the concentration in % w/w. Arrows show the temperature T_c.

The values of E' for 4% and 6% gels are larger in kappa-carrageenan gels than in agarose gels. The values of tan δ for kappa-carrageenan gels are almost a factor of ten larger than those of agarose gels.

Figure 2 shows the temperature dependence of the dynamic Young's modulus E' and mechanical loss tangent tan δ of kappa-carrageenan gels of various concentrations containing sodium thiocyanate. E' decreased gradually with increasing temperature up to a certain temperature T_c and then decreased rapidly beyond T_c. This temperature T_c shifted to higher temperatures with increasing concentration of sodium thiocyanate: T_c ranged from 55°C to 65°C for gels containing 0·2 mol/liter NaSCN (Fig. 2(a)), and from 65°C to 75°C for gels containing 0·5 mol/liter NaSCN (Fig. 2(b)) and from 75°C to 85°C for gels containing 1·0 mol/liter NaSCN (Fig. 2(c)). The loss tangent tan δ increased steeply around the temperature T_c. E' decreased with increasing concentration of added sodium thiocyanate for the same concentration of kappa-carrageenan and at the same temperature.

Figure 3 shows the temperature dependence of the dynamic Young's modulus E' and mechanical loss tangent tan δ of agarose gels of various concentrations containing sodium thiocyanate. E' increased slightly up to 35°C for gels containing 0·2 mol/liter NaSCN (Fig. 3(a)) and 0·5 mol/liter NaSCN (Fig. 3(b)), and then began to decrease gradually, and then rapidly around the temperature T_c. This temperature T_c shifted to lower temperatures with increasing concentration of sodium thiocyanate: T_c ranged from 55°C to 65°C for gels containing 0·2 mol/liter NaSCN (Fig.

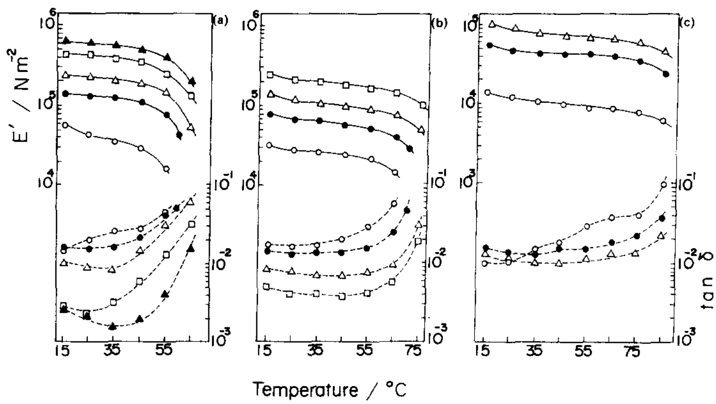

Fig. 2. Dynamic Young's modulus E' (——) and mechanical loss tangent tan δ (– – –) of kappa-carrageenan gels at concentrations of ○, 2%; ●, 3%; △, 4%; □, 5%; ▲, 6% w/w, containing NaSCN at concentrations of (a) 0·2 mol/liter; (b) 0·5 mol/liter; (c) 1·0 mol/liter, as a function of temperature. Arrows show the temperature T_c.

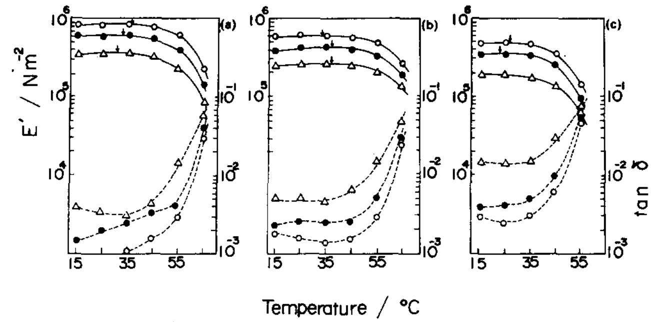

Fig. 3. Dynamic Young's modulus E' (——) and mechanical loss tangent tan δ (- - -) of agarose gels at concentrations of △, 4%; ●, 6%; ○, 8% w/w, containing NaSCN at concentrations of (a) 0·2 mol/liter; (b) 0·5 mol/liter, (c) 0·7 mol/liter, as a function of temperature. Arrows show the temperature T_c.

3(a)), and from 45°C to 55°C for gels containing 0·7 mol/liter NaSCN (Fig. 3(c)). This tendency is contrary to that observed for kappa-carrageenan gels (Fig. 2). E' decreased with increasing concentration of sodium thiocyanate for the same concentration of agarose and at the same temperature.

Heating and cooling DSC curves of kappa-carrageenan and agarose gels of various concentrations are shown in Figs 4 and 5, respectively. In both cases, heating DSC curves showed exothermic peaks at about 30°C, and then showed sharp endothermic peaks accompanying the transition from gel to sol. Small exothermic peaks at about 30°C were attributed to the rearrangement of molecular chains just before the gel melting. The melting temperature T_m ranged from 43°C to 63°C for kappa-carrageenan gels, and shifted to higher temperatures with increasing concentration of kappa-carrageenan, whilst T_m for agarose was about 78°C, and almost independent of concentration. Sharp exothermic peaks in cooling DSC curves were attributed to the transition from sol to gel. This setting temperature, T_s, shifted to higher temperatures with increasing concentration in kappa-carrageenan gels, while T_s was almost independent of concentration in agarose gels. This is consistent with the rheological data shown in Fig. 1. The differences between T_m and T_s were smaller for kappa-carrageenan gels compared with agarose gels.

Heating DSC curves of kappa-carrageenan gels of various concentrations containing sodium thiocyanate are shown in Fig. 6. T_m shifted markedly to higher temperatures with increasing concentration of kappa-carrageenan gels which contain 0·2 mol/liter NaSCN (Fig. 6(a)) and 0·5 mol/liter NaSCN (Fig. 6(b)), but T_m shifted to higher tempera-

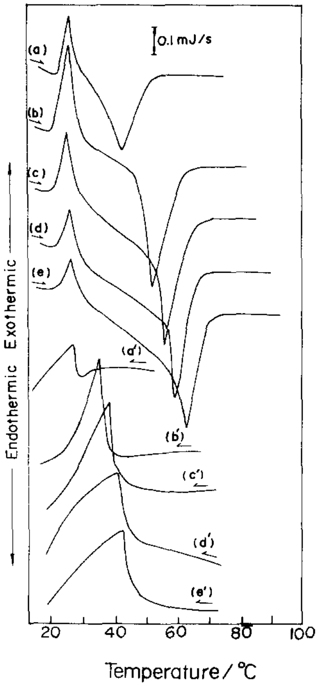

Fig. 4. DSC heating and cooling curves of kappa-carrageenan gels of various concentrations. (a)–(e) Heating curves; (a')–(e') cooling curves. (a), (a') 2%; (b), (b') 3%; (c), (c') 4%; (d), (d') 5%; (e), (e') 6%. Heating and cooling rate: 2°C/min. Reference material: water.

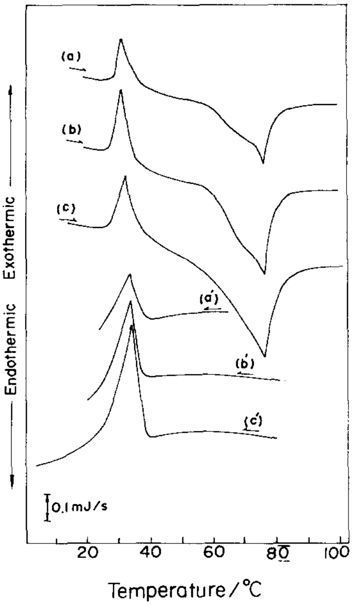

Fig. 5. DSC heating and cooling curves of agarose gels of various concentrations. (a)–(c) Heating curves; (a')–(c') cooling curves. (a), (a') 4%; (b), (b') 6%; (c), (c') 8%. Heating and cooling rate: 2°C/min. Reference material: water.

tures only slightly with increasing kappa-carrageenan concentration for gels which contain 1·0 mol/liter NaSCN (Fig. 6(c)). This behavior is consistent with the rheological behavior shown in Fig. 2; both T_c and T_m shift to higher temperatures with increasing concentration of NaSCN and kappa-carrageenan. This shift is more pronounced in gels with less NaSCN. Around the temperature T_c, gels are not melted completely but some segments are released from junction zones and then E' begins to decrease rapidly.

Cooling DSC curves of kappa-carrageenan gels of various concentrations containing sodium thiocyanate are shown in Fig. 7. T_S shifted to higher temperatures with increasing concentration of NaSCN and kappa-carrageenan.

Heating DSC curves of agarose gels of various concentrations containing sodium thiocyanate are shown in Fig. 8. Sharp endothermic

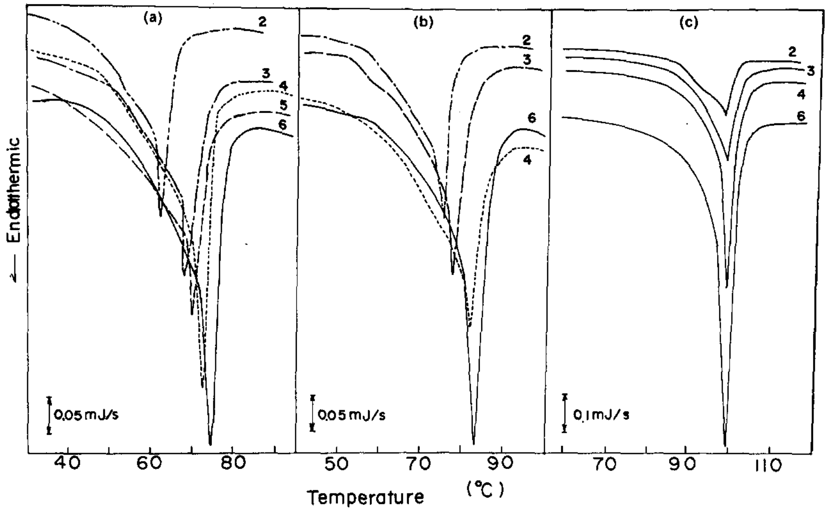

Fig. 6. DSC heating curves of kappa-carrageenan gels of various concentrations containing NaSCN at concentrations of (a) 0·2 mol/liter; (b) 0·5 mol/liter; (c) 1 mol/liter. The numbers beside each curve show the concentration of kappa-carrageenan in % w/w. Heating rate: 2°C/min. Reference material: water.

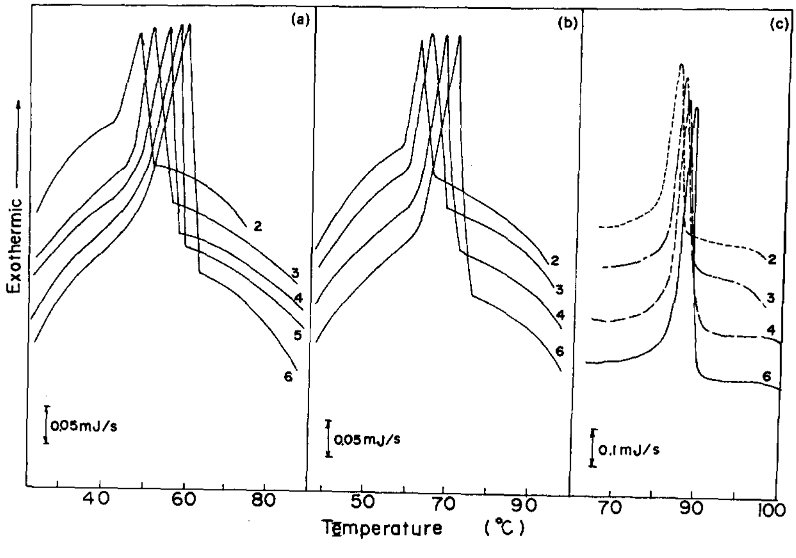

Fig. 7. DSC cooling curves of kappa-carrageenan gels of various concentrations containing NaSCN at concentrations of (a) 0·2 mol/liter; (b) 0·5 mol/liter; (c) 1 mol/liter. The numbers beside each curve show the concentration of kappa-carrageenan in % w/w. Heating rate: 2°C/min. Reference material: water.

peaks accompanying the transition from gel to sol shifted to lower temperatures with increasing agarose concentration in the presence of sodium thiocyanate in contrast to kappa-carrageenan gels (Fig. 6). This shift is more pronounced in gels with less NaSCN. This fact that T_m shifted to lower temperatures with increasing concentration of sodium thiocyanate corresponds well to the similar behavior of T_c observed for the rheological measurements shown in Fig. 3.

Cooling DSC curves of agarose gels of various concentrations containing sodium thiocyanate are shown in Fig. 9. Sharp exothermic peaks have been attributed to gelation, and this exothermic peak temperature T_s shifted to lower temperatures with increasing concentration of sodium thiocyanate. T_s shifted to higher temperatures with increasing concentration of agarose.

The temperature difference, $\Delta T = T_m - T_s$, for both kappa-carrageenan and agarose gels was always positive. ΔT decreased with increasing polymer concentration except in the case of kappa-carrageenan gels without NaSCN. ΔT was larger in kappa-carrageenan gels which contain lower levels of NaSCN, while ΔT was larger in agarose gels which contain higher levels of NaSCN.

The gelation mechanism of agarose or kappa-carrageenan is believed to be as follows (Morris *et al.*, 1980; Rees, 1981; Paoletti *et al.*, 1984; Smidsrod & Grasdalen, 1984): at high temperatures, these molecules assume a random coil conformation. A proportion of them form double

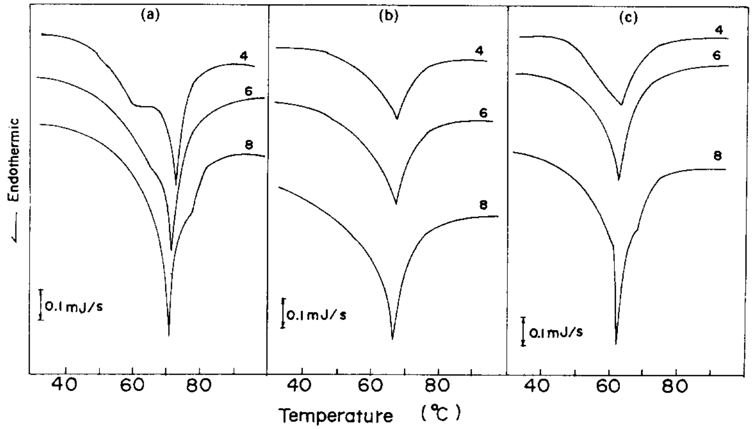

Fig. 8. DSC heating curves of agarose gels of various concentrations containing NaSCN at concentrations of (a) 0·2 mol/liter; (b) 0·5 mol/liter; (c) 0·7 mol/liter. The numbers beside each curve show the concentration of agarose in % w/w. Heating rate: 2°C/min. Reference material: water.

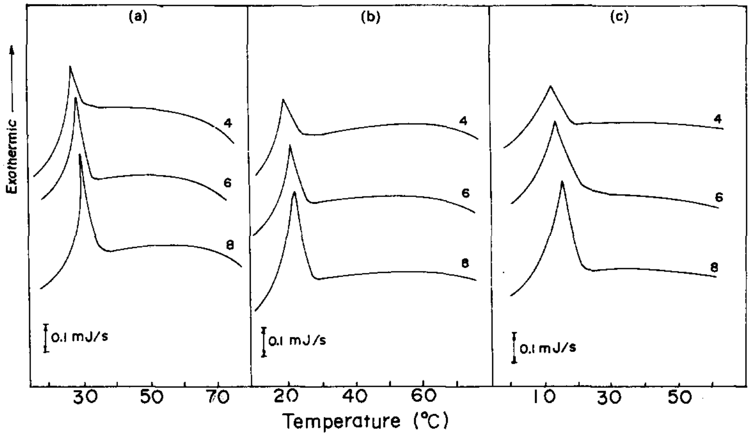

Fig. 9. DSC cooling curves of agarose gels of various concentrations containing NaSCN at concentrations of (a) 0·2 mol/liter; (b) 0·5 mol/liter; (c) 0·7 mol/liter. The numbers beside each curve show the concentration of agarose in % w/w. Heating rate: 2°C/min. Reference material: water.

or single helices when cooled. Further cooling produces aggregation of double helices, which play a role of junction zones, and then gels are formed. For kappa-carrageenan, the presence of cations promotes formation and aggregation of helices.

The structures of kappa-carrageenan and agarose resemble each other. The differences are as follows: (i) kappa-carrageenan has a higher degree of regularity of the alternate repeating units of D-galactose and 3,6-anhydro-L-galactose than agarose; (ii) kappa-carrageenan has a higher content of sulfate groups; and (iii) kappa-carrageenan has a higher molecular weight, as shown by the higher intrinsic viscosity. The interactions of these two polysaccharides with electrolytes or protein molecules are influenced strongly by these structural differences. It is expected that E' of the gel becomes larger and T_m of the gel becomes higher when the molecular weight is higher and the sulfate group content is less (Watase & Nishinari, 1987). Although kappa-carrageenan molecules have a higher content of sulfate groups, E' of kappa-carrageenan gels was larger than that of agarose gels at the same concentration (4% and 6%) at temperatures from 15°C to 45°C (Fig. 1). However, E' of kappa-carrageenan gels was smaller at higher temperatures. Therefore, the effect of molecular weight is dominant at lower temperatures, while the effect of sulfate content becomes important at higher temperatures.

As is shown in Figs 4 and 5, T_m of kappa-carrageenan gels is much lower than that of agarose gels, while T_s is not so different. Although kappa-carrageenan has a higher content of sulfate groups, kappa-carrageenan gels showed a higher T_s because of the higher molecular weight. The effect of molecular weight appeared at the cooling process.

Sodium thiocyanate breaks hydrogen bonds which stabilize the junction zones in the network structure of agarose and kappa-carrageenan gels. But sodium ions shield the electrostatic repulsion between sulfate groups in kappa-carrageenan molecules, and they promote the formation and association of helices (Watase & Nishinari, 1981, 1982*a*, 1982*b*; Morris & Chilvers, 1981, 1982). Since agarose molecules do not contain sulfate groups, the gelation of agarose solutions is not affected by the presence of cations. Therefore, the gelling ability of agarose is weakened by the addition of NaSCN, and then T_m and T_c are shifted to lower temperatures by the addition of NaSCN (Figs 3 and 8). The elastic modulus of agarose gels is decreased by the addition of NaSCN (Fig. 3). However, the reason why T_m is shifted to lower temperatures with increasing concentration of agarose in the presence of 0·2, 0·5 and 0·7 mol/liter NaSCN is not clear at present. Further studies which clarify this are urgently required.

For kappa-carrageenan gels, the situation is more complicated than in the case of agarose gels, because sodium thiocyanate plays two contradictory roles as mentioned above. The fact that T_m and T_c are shifted to higher temperatures with increasing concentration of NaSCN (Figs 2 and 6) is explained in terms of the shielding effect of electrostatic repulsion between sulfate groups by sodium ions. This strengthens the structure of junction zones, and then kappa-carrageenan gels become more heat-resistant. Since the thiocyanate ion is bulky, the shielding effect of sodium ions against the electrostatic repulsion of sulfate groups in kappa-carrageenan is inhibited at lower temperatures. Therefore, E' of kappa-carrageenan gels decreases with increasing concentration of NaSCN at lower temperatures (Fig. 2). However, the molecular motion of water is activated with increasing temperature, and at the same time the shielding effect of sodium ions becomes more effective. Aggregation of kappa-carrageenan is promoted during heating, and the endothermic peak accompanying the transition from gel to sol becomes sharper with increasing concentration of NaSCN (Fig. 6). Similar phenomena are seen in cooling DSC curves (Fig. 7). However, sodium ions do not increase so much the elastic modulus, which is proportional to the number of junction zones. Therefore, the elastic modulus is decreased by the addition of NaSCN which breaks the hydrogen bonds. The fact that T_m and T_s of kappa-carrageenan gels shifted to higher temperatures with increasing concentration of kappa-carrageenan (Figs 6 and 7), in contrast to agarose, is quite natural.

The gelation mechanism of agarose and kappa-carrageenan described briefly above is widely accepted. However, the difference between the gelation mechanism of agarose and kappa-carrageenan cannot be explained only by the existence of sulfate groups in kappa-carrageenan. A more precise picture, which can also explain the reason why T_m of agarose gels shifted to lower temperatures with increasing concentration of agarose in the presence of NaSCN (Fig. 8), is necessary.

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