SHORT COMMUNICATIONS

Spin-Spin Relaxation Time of Water Protons in κ -Carrageenan Gel

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It has been reported that κ -carrageenan aqueous solution transforms to gel in the presence of monovalent cations such as K^+ , Rb^+ , and Cs^+ , as the temperature is lowered. The sol-gel transition is thermo-reversible, and is believed to be associated with the formation of the double helix of the polysaccharide chains.^{1,2} Although much work on the sol-gel transition of κ -carrageenan has been done, detailed mechanism has not been understood yet. With respect to the sol-gel transition, the dynamic behavior of water molecules in κ -carrageenan gel is of interest. In this work, we measured the spin-spin relaxation time of water protons in a temperature range of the sol-gel transition of κ -carrageenan aqueous solution in the presence of K^+ ion.

κ -Carrageenan from *Eucheuma cottonii* was purchased from Sigma Chemical Co. It was purified by dialysis against a 10 mM EDTA aqueous solution and distilled water, and was lyophilized after neutralized by NaOH. The presence of Na^+ ion does not affect the sol-gel transition of κ -carrageenan solutions.^{2,3}

The nuclear magnetic resonance measurement was made on a JEOL FX-60Q spectrometer operating at a frequency of 60 MHz. The spin-spin relaxation time T_2 was measured by the Carr-Purcell-Meiboom-Gill

method.^{4,5} The 10 mm NMR sample tube was used. κ -Carrageenan was dissolved in H_2O - D_2O (1:1) containing a pre-determined amount of KCl at a temperature of 80°C in the NMR sample tube and the solution was rapidly cooled to 0°C to transform to gel. The molar ratio of disaccharide units of κ -carrageenan to water molecules, f , ranges from

Figure 1. The contribution of κ -carrageenan to the spin-spin relaxation rate of water protons $1/T_{2obs} - 1/T_{2f}$ as a function of temperature at various molar ratios of disaccharide units of κ -carrageenan to water molecules f and at various salt concentrations. $\bullet$, $f = 0.94 \times 10^{-3}$, $[KCl] = 50$ mM; $\square$, $f = 1.41 \times 10^{-3}$, $[KCl] = 75$ mM; $\triangle$, $f = 1.88 \times 10^{-3}$, $[KCl] = 100$ mM; $\circ$, $f = 2.35 \times 10^{-3}$, $[KCl] = 125$ mM.

0.94×10^{-3} to 2.3×10^{-3} . Temperature was raised from 30 to 80°C.

At such low concentrations of κ -carrageenan, the NMR signal is originated from only water protons. The decay curve of the spin-echo envelope was found to be single exponential, yielding only one T_2 value. Figure 1 shows the temperature dependence of the contribution of κ -carrageenan to the observed spin-spin relaxation rate of water protons. This is defined as $1/T_{2\text{obs}} - 1/T_{2f}$, where $T_{2\text{obs}}$ is the T_2 value observed in the presence of κ -carrageenan and T_{2f} is the one in the absence of κ -carrageenan. The figure indicates that as temperature is raised the contribution increases and reaches maximum at temperature of the sol-gel transition where it suddenly decreases. The temperature of the maximum shifts to higher temperatures with increasing KCl concentration. This temperature is in agreement with the transition temperature determined by the falling ball method. Similar temperature dependence of T_2 has been reported for agar gels.^{6,7} The results obtained here indicate that dynamic behavior of water molecules markedly changes in the vicinity of the sol-gel transition temperature.

It is reasonable to assume that there are two types of water molecules in gel, *i.e.*, water molecules bound to κ -carrageenan and free water. If water molecules undergo exchange between the bound and free states with an exchange rate faster than the spin-spin relaxation rate in the free state, the contribution of κ -carrageenan to the spin-spin relaxation rate of water protons $1/T_{2p}$ is expressed by the following equation,⁸⁻¹⁰

$$\begin{aligned} 1/T_{2p} &= 1/T_{2\text{obs}} - 1/T_{2f} \\ &= fq/(T_{2b} + \tau) \end{aligned} \quad (1)$$

Here, T_{2b} is the spin-spin relaxation time of the bound water protons, τ is the life time of the bound water, f is the molar ratio of disaccharide units of κ -carrageenan to water

molecules, and q is the number of binding sites for water molecules in a disaccharide unit.

It is seen from eq 1 that $1/T_{2p}$ is determined by two parameters with different temperature dependence, T_{2b} and τ . T_{2b} is an increasing function of temperature. On the contrary, the life time of bound water τ decreases with increasing temperature. As seen in Figure 1, $1/T_{2p}$ increases with increasing temperature in the gel state. Thus, in the gel state $1/T_{2p}$ is governed by τ . In the sol state, $1/T_{2p}$ decreases with increasing temperature, indicating that it is determined by T_{2b} .

It is reasonable to assume that T_{2b} is determined by motion of the polysaccharide. In the gel state, T_{2b} is much small because of slow motion of the polysaccharide. On the other hand, the life time of bound water τ is much longer. Thus, T_{2b} becomes less than τ . Above the gel-sol transition temperature, τ becomes smaller due to rapid exchange between free and bound states, and T_{2b} becomes larger because of rapid motion of the polysaccharide. Thus, T_{2b} becomes greater than τ . This is the case we observed here.

If we assume that a disaccharide unit binds about 10 water molecules,¹¹ it was found that in the gel state water molecules reside on the polysaccharide for a period of $\tau = 50$ ms at room temperature. As temperature is raised, the life time of bound water τ decreases to about 8 ms at the sol-gel transition temperature and in the sol state τ will decrease further. The activation energy of the removal of water molecules from κ -carrageenan in the gel was found to be 18 kJ mol⁻¹, which is comparable to a value of 14 kJ mol⁻¹ reported by Woessner and Snowden for agar gels.⁶

In conclusion, water molecules in κ -carrageenan gel are immobilized by the binding to polysaccharides, and are liberated from the restriction above the transition temperature.

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