

# The effect of the linear charge density of carrageenan on the ion binding investigated by differential scanning calorimetry, dc conductivity, and kHz dielectric relaxation

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## Abstract

The effect of the linear charge density of natural polyelectrolyte, carrageenan, on the ion binding to carrageenan molecules in relation to the gelation was investigated by using the dielectric relaxation spectroscopy, dc conductivity, optical rotation, and differential scanning calorimetry (DSC). Although carrageenan is an anionic polysaccharide, carrageenan molecules in the helix state at low temperatures can bind not only cation, such as potassium and cesium, but also anion, such as iodide. The dc conductivity steeply decreases just below the coil–helix transition temperature, which indicates the binding of ion to the carrageenan molecules in the helix state due to the increase of the linear charge density compared with that in the coil state. The addition of NaI promotes the helix formation, and prevents from aggregation of helices, which was suggested by the results of the dynamic shear modulus and the DSC, and resulted in an increase of the relaxation amplitude of the lowest frequency relaxation ( $\sim$ kHz) attributed to the fluctuation of the tightly bound counter ions along the high charge density region (helix). It is concluded that binding of iodide induces (1) the increase in the amount of tightly bound counterions to carrageenan molecules and (2) the formation of non-aggregated helix.

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## 1. Introduction

For the gelation of polyelectrolyte gels, counterions play an important role, because binding of counterions to polymer decreases the effective charge density of the polymer. Therefore, the formation of an aggregation among polymers, an essential component of gel, becomes easier. The aggregation among helices acts as a cross-link for physically cross-linked gels, such as carrageenan [1], gellan gum [2], and agarose [3]. In the case of physically cross-linked gels strongly coupled to the coil–helix transition, such as carrageenan, there are two essential factors, the number of helix bundled in a

cross-link region and the length of helix [4]. These two factors dominate the gel network structure and the macroscopic properties of gels. As for carrageenan gels, a number of aggregated helical molecules is essential, and increases with decreasing temperature [5].

Carrageenan, natural polysaccharides extracted from red algae, is based on an alternating disaccharide-repeating unit of 1,3-linked  $\beta$ -D-galactose and 1,4-linked 3,6-anhydro- $\alpha$ -D-galactose. These aqueous solutions form physically cross-linked thermo-reversible polyelectrolyte gels in the presence of specific kinds of cations. It is believed that a side-by-side aggregation of helical molecules acts as a cross-link, and it forms a three dimensional structure of gel network [1,6]. The macroscopic properties, such as shear and Young's modulus, of carrageenan gel are strongly affected by cation species [7–10]. One of the most interesting features of carrageenan

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is an ion selectivity [11]. The structure of the cross-link of gels depends on the counterion species and amount of added salt [5]. The charge density of the carrageenan chain increases with decreasing temperature due to the temperature induced coil to helix transition and the aggregation of helices. Therefore, the amount of the bound counterions increases. Since the binding of cations and/or anion to carrageenan, the anionic polysaccharide, should be important for the electrostatic repulsion among helices, it is expected that a new physical insight into the origin of the aggregation among helices, an essential factor of the physically cross-linked gels will be obtained through the investigation on the linear charge density of polymer.

It was suggested that not only cations but also anions are bound to polymer and change the physical properties of gel network, which can be interpreted as the change of the number of helix bundled in a cross-link region due to the change of the charge density of polymer. It is known that an addition of salt to helix-aggregation type polyelectrolyte gels such as carrageenans increases the shear and Young's modulus, which suggests that bound cations to polymer decrease the charge density and the electrostatic repulsion between helices and promote the aggregation among helices. Viebke et al. [12], investigated the effect of specific anion ( $I^-$ ) and cation ( $Cs^+$ ) separately under a series of systematic conditions. To investigate the effect of the different kinds of ion, they chose the condition as follows: (1) A polymer concentration is fixed to 1 wt.% for all samples. (2) The mixture of salts, NaCl and NaI, was added to the solutions. The molar ratio between NaI and NaCl was varied under the condition of the fixed total concentration (100 mM). For example, (a) 100 mM NaCl + 0 mM NaI, (b) 75 mM NaCl + 25 mM NaI, (c) 50 mM NaCl + 50 mM NaI, (d) 25 mM NaCl + 75 mM NaI, and (e) 0 mM NaCl + 100 mM NaI. These conditions make it possible to investigate the effect of iodide under the fixed Na concentration. They calculated the charge density of helix with the specifically bound ions based on the Poisson–Boltzmann equation. However, the calculated charge density and the effect of bound ions have not been confirmed by the experiments directly.

Conductmetric measurements are useful to investigate the binding of ions, since dc conductivity directly reflects the amount of freely movable counterions. Dielectric relaxation spectroscopy is a strong tool to investigate the binding state of ions, because the kHz relaxation is sensitive to the linear charge density of polymer [13], and the dielectric relaxations with different relaxation times reflect different binding states of ion [14]. The relaxation time  $\tau$  of the kHz relaxation ascribed to the counterion fluctuation of tightly bound to polyion is characterized by the relation,  $\tau \propto d^2/\mu$ , where  $d$  is the fluctuation distance of the counterion and  $\mu$  is the mobility of the counterion, which is proportional to the diffusion coefficient [15]. The fluctuation distance estimated from the kHz relaxation process reflects the size of the high charge density region.

In this study, we investigated the effect of the linear charge density on the aggregation of helices, i.e., the cross-link struc-

Table 1

Molar fraction of cations estimated by atomic absorption spectroscopy (for Na) and flame analysis (for the others)

	Kappa
K (%)	3.84
Na (%)	100
Ca (%)	0.38
Cs (%)	0.09

Table 2

The estimated amount of kappa-, iota-, and lambda-carrageenans included in the samples, kappa-carrageenan from CP Kelco and iota-carrageenans from SKW-Industries

	Kappa (%)	Iota (%)	Lamba (%)
Kappa (CPKelco)	95.8	4.1	0.0
Iota (SKW Industries)	2.4	90.8	6.8

ture of carrageenan gels, in the same way, an addition of the mixture of two kinds of salt ( $NaCl \rightarrow NaI$ ,  $NaI \rightarrow CsI$ ,  $CsI \rightarrow CsCl$ , and  $CsCl \rightarrow NaCl$ ), used by Viebke et al. [12], focusing on the effect of bound ions on the linear charge density of the carrageenan molecules in the helix state.

## 2. Experiments

### 2.1. Samples

Carrageenan samples, Na-form kappa-carrageenan (CP Kelco-X0909) and iota-carrageenan (SKW Industries), were gift from Unilever. Ion contents estimated by using atomic absorption spectroscopy and flame analysis of sodium, calcium, cesium, and potassium are listed in Table 1. It is reported that only small amount of kappa-carrageenan included in iota-carrageenan samples can affect the physical properties, such as ion-selectivity [16] and rheology [17]. The fraction of the kappa- iota-, and lambda-carrageenan in each sample was checked by the  $^1H$  NMR spectrum with the method reported by Tojo et al. [18] and is listed in Table 2.

### 2.2. Apparatus

For the dc conductivity and dielectric measurements, a LCR meter, Hewlett-Packard HP4284A, was used with a 2-electrodes cell made of platinum plated with platinum black. Differential scanning calorimetry (DSC) measurements were performed with a high sensitivity calorimeter, micro DSC-III (Setaram, France). A 800 mg solution sample was poured into DSC cell, and exactly the same weight of solvent within an error of 0.1% was poured into a reference cell. Optical rotation data at 589 nm were collected by using a polarimeter, Jasco P-1030. The temperature was controlled with a jacketed cell. The optical path length is 100 mm.  $^1H$  NMR spectrum for the quantitative analysis of kappa- and iota-carrageenan was measured by Varian unity plus 500 (working at 499.864 MHz). All the measurements were performed

Table 3  
Experimental conditions, salt composition and polymer concentrations, used in this study

Sample	Concentration of added salt [mM]					Total salt concentration [mM]	Polymer type and concentration [wt.%]
	NaCl	NaI	CsI	CsCl	NaCl		
1	50.00	0.00	0.00	0.00	0.00	50.00	Kappa, 2.0
2	33.33	16.67	0.00	0.00	0.00	50.00	Kappa, 2.0
3	16.67	33.33	0.00	0.00	0.00	50.00	Kappa, 2.0
4	0.00	50.00	0.00	0.00	0.00	50.00	Kappa, 2.0
5	0.00	30.00	20.00	0.00	0.00	50.00	Kappa, 2.0
6	0.00	20.00	30.00	0.00	0.00	50.00	Kappa, 2.0
7	0.00	10.00	40.00	0.00	0.00	50.00	Kappa, 2.0
8	0.00	0.00	50.00	0.00	0.00	50.00	Kappa, 2.0
9	0.00	0.00	33.33	16.67	0.00	50.00	Kappa, 2.0
10	0.00	0.00	16.67	33.33	0.00	50.00	Kappa, 2.0
11	0.00	0.00	0.00	50.00	0.00	50.00	Kappa, 2.0
12	0.00	0.00	0.00	25.00	25.00	50.00	Kappa, 2.0
13	0.00	50.00	0.00	0.00	0.00	50.00	Kappa, 3.0
14	0.00	30.00	20.00	0.00	0.00	50.00	Kappa, 3.0
15	0.00	20.00	30.00	0.00	0.00	50.00	Kappa, 3.0
16	0.00	10.00	40.00	0.00	0.00	50.00	Kappa, 3.0
17	0.00	0.00	50.00	0.00	0.00	50.00	Kappa, 3.0
18	0.00	50.00	0.00	0.00	0.00	50.00	Kappa, 1.0
19	0.00	30.00	20.00	0.00	0.00	50.00	Kappa, 1.0
20	0.00	20.00	30.00	0.00	0.00	50.00	Kappa, 1.0
21	0.00	0.00	50.00	0.00	0.00	50.00	Kappa, 1.0
22	50.00	0.00	0.00	0.00	0.00	50.00	Iota, 2.0
23	0.00	50.00	0.00	0.00	0.00	50.00	Iota, 2.0
24	0.00	0.00	50.00	0.00	0.00	50.00	Iota, 2.0
25	0.00	0.00	0.00	50.00	0.00	50.00	Iota, 2.0
26	0.00	20.00	30.00	0.00	0.00	50.00	Kappa, 0.5
27	0.00	20.00	30.00	0.00	0.00	50.00	Kappa, 4.0

under the same condition of the temperature control. The sample was cooled from 80 to 5 °C at a rate of 0.25 °C/min, and kept at 5 °C for 1 h, and then heated from 5 °C to 80 °C at a rate of 0.25 °C/min.

### 3. Results and discussion

#### 3.1. DSC

We investigated thermal properties of 2 wt.% Na-form kappa-carrageenan aqueous solutions in the presence of

50 mM salt (the mixture of two salts; NaCl + NaI, NaI + CsI, CsI + CsCl, and CsCl + NaCl. See introduction). With these conditions we can focus on the effect of a single kind of ion, for instance, the effect of I<sup>−</sup> in the range of the mixture of NaCl and NaI with varying the ratio between them. That is, we used the conditions of NaCl → NaI, (1) 50 mM NaCl + 0 mM NaI, (2) 33.3 mM NaCl + 16.7 mM NaI, (3) 16.7 mM NaCl + 33.3 mM NaI, and (4) 0 mM NaCl + 50 mM NaI. All the conditions we used in this study are summarized in Table 3. These conditions include four different kinds of ions, Na<sup>+</sup>, Cs<sup>+</sup>, Cl<sup>−</sup>, and I<sup>−</sup>. These ions are classified into

Table 4  
DSC peak temperatures,  $T_{\text{peak, cooling}}$  and  $T_{\text{peak, heating}}$ , onset and end temperatures,  $T_{\text{onset, cooling}}$  and  $T_{\text{end, heating}}$ , the onset and end temperatures of the dc conductivity,  $T_c$  and  $T_h$ , and the thermal hysteresis,  $T_h - T_c$ , estimated from DSC ( $=T_{\text{end, heating}} - T_{\text{onset, cooling}}$ ) and from dc conductivity

Sample	DSC					dc conductivity		
	$T_{\text{peak, cooling}}$	$T_{\text{onset, cooling}}$	$T_{\text{peak, heating}}$	$T_{\text{end, heating}}$	$T_h - T_c$	$T_c$	$T_h$	$T_h - T_c$
1	9.6	10.72	10.5	11.98	0.9	10.0	10.0	0.0
2	20.0	21.49	20.7	22.69	0.7	19.5	20.2	0.7
3	24.5	25.92	25.5	26.98	1.0	23.6	24.2	0.6
4	27.8	29.21	28.8	30.35	1.0	28.7	29.2	0.5
5	38.0	39.42	38.6	44.76	0.6	39.8	39.8	0.0
6	40.3	44.40	43.0	51.78	2.7	43.7	50.0	6.3
7	46.0	47.46	51.9	56.64	5.9	47.1	55.9	8.8
8	48.5	49.73	55.6	60.01	7.1	50.6	61.8	11.2
9	44.6	45.74	54.2	57.86	9.6	45.2	58.0	12.8
10	42.4	43.52	54.9	58.26	12.5	43.1	57.9	14.8
11	37.8	39.20	55.1	57.92	17.4	40.5	58.2	17.7
12	29.4	30.54	43.7	46.87	16.3	32.1	47.1	15.0

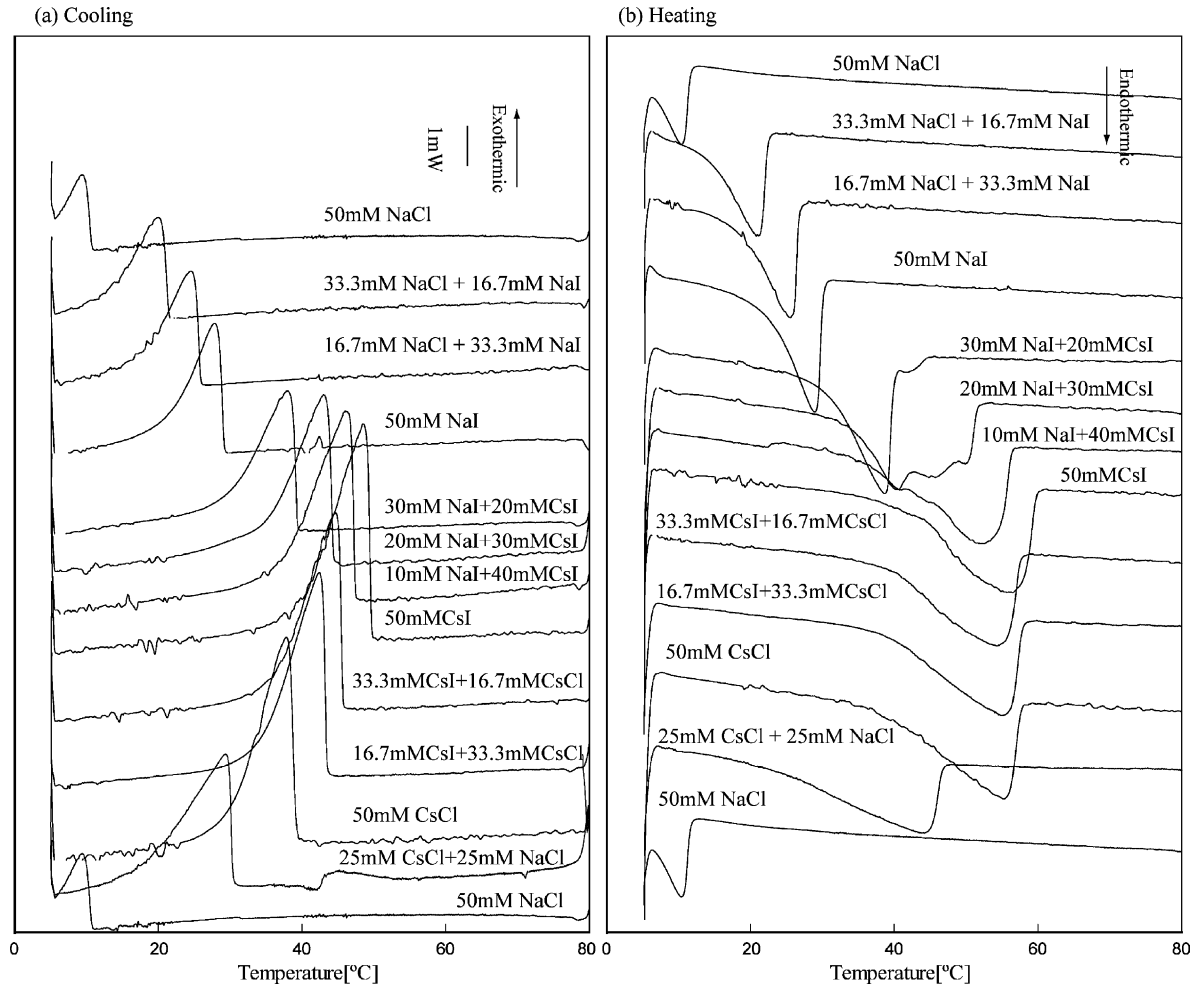


Fig. 1. The salt composition dependence of DSC thermograms of 2 wt.% Na-form kappa-carrageenan (samples 1–12).

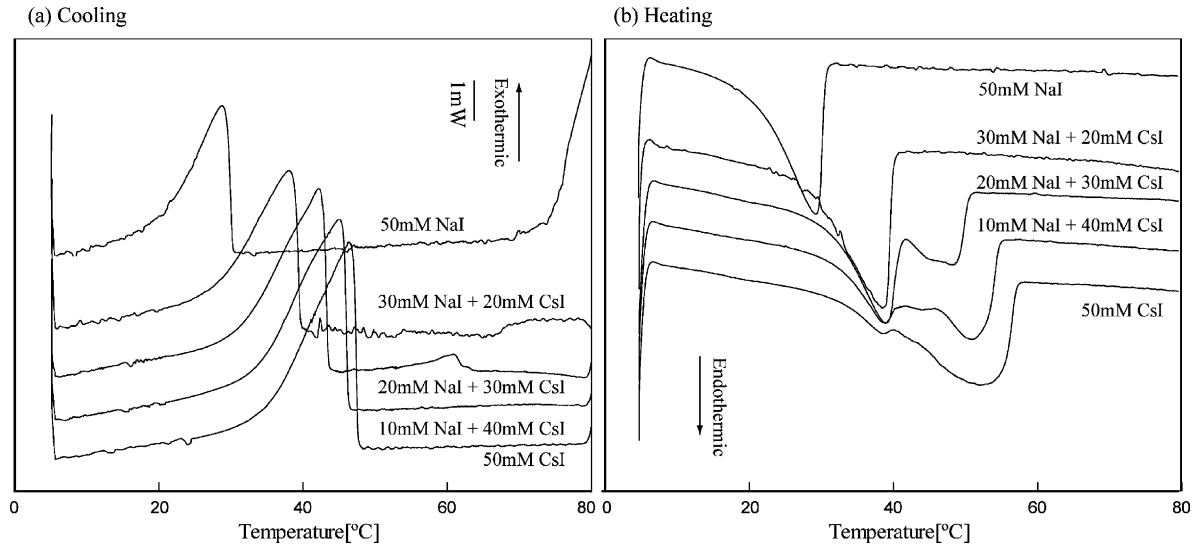


Fig. 2. The salt composition dependence of DSC thermograms of 3 wt.% Na-form kappa-carrageenan (samples 13–17).

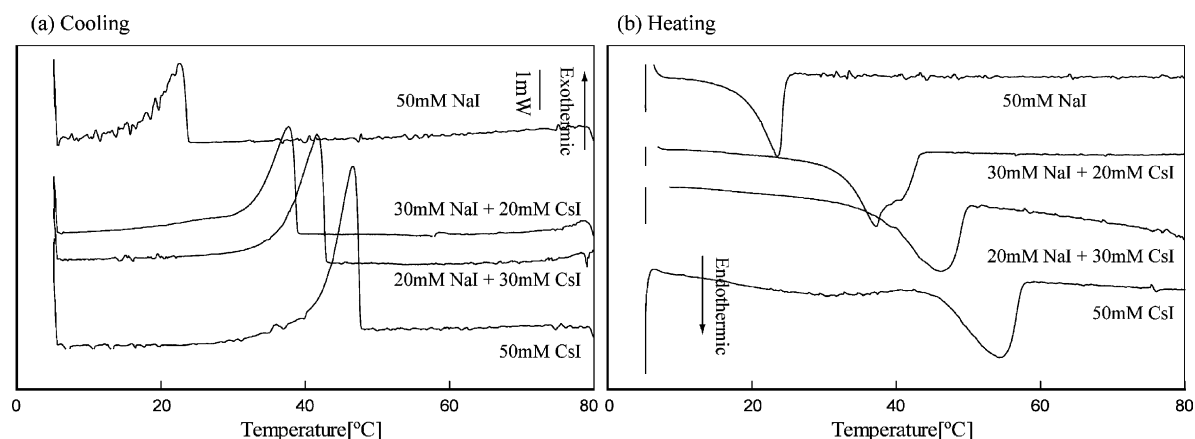


Fig. 3. The salt composition dependence of DSC thermograms of 1 wt.% Na-form kappa-carrageenan (samples 18–21).

the two groups, the inert and the specifically bound ion [12]. It was considered that  $\text{Na}^+$  and  $\text{Cl}^-$  are the inert ions (the interaction between ion and carrageenan polymer is only the general electrostatic interaction), and  $\text{Cs}^+$  and  $\text{I}^-$  are classified into the specifically bound ions to polymer. Since the ionic concentration is also important for the formation of helix and aggregation [11], we kept the total ion concentration at 0.05 M.

As shown in Fig. 1 for all the samples, one peak was found in both cooling and heating curves, except for DSC curves on heating in the  $\text{NaI} \rightarrow \text{CsI}$  region. The exothermic peak observed on cooling is ascribed to the formation of the helix (coil to helix transition) and aggregation of helix, and the endothermic peak on heating is ascribed to the melting

of aggregation and helix [19–21]. The peak temperatures on cooling,  $T_c$ , and on heating,  $T_h$ , are listed in Table 4.  $T_c$  and  $T_h$  strongly depend on the salt composition. The overall behavior of the salt composition dependence of  $T_c$  and  $T_h$  for the 2 wt.% Na-form kappa-carrageenan with added 50 mM salt is quite similar to that reported for 1 wt.% Na-form carrageenan in the presence of 100 mM salt [12].

In  $\text{NaCl} \rightarrow \text{NaI}$  range,  $T_c$  increases with increasing the amount of iodide, which is explained by the increase of the stability of helix by an addition of iodide [12]. In  $\text{NaI} \rightarrow \text{CsI}$  range, the difference between  $T_c$  and  $T_h$  corresponding to the thermal hysteresis increases with increasing the fraction of  $\text{CsI}$ . The addition of cesium causes the decrease of the charge density and promotes the aggregation of helices.

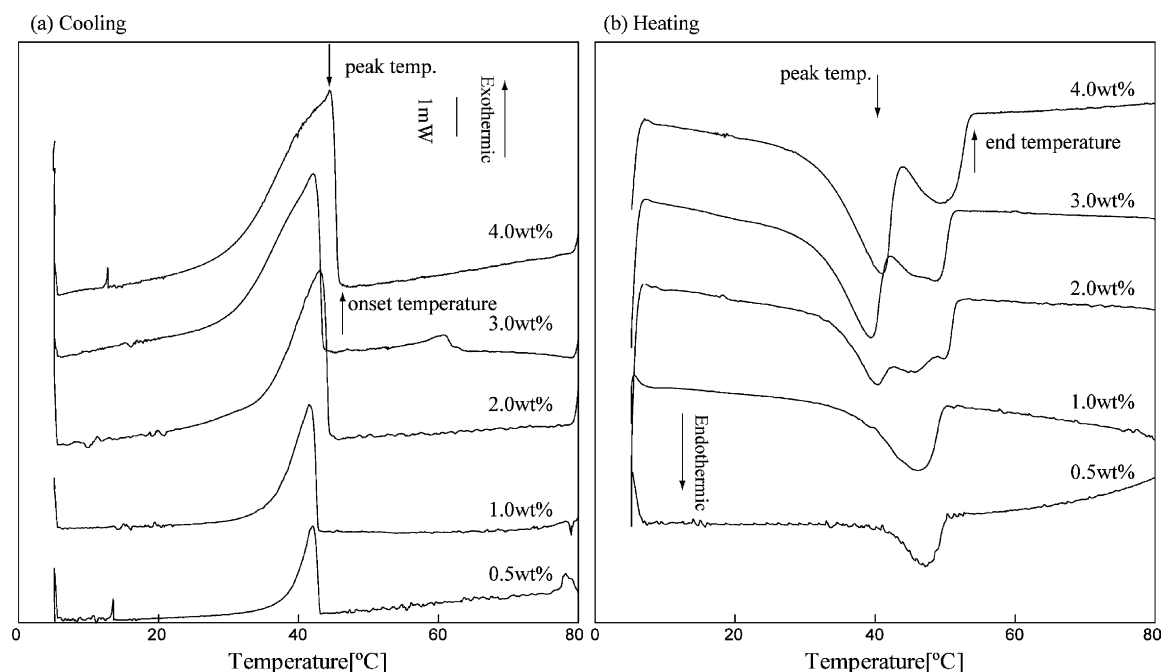


Fig. 4. The concentration dependence of DSC thermograms of Na-form kappa-carrageenan in the presence of 20 mM NaI and 30 mM CsI (samples 26, 20, 6, 15 and 27). The arrows represent DSC peak temperatures on cooling,  $T_{\text{DSCpeak, cooling}}$ , and on heating,  $T_{\text{DSCpeak, heating}}$ , the onset temperatures of the DSC on cooling,  $T_{\text{DSC, onset, cooling}}$ , and end temperatures on heating,  $T_{\text{DSC, end, heating}}$ .

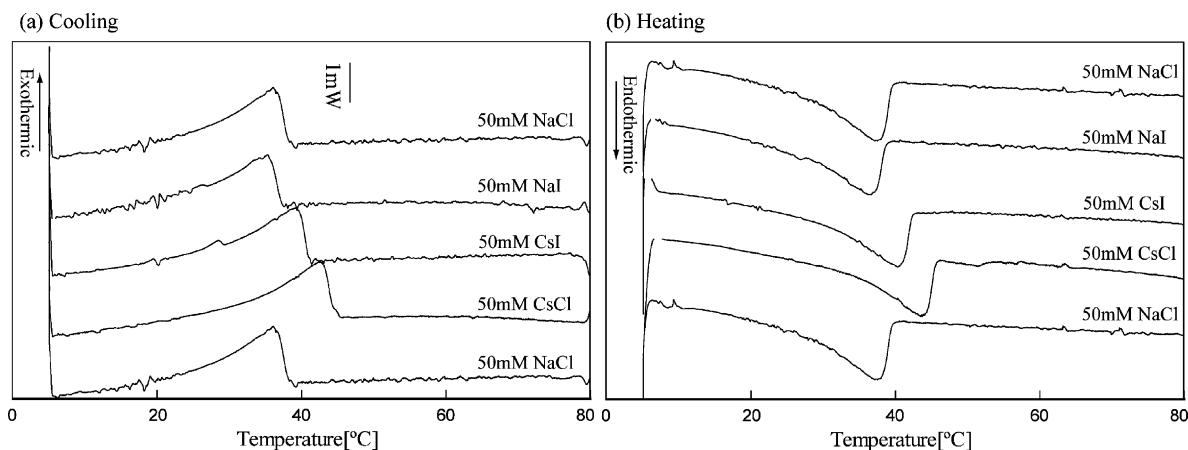


Fig. 5. The salt composition dependence of DSC thermograms of 1 wt.% Na-form iota-carrageenan (samples 22–25).

As will be seen later in Fig. 7, a large thermal hysteresis was observed under the conditions of high cesium content. The increase of the thermal hysteresis with increasing the amount of cesium is interpreted as the bound cesium shield the electrostatic repulsion between helices, since it is considered that the thermal hysteresis is caused by an increase of the thermal stability of the aggregation of helices.

We found that only in the limited region (NaI  $\rightarrow$  CsI) the endothermic peak on heating was split into two peaks. The peak separation on heating was reported for potassium-form kappa-carrageenan without salt [19] and gellan gum [22]. This peak separation always appears in the presence of excessive salts. According to Rochas and co-workers [19,20], these two peaks observed at low and high temperature are attributed to the melting of isolated segments of the helix and the melting of aggregation of helical segments, respectively. Morris et al. [1] also got the similar conclusion for iota-carrageenan. This splitting means that they are different ordered structure with different thermal hysteresis, and in our case tightly bound helices and partially disordered helices may be candidates.

As far as we know, no one paid attention on the effect of the salt composition on this peak separation. We have confirmed that the clear peak separation was not observed on heating under the condition, 1 wt.% Na-form kappa-carrageenan in the presence of 100 mM added salt. The total salt concentration chosen by them, 100 mM, is higher than that in this study, and polymer concentration is 1 wt.%. We chose 2 wt.% as the polymer concentration and 50 mM for the salt concentration, because these conditions increase  $\epsilon'$ , the real part of the complex permittivity  $\epsilon^*$  and decrease  $\epsilon''$ , the imaginary part of  $\epsilon^*$ , and thus we can obtain better results of the dielectric measurements from the point of view of the S/N ratio. We investigated the effect of polymer concentration on this peak separation. Fig. 2 and Fig. 3 show 3 and 1 wt.% Na-form kappa-carrageenan with 50 mM added salt (NaI  $\rightarrow$  CsI), respectively. In the case of 1 wt.% solutions, only one peak was observed on heating. Although a slight peak separation was observed in the case of the samples with 30 mM NaI

+ 20 mM CsI, it was not clearer than that for 2 wt.% solutions. The separation of two peaks for 3 wt.% samples was clearer compared to that for 2 wt.% samples. The separation of these two peaks becomes clearer with increasing polymer concentration as shown in Fig. 4.

In this range, NaI  $\rightarrow$  CsI, the isolated helices are easily formed, because the electrostatic repulsion among helices with bound iodide should be higher than that of the other conditions due to the high charge density. Additionally, an aggregation among helices will be formed in high cesium content. Therefore, it is considered that this region promotes the coexistence of the isolated helix and an aggregation among helices.

As can be seen in Fig. 5, the effect of the salt composition on the thermal properties was not remarkable for iota-carrageenan compared to that for kappa-carrageenan shown in Fig. 1. It was reported that the enzymatically purified iota-carrageenan does not show the specific binding in terms of the line width of the  $^{87}\text{Rb}$  NMR spectra [16]. Since the iota-carrageenan samples used in this study contain small amount of kappa-carrageenan,  $\sim 2.4\%$  against kappa-carrageenan, as listed in Table 2, it is considered that these effects of salts on the coil-helix transition temperature for iota-carrageenan are much smaller than those for kappa-carrageenan.

### 3.2. dc conductivity

Fig. 6 shows the temperature dependence of dc conductivity of 2 wt.% Na-form kappa-carrageenan aqueous solutions with 50 mM salt. The salt compositions are the same as those for DSC measurements. The samples with high iodide and cesium content exhibit large decrease of the dc conductivity just below the coil-helix transition temperature, which suggests binding of iodide and cesium just below the coil-helix transition temperature. dc conductivity  $\sigma$  is given by,

$$\sigma = \frac{F^2}{kT} \sum |z_i| c_i D_i, \quad (1)$$



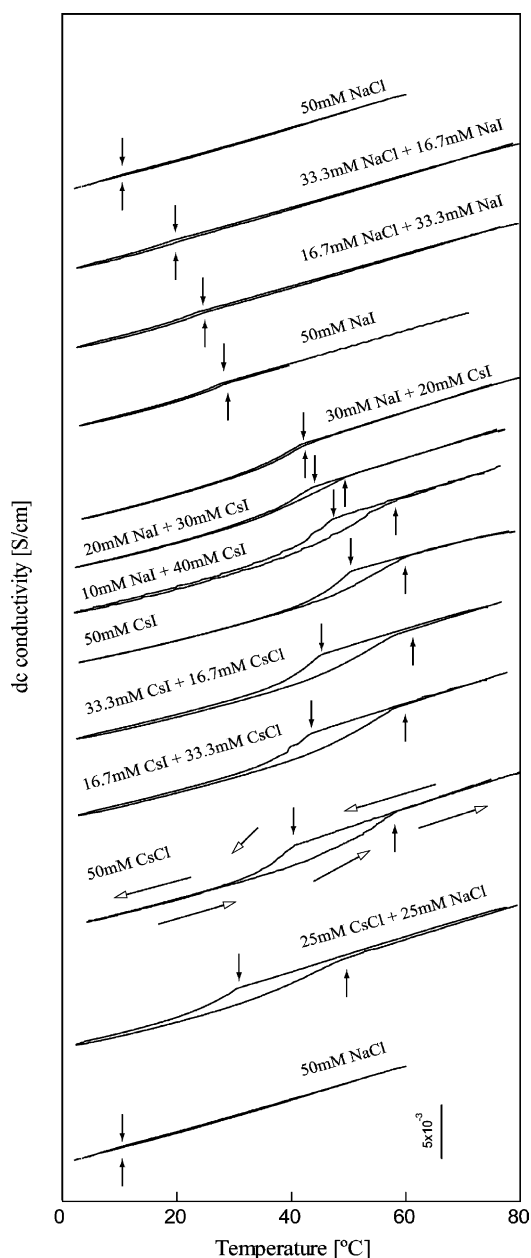


Fig. 6. The temperature dependence of dc conductivity  $\sigma$  for a 2 wt.% Na-form kappa-carrageenan with different composition of salt. The arrows of the upper and lower directions in the figures represent the onset temperatures on cooling,  $T_{\sigma, \text{onset, cooling}}$ , and end temperatures on heating,  $T_{\sigma, \text{end, heating}}$ , respectively. The open arrows around the data in the presence of 50 mM CsI represent the direction of cooling and heating.

where  $F$  is the Faraday constant,  $k$  the Boltzmann constant,  $T$  the absolute temperature,  $z_i$  the valence,  $c_i$  the local concentration, and  $D_i$  is the diffusion coefficient of  $i$ th ion. The summation covers all the ionic species. The decrease of the dc conductivity is caused by the decrease of the amount of freely movable counterions. In other words, it is caused by the decrease of the diffusion coefficient of counterions. Namely, it is caused by the binding of counterions to the polymer because the diffusion coefficient of bound ion is almost similar to that of polymer, and is almost negligible ( $\sim 1/100$ ) com-

pared with that of ion in the free state [23]. These conductivity results indicate the binding of cesium and iodide ions.

The salt composition dependence of the DSC peak temperature and the onset and end temperatures of dc conductivity and DSC heat flow at which the values start to change or end to change for 2 wt.% Na-form kappa-carrageenan are shown in Fig. 7. According to Rinaudo et al., DSC, dc conductivity, and optical rotation reflect basically quite similar information, the amount of helix. Although the clear peak separation was observed only for the DSC, the estimated onset temperatures by both method, DSC and dc conductivity, coincide with each other.

### 3.3. Dielectric properties

Since the contribution of the bound ions to the dc conductivity is quite small as mentioned above, and total conductivity is the summation of each contribution from free and bound counterions as formulated in Eq. (1), the binding of ions decreases the total dc conductivity just below the coil-helix transition. On the other hand, the binding of ions increases the contribution to the real part of the complex permittivity.

Fig. 8 shows the temperature dependence of the dielectric dispersion (the real part,  $\epsilon'$ , of the complex permittivity  $\epsilon^*$ ) spectrum of 2 wt.% Na-form kappa-carrageenan in the presence of (a) 50 mM NaCl and of (b) 50 mM NaI on cooling. Both samples exhibit the coil-helix transition, and the transition temperatures estimated from dc conductivity (onset temperature) are 10.0 and 28.7 °C, respectively. As for 2 wt.% Na-form kappa-carrageenan in the presence of 50 mM NaI, a significant change of the spectrum was observed just below the coil-helix transition temperature.  $\epsilon'$  around 10 kHz steeply increases with decreasing temperature just below the coil-helix transition temperature. In contrast, the change of the sample with 50 mM NaCl at the coil-helix transition temperature is smaller than that with NaI.

Takemasa has already reported that kHz relaxation appearing just below the coil-helix transition temperature is ascribed to the fluctuation of ions tightly bound to the high charge density region (helix) [13]. Within the framework of the Manning's counterion condensation theory, tightly bound counterions exist only in the case that the linear charge density parameter  $\xi$  is higher than unity. In the case of kappa-carrageenan aqueous solutions, the kHz relaxation attributed to the tightly bound ions fluctuating along the high charge density region, helix, was observed only in the helix state. We showed that the equation,

$$\epsilon^* = \frac{1}{1/(\epsilon_\infty + (\Delta\epsilon/(1 + (i\omega\tau)^\beta)) + \sigma/i\omega) + 1/(\epsilon_{ep}/(i\omega)^\gamma)}, \quad (2)$$

$$\Delta\epsilon \equiv \epsilon_0 - \epsilon_\infty, \quad (3)$$

where  $\epsilon_0$  is the static permittivity,  $\tau$  the relaxation time,  $\beta$  the distribution parameter of the relaxation time in the Cole-Cole

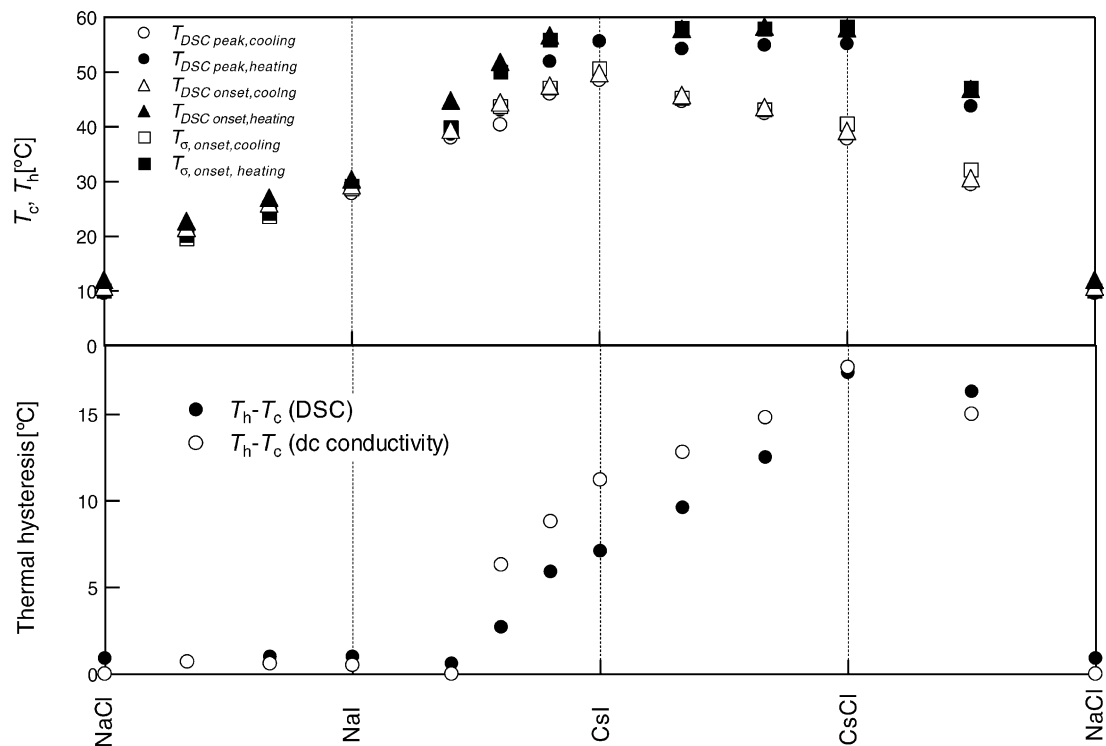


Fig. 7. The salt composition dependence of the DSC peak temperatures on cooling,  $T_{\text{DSCpeak, cooling}}$ , and on heating,  $T_{\text{DSCpeak, heating}}$ , the onset temperatures of the DSC on cooling,  $T_{\text{DSC, onset, cooling}}$ , and end temperatures on heating,  $T_{\text{DSC, end, heating}}$ , the onset temperatures of the dc conductivity on cooling,  $T_{\sigma, \text{onset, cooling}}$ , and end temperatures on heating,  $T_{\sigma, \text{end, heating}}$ , and the thermal hysteresis estimated from the DSC,  $T_h - T_c(\text{DSC}) = T_{\text{DSC, end, heating}} - T_{\text{DSC, onset, cooling}}$ , and from the dc conductivity,  $T_h - T_c(\text{dc conductivity}) = T_{\sigma, \text{end, heating}} - T_{\sigma, \text{onset, cooling}}$ .

dielectric relaxation function [24], and both  $\varepsilon_{\text{ep}}$  and  $\gamma$  the electrode polarization parameters, is suitable to reproduce the spectrum.[1,13] Eq. (2) concerns the effect of dc conductivity, the electrode polarization, and the effect of the fluctuating bound ions.

Fig. 9 shows the temperature dependence of the relaxation strength  $\Delta\varepsilon$  of the kHz relaxation for Na-form kappa-carrageenan with 50 mM NaI and NaCl estimated by using a non-linear least square fitting procedure based on Eq. (2). The increase of the  $\Delta\varepsilon$  with decreasing temperature just below the coil–helix transition temperature was observed for

both salt conditions, which suggests the increase in the linear charge density due to the coil–helix transition.  $\Delta\varepsilon$  at low temperatures of the samples in the presence of NaI is much higher than that of NaCl. This is the clear evidence that the linear charge density for the sample with NaI is higher than that with NaCl.

### 3.4. Optical rotation

Judging from the DSC thermograms on cooling, it is suggested that the two essential events of the gelation, the

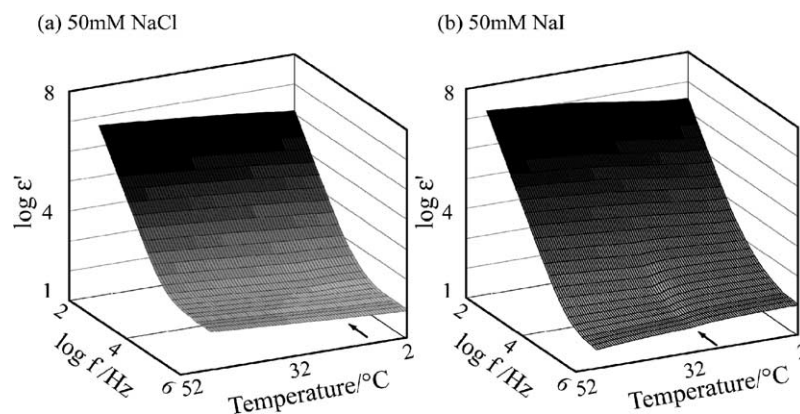


Fig. 8. The temperature dependence of the dielectric spectrum  $\varepsilon'(f)$  of 2.0 wt.% Na-form kappa-carrageenan in the presence of (a) 50 mM NaCl and (b) of 50 mM NaI on cooling. Arrows show the coil to helix transition temperature.



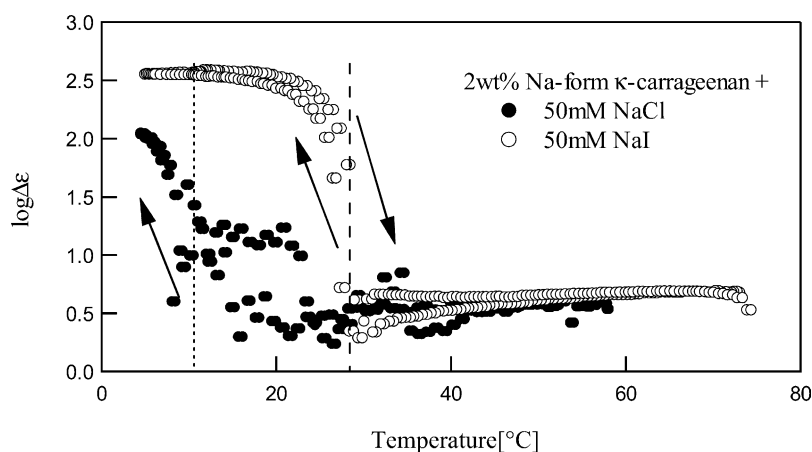


Fig. 9. The temperature dependence of the dielectric relaxation strength,  $\Delta\epsilon$  of 2 wt.% Na-form kappa-carrageenan in the presence of 50 mM NaI and of 50 mM NaCl on cooling and heating (arrows show the directions).

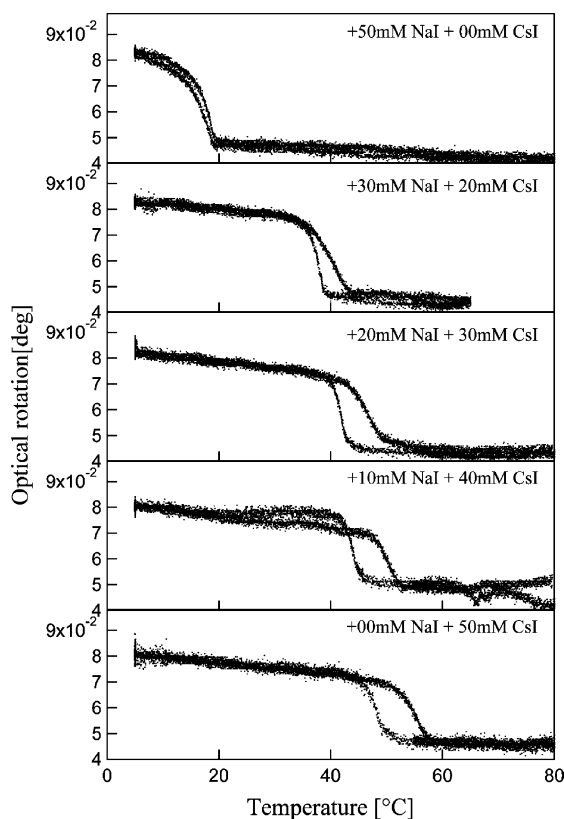


Fig. 10. The temperature dependence of the optical rotation for the 0.1 wt.% Na-form kappa-carrageenan aqueous solutions with 50 mM salts.

increase of length of helix and the formation of the aggregation among helices, progress simultaneously as mentioned above. In this case, there is a possibility that the aggregation formed at the initial stage of gelation prevents the formation of helix. For example, if both the coil and helix states coexist in the single molecules, and the aggregation among helices is formed even in the case that total fraction of helix is less than 100%, the formation of the helix (an increase of the helical amount) may be restricted by the topological restriction of

the network structure of gel. In order to focus on the effect of the aggregation on the total amount of helix at low temperatures far below the coil–helix transition temperature, we studied the temperature dependence of the optical rotation in the range of NaI  $\rightarrow$  CsI. The temperature dependence of the optical rotation of the 0.1 wt.% kappa-carrageenan with salt (NaI  $\rightarrow$  CsI) is shown in Fig. 10. At low and high temperatures in the helix and coil state, the values of the optical rotation of all the samples are almost the same,  $\sim 0.082^\circ$ , which means that the formation of the helix is not affected by the topological restriction of the aggregation.

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