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Mechanical and Dielectric Studies of Carrageenan Sols and Gels

Makoto Takemasa,[*1] *Akio Chiba,*[1] *Munehiro Date*[2]

[1] Department of Applied Physics, School of Science and Engineering, Waseda University, 3-4-1 Okubo, Shinjuku-ku, Tokyo, 169-8555 Japan
[2] Kobayasi Institute of Physical Research, 3-20-31 Kokubunji, Tokyo, 185-8533, Japan

Summary: The temperature dependence of the dynamic shear modulus, strain optical coefficient, DC conductivity, and complex dielectric spectrum of κ- and ι-carrageenan aqueous solutions with K, Ca, Cs, and Na were measured in order to clarify the formation process of the cross-linking region and the gel network structure. From the correlation analysis between the shear modulus and the strain optical coefficient, we found that the stress inducing unit orientation increases with decreasing temperature, which strongly suggests that the branching number in a cross-linking region increases with decreasing temperature, which depends on counterion species. In terms of the correlation parameters, an increasing scheme of the branching number depends on counterion species. Just below the coil-helix transition temperature, dielectric relaxation arises, with relaxation time ~100μs and relaxation strength ~10^3.Dielectric relaxation can be assigned to the counterion fluctuation in the parallel direction to the helical axis. The fluctuation distance of the counterion estimated from the relaxation time increases sharply in the initial stage of gelation and gradually reaches a constant value. We concluded that the longitudinal length of the aggregated region increases sharply at the initial state of gelation while the number of helical molecules bundled in a cross-linking region increases successively with decreasing temperature.

Introduction

Carrageenans are natural polysaccharide extracted from red algae. Their primary structures are based on an alternating disaccharide repeating unit of 1,3-linked *β*-D-galactose and 1,4-linked 3,6-anhydro-*α*-D-galactose. These aqueous solutions with specific cations form physically cross-linked thermo-reversible polyelectrolyte gels. The macroscopic properties of carrageenan gels are strongly affected by the concentration and species of cations. Although a number of studies on the gelation mechanism have been performed over the last 3 decades using various methods, such as X-ray diffraction,[1] electron microscopy,[2] and light scattering,[3,4,5] both the formation process of the crosslinks and the gel network structure remain uncertain, and a detailed model has not been constructed. Undoubtedly, the structure of the branching and the cross-link

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points are essential features governing the macroscopic properties of carrageenan gels. The purpose of this study is to clarify the formation process and growth. Measurement f multiple properties, has provided a physical insight into the cross-linking mechanism. We investigate responses to mechanical deformation, i.e. the dynamic shear modulus and the strain optical coefficient, and electrical properties, i.e. DC conductivity and dielectric properties.

The response to mechanical deformation provides information about the cross-link structure and the polymers connecting between cross-linking points. From the correlation analysis between shear modulus and strain optical coefficient, we estimate the optical anisotropy of polymer chains and the stress required to induce orientation, and discuss the increasing scheme of the branching number in a cross-linking region.

On the other hand, the electrical properties are sensitive to the change of binding nature of the counterions around the aggregated region of highly charged helical molecules and the abundance of freely movable counterions. According to the Manning's counterion condensation theory, bound counterions are classified into two types; loosely and tightly bound counterions, of the relative abundance of which depends on charge density along the polymer axis. 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. Then, the number of the bound counterions increases, and the binding state of counterions changes in the sol-gel transition.

Linear polyelectrolyte solutions exhibit two dielectric relaxation processes appearing at the low frequency (~kHz) and the high frequency (~MHz) in the semi-dilute concentration region,[6,7] and it is suggested that these relaxations arise from the counterion fluctuations in the directions parallel and perpendicular to the polymer chain axis, in other words, tightly and loosely bound to polyions,[8,9] respectively. We investigated the formation and the longitudinal length of the high charge density region by using the low frequency dielectric relaxation analysis. Under the assignment of the relaxation process, the fluctuation distance can be obtained from the relaxation time τ,[10] which is expected to reflect the size of the high charge density region. The fluctuation distance of counterions reflects the size of the associated region of helical molecules. It is expected that a new physical insight into the cross-linking region of aggregated carrageenan helices will be obtained by investigating the nature of free and bound counterions.

Experiments

***Sample preparation*:** κ- and ι-carrageenan from *Eucheuma cottonii*, were purchased from Sigma Chemical Co. Ltd.. We prepared the potassium, sodium, calcium, and cesium-form κ- and ι-carrageenan. Purification was performed by dialysis, and the carrageenans were de-ionized by passage through Amberlite IR-120 (H^+) cation exchange resin, and neutralized with KOH, NaOH, CsOH, or $Ca(OH)_2$ aqueous solutions for each cation-form solution, and then freeze-dried. The cation content was confirmed by ion chromatography, and the sulphur content was estimated by inductively coupled plasma spectrometry.

***Apparatus*:** In this study, we particularly focused on the two groups of property, the responses to the mechanical deformation, and electrical properties. We have custom-built apparati for the measurement of complex shear modulus, strain optical coefficient, and dielectric relaxation at low frequencies (~kHz).[11,12] The special feature of the apparatus measuring dynamic shear modulus is its ability to make an accurate measurements over the ~7 decades in a concentric cylindrical cell applying a small amplitude of the strain.[11] The strain optical coefficient can be measured separately from the rotation of the optical azimuth of the gelled sample.[11] Using the other custom built apparatus, we can obtain the dielectric spectrum at the low frequency (~kHz) with such quality that the separation of the relaxation process can be successfully done even in samples exhibiting high DC conductivity.[12]

Measurements of the dynamic shear modulus and the strain optical coefficient were performed in the frequency range 1.0^{-2}-10^{0} Hz and the temperature range 2°C-60°C. All the measurements were performed under the same thermal condition. The sample was cooled from 60°C to 2°C at a rate of 0.5°C/min, and kept at 2°C for 120 minutes, and then heated from 2°C at a rate of 0.5°C/min.

Results and Discussion

Figure 1 shows the typical temperature dependence of the multiple properties for 2.0wt% Cs-form κ-carrageenan aqueous solution; dynamic shear modulus (G' and G''), the optical transmission (I/I_0), the strain optical coefficient ($\Delta n/\Delta\gamma$), the DC conductivity (σ), the dielectric relaxation time (τ) of the low frequency (~kHz) process, and the dielectric relaxation strength ($\Delta\varepsilon$), where I is the intensity of the transmitted light through the cell with sample and the I_0 is the intensity without sample. All the properties showed a characteristic change at the onset temperature T_o at which a marked change starts in the cooling process. Since the temperature T_{cross} at which G' crosses with G'' depends on frequency and does not agree with the gelation temperature estimated with the commonly accepted method proposed by Winter and Chambon,[13,14] we think that T_o does not correspond to the sol-to-gel transition temperature, but neither corresponds to the coil-to-helix conformational transition temperature. For precise estimation of the gelation temperature T_{gel} using a mechanical method, measurement of the frequency dependence of G^* at a fixed temperature is employed. Under the condition of constant cooling rate, the temperature T_{cross} at which G' crosses with G'' becomes a guidepost of the sol to gel transition temperature T_{gel}. The temperature at which both the mechanical properties increase significantly lies very close to the coil helix transition temperature, which is evaluated by dc conductivity just corresponding to the optical rotation result.18) As shown in Figure 1, T_{cross} lies very close to T_o, which indicates that T_{gel} in this study lies very close to the coil-helix transition temperature T_{CH} under this experimental condition.

In the cooling process, both the responses to the mechanical deformation, $\Delta n/\Delta\gamma$, G', and G'' increase with decreasing temperature, which apparently shows that the formation of the gel network, such as an increase of the number of the cross-link points and an increase of the rigidity of the polymers connecting between cross-link points, successively proceeds. On the other hand, the decrease of the DC conductivity means a decrease of the amount of the freely movable counterions, and the increase of the dielectric relaxation strength with the relaxation time ~100 μs means an increase of the amount of the bound counterions to polyions fluctuating along the polymer axis.

We have carefully checked that a frequency dependence of G^* and $\Delta n/\Delta\gamma$ observed just below the sol-gel transition temperature does not affect the conclusion of the growth process of the gel network.[11] The data of G^* and $\Delta n/\Delta\gamma$ shown in Figure 1 were measured at a constant frequency 0.5Hz. Since we confirmed that almost no apparent frequency dependence was observed for G^* and $\Delta n/\Delta\gamma$ far below the gelation temperature T_{gel}, we consistently used the data at a constant frequency of 0.5Hz.

Mechanical properties: In the case of a rubber-like network, the temperature and frequency dependence of viscoelasticity offers information about the formation of cross-link points and the number of polymer chains in the gels. In contrast, in the case of gels

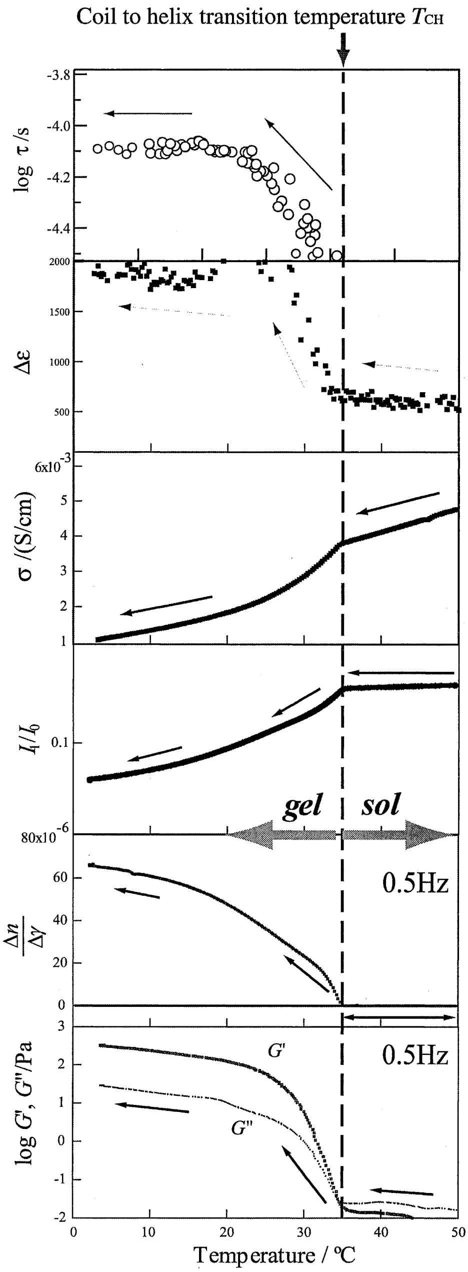

Figure 1 A typical temperature dependence of the relaxation time τ, the dielectric relaxation strength $\Delta\varepsilon$ of the dielectric relaxation process observed at the low frequencies (~kHz), DC conductivity σ, the optical transparency I_1/I_0, the strain optical coefficient $\Delta n/\Delta\gamma$, and the shear modulus G', G'' of 2.0wt% Cs-form κ-carrageenan. (The figures of I_1/I_0, $\Delta n/\Delta\gamma$, and G^* are reconstructed with permission from Fig.7 in *Macromolecules*. 2001, *34*, 7427-7434.)

made of rigid segments, these properties are affected by the structure of the cross-link region, and the topological structure of the gel network. However, it is difficult separate these effects using only the viscoelastic experiments. Moreover, the viscoelasticity of the κ-carrageenan solutions does not exhibit significant frequency dependence far below the gelation temperature. On the other hand, the investigation of the strain optical coefficient offers information about the anisotropy of the refractive index of the polymer chain and the polymer chain orientation caused by mechanical deformation of gel network. Combining *G*' with $\Delta n/\Delta\gamma$, we have obtained new information about the growth process of the gel network through the change in the orientation of the polymer chains and the optical anisotropy of molecules during gelation. Figure 2 shows the correlation between log *G*' and log $\Delta n/\Delta\gamma$, as deduced from the data of the temperature dependence of *G*' and $\Delta n/\Delta\gamma$, eliminating temperature. As an important finding in this

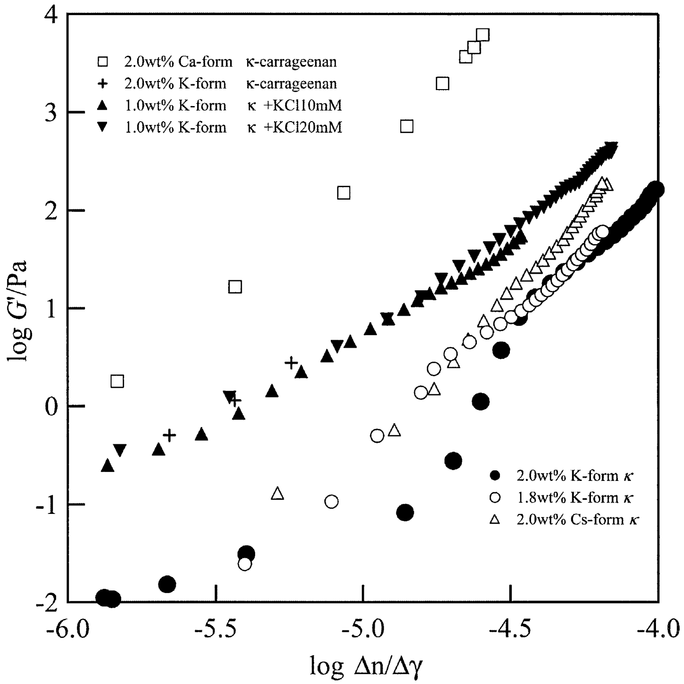

Figure 2 The correlation between the strain optical coefficient and the storage modulus. Upper right region corresponds to lower temperature. (+Ca-form κ 2.0wt%, □ Cs-κ 2.0wt%, ■K-κ 1.5wt%, ●K-κ 1.8wt%, ○ K-κ 2.0wt%, ▲ K-κ 1.0wt%+KCl10mM, ▼ K-κ 1.0wt%+KCl20mM) (Reproduced with permission from Figure 12, *Macromolecules* **2001,** *34,* 7427-7434. Copyright 2001 Am. Chem. Soc.)

study, a linear relation between log G' and log $\Delta n/\Delta\gamma$ was observed for all the samples at the temperatures far below the gelation temperature T_{gel}. The linear relation is formulated as,

$$G' = \left(\frac{d\sigma}{d\gamma}\right) = \alpha\left(\frac{d\Delta n}{d\gamma}\right)^{\beta}. \tag{1}$$

The two parameters α and β characterizing the correlation between G' and $\Delta n/\Delta\gamma$ have an important physical meaning. α corresponds to the optical anisotropy of the segment, and β characterizes the growth scheme of the gel network, reflecting the stress required for inducing the unit stress.[11] The value of β is larger than unity for all samples, which indicates that the stress required for inducing unit orientation increases with decreasing temperature over a wide temperature range. The topological structure of gel network made of rigid segment such as helical molecules should largely determine the mechanical properties. The change in topological structure of the gel network is explained by an increase of the branching number in a cross-link region. An increase of the branching induces an increase of the number of the polymer chain acting as a brace. Based on the commonly accepted model of a crosslink structure for κ-carrageenan gel, which indicates that the branching is an interhelical association,[15] we conclude that the parameter β is the characteristic parameter connected with the increasing scheme of the branching number in a cross-linking region.

Logα represents the offset of the vertical axis at $\log(\Delta n/\Delta\gamma)=0$ in Figure 2, and strongly depends on the value of β. Since the values of $\Delta n/\Delta\gamma$ are much smaller than 10^0, the value of α reflects the optical anisotropy expanded by the value of β. For the more adequate evaluation of optical anisotropy in eq 1, we use the value of the horizontal offset α' defined as,

$$\begin{aligned} \log G' &= \beta\left(\frac{d\Delta n}{d\gamma} - \alpha'\right), \\ \alpha' &= \frac{\log\alpha}{\beta}. \end{aligned} \tag{2}$$

As listed in Table 1, the values of α' with different cation species (Cs, K, and Ca-form κ-carrageenan) are quite different. We think that the difference of α' is dominated by the effects of cations bound to helical molecules, rather than the change in the optical anisotropy during conformational transition of the polymer chain. This indicates that the structure inside the cross-link region and the conformation of the chains connecting

Table 1. The correlation exponents α and β in eq 1 estimated from the data in Figure 2 by a least squares fitting procedure. (Reproduced with permission from Table 2, *Macromolecules* 2001, *34,* 7427-7434. Copyright 2001 Am. Chem. Soc)

sample	polymer concentration(wt%)	logα	β	$-\frac{\log a}{b}$
κ K-form + KCl 10mM	1.0	8.8	1.63	-5.42
κ K-form + KCl 20mM	1.0	9.0	1.63	-5.51
ι K-form	2.0	8.5	1.54	-5.50
κ K-form	1.8	12.1	2.48	-4.88
κ K-form	2.0	12.8	2.65	-4.82
κ Ca-form	2.0	16.6	2.83	-5.89
κ Cs-form	2.0	16.7	3.46	-4.84

cross-link regions change successively in the low temperature region.

We found that in the case of K-form ι-carrageenan, the value of the exponent β is much smaller than that for K-form κ-carrageenan and without added salt it is rather close to unity. This suggests that the growth mechanisms of κ- and ι-carrageenan are quite different. Piculell *et al.* pointed out the possibility that aggregation is essential for the gelation of κ-carrageenan, but is not essential for that of ι-carrageenan.[16] We think that the difference of the values of β for κ- and ι-carrageenan is caused by the difference in cross-linking mechanism, i.e., the branching number in the cross-link region increases during gelation in the case of κ-carrageenan gel, but hardly increases in the case of ι-carrageenan gel.

Light scattering studies focusing on change of the molecular weight during coil-helix transition and aggregation of helices showed a continuous increase of the molecular weight,[5,17] indicating an increase of the number of helices associated in a cross-link region. We think that our results are consistent with those results.

***The electrical properties*:** Knowledge about the formation of the cross-linking region obtained from the correlation analysis between *G'* and $\Delta n/\Delta\gamma$ is reinforced by the estimation of the longitudinal length of the aggregated region using low frequency dielectric relaxation measurements. We focused on the dynamics of counterions bound to the high charge density region reflecting the cross-linking region in relation to the conformational transition.

Figure 3 shows the temperature dependence of the dielectric dispersion (ε') spectrum for

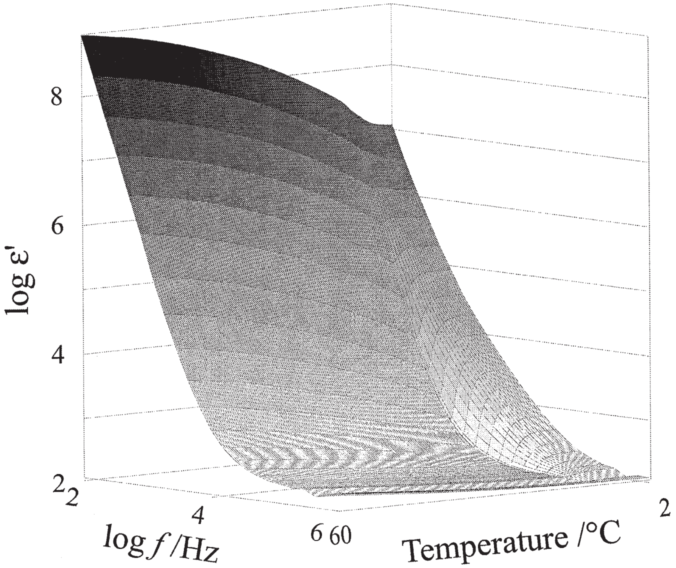

Figure 3 The temperature dependence of the dielectric dispersion ε' spectrum of 1.0wt% Ca-form κ-carrageenan. (Reproduced with permission from 2002**,** *35,* 5595. Copyright 2001 Am. Chem. Soc)

1.0wt% Ca-form κ-carrageenan solutions in the frequency range 10^2-10^6Hz in the temperature range 2°C-60°C. The shape of the ε' spectrum shows the characteristic change at the coil-helix transition temperature, and is quite different below and above the coil to helix transition temperature T_{CH}. Since in the case of the sample with non-gelling promoting cation of Na, which does not show the coil-helix transition at this ionic concentration,[18] such a behavior was not observed, the increase of ε' near the 10kHz must be connected with the coil-helix transition.

Figure 4 shows that the ε' and ε" spectrum of 1.0wt% Ca-form κ-carrageenan solution at 60°C and 5°C, corresponding to the coil and helical state, respectively. It is seen that ε" obeys ε" ~$1/\omega$ over the wide frequency range, 10^0-10^6Hz, which means that the dominant effect in this range is DC conductivity. An increase in ε' with decreasing temperature at the frequencies lower than 10^3Hz is due to the electrode polarization effect ε_{ep}^*, which is represented by the empirical function,[19]

$$\varepsilon_{el}^{*} = \frac{\varepsilon_{ep}}{(i\omega)^{\gamma}}, \tag{3}$$

where ω is the angular frequency and $\varepsilon_{\mathrm{ep}}$ and γ the electrode polarization parameter. We can well reproduce the data at sol state (60°C) by using the effects of DC conductivity and electrode polarization to reproduce the experimental data,

$$\varepsilon^{*} = \varepsilon_{\infty} + \frac{1}{\dfrac{i\omega}{\sigma} + \dfrac{(i\omega)^{\gamma}}{\varepsilon_{ep}}}, \tag{4}$$

where ε_{∞} is the instantaneous permittivity and σ the DC conductivity. In contrast, we cannot reproduce the data with eq 4 at 5°C. Only an addition of the Cole-Cole relaxation term gave an excellent fit, i.e.

$$\varepsilon^{*} = \frac{1}{\dfrac{1}{\varepsilon_{\infty} + \dfrac{\Delta\varepsilon}{1+(i\omega\tau)^{\beta}} + \dfrac{\sigma}{i\omega}} + \dfrac{1}{\dfrac{\varepsilon_{ep}}{(i\omega)^{\gamma}}}}, \tag{5}$$

where $\Delta\varepsilon$ is the relaxation strength defined as ε_0-ε_{∞}, τ the relaxation time, and β is the distribution parameter of the relaxation time in the Cole-Cole dielectric relaxation function. It is confirmed that the electrode polarization parameters are nearly constant in the entire temperature range.[12] The residual resulting from the fitting procedure with eq

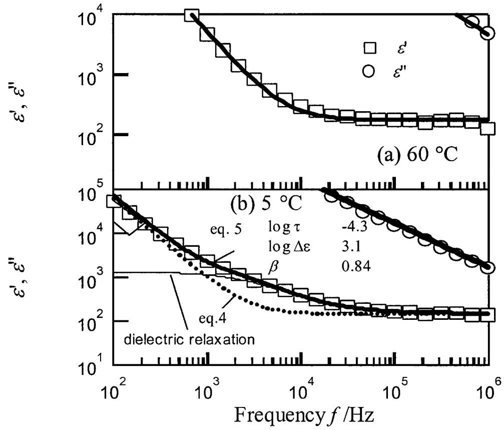

Figure 4 The temperature dependence of the dielectric dispersion ε' and loss ε'' of 1.0wt% Ca-form κ-carrageenan at (a) 60°C and (b) 5°C. The thick solid, the dotted, and the thin solid lines represent the best fit curve with eq 5, the fitted curve with eq 4, and the relaxation term in eq 5, respectively.

3 is much larger than that with eq 4, especially at the temperatures lower than T_{CH}. We therefore used eq 4.

As shown in Figure 1, the dielectric relaxation parameters, τ and $\Delta\varepsilon$ of Cs-form κ-carrageenan estimated by the least square fitting procedure show the characteristic change at T_{CH}. σ decreases sharply just below the T_{CH}. This means that the sharp decrease of the σ is caused by a decrease of the amount of counterions contributing to σ. This is a strong evidence that the dielectric relaxation arises due to a change of the binding nature of the counterions linked with the coil to helix transition.

According to the Manning's counterion condensation theory,[20] the bound counterion to the polyion is classified as loosely and tightly bound counterions, in terms of the dimensionless charge density parameter ξ defined as

$$\xi = \frac{e^2}{\varepsilon bkT}, \tag{6}$$

where b is the charge separation distance, e the elementary of electric charge, ε the dielectric constant of solvent, k the Boltzmann constant, and T the temperature. The tightly bound counterions arise when ξ is higher than $1/z$, where z is the counterion valence.[20]

The charge separation distance b of the helix state is shorter than that of the coil state, and the helical content increases with decreasing temperature below T_{CH}. For κ-carrageenan aqueous solutions, the values of ξ for the coil and helical state, estimated from the chemical structure of the monomers and the X-ray data.[1,21] are ~0.68 and 1.65, respectively. The value of ξ in the helical part in the polymer chains exceeds unity. An increase of the amount of tightly bound counterions with decreasing temperature is anticipated.

As mentioned in the introduction, low frequency dielectric relaxation is ascribed to the fluctuation of counterions tightly bound to polyions along the polyion axis.[8,9] The relaxation time τ for the process is proportional to d^2/μ, where μ is the mobility of the counterion and d is the fluctuation distance of the counterion.[10] Based on the cross-linking mechanism of carrageenan gels, a side-by-side aggregation of helical molecules,[2,15] it is expected that the fluctuation distance d of tightly bound counterions estimated from the relaxation time of the low frequency process will reflect the size of the high charge density region. In other words, d reflects the longitudinal length of the aggregated region of helices.

We think that the cross-linking and the gel network structure are dominated by whichever process, growth of the length of helices or aggregation of helices, precedes in the initial stage of gelation, since $\Delta\varepsilon$ increases sharply just below T_{CH}, and gradually reaches a constant value at low temperatures. At the initial stage of gelation, in other words, just below T_{CH}, some short helices appear in a chain. When the aggregation rapidly occurs just below T_{CH} and the gel network is formed before the growth of the long helices, the longitudinal length of the aggregated region is difficult to increase due to the constraint by the other chains in the network. The long helices can be formed when the growth of the long helix occurs before the helices take part in forming the gel network.

The length of the rigid segment, that is, the helical molecule, is important for enlarging the stress for the orientation. In terms of the dielectric relaxation time, the longitudinal length of the helices and their aggregated region sharply increase in the initial stage of gelation and gradually reaches a constant value at low temperatures. We think that this result reinforces the suggestion that the enlargement of the stress inducing polymer orientation is caused by the increase of the number of helical molecules bundled in a cross-linking region.

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