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Effect of thermal history on kappa-carrageenan hydrogelation by differential scanning calorimetry

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Abstract

The effect of thermal history on gel–sol transition was investigated by highly sensitive differential scanning calorimetry (DSC) in order to clarify the non-equilibrium state of κ-carrageenan hydrogels. κ-Carrageenan with a concentration from 0.5% to 5.0% was used. When concentration of solution was lower than 2.0%, homogeneous κ-carrageenan gel was formed when aqueous solution was fully equilibrated. When concentration exceeded 3.0%, a sub-peak could be observed at the low temperature side of the main peak. It was indicated that helices having various sizes and different kinds of defects are present in the junction zone. Thermal histories, such as cooling rate from the sol state or annealing at around gel–sol transition temperature, markedly affect the junction zone formation. A large junction zone is formed at a slow gelation rate, and also that many small junction zones form at a fast gelation speed.

Keywords: κ-Carrageenan; Hydrogel; DSC; Gel–sol transition; Thermal history

1. Introduction

It is well known that the first order phase transition of polymeric materials in the solid state is markedly affected by thermal history and that equilibrium state is not easily attained due to the complex higher order structure [1]. Although efforts have been made to attain the equilibrium melting temperature of polymers and obtain basic thermodynamic data, the transition temperatures obtained by ordinal thermal analytical (TA) methods are necessarily affected by thermal history, accordingly molecular relaxation phenomena accompany the thermal transition data.

Recently, hydrogelation of bio-macromolecules, including various kinds of polysaccharides, has received particular attention, since the bio-compatible nature of polysaccharides has a potential for medical applications [2,3]. On this account, the thermal transition of highly concentrated aqueous polysaccharide solution has been extensively studied by TA, especially by differential scanning calorimetry (DSC) [4–8]. Among DSC data concerning the gel–sol transition, double or triple transition peaks are frequently reported and these peaks are mainly attributed to the complex characteristics of the samples. In the case of solid polymers, DSC melting or crystallization peaks having several peaks are attributed to not only polymorphic structure, but also size and regularity of crystalline region which are controllable by the relaxation process of the sample [1].

In this study, the effect of thermal history on gel–sol transition is investigated by DSC in order to clarify the non-equilibrium state of polysaccharide hydrogels. κ-Carrageenan is chosen as a representative polysaccharide showing clear gel–sol or sol–gel transition.

2. Experimental

2.1. Sample preparation

κ-Carrageenan in powder form was purchased from SIGMA. The M_w of κ-carrageenan was 3.71×10^5 g mol^{-1} by dynamic light scattering [9]. Carrageenan was solved in deionised water

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at 25 °C and solutions with concentration from 0.5% to 5.0% were prepared. The solutions were annealed at 105 °C for 2 h. The sol sample was poured into a DSC pan. The samples were maintained at below 25 °C for 10–30 min. Transparent gels were formed.

2.2. Measurements

A Seiko Instruments Inc. highly sensitive differential scanning calorimeter (DSC) DSC 120 equipped with a cooling apparatus was used. Temperature and enthalpy calibrations were carried out using water. A silver sealed type sample pan with a volume of 70 μl was used. Sample mass was ca. 50 mg. A Sartorius ultramicro-balance ($\pm 1.0 \times 10^{-5}$ g) was used for sample mass measurements. The sample pan was hermetically sealed and the total mass of the carrageenan gel was recorded. Liquid nitrogen was used as a coolant. Distilled water was used as a reference in order to balance the thermal conductivity of the reference and sample holders. The sample was heated from 25 °C to 90 °C at sol state in the sample pan. Then the sample was cooled from 90 °C to −10 °C and heated from −10 °C to 90 °C (first-run measurement). Next, the sample was cooled from 90 °C to −10 °C and heated from −10 °C to 90 °C (second-run). The heating rate was 0.5 °C min^{-1} and cooling rate was varied from 0.1 °C min^{-1} to 5 °C min^{-1}. At the same time, a series of κ-carrageenan hydrogels were annealed at 50–70 °C for 1–120 min in the DSC sample holder.

From heating curves, the starting temperature of gel–sol transition ($T_{i,g–s}$), peak ($T_{p,g–s}$) and end temperature of gel–sol transition ($T_{e,g–s}$) were defined. $T_{i,g–s}$ was defined as the temperature where DSC curves deviate from the baseline. $T_{e,g–s}$ was defined as the temperature where DSC curves return to the baseline. From cooling curves, the starting temperature of sol–gel transition ($T_{i,s–g}$), peak ($T_{p,s–g}$) and end temperature of sol–gel transition ($T_{e,s–g}$) were defined. The gel–sol transition enthalpy ($\Delta H_{g–s}$) and sol–gel transition enthalpy ($\Delta H_{s–g}$) were calculated from the area of gel–sol transition endothermic peak and that of sol–gel transition exothermic peak, respectively. $\Delta H_{s–g}$ values were calibrated using ΔH_c values of water.

3. Results

Fig. 1 shows stacked DSC curves of κ-carrageenan hydrogels with various concentrations. Fig. 1(a) shows cooling curves and (b) heating curves. The patterns of DSC heating and cooling curves were reproducible by repeated runs. In DSC cooling curves, an exothermic peak due to sol–gel transition was clearly observed. The exothermic peak became broad and a shoulder was observed in the low temperature side of exothermic peak when concentration exceeded about 3.0%. A double peak was distinctly observed when concentration was 3.0%, 4.0% and 5.0%. Temperature of transition region (T_{ig}–T_{es}) increased with increasing concentration, i.e. ca. 15 °C for 0.5 wt% and ca. 25 °C for 5.0% sample. In DSC heating curves, DSC baseline gradually shifted to endothermic direction at around 25 °C regardless of concentration, and an endothermic peak due to gel–sol transition was observed. The endothermic peak of gel–sol transition was

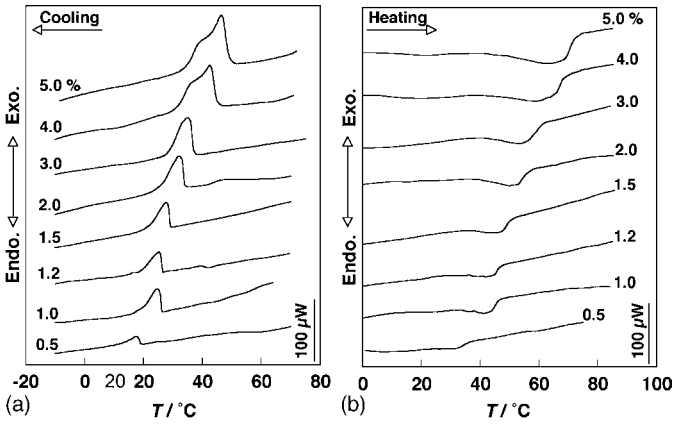

Fig. 1. Stacked DSC curves of κ-carrageenan hydrogels with various concentrations: (a) cooling curves and (b) heating curves.

broad and a shoulder was observed in the low temperature side of the endothermic peak for 4.0 wt% and 5.0 wt% samples. When heating and cooling DSC curves are compared, the exothermic peak due to sol–gel transition was narrow and the endothermic peak broad.

Fig. 2(a) shows peak temperature of sol–gel ($T_{s–g}$) and gel–sol transition ($T_{g–s}$) as a function of concentration. Peak temperature increases with increasing concentration and thermal histeresis is clearly observed. When temperature of thermal histeresis is defined as $\Delta T = T_{p,g–s} - T_{p,s–g}$, ΔT was ca. 18 °C in a whole range of concentrations. Fig. 2(b) shows relationships between sol–gel transition enthalpy ($\Delta H_{s–g}$) or gel–sol transition enthalpy ($\Delta H_{g–s}$) and concentration. A linear relationship can be observed in a whole range of concentration, however, if ΔH values are examined precisely, it is found that $\Delta H_{g–s}$ is smaller than $\Delta H_{s–g}$ at a concentration lower than 3.0%. In contrast, $\Delta H_{g–s}$ is larger than $\Delta H_{s–g}$ when concentration is higher than 3.0%.

In order to examine the effect of thermal history on sol–gel transition, 5.0% κ-carrageenan sol was cooled at various cooling rates, as shown in Fig. 3(a). In cooling curves, exothermic double peaks by sol–gel transition are observed and the peaks widen

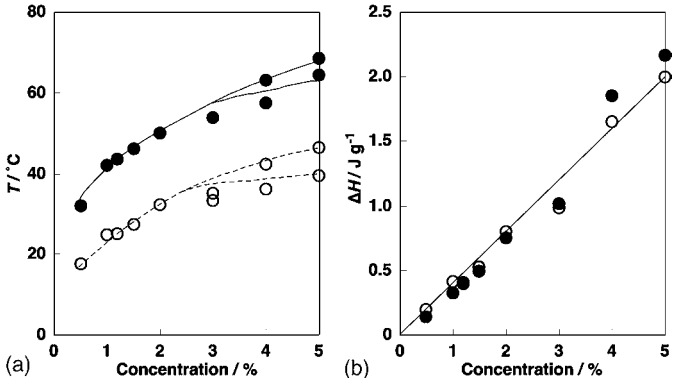

Fig. 2. (a) Relationships between sol–gel transition temperature ($T_{s–g}$) or gel–sol transition temperature ($T_{g–s}$) and concentration of κ-carrageenan hydrogels: (○) $T_{p,s–g}$ and (●) $T_{p,g–s}$. (b) Relationships between sol–gel transition enthalpy ($\Delta H_{s–g}$) or gel–sol transition enthalpy ($\Delta H_{g–s}$) and concentration of κ-carrageenan hydrogels: (○) $\Delta H_{s–g}$ and (●) $\Delta H_{g–s}$.

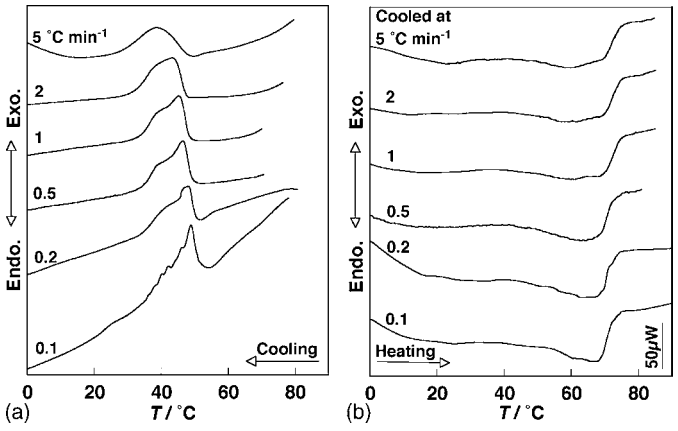

Fig. 3. Stacked DSC curves of 5.0% κ-carrageenan hydrogels with various cooling rates: (a) cooling curves and (b) heating curves.

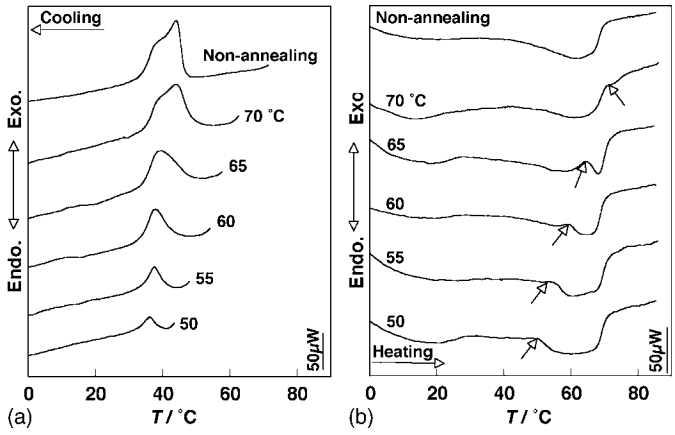

Fig. 5. Stacked DSC curves of 5.0% κ-carrageenan hydrogels annealed at various temperatures for 60 min: (a) cooling curves and (b) heating curves.

with increasing cooling rate. $T_{i,s-g}$ and $T_{e,s-g}$ decreased with increasing cooling rate. When cooling rate was 5 °C min^{-1}, the high temperature side peak was merged into the low temperature side peak. DSC heating curves of gels formed at various cooling rates are shown in Fig. 3(b). Heating rates are 0.5 °C min^{-1} for all samples. In heating curves, two endothermic peaks can clearly seen for samples cooled faster than 1 °C min^{-1}. It is notable that $T_{i,g-s}$ and $T_{e,g-s}$ are maintained at 25 and 78 °C regardless of cooling rate.

Peak temperatures of gel–sol transition T_{g-s} are shown as a function of cooling rate in Fig. 4(a). Enthalpies of sol–gel and gel–sol transition are also shown in Fig. 4(b). Both ΔH_{s-g} and ΔH_{g-s} gradually decrease with increasing cooling rate. The values of ΔH_{g-s} are larger than those of ΔH_{s-g}. The enthalpy difference ($=\Delta H_{g-s} - \Delta H_{s-g}$) of both transitions is larger in a range of slow cooling rate.

Sol–gel transition of 2.0% κ-carrageenan was also measured by DSC at various cooling rates, although this data is not shown. The transition behaviour was quite different from the sample of 5.0%, i.e. $T_{i,g-s}$, $T_{p,g-s}$ and $T_{e,g-s}$ were maintained at a constant at 27 °C, 50 °C and 60 °C, respectively, regardless of cooling rate. ΔH_{g-s} was a constant value at 0.76 J g^{-1}. This suggests

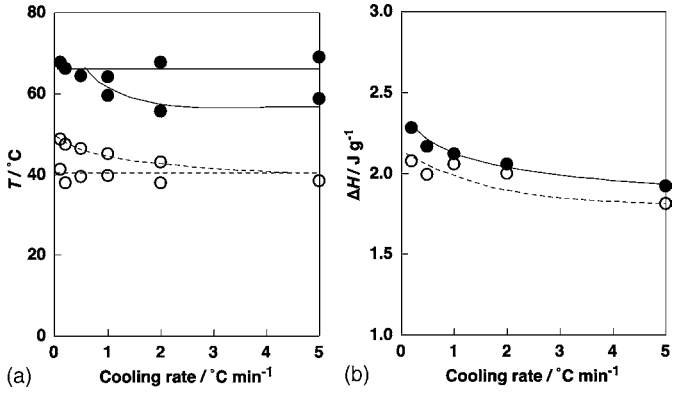

Fig. 4. (a) Relationships between gel–sol transition temperature (T_{g-s}) or sol–gel transition temperature (T_{s-g}) and cooling rate of 5.0% κ-carrageenan hydrogels: (○) $T_{p,s-g}$ and (●) $T_{p,g-s}$. (b) Relationships between gel–sol transition enthalpy (ΔH_{g-s}) or sol–gel transition enthalpy (ΔH_{s-g}) and cooling rate of 5.0% κ-carrageenan hydrogels: (○) ΔH_{s-g}, and (●) ΔH_{g-s}.

that transition temperature and enthalpy were scarcely affected by cooling rate when concentration was low.

In order to investigate the effect of annealing on gelation, 5.0% κ-carrageenan samples in sol state were annealed in a temperature range of gel–sol transition. Annealing was carried out at 50 °C, 55 °C, 60 °C, 65 °C and 70 °C for 60 min. Fig. 5(a) shows stacked DSC cooling curves of 5.0% κ-carrageenan hydrogels annealed at various annealing temperatures. In cooling curves, the high temperature side peak of annealed samples decreased with decreasing annealing temperature. When annealing temperature was lower than 65 °C, a single exothermic peak was observed. Enthalpy decreased with decreasing annealing temperature. DSC heating curves of gels formed after annealing are shown in Fig. 5(b). The pattern of the endothermic peak varied according to annealing temperature. It is observed that the gel–sol transition peak is divided into two parts at a characteristic point, as indicated by the arrows. As shown in Fig. 5(b), the temperature indicated as a characteristic point between two endothermic peaks accorded well with annealing temperature. It is also seen that the high temperature side became sharper with increasing annealing temperature. The endothermic peak temperature, $T_{p,g-s}$, shifted to the high temperature side with increasing annealing temperature.

The endothermic peak was divided into two parts, one is the peak area observed at a temperature lower than the annealing temperature and the other is the peak area higher than annealing temperature. From each peak area, the enthalpy of the high temperature side $\Delta H_{g-s\,1}$, and that of the low temperature side $\Delta H_{g-s\,2}$ were calculated. Fig. 6 shows relationships between ΔH_{g-s} and annealing temperature of 5.0% κ-carrageenan hydrogels annealed for 60 min. Fig. 6 shows $\Delta H_{g-s\,1}$, $\Delta H_{g-s\,2}$ and the summation of $\Delta H_{g-s\,1}$ and $\Delta H_{g-s\,2}$ (ΔH_{g-s}). $\Delta H_{g-s\,1}$ linearly decreased and $\Delta H_{g-s\,2}$ increased with increasing annealing temperature. At the same time, it was found that ΔH_{g-s} was observed at 2.1 J g^{-1}, regardless of annealing temperature.

The effect of annealing time of gelation was also investigated. Annealing was carried out at 60 °C for various times from 1 min to 120 min. Fig. 6(a) shows stacked DSC cooling curves of 5.0% κ-carrageenan sols annealed at various times. In cooling curves, a single exothermic peak due to sol–gel transition

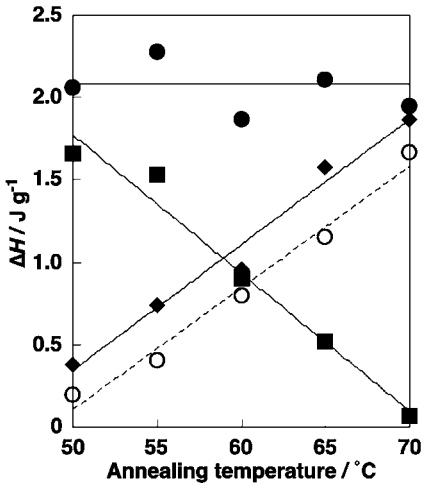

Fig. 6. Relationship between gel–sol transition enthalpy ($\Delta H_{g–s}$) or sol–gel transition enthalpy ($\Delta H_{s–g}$) and annealing temperature of 5.0% κ-carrageenan hydrogels annealed for 60 min: (●) $\Delta H_{g–s}$, (♦) $\Delta H_{g–s\,1}$, (■) $\Delta H_{g–s\,2}$, and (○) $\Delta H_{s–g}$.

was observed at 38 °C. Fig. 7(b) shows heating curves of hydrogels formed after annealing at 60 °C for various times. Heating rate was 1 °C min^{-1}. No large differences were found in heating curves, although the endothermic peak is separated into two parts at 60 °C.

4. Discussion

It is known that carrageenan gel is formed after molecular conformation changes from coil to helix [10], although the helical structure associated with the above conformational change is a matter of discussion [11–14]. It has also been reported that gel formation is affected by various factors such as the kind of alkali metal ions, the temperature, the pH and the concentration [15–19]. In relation to gelation mechanism, the structure of carrageenan in aqueous media has been investigated by optical rotation [20,21], light scattering [22,23], X-ray diffraction [24], thermal analysis [25–30], dielectric relaxation [31], small angle X-ray scattering (SAXS) [9,32], rheology [33] and atomic force microscopy (AFM) [34]. Among various measurement techniques, DSC is a useful method when molecular relaxation in the sol state is concerned with the gelation, since thermal history of sol state can be easily and precisely controlled in a sample holder.

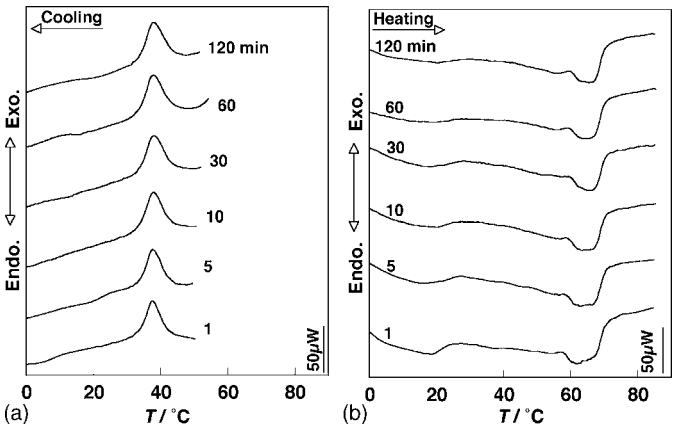

Fig. 7. Stacked DSC curves of 5.0% κ-carrageenan hydrogels annealed at various times at 60 °C: (a) cooling curves and (b) heating curves.

Homogeneous κ-carrageenan gel is formed from well equilibrated aqueous solution, when concentration of solution is lower than 2.0%. As described in the results section, when concentration was 2.0%, transition behaviour showed no significant change by annealing at a temperature from 50 °C to 70 °C. The gel–sol transition accorded well with those reported by other researchers [27–30].

When concentration exceeds 3.0%, a sub-peak can be observed at the low temperature side of the main peak. As shown in Fig. 1, the sub-peak was distinctly observed in exothermic transition to a greater degree than that of the endothermic peak. It is noteworthy that clear sol–gel transition was observed in this study, since no quantitative data on sol–gel transition from cooling DSC curves has been reported [25–27,35,36], although thermal data on gel–sol transition of κ-carrageenan hydrogels has been widely investigated [28–30]. As shown in Fig. 2(b), thermal hysteresis was clearly seen i.e. the temperature difference between ΔT ($T_{p,g–s} - T_{p,s–g}$) values maintained a constant value, ca. 18 °C, regardless of concentration. This ΔT value accords with the data obtained by optical rotation [37] and by photon transmission techniques [38,39].

As shown in Fig. 2(b), $\Delta H_{s–g}$ and $\Delta H_{g–s}$ of κ-carrageenan ranged from 0.5% to 5.0% were about 0.15–2.17 J g^{-1}. Both $\Delta H_{s–g}$ and $\Delta H_{g–s}$ linearly increased with increasing concentration, however when concentration exceeded 3.0%, the values of $\Delta H_{s–g}$ became larger than that of $\Delta H_{g–s}$. The enthalpy difference of $\Delta H_{s–g}$ and $\Delta H_{g–s}$ observed for 4.0% and 5.0% is not negligible. This suggests the gelation mechanism changes at around this concentration range [25].

In the cooling process, when concentration exceeds 3.0%, carrageenan molecules aggregate with adjacent molecules and regular helices are formed. This junction zone formation is observed as a high temperature side exothermic peak in DSC curve. By successive cooling, due to the existence of an excess number of molecular chains, residual carrageenan molecules aggregate with other molecules located at certain distances. This formation of helices is observed as a low temperature side peak. The fact that $\Delta H_{g–s}$ is larger than $\Delta H_{s–g}$ in a concentration more than 3.0% (Fig. 2(b)) suggests that not all carrageenan molecules are involved in junction zone formation in the cooling process, but rearrange in the heating process. The fact that starting temperature of gel–sol transition was hardly detected in a high concentration range (Fig. 1(b)), indicates that helices having various sizes and different kinds of defects are present in the junction zone. From the results of DSC curves cooled at various rates, it is thought that a large junction zone is formed at a slow gelation rate, and also that many small junction zones form at a fast gelation speed (Fig. 3). Fig. 8 shows schematic illustration of κ-carrageenan gel formation by fast and slow cooling [40]. Cooling dependency of $\Delta H_{g–s}$ for 5.0% gel (Fig. 4(b)) showed that the number of molecules involved in the junction zone depends on cooling rate and random sizes helices in the junction zone increases with increasing cooling rate.

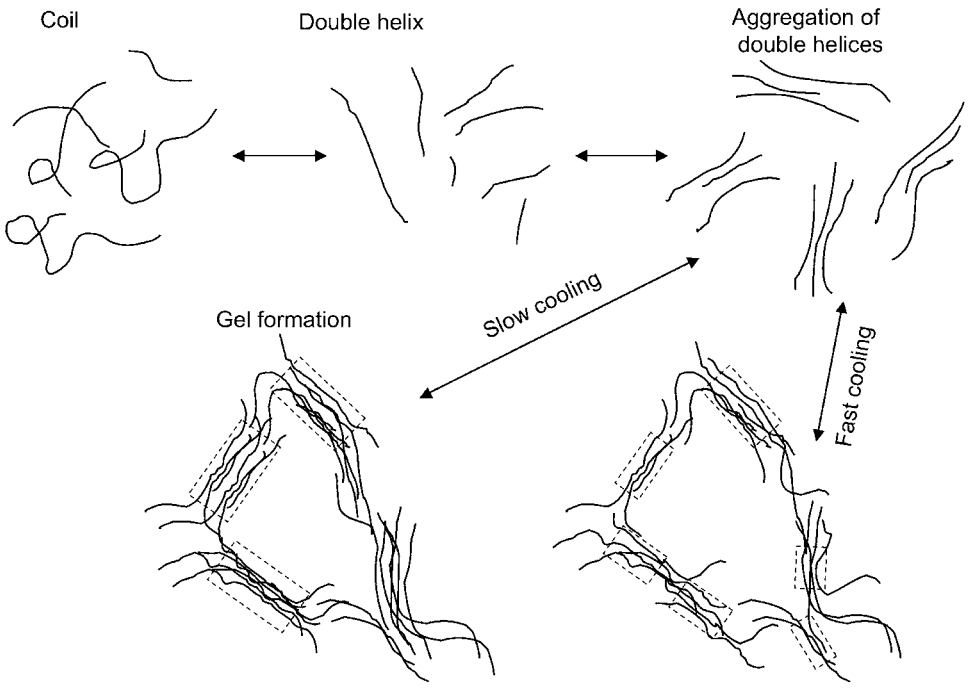

Fig. 8. Schematic illustration of κ-carrageenan gel formation.

By annealing, the junction zone clearly divided into two portions since the characteristic temperature that separated the endothermic peak exactly corresponded to the annealing temperature (Fig. 5). Enthalpy variation by annealing at sol–gel transition indicates that $\Delta H_{g-s\,1}$ corresponds to gel–sol transition of a portion of hydrogel transformed from sol state during annealing, and also that $\Delta H_{g-s\,2}$ is attributable to a residual portion of hydrogel after annealing (Fig. 6). The ΔH_{g-s} showed that the total amount of junction zone is constant, although the fraction of each portion can be varied by appropriate annealing. It is also confirmed that the time of annealing is not a significant factor in affecting the gelation (Fig. 7). Hydrogels having two distinct junction zones could be prepared by annealing at a temperature around gel–sol transition, which was observed as two distinct endothermic peaks in heating curves after gelation.

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