Title: NMR Studies of Water Dynamics during Sol-to-Gel Transition of Poly (N-isopropylacrylamide) in Concentrated Aqueous Solution

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Abstract

The focus of this work was on the significant changes in the water dynamics of aqueous poly(N-isopropylacrylamide) (PNIPAM) solution during sol-to-gel transition. Through the use of NMR (particularly two-dimensional ^2H NMR T_1 - T_2 relaxation) and rheology, we were able to show that below 34 °C fast exchange occurs among free water and water molecules adsorbed on the surface of PNIPAM molecules. At 34 °C, PNIPAM becomes aggregated; most of the water molecules are trapped in the PNIPAM aggregates, where water molecules with different dynamics are found. Above 34 °C, PNIPAM molecules aggregate further to form a gel network; the free bulk water then becomes dominant at this stage. On the basis of these observations, a model where water molecules interact with PNIPAM in different ways during the transition was proposed. We believe that our experimental approach provides new information and fresh perspectives on the sol-to-gel transition of PNIPAM.

Introduction

Poly(N-isopropylacrylamide) (PNIPAM) is a well-known thermo-sensitive polymer and undergoes a sol-gel transition around the body temperature[1-3]. These transitions are important in a number of practical applications, including drug delivery, medical diagnostics, tissue engineering, and electrophoresis[4-8]. Many papers have been published using light scattering[9-12], NMR[13-18], IR[19-23], Raman[24,25], DSC[26-29] and dielectric

relaxation[30-32] to study PNIPAM's thermal behavior in aqueous media together with theoretical approaches like molecular dynamics (MD) simulation[33-39]. Because of the amphiphilic character of PNIPAM molecule with polar amide and non-polar backbone and isopropyl groups, the polymer-water interaction is modulated by temperature. Thus, below the lower critical solution temperature (LCST), the hydrophilic groups of PNIPAM are exposed to water and form hydrogen bonds with proximal water molecules, thereby leading to the observed random coil form. Once the temperature exceeds LCST, the hydrophobic moieties of PNIPAM start to aggregate, and the polymer assumes a swollen gel structure.

Because the sol-gel transition of PNIPAM occurs in water, it is important to clarify the polymer-water interactions and the dynamics of water molecules during the transition in order to improve our understanding of the sol-gel transition. Currently, only some information on water molecules in PNIPAM solution is available because the number of applicable experimental methods is limited [13-18, 30-32]. As regards to NMR data, Ohta et al measured T_2 for water in PNIPAM/water solution at different pressures [13]. Tanaka et al studied the dynamics of water in cross-linked PNIPAM [14]. Starovoytova and Spevacek followed the time-dependence of T_2 of HDO in aqueous PNIPAM solutions [15]. Spevacek reviewed NMR phase transition studies in aqueous polymer solutions and gels [16]. Two related papers dealt with poly(vinyl methyl ether) [17] and PNIPAM graft copolymers [18].

Recently, two-dimensional deuterium T_1 - T_2 ($2D\ ^2H\ T_1$ - T_2) NMR relaxation spectroscopy,

coupled with inverse Laplace transform (ILT), was used to monitor the dynamics of the thermo-responsive behavior of a poly(asparagine) derivative in $^2\text{H}_2\text{O}$ through the changes in the dynamics and distribution of the mobility of water molecules during its phase transition[40]. Here T_1 is spin-lattice relaxation time and T_2 spin-spin relaxation time. Through the 2D ^2H T_1 - T_2 ILT correlation map, water was found to be in separate reservoirs, which were detected via the differences in water relaxation and dynamics. Previously, this relaxation approach was also used to study the dynamics of water molecules in numerous multi-compartment systems, such as saturated sedimentary rock, processed starch and potato tissue, a variety of food products such as cheese and yogurt, and cement paste[41-45]. In addition, 2D ^2H T_1 - T_2 NMR was utilized to probe the behavior of water molecules in mechanically strained elastin [46-48]. This relatively new experimental technique correlates the measured T_1 and T_2 relaxation times in a 2D map, analogous to chemical shift correlation methods (such as COSY) that are based on Fourier transform. Thus, 2D ^2H T_1 - T_2 NMR experiment is a promising analytical method to study sol-gel transition of PNIPAM from the viewpoint of water dynamics. In particular, at the gel state of PNIPAM in water, the water molecules are expected to exist in several different kinds of dynamical states with restricted motion. The ^2H T_1 - T_2 NMR experiment should be able to sort out these dynamical states of water and estimate the fraction of each state at the temperature studied.

In this work, we have applied the ^2H T_1 - T_2 NMR technique to study the interactions between PNIPAM and water molecules through the change in the dynamics of water molecules during the sol-gel transition of PNIPAM in concentrated aqueous solution. Moreover, the ^1H pulsed NMR and ^{13}C solution NMR techniques have also been used at the same time to monitor the behavior of PNIPAM polymer during the transition.

Experimental

Samples

Two PNIPAM samples with different racemic (r) diad, r-57 and r-66, were synthesized in toluene at $-60\text{ }^\circ\text{C}$ in the presence of methanol and 3-methyl-3-pentanol, respectively, as reported previously[49]. The tacticities of the samples were determined in DMSO- d_6 solutions by ^{13}C solution NMR using JEOL ECX 400 MHz NMR spectrometer[50, 51]. The samples were named according to the r-diad level as calculated from the triad intensities with the equation $r = rr + mr/2$. The weight-average molecular weight, M_w , and the polydispersity, M_w/M_n , were determined by size exclusion chromatography (SEC); the chromatograph was calibrated with standard poly(methyl methacrylate) samples. SEC was performed on a Tosoh HLC 8220 chromatograph equipped with TSKgel columns [SuperHM-M (6.5 mm inner diameter $\times$ 150 mm) and SuperHM-H (6.5 mm inner diameter $\times$ 150 mm)]. N,N-Dimethylformamide containing 10 mmol L^{-1} LiBr was used as the eluent at

40 °C at a flow rate of 0.35 mL min⁻¹. The initial polymer concentration was set at 1.0 mg mL⁻¹. The data are summarized in Table 1. For NMR and viscosity measurements, the samples were dissolved in 99.9% ²H₂O (Wako Pure Chemical Industries Co. Ltd., Japan).

Viscosity Measurements

The viscosities of the PNIPAM samples were measured in 15 wt% ²H₂O solutions using an Anton-Paar MCR302 rheometer equipped with a parallel plate accessory (25 mm diameter) at a heating rate of 1.0 °C /min and a constant frequency of 1.0 Hz. The temperature in all viscosity measurements was controlled within 0.5 °C by a Peltier element. We used the dynamic strain method in order not to break the PNIPAM gel during complex viscosity observations.

¹³C NMR Experiments

The ¹³C solution NMR spectra of 15 wt% ²H₂O polymer solutions were obtained using ECX 400 spectrometer (JEOL, Tokyo, Japan) with the temperature increasing from 30 °C to 40 °C in 2 °C steps.

¹H pulsed NMR Experiment

The ^1H pulsed NMR experiments were performed on 15 wt% $^2\text{H}_2\text{O}$ solutions of PNIPAM in a 10-mm O.D. NMR sample tube using a JEOL Mu-25 NMR spectrometer. The solid-echo pulse sequence was used to measure the short T_2 component of PNIPAM samples in the gel state. The observed temperature was changed in one-degree steps from 30 °C to 40 °C. The FID curves were fitted according to eq. 1.

$$M(t) = M_0 \exp\left(-\frac{t}{T_{2(\text{short})}}\right) + (1 - M_0) \exp\left(-\frac{t}{T_{2(\text{long})}}\right) \quad (1)$$

2D ^2H T_1 - T_2 Correlation Experiment

The ^2H T_1 - T_2 of the water in the PNIPAM samples (15 wt% in $^2\text{H}_2\text{O}$) in 5-mm NMR tubes were measured on a JEOL ECX 400 MHz NMR spectrometer with a liquid state NMR probe without spinning. The variable temperature ^2H T_1 - T_2 experiments were conducted at 21 °C, 30 °C, 32 °C, 34 °C, 36 °C, 38 °C and 40 °C. The pulse sequence used for the ^2H T_1 - T_2 experiments is shown in Fig. S-1 in the Supporting Information. The magnetization was inverted by an initial 180° pulse and then recovered to thermal equilibrium during the variable delay t_1 through T_1 relaxation. The 90° pulse following the t_1 delay rotated the magnetization into the transverse plane. The NMR echo signal was stroboscopically detected at $2n\tau$ time with a Carr-Purcell-Meiboom-Gill (CPMG) pulse train. The number of scans accumulated in these experiments was 8. Through ILT of the 2D data, a T_1 - T_2 correlation map was obtained.

Results

Complex Viscosity Determination

Change in the complex viscosity, η^* during the sol-gel transition was observed for the aqueous PNIPAM solutions (15 wt %) at temperatures that ranged from 30 °C to 40 °C (Fig.1). Below 33 °C, the viscosities were very low for both PNIPAM samples because they were in the sol state. For sample r-57, the viscosity started to increase at 33 °C and continued to increase gradually with increasing temperature. This viscosity increase was due to the sol-gel transition. As for sample r-66, the temperature dependence was different. The viscosity started to increase at 34 °C and escalated rapidly with increasing temperature until 36 °C. It then increased gradually to reach a higher viscosity level at 40 °C than sample r-57. These observations were essentially the same as the reported experimental results of the sol-gel transition of PNIPAM: the increase in r diad content delays the transition temperature slightly and sharpened the transition behaviour [12, 49, 52].

¹³C NMR Analysis

Next, we used the ¹³C solution NMR to investigate the sol-gel transition from the polymer's perspectives. Fig.2 shows the expanded ¹³C solution NMR spectra (30-50 ppm) of samples r-57 and r-66 as a function of temperature. The peaks at around 43 ppm were assigned to the backbone CH carbon and the multiplet peaks at around 35 ppm to the

backbone CH₂ carbon. The peaks at 42 ppm were assigned to the side chain CH carbon.

Both PNIPAM samples yielded high resolution NMR spectra at 30 °C and 32 °C with similar spectral intensities. However, at 34 °C, the peak intensities started to decrease, and above 36 °C, the backbone CH₂ peaks became very broad and mostly disappeared. This means that the PNIPAM samples were in the sol state below 32 °C and started to be in the gel state at 34 °C.

Note that the signal intensities in the spectrum of sample r-57 were weaker than those of sample r-66 at 34 °C. This is likely because of the slightly lower transition temperature of sample r-57 relative to r-66 as shown in Fig.1. Above 36 °C, the CH₂ peaks disappeared because of highly restricted motion of PNIPAM molecules in the gel state. This trend was similar for both PNIPAM samples r-57 and r-66. For convenience, we focus on the CH₂ peaks of the backbone chain which exhibited splitting due to tacticity. The difference in the line shape between r-57 and r-66 samples was due to the difference in tacticity. However, the tacticity assignment of the CH₂ peaks was not previously reported although hexad splitting was speculated rather than tetrad because the number of the peaks observed exceeded the number expected from tetrad splitting. An important point to note is the decrease of peak intensities from 30 °C to 34 °C without changing the line shape, which was independent of the samples. This means that the sol-gel transition occurred without changing the local conformation of the remaining PNIPAM molecules in the random coil form. It has been proposed previously[53, 54] that during the transition, disruptions and formations of intra- or

intermolecular hydrogen bonding occur through the side chain amide groups of PNIPAM molecules.

¹H Pulsed NMR Analysis

In order to gain further information on the dynamical behavior of PNIPAM during the sol-gel transition, we employed ¹H pulsed NMR analysis. The solvent was ²H₂O and therefore the observed ¹H signal with low mobility came exclusively from the ¹H nuclei of PNIPAM samples. Fig.3 shows the plots of the T₂ and the percentage of the shorter T₂ component in eq. 1 determined by ¹H pulsed NMR method as a function of temperature for the two PNIPAM samples (The T₂ values and the percentage of the longer T₂ component are shown in Fig.S-2 in the Supporting Information). The shorter T₂ component in the ¹H pulsed NMR could be observed at 35°C, indicating that the PNIPAM molecules started to aggregate near 35°C. At 35°C, the T₂ value of the sample r-57 was shorter and their % fraction was higher than those of sample r-66. Yet, at 40 °C the two PNIPAM samples showed almost the same values of T₂ and % fraction. These data indicate that the gelation of sample r-66 occurs at a narrower temperature range than that of the sample r-57, i.e., the same trend as shown by the viscosity data.

2D ²H solution T₁-T₂ ILT Correlation Maps

The ^2H solution T_1 - T_2 NMR experiment was aimed to investigate the dynamics of the water molecules that interacted with PNIPAM molecules during the sol-gel transition. The 2D ^2H T_1 - T_2 correlation maps derived from ILT of the PNIPAM samples, r-57 and r-66, were studied as a function of temperature (Figs.4 and 5). In all cases, the horizontal axis was T_1 and vertical axis T_2 , both in log scale. The diagonal line in each map indicates $T_1=T_2$ and therefore T_1 and T_2 of free bulk water should be observed on the diagonal. The T_1 and T_2 values of free water observed independently were 0.37 sec at 21°C, 0.47 sec at 30 °C and 0.60 sec at 40 °C.

In order to compare the data between two PNIPAM samples, we also extracted selected T_1 and T_2 projections from the 2D ^2H T_1 - T_2 ILT correlation map. These projections are probably easier to compare than the full 2D ^2H T_1 - T_2 NMR maps. The projections for the PNIPAM samples r-57 and r-66 are shown as Figs. 6 and 7, respectively. In these figures, we use the word “peak” for one reservoir in the projection. The peaks with the same color between T_1 and T_2 projections are the same reservoir in the 2D ^2H T_1 - T_2 correlation maps of Figs. 4 and 5.

From 21 °C to 32 °C, there was only one reservoir in the 2D T_1 - T_2 maps. However, the T_1 and T_2 values ($T_1 = 0.18$ s, $T_2 = 0.18$ s) are considerably smaller than those of free water ($T_1 = 0.37$ s, $T_2 = 0.37$ s) even at 21 °C. This suggests the presence of fast exchange in the NMR time scale among free bulk water and the water molecules that were involved with the hydration of PNIPAM. In other words, there was a small amount of water molecules that interacted with PNIPAM molecules, which could be inferred through the T_1 and T_2 values of

water molecules at 21 °C. This water could be detected in a macroscopic observation like viscosity (Fig.1). At 34 °C, several more reservoirs (i.e., water molecules in several different dynamical states) appeared suddenly. These observations were the same for the two PNIPAM samples. However, the number of the reservoirs and their % fractions were different at 34 °C.

A closer examination can be made of the reservoirs observed in the map and projections for sample r-57 at 34 °C as shown in Figs.4 and 6, respectively. Whereas the map at 32 °C had one reservoir, three reservoirs (labeled A, B and C) suddenly appeared at 34 °C. The T_1 and T_2 values of the reservoir A were considerably larger than those of the single reservoir at 32 °C, and stayed as the dominant peak even above 36 °C . These water molecules are considered mostly free bulk water molecules which are produced by the dehydration of PNIPAM molecules in the gel state by the sol-gel transition. The other two reservoirs (B, C) with remarkably small T_1 and T_2 values are attributed to water molecules that interacted with PNIPAM strongly. The degree of such interactions is considered to be different between two reservoirs B and C, and the latter interaction is stronger. With increasing temperature, more reservoirs appeared. The T_1 , T_2 and % fraction data of two PNIPAM samples are summarized in Table 2. Above 34 °C, the exchange among free bulk water and water molecules involved with the hydration of PNIPAM became slow, and the individual water peaks that interacted with PNIPAM molecules in the gel state were observed as peak “reservoirs” in this slow NMR time scale. A similar tendency was observed for sample r-66 as shown Figs. 5 and 7.

However, a significant difference was noted on the ^2H T_1 - T_2 correlation map and projections at 34 °C; i.e., the dominant peak at Figure 7 was peak C, not peak A, although peak A became dominant above 36 °C. This observation seems to fit with the behavior of the complex viscosity shown in Fig.1, where gel-sol transition is ongoing at 34 °C for sample r-57 but just gets started for sample r-66. Thus the appearance of the dominant reservoir A corresponds to the start of the sol-gel transition, which is influenced by the tacticity of the sample: the increase in r diad content delays the transition temperature slightly[49,52]. From the complex viscosity, ^{13}C solution NMR and ^1H pulsed-NMR data presented earlier, PNIPAM molecules clearly aggregate at > 34 °C, and therefore the appearance of several reservoirs must be closely related with the different aggregation modes of PNIPAM in the gel state. These data indicate that the aggregated states of PNIPAM molecules are complex and can change depending on the temperature. In order to discuss the mobility of water molecules in the gel state of PNIPAM more quantitatively, we will calculate the correlation times of water molecules from both the T_1 and T_2 values in the next section.

Change in the correlation times of water molecules during sol -gel transition of PNIPAM

For deuterium atom with nuclear spin $I = 1$, the ^2H - ^2H and ^1H - ^2H dipolar interactions are usually negligible relative to the quadrupolar interaction. In order to check the effect of dipolar coupling in this 2D ^2H T_1 - T_2 NMR experiment, exponential decay curves in the T_1 - T_2

map were plotted as shown in Fig.S-3 of Supporting Information. The experimental data were perfectly reproduced in this T_1 - T_2 map. If there are any contributions from residual dipolar couplings which cause Gaussian-like decay, the appearance of some artificial peaks in this map is expected. However, this is not the case and therefore it is concluded that the contribution of the residual dipolar coupling was negligible in the resultant T_1 - T_2 map.

Thus the expressions for T_1 and T_2 are given by the following equations [55].

$$\frac{1}{T_1} = \frac{3}{40} \left(1 + \frac{\eta^2}{3} \right) C_q \{ J(\omega) + 4J(2\omega) \} \quad (2)$$

$$\frac{1}{T_2} = \frac{1}{80} \left(1 + \frac{\eta^2}{3} \right) C_q \{ 9J(0) + 15J(\omega) + 6J(2\omega) \} \quad (3)$$

where the quadrupolar coupling constant $C_q = \{ [eQ/\hbar][\partial^2 V/\partial^2 z] \}^2$, η is the asymmetry parameter of the potential V ($\eta = 0$ for ^2H nucleus in water), and $J(\omega)$ is the spectral density function. $J(\omega)$ can be expressed in Eq. 4 by assuming a single distribution of the correlation time, τ_c ,

$$J(\omega) = \frac{\tau_c}{1 + (\omega\tau_c)^2} \quad (4)$$

Here τ_c is related to the interaction of the nuclear quadrupolar moment with the electric field gradient. For the case of ^2H in $^2\text{H}_2\text{O}$, the quadrupolar interaction is intramolecular in origin and the correlation time quantifies the tumbling motion of the water molecule. Thus, a larger correlation time corresponds to reduced tumbling motion of an individual water molecule.

With the use of the above equations, the ratio of T_1 and T_2 then gives the correlation time, τ_c .

[48] It is not appropriate to determine the correlation time for the single peak below 32 °C

because of fast exchange among free bulk water and the water molecules involved with the hydration of PNIPAM. In addition, when $T_1=T_2$, the correlation time cannot be calculated from the ratio of T_1 and T_2 ; this is the case for the dominant peak A at temperatures above 36 °C in Figs. 6 and 7. The correlation times calculated from the ratio of T_1 and T_2 are also included in Table 2. It is instructive to scrutinize the data of both samples at 34 °C. For sample r-57, the correlation time of the predominant peak A is 6.3×10^{-9} s and that of peaks B and C is $1.4\text{-}1.5 \times 10^{-8}$ s, and thus, the correlation times of the latter peaks are about 2 times larger than that of the predominant peak A. In contrast, for sample r-66 the correlation times of all six peaks are in the range of $1.0\text{-}2.5 \times 10^{-8}$ s. Thus, although these peaks appeared suddenly at 34 °C, the correlation times of all the peaks are similar; thus it is likely that the sol-to-gel transition has just begun at this temperature. Note that the correlation time of peak C in sample r-66 is almost 3 times larger than that of peak A in sample r-57. At 36 °C, both samples show a similar trend. The dominant peaks A in these samples have the smallest correlation times among the peaks and close to the correlation time of free water ($T_1=T_2$ = about 0.6 s). In addition, at 36 °C a relatively large peak with the largest correlation time of $3.0\text{-}3.6 \times 10^{-8}$ s was newly observed in each sample, i.e., peak D for sample r-57 and peak E for sample r-66. These peaks also turned up as the peak C ($\tau_c = 3.0 \times 10^{-8}$ s) and peak E ($\tau_c = 2.4 \times 10^{-8}$ s) at 38 °C, and as peak C ($\tau_c = 2.9 \times 10^{-8}$ s) and peak C ($\tau_c = 3.0 \times 10^{-8}$ s) at 40 °C for samples r-57 and r-66 respectively. These peaks are of interest because they suggest the

presence of a significant amount of water molecules that interacted with PNIPAM strongly in the gel state. During the sol-gel transition, there seems to be no increase in the mobility of water molecules even for the dominant peaks A in spite of increased temperature from 34 °C to 40 °C.

DISCUSSION

Because the sol-gel transition is an inherently complex phenomenon, we chose in this work to study it with a combination of NMR and viscosity methods. The viscosity and ^1H pulse NMR experiments of the aqueous PNIPAM solutions showed differences with respect to polymer tacticity although there was no significant difference in the appearance of the ^{13}C solution NMR spectra. Below 34 °C only one reservoir in the 2D ^2H T_1 - T_2 ILT correlation maps could be observed, but several more reservoirs appeared suddenly at 34 °C. At 34 °C, the ^2H T_1 - T_2 behavior of water molecules was different between two PNIPAM samples. However, above 34 °C, the relaxation behavior was similar for both samples. The change in the water dynamics during the sol-gel transition of PNIPAM in concentrated aqueous solution was analyzed more quantitatively by comparing the correlation times of water molecules in different reservoirs, as calculated from the T_1/T_2 ratio in deuterium solution NMR.

In view of the results obtained in this work, we propose a schematic model (Fig. 8) that portrays the interaction of water with PNIPAM at different temperatures during the sol-gel

transition. In conformity with our ^2H T_1 - T_2 results, the proposed model consists of three stages: “below”, “at” and “above” 34 °C. At the “below 34 °C” stage, there is fast exchange among free bulk water and water molecules that are associated with PNIPAM in the sol state. In the latter case, the water molecules are in the adsorbed state on the surface of PNIPAM molecules rather than being trapped in PNIPAM molecules. Thus, the arrows in Fig.8a indicate fast exchange among free bulk water and water molecules adsorbed on the surface of PNIPAM molecules. This “below 34 °C” model is basically the same for the two PNIPAM samples.

At 34 °C, the exchange among free bulk water and water molecules associated with PNIPAM becomes slow. However, a significant difference can be observed between two samples. A schematic model of the sol-gel transition for sample r-66 is proposed in Fig.8b. The water molecules are mainly trapped in the polymer aggregates with small sizes although several discontinuous states of water molecules with different dynamics are also present, including free bulk water, as shown in Fig.7. Thus, for sample r-66 the sol-gel transition is just starting as is expected from the complex viscosity shown in Fig.1. However, for sample r-57 at 34 °C (Fig.6), the free bulk water molecules are dominant, indicating that the interactions among water and PNIPAM molecules are relatively weak because of the formation of larger aggregated PNIPAM networks, and only a small amount of water molecules that strongly interacted with the polymer molecules can be observed. At 36 °C,

the fraction of free bulk water increases and becomes dominant for sample r-66 where larger aggregated PNIPAM networks form, which is basically the same as the case of sample r-57 at 34 °C. Only a small amount of water molecules that interact strongly with the PNIPAM molecules is present, and this fraction decreases slightly with increasing temperatures. For both samples, the free water molecules stay dominant, and only a small amount of water molecules that interacted strongly with the r-57 molecules is present at 40 °C. The viscosity is still high at 40 °C as shown in Fig.1 for the two aqueous PNIPAM solutions, which means that the gel states of these PNIPAM solutions are still maintained at 40 °C. With a further increase of temperature, phase separation finally occurs, and the networks of PNIPAM molecules precipitate out, as evidenced by the rapid decrease in viscosity (data not shown).

Starovoytova and Spevacek [15] and Rusu et al.[16] reported that there was a fast exchange between free and bound water in the transition region and above the transition as shown by mono-exponential relaxation curves of the aqueous solution of PNIPAM. In this paper, a slow exchange at (and above) the phase transition was observed. This seeming discrepancy is considered to arise from different polymer concentrations used: 5 wt% (for Starovoytova and Spevacek), 0.8 wt% (for Rusu et al.), and 15 wt% in the present study. We actually tried to observe 2D ^2H T_1 - T_2 NMR spectra for the 1 wt% solution of sample r-66 from 30 °C to 40 °C. The data are shown in Fig.S-4 of Supporting Information. There is only one peak in the T_1 - T_2 maps over the observed temperatures. Thus, a fast exchange between free and bound

water was observed in the transition region and above the transition for the 1 wt% aqueous solution of PNIPAM. These observations are similar to an earlier situation reported for another thermosensitive polymer, poly(vinyl methyl ether) , where fast and slow exchange regimes between bound and free water were reported above the LCST transition for polymer concentrations 2-10 wt% and 20-80 wt%, respectively [17,18].

Conclusions

This study aims to elucidate the sol-gel transition of PNIPAM using selected NMR and viscosity techniques. On the basis of the data obtained, we have proposed a model where water molecules interact differently with PNIPAM samples during the transition as shown in Fig.8. Through the ^2H T_1 - T_2 experiments, several water reservoirs have been found at 34 °C for two PNIPAM samples in contrast to a single reservoir found below 34 °C. However, at temperatures above 34 °C, the water molecules exhibit different ^2H T_1 - T_2 relaxation behavior. These changes in the water dynamics during the sol-gel transition of PNIPAM can be more quantitatively analyzed by comparing the correlation times of water molecules as calculated from deuterium solution NMR. Thus far, experimental data on the dynamics of water molecules in aqueous PNIPAM solution are quite scarce. Our NMR approach, particularly ^2H T_1 - T_2 two-dimensional relaxation spectroscopy, can provide detailed information on water dynamics and

appears attractive in clarifying the sol-gel transition phenomenon in PNIPAM. This approach should also be useful in studying other water-based stimuli-responsive polymers.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at

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Table 1 Tacticity, weight-average molecular weight, and polydispersity of the PNIPAM samples.

Sample	Tacticity(%)				$M_w(\times 10^{-4})$	M_w/M_n
	<i>rr</i>	<i>mr</i>	<i>mm</i>	<i>r</i> -diad		
r-57	26.4	60.5	13.1	57	8.83	1.6
r-66	43.3	45.8	10.9	66	6.76	1.9

Table.2 ^2H T_1 and T_2 values, correlation times (τ_c), fractions (in %) of the reservoirs calculated from the 2D ^2H T_1 - T_2 ILT correlation maps for the PNIPAM samples, r-57 and r-66 (Figs.4,5)

Sample r-57						Sample r-66					
Temp	T ₁ (s)	T ₂ (s)	τ _c	%	Peak	Temp	T ₁ (s)	T ₂ (s)	τ _c	%	Peak
21 °C	0.18	0.18	—	100		21 °C	0.18	0.18	—	100	
30 °C	0.23	0.18	—	100		30 °C	0.23	0.18	—	100	
32 °C	0.23	0.18	—	100		32 °C	0.23	0.15	—	100	
34 °C	0.47	0.37	6.3×10 ⁻⁹	85.3	A	34 °C	0.47	0.29	1.0×10 ⁻⁸	9.4	A
	0.15	0.072	1.5×10 ⁻⁸	8.6	B		0.37	0.18	1.5×10 ⁻⁸	1.9	B
	0.014	0.0069	1.4×10 ⁻⁸	6.2	C		0.18	0.072	1.8×10 ⁻⁸	85.2	C
							0.12	0.036	2.5×10 ⁻⁸	1.9	D
							0.036	0.014	1.9×10 ⁻⁸	1.0	E
	0.0069	0.0034	1.4×10 ⁻⁸	0.6	F						
36 °C	0.6	0.6	—	87.8	A	36 °C	0.6	0.6	—	83.0	A
	0.15	0.12	6.0×10 ⁻⁹	2.7	B		0.23	0.12	1.3×10 ⁻⁸	8.5	B
	0.045	0.028	1.0×10 ⁻⁸	2.3	C		0.057	0.022	1.9×10 ⁻⁸	1.4	C
	0.022	0.0042	3.6×10 ⁻⁸	7.1	D		0.022	0.0069	2.4×10 ⁻⁸	1.2	D
							0.014	0.0034	3.0×10 ⁻⁸	5.9	E
38 °C	0.6	0.6	—	90.3	A	38 °C	0.6	0.6	—	87.6	A
	0.12	0.072	1.1×10 ⁻⁸	3.0	B		0.18	0.12	9.0×10 ⁻⁹	4.1	B
	0.018	0.0043	3.0×10 ⁻⁸	6.7	C		0.057	0.036	9.9×10 ⁻⁹	1.3	C
							0.036	0.0034	5.6×10 ⁻⁸	1.8	D
							0.014	0.0043	2.4×10 ⁻⁸	5.2	E
40 °C	0.6	0.60	—	91.0	A	40 °C	0.6	0.6	—	92.1	A
	0.057	0.036	7.1×10 ⁻⁸	2.3	B		0.37	0.15	1.8×10 ⁻⁸	1.4	B
	0.028	0.0069	2.9×10 ⁻⁸	6.6	C		0.018	0.0043	3.0×10 ⁻⁸	6.6	C

Fig.1 Complex viscosity, η^* of two PNIPAM samples, r-57 (solid circle) and r-66 (circle), plotted against temperature.

Fig.2 ^{13}C solution NMR spectra (the 30-50 ppm spectral range was expanded) of two PNIPAM samples, r-57 and r-66, in 15 wt% $^2\text{H}_2\text{O}$ aqueous solutions as a function of temperature. The peak labelled bCH is the backbone methine carbon; sCH corresponds to the side chain methine carbon; and CH_2 corresponds to the backbone methylene carbon of PNIPAM.

Fig.3 ^1H pulsed-NMR spin-spin relaxation time T_2 in μs (upper plot) of the short T_2 component and their percentage (lower plot) for two PNIPAM samples, r-57 (solid circle, solid line) and r-66 (circle, dashed line) plotted against temperature. The experimental errors of T_2 and fraction are $\pm 5\%$ and $\pm 3\%$, respectively.

Fig.4 2D ^2H T_1 - T_2 ILT correlation maps for PNIPAM sample r-57 as a function of temperature. The reservoirs are marked by letters with different colors. The fraction of each reservoir is plotted against the T_1 and T_2 projections in Fig.6. The T_1 and T_2 values together with the fraction of each reservoir are also listed in Table 2.

Fig.5 2D ^2H T_1 - T_2 ILT correlation maps for PNIPAM sample r-66 as a function of temperature. The reservoirs are marked by letters with different colors. The fraction of each reservoir is plotted against the T_1 and T_2 projections in Fig.7. The T_1 and T_2 values together with the fraction of each reservoir are also listed in Table 2.

Fig.6 Change in the T_1 and T_2 projections of 2D ^2H T_1 - T_2 ILT correlation maps of PNIPAM sample r-57 with increasing temperature. The color of each peak is the same as that of the letter in Fig.4. The same color between T_1 and T_2 means the same spot in Fig.4. The T_1 and T_2 values together with the fraction of each peak are also listed in Table 2.

Fig.7 Change in the T₁ and T₂ projections of 2D ²H T₁-T₂ ILT correlation maps of PNIPAM sample r-66 with increasing temperature. The color of each peak is the same as the letter in Fig.5. The same color between T₁ and T₂ means the same spot in Fig.5. The T₁ and T₂ values together with the fraction of each peak are also listed in Table 2.

Fig.8 Schematic model of water molecules that interact with PNIPAM in Sample r-66 at different temperatures during the sol-to-gel transition; a) “Below 34 °C” refers to temperatures between 21 °C - 32 °C; b) at 34 °C; and c) “Above 34 °C” refers to 36-40 °C, although the interactions may change slightly with changing temperature.

The arrows in Fig. 8a mean fast exchange in the NMR time scale among free bulk water and water molecules adsorbed on the surface of PNIPAM molecules in the sol state below 34 °C. At 34 °C, the PNIPAM molecules aggregate suddenly; water molecules are mostly trapped in the aggregated PNIPAM molecules, and several discontinuous states of water molecules with different dynamics appear, including free bulk water. Above 34 °C, the aggregates of the PNIPAM molecules grow and form a gel network. The free bulk water molecules become dominant at this stage.

a) Below 34 °C

b) At 34 °C

c) Above 34 °C