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Molecular mobility and microscopic structure changes in κ-carrageenan solutions studied by gradient NMR

Qiuhua Zhao, Tom Brenner, Shingo Matsukawa*

Department of Food Science and Technology, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan

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ABSTRACT

Changes in the molecular mobility of κ-carrageenan were observed by the pulsed field gradient stimulated echo (PGSTE) and Carr–Purcell–Meiboom–Gill (CPMG) methods for elucidating the molecular aspect of the sol-to-gel transition. The echo signal intensity of κ-carrageenan without a gradient, $I_{kap}(0)$, decreased steeply near the sol-to-gel temperature (T_{sg}), suggesting that κ-carrageenan chains formed aggregates and a network structure. Below T_{sg}, the spin–spin relaxation time T_2 and the diffusion coefficient of κ-carrageenan (D_{kap}) increased with decreasing temperature, indicating that the solute κ-carrageenan chains have a lower molecular weight M_w than chains involved in the aggregation. The diffusion coefficient of pullulan (D_{pul}) added as a probe molecule in κ-carrageenan solutions was measured, and the characteristic hydrodynamic screening length, ξ, was then estimated from the degree of diffusion restriction. Below a certain temperature, D_{kap} reached a higher value than that of D_{pul}, suggesting that the M_w of solute κ-carrageenan became lower than that of pullulan. GPC measurements confirmed the presence of κ-carrageenan chains with a lower M_w than that of pullulan. A simple physical model of the structural change in κ-carrageenan solution was proposed with a bimodal distribution of κ-carrageenan with higher and lower M_w than the pullulan probe. The higher M_w chains form the gel network restricting the probe's diffusion, and the lower M_w chains increase the effective viscosity. The concentration of the high M_w solute κ-carrageenan chains in 1%, 2% and 4% κ-carrageenan solutions was estimated from $I_{kap}(0)$ and the total κ-carrageenan concentration, and the relation with pullulan diffusion was studied.

1. Introduction

κ-Carrageenan comes from a family of linear water-soluble polysaccharides extracted from different species of marine red algae. The chemical structure of κ-carrageenan is characterized by an alternating disaccharide composed of α-1,3-linked galactose-4-sulphate and β-1,4-linked 3,6-anhydrogalactose. It is largely used in the food (Imeson, 2000), pharmaceutical (Garcia & Ghaly, 1996; Makino, Idenuma, Murakami, & Ohshima, 2001) and cosmetic (Campanella, Roversi, Sammatrtino, & Tomassetti, 1998) industries as a gelling and thickening agent, texture enhancer or stabilizer. The gelling properties of κ-carrageenan have been extensively studied by many researchers (Hjerde, Smidsrød, & Christensen, 1999; MacArtain, Jacquier, & Dawson, 2003; Mangione, Giacomazza, Bulone, Martorana, & San Biagio, 2003; Mangione et al., 2005; Nono, Nicolai, & Durand, 2010; Sankalia, Mashru, Sankalia, & Sutaria, 2006). The gelling process is generally accepted as a two-step model involving a coil to helix conformational transition followed by aggregation of the ordered molecules to form an infinite network (Rochas & Rinaudo, 1984; Takemasa & Chiba, 2001). This aggregation leads to a decrease in the solute (random-coil) concentration of κ-carrageenan, and therefore, molecular diffusion of probe molecules should be enhanced. This diffusion behavior can provide microscopic insights into the gelation mechanism of κ-carrageenan. However, there is a lack of systematic research on molecular mobility of κ-carrageenan in terms of variation of diffusion coefficients during the entire gelation process.

Recently, the diffusion of probe molecules in κ-carrageenan gels has attracted much attention since it is closely related to a variety of applications, such as release of drugs from gels and release of flavor compounds in foods. Reports on the influence of κ-carrageenan gel structures on the diffusion of a dendrimer probe (Walther, Lorén, Nydén, & Hermansson, 2006) and a polymer (ethylene glycol) probe (Lorén et al., 2009) have been published, where the gel structures were controlled by varying the salt content and the cooling rate. The diffusion of probe molecules in polymer matrices can be affected by hydrodynamic interactions (Phillips, 2000). The probe molecules in polymer solutions and gels experience an additional frictional force exerted by the polymer network (Amsden, 1998). Recognizing the increasing number of reports on the effect of polymer concentration and mesh size on probe diffusion, we aimed at elucidating in the present study the diffusion behavior of a probe molecule and

* Corresponding author. Tel.: +81 3 5463 0581; fax: +81 3 5463 0581.
E-mail address: matsukaw@kaiyodai.ac.jp (S. Matsukawa).

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the structural changes in κ-carrageenan solutions during gelation through use of the PGSTE method. We could measure the diffusion coefficient of a probe molecule and therefore quantify the hydrodynamic screening length in gelling κ-carrageenan solutions.

2. Materials and methods

2.1. Materials and sample preparation

The κ-carrageenan powder was purchased from Sigma Chemical Co. and was used without any purification. The concentrations of K^+, Na^+, and Ca^{2+} contained in the original sample were 6.8%, 0.6%, and 2.4%, respectively. No salt was added during sample preparation. A pullulan sample with a peak molecular weight, M_p, of 10.7×10^4 g/mol was purchased from Showa Denko Co. (Tokyo, Japan). Three solutions, containing 0.1% pullulan and variable concentrations of κ-carrageenan (1%, 2% and 4%) were prepared as follows: appropriate amounts of κ-carrageenan were dispersed in D_2O (deuterium isotopic content: 99.0%) by stirring for 1 h at room temperature, and 0.5 h at 80 °C. Thereafter, the same amount of 0.2% pullulan/D_2O solution was added and the mixture was stirred at 80 °C for 0.5 h to obtain a final homogenous 1%, 2% or 4% κ-carrageenan solution containing 0.1% pullulan. The concentrations of κ-carrageenan and pullulan are expressed in w/w% of total weight of the solution. The resulting solution was immediately transferred into a preheated 10 mm NMR tube.

2.2. Methods

2.2.1. Diffusion measurements

Self-diffusion coefficient measurements using a PGSTE pulse sequence (Price, 2009) were performed on a Bruker Avance II 400WB spectrometer equipped with a gradient probe. The temperature was varied using Bruker BVT-3200 and was monitored with an optical fiber thermometer (Takaoka Electric Manufacturing Co., Tokyo, Japan) placed in the sample tube. Each measurement was carried out after waiting for 30 min at each temperature. The diffusion coefficient values were determined from the decay of echo signal intensities, expressed as follows:

$$I(2\tau_2 + \tau_1, g\delta) = I(1\tau_2 + \tau_1, 0)\exp[-\gamma^2\delta^2 g^2(\Delta - \delta/3)D] \quad (1)$$

where $I(2\tau_2 + \tau_1, g\delta)$ and $I(2\tau_2 + \tau_1, 0)$ are echo signal intensities at $t = 2\tau_2 + \tau_1$ with and without the field gradient pulse, respectively, γ is the gyromagnetic ratio of ^1H, g is the field gradient strength, δ is the duration of the gradient pulse and Δ is the diffusion time that corresponds to the time interval between two field-gradient pulses. Note that $I(2\tau_2 + \tau_1, 0)$ has decayed from the initial intensity, $I(0, 0)$, (i.e., the signal intensity immediately after the first $\pi/2$ rf pulse) by T_1 and T_2 relaxations:

$$I(2\tau_2 + \tau_1, 0) = I(0, 0)\exp[-2\tau_2/T_2 - \tau_1/T_1] \quad (2)$$

From Eq. (2) it follows that a decrease in the relaxation times leads to a decrease in $I(2\tau_2 + \tau_1, 0)$.

In all experiments, δ and Δ were fixed to 1 ms and 10 ms, respectively. The g values were varied from 2 to 16 T/m for each diffusion measurement. The $\pi/2$ pulse length for ^1H was 16 μs and the relaxation delay was set to 3.5 s. All chemical shifts were referenced to the peak at 5.36 ppm, which is attributed to the anomeric proton of pullulan.

The diffusion coefficient of pullulan in a dilute D_2O solution at 25 °C was measured as described previously (Zhao & Matsukawa, 2012). The hydrodynamic radius R_H of pullulan was assumed to be independent of temperature (Nordmeier, 1993; Viebke & Williams, 2000), and the diffusion coefficient of pullulan in dilute D_2O ($D_{pul,0}$) at all temperatures was calculated from the Stoke–Einstein equation:

$$D_{pul,0} = \frac{k_B T}{6\pi\eta_{(T)}R_{(H)}} \quad (3)$$

where k_B is Boltzmann's constant, T is the absolute temperature, and $\eta_{(T)}$ is the viscosity of D_2O.

2.2.2. Spin-spin relaxation time (T_2) measurements

The spin relaxation times were measured using the pulse sequence. Two gradient pulses were added to the standard Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence to eliminate the water peak, which affects the analysis of polymers in some cases. The pulse space (2τ) between π pulses was 4 ms. Values of T_2 were obtained from the following relationship:

$$I_{2n\tau} = I_0 \exp(-2nt/T_2) \quad (4)$$

where n is the echo number.

2.2.3. GPC measurement

Gel permeation chromatography (GPC) of κ-carrageenan was performed on a chromatograph equipped with a PerkinElmer Series 200 pump, a Knauer Smartline 2300 refractive index detector, a Knauer Smartline column thermostat, and two Shodex OHpak SB-806MHQ columns in series protected by a Shodex OHpak SB-G guard column. Elution was carried out using a 0.1 M $NaNO_3$ solution as the mobile phase at a flow rate of 0.8 ml/min. The temperature of the columns was maintained at 60.0 °C. A calibration curve was constructed using pullulan standards with peak molecular weights, M_p, of 2400, 1560, 710, 380, 200, 106, 45.9, 22.0, 11.2, 5.6, 1.08 and 0.342 kDa. the elution volume was corrected to the internal marker of ethylene glycol (0.01% in sample) at 23.04 ml. The κ-carrageenan concentration used was 0.07% and the sampling volume was 100 μl. To obtain more reliable results, the κ-carrageenan was dissolved in the same solvent used as an eluent in the GPC system. The hot κ-carrageenan solution (60 °C) was filtered through a 0.45-μm membrane (Spartan 30/0.45 RC) and injected into the HPLC system.

3. Results and discussion

3.1. Translational and rotational mobility of κ-carrageenan

Fig. 1 shows changes in the PGSTE ^1H spectra of a 2% κ-carrageenan solution containing 0.1% pullulan at a gradient strength of 2.0 T/m during cooling from 53 °C to 28 °C. For the translational mobility analysis, the peaks at 5.36 and 5.17 ppm, which are assigned to the anomeric proton of pullulan and the C1 proton on the β-1, 4-linked 3,6-anhydrogalactose unit of κ-carrageenan, respectively, were chosen. The corresponding signals are indicated by p and κ, respectively. Detailed information on the signal assignments of pullulan and κ-carrageenan can be found in the literature (McIntyre & Calgary, 1993; Tojo & Prado, 2003). The water peak at 4.7 ppm was completely eliminated due to the fast diffusion of water. The signal intensity of κ-carrageenan (5–5.25 ppm) started to decrease at 33 °C, indicating a structural change of κ-carrageenan chains, which will be discussed in detail later. Because the signal intensity of pullulan remained roughly constant during cooling and showed no change around 33 °C, we conclude that pullulan was not involved in any way in the aggregation and gelation process of κ-carrageenan.

Measurements of the diffusion coefficient are performed by measuring the echo signal intensities at different g while Δ and δ are kept constant. Fig. 2a shows the echo signal intensities for κ-carrageenan, $I_{kap}(g)$, and pullulan, $I_{pul}(g)$, against $\gamma^2\delta^2 g^2(\Delta - \delta/3)$ measured at 38 °C in a semi-logarithmic representation. The linear decrease observed for the signal intensities of signals from both

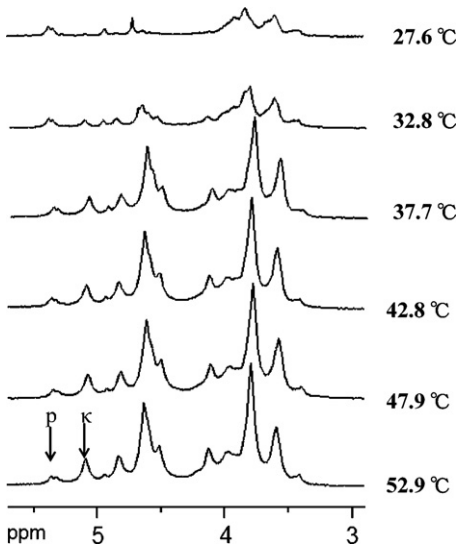

Fig. 1. PGSTE ^{1}H NMR spectra of a 2% κ-carrageenan solution containing 0.1% pullulan at a gradient strength of 2.0 T/m as a function of temperature. The peaks selected for the diffusion analysis of the pullulan and κ-carrageenan are indicated by "p" and "κ", respectively.

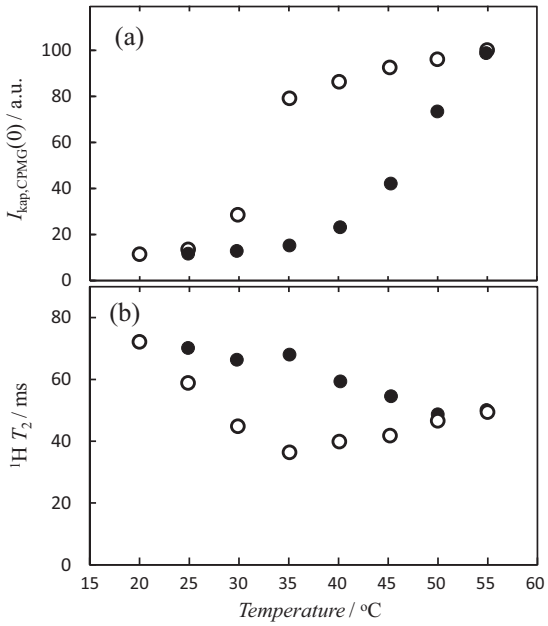

Fig. 3. The temperature dependence of (a) signal intensity of κ-carrageenan at echo time $\tau = 0$ of the CPMG measurements, and (b) ^{1}H spin-spin relaxation times T_2 of a 2% κ-carrageenan solution during cooling (○) and heating (●).

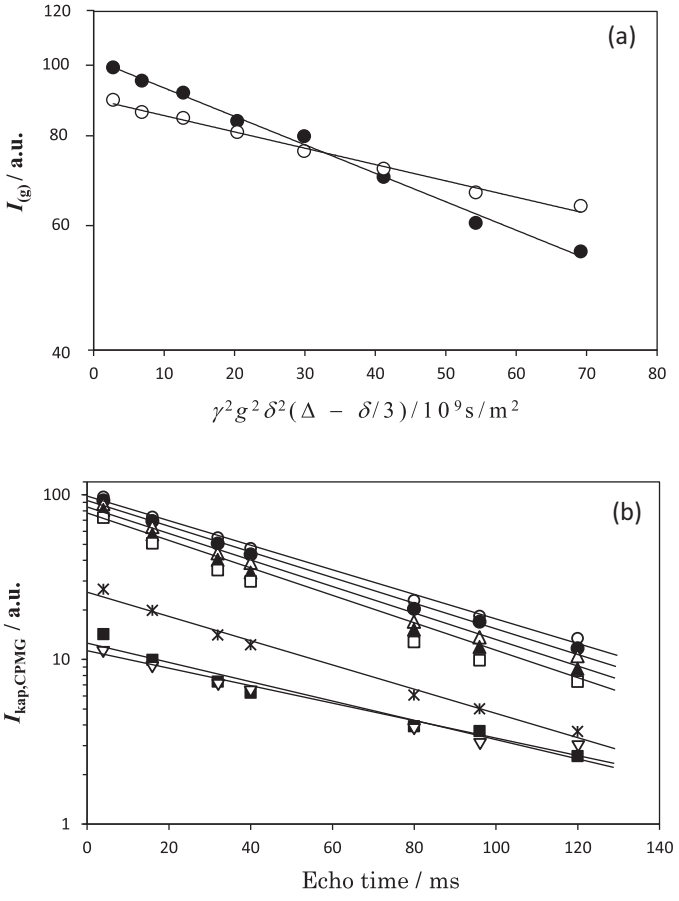

Fig. 2. (a) The signal decay of pullulan (●) and κ-carrageenan (○) in a 2% κ-carrageenan solution containing 0.1% pullulan at 38 °C. The lines are fits to Eq. (1). (b) Semi-logarithmic plots of the peak intensity in the CPMG echoes against the echo time for a 2% κ-carrageenan solution at 55 °C (○), 50 °C (●), 45 °C (△), 40 °C (▲), 35 °C (□), 30 °C (*), 25 °C (■) and 20 °C (▽). The lines are fits to Eq. (4).

pullulan and κ-carrageenan indicates a single-mode diffusional process of both macromolecules. Pullulan, which was a grade of GPC standard, has a quite narrow molecular distribution. On the other hand, the κ-carrageenan chains were known to be polydisperse. Nevertheless, deviations of the experimental data from the straight line fits were small at all temperatures. For this reason, we determined a single-value of the diffusion coefficient of κ-carrageenan using Eq. (1). The fit also provided us with the signal intensity of κ-carrageenan without the gradient $I_{kap}(0)$.

The spin–spin relaxation time T_2 of κ-carrageenan was determined by the CPMG method. The semi-logarithmic plots of the peak intensity against the echo time in the CPMG measurements at various temperatures are given in Fig. 2b. The data at all temperatures lie on a straight line and were therefore analyzed assuming a monomodal relaxation, and the fits to Eq. (4) yield the ^{1}H T_2 values as well as the signal intensity at echo time $\tau = 0$ in CPMG measurements, $I_{kap,CPMG}(0)$. The corresponding results of $I_{kap,CPMG}(0)$ and ^{1}H T_2 are shown in Fig. 3a and b, respectively. Values of $I_{kap,CPMG}(0)$ decreased slightly in the temperature range from 55 °C to 35 °C (stage 1). In this stage, the T_2 values are in the range 35–50 ms, indicating that the κ-carrageenan chains have high segmental mobility. The values of $I_{kap,CPMG}(0)$ decreased steeply with decreasing temperature from 35 °C to 25 °C (stage 2), but the semi-logarithmic plots still showed a monomodal relaxation, as shown in Fig. 2b. The gelation temperature T_{sg} determined by the falling ball method for a 2% κ-carrageenan solution is 35 °C. We therefore concluded that the steep decrease in the signal intensity in stage 2 is due to the formation of aggregated bundles with highly restricted segmental mobility and extremely short T_2 values reflecting their rigid structure. Consequently, the corresponding signal of aggregates was completely decayed before the first echo time of 4ms. Namely, only the non-aggregated κ-carrageenan chains (solute κ-carrageenan) contribute to the CPMG echo signal intensities. From this assumption it follows that $I_{kap,CPMG}(0)$ is proportional to the solute fraction of κ-carrageenan chains, so that the fraction of soluble κ-carrageenan chains at each temperature can be estimated by comparing their corresponding value of $I_{kap,CPMG}(0)$ to the value of $I_{kap,CPMG}(0)$ at the highest temperature. The aggregation

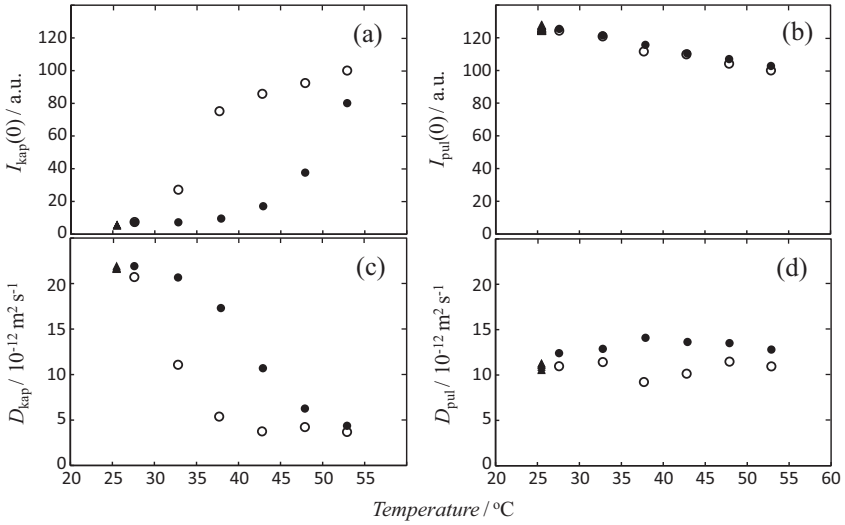

Fig. 4. Temperature dependence of (a) the echo signal intensity of κ-carrageenan $I_{kap}(0)$, (b) the echo signal intensity of pullulan $I_{pul}(0)$, (c) diffusion coefficient of κ-carrageenan D_{kap} and (d) diffusion coefficient of pullulan D_{pul} (2% κ-carrageenan,0.1% pullulan during cooling (○), keeping at 25°C (▲) and heating (●).

of κ-carrageenan chains continued with decreasing temperature and at the lowest temperature measured, about 10% of soluble κ-carrageenan chains were left in the solution. In stage 2, the T_2 values of the solute increased with decreasing temperature, indicating an increase in molecular mobility. The most plausible explanation for this increase in T_2 is a decrease in the microscopic viscosity following the incorporation of κ-carrageenan chains aggregates into the gel network with a concomitant decrease in soluble κ-carrageenan concentration. On heating process, $I_{kap,CPMG}(0)$ showed an increase with increasing temperature above the melting temperature T_{gs} (ca. 43 °C, determined by the falling ball method), indicating melting of the network and aggregates. This was accompanied with a decrease in T_2, attributed to the increase in microscopic viscosity.

The temperature dependence of the echo signal intensities without a gradient I_{kap} (0) and diffusion coefficients of κ-carrageenan D_{kap} determined from PGSTE measurement are shown in Fig. 4a and c, respectively. During cooling, $I_{kap}(0)$ decreased slightly in stage 1, which is most likely a result of an increase in the viscosity upon cooling in this temperature range. This decrease is therefore incurred through a decrease in the relaxation times T_1 and T_2, cf. Eq. (2). The same conclusion arises from Fig. 3a. $I_{kap}(0)$ decreased sharply in stage 2, which is a consequence of the formation of aggregated bundles and the gel-network. We assume that the T_2 values of aggregates are far shorter than the echo time $\tau_1 + 2\tau_2$ (ca. 12 ms). Consequently, the echo signals of aggregates disappear by their fast decay during the echo times. In previous reports, a similar behavior of the echo signal intensity was observed in agar solutions and gellan solutions (Dai & Matsukawa, 2012, 2013; Shimizu, Brenner, Liao, & Matsukawa, 2012). After cooling, the sample was kept for at 12 h during which no measurable change was found in the echo signal intensity.

Fig. 4c shows that the diffusion coefficient of κ-carrageenan (D_{kap}) increased markedly with decreasing temperature below T_{sg} during the cooling process. Obviously, the concentration of solute κ-carrageenan is decreased during aggregation. The local viscosity was thus decreased and led to an increase in D_{kap}. However, the increase of D_{kap} is more pronounced than that expected from the decrease of local viscosity. We therefore concluded that the diffusing κ-carrageenan chains are mainly the shorter chains, with the aggregating chains being mainly the higher M_w chains. During the heating process, D_{kap} decreased with increasing temperature above T_{gs}, indicating an increase in the solute κ-carrageenan concentration accompanied with the increase of average *M*w and an increase in microscopic viscosity.

The molecular weight distribution of the κ-carrageenan solution is shown in Fig. 5. The *x*-axis is given in form of elution volume instead of molecular weight, because elution volumes below 14.5 ml are below the highest molecular-weight standard marker. The κ-carrageenan under study has a quite wide molecular weight distribution in the main peak even though the experimental data for diffusion measurements were fairly well analyzed as single-mode diffusions probably because of the averaging effect in semi-dilute solutions (Willis, Dennis, Zheng, & Price, 2010). The peak has a small shoulder at larger retention volume but also has no effect on the single-mode analysis because of the small fraction. The M_p of the κ-carrageenan determined by GPC was 14.8×10^5 g/mol, which is much higher than that of the pullulan probe used (10.7×10^4 g/mol). For construction of a simple physical model, we divide the κ-carrageenan into 2 populations. One

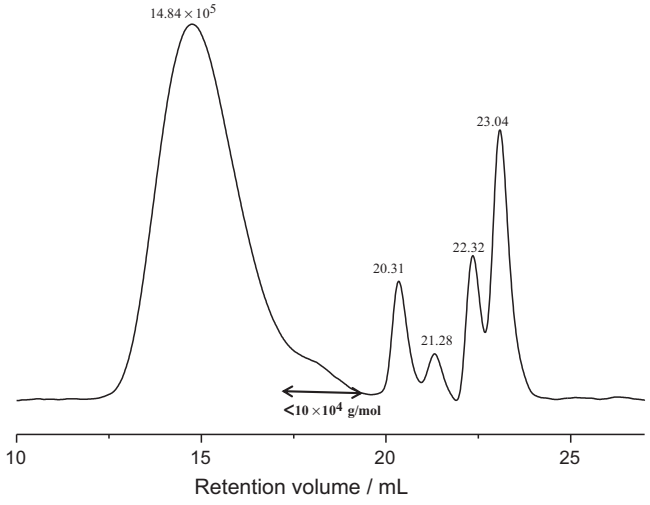

Fig. 5. Gel permeation chromatography (GPC) retention profiles for κ-carrageenan. The elution volume was corrected to the internal marker of ethylene glycol at 23.04 ml. The molecular weight values are given relative to pullulan standards. Peaks at 20.31, 21.28 ml correspond to the free SO_4^{2-} and CL^{-1} contained in the original sample, respectively. Peak at 22.32 ml is due to the small differences in $NaNO_3$ concentration between the HPLC eluent and the sample solution.

population has a higher and the other a lower M_w than the pullulan probe. The portion of κ-carrageenan chains with a lower M_w than pullulan (10×10^4 g/mol) is indicated in Fig. 5, and corresponds to about 7% by weight of the κ-carrageenan chains. As mentioned earlier, the higher M_w κ-carrageenan chains are preferably involved in the aggregation process.

3.2. Molecular mobility of pullulan

Temperature dependences of $I_{kap}(0)$, D_{kap} and T_2 of κ-carrageenan chains, have provided an insight into the gelation mechanism of the κ-carrageenan solution, and indicated a decrease of solute (non-aggregated) κ-carrageenan chains during the gelation process. To further elucidate the changes in the network structure during gelation, the diffusion coefficient of the pullulan, added as a probe polymer, was measured during cooling by means of PGSTE.

Fig. 4b indicates the change in pullulan echo signal intensities without gradient of $I_{pul}(0)$, which showed a gradual and continuous increase with decreasing temperature. The increase of $I_{pul}(0)$ is most likely caused by a little increase of magnetization recovery during the repetition time of 0.7 s. The repetition time should be five times longer than T_1 to recover more than 98% of magnetization and was not long enough for pullulan in the κ-carrageenan solution at high temperature (T_1 was ca. 0.73 s), but allowed for higher recovery at lower temperature since T_1 decreased with decreasing temperature. The echo signal intensity of pullulan didn't decrease steeply around T_{sg}. The implication is that pullulan is not involved in the aggregation and gelation process of κ-carrageenan, as already mentioned earlier.

D_{pul} at various temperatures are shown in Fig. 4d. Below T_{sg}, D_{pul} increased with decreasing temperature from 38 °C to 33 °C, indicating an increase in molecular mobility. In this work, we assumed that the restriction of the probe's diffusion was mainly due to hydrodynamic drag exerted by the κ-carrageenan network as well as solute chains. We therefore expect this hydrodynamic drag to become smaller as the solute κ-carrageenan concentration is decreased markedly concomitant with gelation in this temperature range (Fig. 4a). As temperature further decreased, D_{pul} decreased slightly with decreasing temperature. This may be an indication that the effect of hydrodynamic interaction became weaker as the change in signal intensity for κ-carrageenan became smaller. So that the temperature became the dominant factor and led to a decrease in D_{pul}.

For quantifying the degree of restriction on the probe diffusion, we calculate the ratio $D_{pul}/D_{pul,0}$, i.e., the ratio of pullulan's diffusion coefficient to its value in the pure solvent. These values are shown in Fig. 6. Within the frame of the hydrodynamic model, the ratio $D_{pul}/D_{pul,0}$ is given by (Cukier, 1984; Matsukawa & Ando, 1996):

$$D_{pul}/D_{pul,0} = \exp(-R_H/\xi) \quad (5)$$

where R_H is the hydrodynamic radius of the probe polymer, and ξ is the hydrodynamic screening length of the host polymer, i.e., the hydrodynamic mesh size of the network. It is clear from Eq. (5) that an increase in $D_{pul}/D_{pul,0}$ indicates an increase in ξ. During the cooling process, $D_{pul}/D_{pul,0}$ was almost constant at high temperature, and increased with decreasing temperature below T_{sg}, indicting a decrease of restriction on diffusion of pullulan. This is thought to be caused by the addition of κ-carrageenan chains into aggregates and a network structure, which reduces the concentration of solute κ-carrageenan chains. During the reheating process, $D_{pul}/D_{pul,0}$ decreased with increasing temperature at high temperature, suggesting that the gel-network gradually melted.

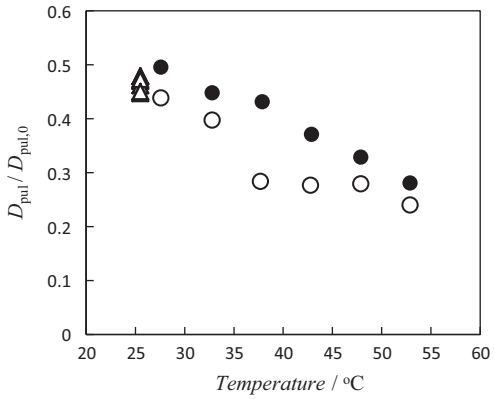

Fig. 6. Temperature dependence of $D_{pul}/D_{pul,0}$ for pullulan in the 2% κ-carrageenan solution during cooling (○), keeping at 25 °C (△) and heating (●).

3.3. A simple physical model of the structural change in κ-carrageenan solution during gelation process

By comparing D_{pul} with D_{kap}, the change in average molecular weight for solute κ-carrageenan after gelation can be clarified. In Fig. 4c and d, it is seen that D_{kap} is smaller than D_{pul} above the temperature around T_{sg} and became larger than D_{pul} below the temperature. This indicates that the average molecular weight of solute κ-carrageenan was larger than that of pullulan above the temperature around T_{sg} and became smaller than that of pullulan below the temperature because the higher molecular weight of κ-carrageenan was involved into the aggregates. Similar trends have been found in previous studies on agarose solution (Dai & Matsukawa, 2012, 2013) and gellan solution (Shimizu, Brenner, Liao, & Matsukawa, 2012). Here, we will emphasize the physical model of this study based on the above discussions, which is summarized in Fig. 7. As illustrated above, we assume that the κ-carrageenan chains can be divided into two groups. One group contains κ-carrageenan chains with a higher molecular weight than that of pullulan, and form a network of overlapped chains retarding the diffusion of probe molecules by hydrodynamic interactions. The second group is κ-carrageenan with a lower molecular weight than that of pullulan. These chains cannot form network junctions that hydrodynamically affect the diffusion of probe molecules, but they increase the microviscosity. We define the temperature at which D_{kap} exceeded the value of D_{pul} as the so-called "reference temperature", T_{ref}. At high temperature, all the κ-carrageenan chains exist as random coils, and both ξ, the hydrodynamic correlation length of the overlapped chain network of the high M_w κ-carrageenan chains, and the microviscosity, which is affected by the low M_w κ-carrageenan chains, remain constant. As the temperature decreased, the high M_w κ-carrageenan chains start to form aggregates, which results in a decrease of the number concentration of chains forming the overlapped network, and of course an increase in the network mesh size. We assume that at around T_{ref}, all the long κ-carrageenan chains have formed aggregated. As the temperature further decreased, the shorter κ-carrageenan chains also aggregate into the gel network, which results in a decrease of the microviscosity.

At temperatures above T_{ref}, the solute concentration of long κ-carrageenan chains C_s' can be estimated from $I_{kap}(0)$ and the initial total solute κ-carrageenan concentration C_0 based on the assumption that all the long chains have formed aggregates at T_{ref}:

$$C_S' = C_0 - \frac{I_{kap}(0) - I_{kap,T_{ref}}(0)}{I_{kap,T_{hig}}(0)} \quad (6)$$

where $I_{kap,T_{hig}}(0)$ is the signal intensity of κ-carrageenan at the highest temperature.

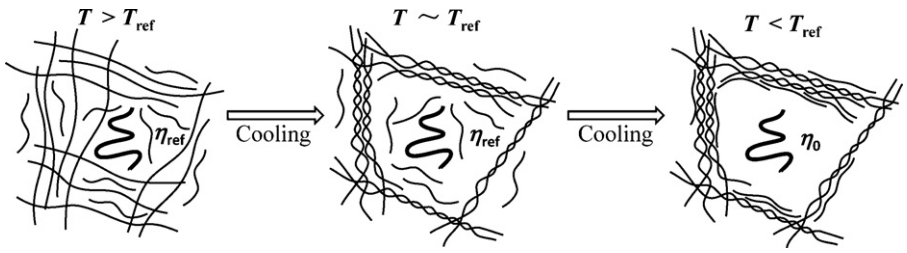

Fig. 7. Schematic model for the cooling process of κ-carrageenan.

For the quantification of the restriction of probe diffusion, $D_{pul,0}$ in Eq. (5) should be replaced by $D_{pul,s}$, the diffusion coefficient of pullulan in the solution of the short κ-carrageenan chains without network. In other words, we decouple the effect of the hydrodynamic effect and that of the microviscosity, by replacing the viscosity of pure D_2O with the viscosity of the lower M_w κ-carrageenan solution. The ratio of $D_{pul,s}$ and $D_{pul,0}$ is given by the ratio of the viscosities as follows:

$$D_{pul,s}/D_{pul,0} = \eta_0/\eta_s \quad (7)$$

where η_0 is the viscosity of pure D_2O and η_s is the viscosity in the solution of the short κ-carrageenan chains. Replacing $D_{pul,0}$ in Eq. (5) by $D_{pul,s}$, we have

$$D_{pul}/D_{pul,0} = \eta_0/\eta_s \exp(-R_H/\zeta) \quad (8)$$

To calculate the value of η_0/η_s, we focus on the temperature range below T_{ref}. In this stage, we assume that the short κ-carrageenan chains are gradually aggregated embedded into gel network with no effect on ξ (see Fig. 7). As seen in Fig. 4a, $I_{kap}(0)$ became very small indicating the concentration of the short κ-carrageenan chains was very low at the end of the cooling process (25 °C). Thus, for 25 °C, we assume $\eta_s \approx \eta_0$. Therefore, $\exp(-R_H/\xi)$ below T_{ref} is given by $D_{pul}/D_{pul,0}$ at the lowest temperature T_{low}. Then Eq. (8) can be rewritten to give η_0/η_s at T_{ref}.

$$\frac{\eta_{0(\text{at } T_{ref})}}{\eta_{s(\text{at } T_{ref})}} = \frac{D_{pul\,(at\,T_{ref})}}{D_{pul,0(\text{at } T_{ref})}} / \exp(-R_H/\xi) = \frac{D_{pul(\text{at } T_{ref})}}{D_{pul,0(\text{at } T_{ref})}} \frac{D_{pul,0(\text{at } T_{low})}}{D_{pul\,(\text{at } T_{low})}} \quad (9)$$

Above T_{ref}, the short κ-carrageenan chains are assumed to all be in random coil conformation, meaning that the solute concentration of short κ-carrageenan chains is constant above T_{ref}. Under this assumption, the change of η_s above T_{ref} is only due to the change of η_0 with temperature. Then, η_0/η_s above T_{ref} should be a constant value and equal to the value at T_{ref}, which is given by Eq. (9). By using Eqs. (8) and (9), ξ may be calculated at temperatures above T_{ref}.

3.4. Effect of κ-carrageenan concentration on the molecular mobility of pullulan

Here, we concentrate on the temperature range above the T_{ref}. The solute κ-carrageenan concentration of long κ-carrageenan chains C_s' with higher M_w than that of the pullulan probe were calculated from $I_{kap}(0)$ and the total κ-carrageenan concentration by Eq. (6), and the results are shown in Fig. 8a. Values of C_s' provide a quantitative perspective on how much long random-coil (solute) chains are left in the solution during the cooling process. Fig. 8b shows values of D_{pul} measured in 1%, 2%, and 4% κ-carrageenan solutions. At high temperature, the value of D_{pul} in higher initial κ-carrageenan solution is smaller, which is caused by larger hydrodynamic interactions with the long solute κ-carrageenan chains. It can also be seen that the difference in D_{pul} obtained from 1%, 2%, and 4% κ-carrageenan solutions decreased with decreasing temperature concomitant with aggregation of the long κ-carrageenan chains. The values of $D_{pul}/D_{pul,0}$ are shown in Fig. 8c. As is evident from the figure, lower values of $D_{pul}/D_{pul,0}$ were found when C_0 κ-carrageenan of was higher. By using Eqs. (8), (9) and R_H of the pullulan probe, the values of ξ in 2% κ-carrageenan solution above their respective T_{ref} were calculated from $D_{pul}/D_{pul,0}$ and are indicated on the right-hand axis in Fig. 8c. As mentioned earlier, ξ in this temperature range is considered to represent the hydrodynamic mesh size of the overlapped long κ-carrageenan chains' network. The hydrodynamic mesh size ξ was found to be smaller for the higher κ-carrageenan concentrations. It should be noted that $D_{pul}/D_{pul,0}$ of the 4% κ-carrageenan solution became smaller than $1/e$ (~0.37), indicating that ξ became smaller than R_H of the pullulan probe. In this case, the restriction on probe diffusion by entanglements with network is not negligible, i.e., a more complete description of the probe translational movements should be given within the frame of the reputation model. And the corollary is that when Eq. (8) is used, the restriction by hydrodynamic interactions is overestimated and a smaller ξ is calculated. Nevertheless, values of ξ give insight into the microscopic gelation mechanism, and for most conditions, the probe diffusion was dominantly restricted by hydrodynamic interactions with the long κ-carrageenan chains.

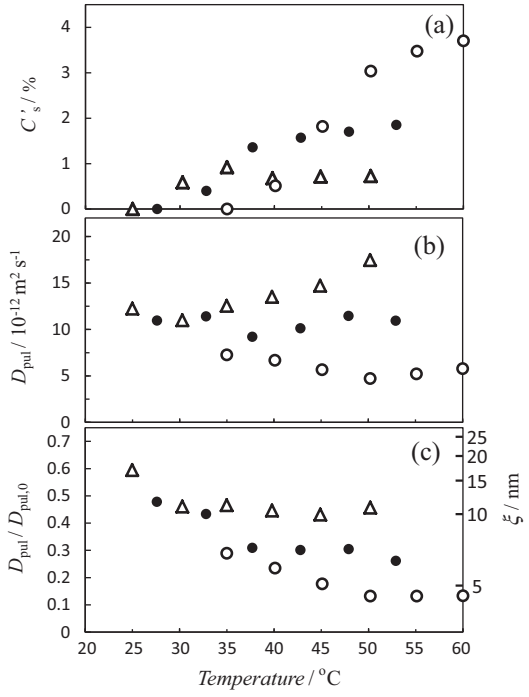

Fig. 8. Temperature dependence of (a) solute long κ-carrageenan chain concentration, (b) diffusion coefficient of pullulan D_{pul}, (c) the ratio $D_{pul}/D_{pul,0}$ in the 1% (△), 2% (●) and 4% (○) κ-carrageenan solution. The corresponding ξ values, calculated using Eq. (8), are displayed on the right-hand axis.

4. Conclusions

The rotational and translational motions of the κ-carrageenan chains were studied using NMR techniques. Below T_{sg}, the spin-spin relaxation time T_2 of κ-carrageenan increased with decreasing temperature as the concentration of solute κ-carrageenan decreased following aggregation. The behavior of D_{kap} and the wide molecular weight distribution of the κ-carrageenan allowed us to conclude that longer κ-carrageenan aggregate preferentially, that is, at higher temperature where the shorter chains remain unaggregated. The extent of aggregation and gelation can be evaluated from the change of solute κ-carrageenan concentration, which can be directly calculated from the value of $I_{kap}(0)$. Pullulan was found to be sensitive to changes in the microscopic environment during the gelation process. The hydrodynamic screening length ξ was calculated from the value of $D_{pul}/D_{pul,0}$. In summary, our results provided microscopic information about the structural changes and the molecular mobility of both the host polymer and a probe polymer during gelation. This methodology can be generally developed to examine and establish the gel structure and the molecular motion of solutes in polymer matrices. Furthermore, the effect of κ-carrageenan concentration on the diffusion of probe molecule was investigated. The solute concentration of κ-carrageenan chains that have a higher M_w than that of the pullulan probe was estimated based on the physical model proposed in the present study. It was also shown that the diffusion coefficients of pullulan decreased with increasing solute κ-carrageenan concentration, which we interpreted as a consequence of the increase in hydrodynamic interactions. The understanding of the gel structure and its effect on the diffusion of guest molecules can be applied in products where diffusion control is of importance.

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