Notes

Hydration and Dynamic Behavior of a Cyclic Poly(*N*-isopropylacrylamide) in Aqueous Solution: Effects of the Polymer Chain Topology

Yuichi Satokawa,[†] Toshiyuki Shikata,*[,†] Fumihiko Tanaka,[‡] Xing-ping Qiu,[§] and Françoise M. Winnik[§]

Department of Macromolecular Science, Osaka University, Toyonaka, Osaka 560-0043, Japan; Department of Polymer Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Kyoto 615-8510, Japan; and Faculty of Pharmacy and Department of Chemistry, Universite de Montreal, CP 6128 Succursale Centre Ville Montreal QC H3C 3J7, Canada

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The coil-to-globule collapse of a macromolecule, which takes place as its solution is heated through its lower critical solution temperature (LCST), has been scrutinized by polymer scientists for many years.[1−4] In the case of aqueous polymer solutions, the heat-induced phase transition is accompanied by the release into bulk of water molecules bound to the polymer chain in its extended form. Thus, thermosensitive waterborne materials tend to undergo a large change in volume and/or hydrophilicity when subjected to a small change in temperature. Thermoresponsive materials are major components of many microfluidics devices,[5] sensors,[6] supports for cell culture and tissue engineering,[7] chromatography columns,[8] and drug delivery systems.[9] Poly(*N*-isopropylacrylamide) (PNIPAM) is the best known thermoresponsive water-soluble polymer. The LCST of aqueous PNIPAM solutions is ∼32 °C. For polymers of sufficiently high molecular weight ($M_n > 15.0 \times 10^3$ g mol^{-1}), the LCST has a very weak dependence on solution concentration (c) and on the sample molecular weight. The LCSTs of PNIPAM oligomers, however, depend significantly on the number of *N*-isopropylacrylamide (NIPAM) repeating units and on the chemical structure of the chain ends.[10] Recent studies of aqueous solutions of cyclic PNIPAMs have revealed that the cyclic topology also significantly affects the temperature and the enthalpy of the phase transition as well as the size, stability, and density of the mesoglobules formed above the LCST.[11−13] These observations suggest that the cyclic topology affects the hydration and/or dehydration characteristics of PNIPAM. However, there is no direct evidence that the hydration state of cyclic PNIPAM differs from that of its linear counterpart.

Dielectric relaxation (DR) measurements, which yield with high precision the relaxation times of electric dipoles in solution, are a powerful means to probe the dynamics of any system containing molecules and/or groups bearing electric dipoles.[4] This technique was applied recently to study aqueous solutions of linear PNIPAMs of $M_w = 7.5 \times 10^3$ and 300×10^3 g mol^{-1}. The experiments provided information on the temperature-dependent relaxation behavior of both the PNIPAM chains and the solvent, water, molecules. It was estimated that the hydration number per NIPAM unit (m) was ∼11 for PNIPAM chains in their extended conformation in cold water. It was demonstrated also that, of the 11 molecules of water, 5−6 are bound to the amide functional group of each NIPAM unit via hydrogen bonds.[4] The additional 5−6 water molecules form hydrogen bonds with the water molecules closest to the amide groups. The total hydration number decreases sharply as the solution approaches the phase transition temperature. The exact hydration level of the NIPAM unit in phase-separated PNIPAM solutions cannot be determined by DR measurements due to difficulties in the analysis of data obtained from inhomogeneous solutions.

We report here the results of a DR study of aqueous solutions of a cyclic PNIPAM (*c*-PNIPAM, $M_n = 12.8 \times 10^3$ g mol^{-1} and $M_w/M_n = 1.1$) and a linear PNIPAM sample of comparable molecular weight (*l*-PNIPAM, $M_n = 10.0 \times 10^3$ g mol^{-1} and $M_w/M_n = 1.1$) bearing an alkyne residue on one end and an azide on the other end (see Figure 1). The two functional end groups are able to couple via "click chemistry", leading to cyclic polymers.[11] This α-azido-ω-propargyl-PNIPAM was prepared by reversible addition−fragmentation transfer (RAFT) polymerization of NIPAM, following a reaction sequence similar to a procedure described previously[11] (see Supporting Information). As a consequence of this reaction sequence, each PNIPAM cycle contains a triazole group. In order to minimize the effect of this moiety on the hydration and dynamics of *c*-PNIPAM in water, we synthesized a cyclic polymer having as high a molecular weight as possible given the experimental constraints of end-to-end polymer cyclization reactions.

The changes with angular frequency (1.0×10^7 s$^{-1} < \omega < 1.3 \times 10^{11}$ s^{-1}) of the real and imaginary parts (ε' and ε'') of the complex permittivity were determined for aqueous solutions of *c*-PNIPAM and *l*-PNIPAM kept at various temperatures (T) between 15 °C and the solutions' LCST. The total spectra of the solutions, ε' and ε'', were decomposed into a number of Debye-type relaxation modes and a constant permittivity (ε_∞) in order to determine the exact dielectric contribution of PNIPAMs in water ($\Delta\varepsilon'$ and $\Delta\varepsilon''$) according to the following standard procedure. The real and imaginary parts, ε' and ε'', were decomposed into the summation of the relaxation functions of the mode i

$$\varepsilon' = \sum_{i=1} \frac{\varepsilon_i}{1 + \omega^2\tau_i^2} + \varepsilon_\infty, \quad \varepsilon'' = \sum_{i=1} \frac{\varepsilon_i \omega\tau_i}{1 + \omega^2\tau_i^2} \qquad (1)$$

where τ_i and ε_i represent respectively the relaxation time and strength for the mode i.[4]

The fastest relaxation mode was assigned to bulk water molecules in the solutions (ε'_w and ε''_w). It was the strongest signal in all spectra and had a relaxation time close to that of

* Corresponding author. E-mail: shikata@chem.sci.osaka-u.ac.jp.
[†] Osaka University.
[‡] Kyoto University.
[§] Universite de Montreal.

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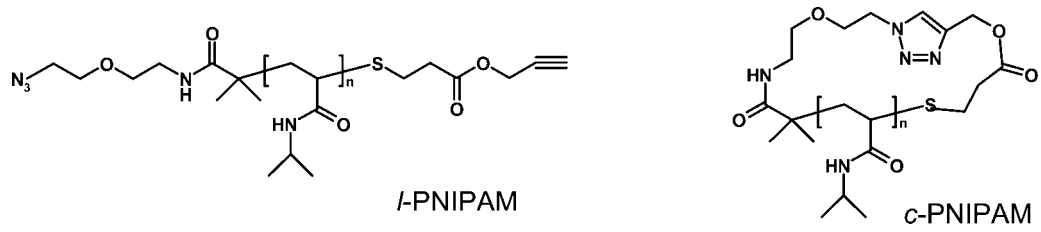

Figure 1. Chemical structures of *c*-PNIPAM and *l*-PNIPAM used in this study.

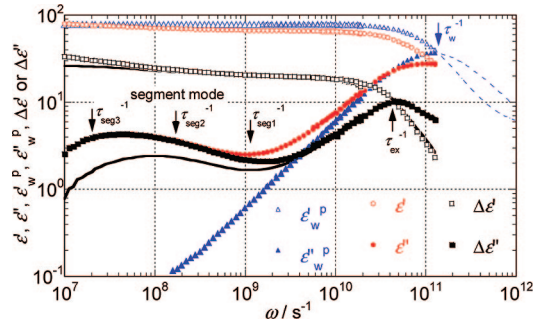

Figure 2. Dependence of ε' and ε'' on ω for an aqueous solution of the *c*-PNIPAM at $c = 1.2$ M and 24.6 °C. This figure also contains the contribution of the polymer, $\Delta\varepsilon'$ and $\Delta\varepsilon''$, and that of pure water, $\varepsilon'_w{}^p$ and $\varepsilon''_w{}^p$; thin broken lines represent the calculated curves due to the literature.[14] $\Delta\varepsilon'$ and $\Delta\varepsilon''$ for an aqueous solution of the *l*-PNIPAM at the same conditions are also shown with thick solid lines.

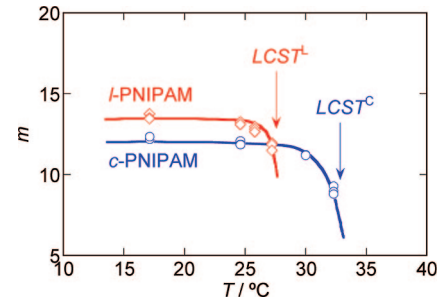

Figure 3. *T* dependencies of *m* for aqueous solutions of *c*-PNIPAM and *l*-PNIPAM.

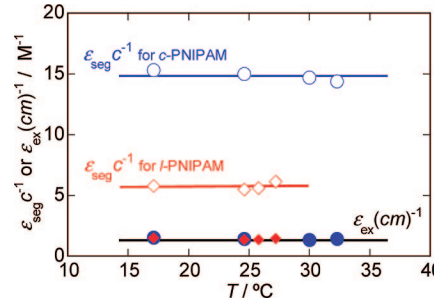

Figure 4. *T* dependencies of $\varepsilon_{ex}c^{-1}$ and $\varepsilon_{seg}c^{-1}$ for aqueous solutions of *c*-PNIPAM and *l*-PNIPAM.

pure water ($\varepsilon'_w{}^p$ and $\varepsilon''_w{}^p$).[14] In Figure 2, we present the dielectric spectra, ε' and ε'', $\Delta\varepsilon' = \varepsilon' - \varepsilon'_w - \varepsilon_\infty$, $\Delta\varepsilon'' = \varepsilon'' - \varepsilon''_w$, $\varepsilon'_w{}^p$, and $\varepsilon''_w{}^p$ as functions of the frequency, ω, recorded for an aqueous *c*-PNIPAM solution ($c = 1.2$ M in monomer units or 121 g L^{-1}) at 24.6 °C as a typical result; we also carried out dielectric relaxation experiments for solutions of $c = 0.47$ and 0.84 M. The $\Delta\varepsilon'$ and $\Delta\varepsilon''$ spectra involved two major relaxation processes, a sharp one and a broader one, at frequencies of 4×10^{10} and $\sim 10^8$ s^{-1}, respectively. Similar dielectric spectra were obtained for all the samples examined below their LCSTs. The data of $\Delta\varepsilon'$ and $\Delta\varepsilon''$ for an aqueous solution of the *l*-PNIPAM in the same condition are shown in Figure 2 with thick solid lines. The ω dependences of $\Delta\varepsilon'$ and $\Delta\varepsilon''$ for both the *c*-PNIPAM and *l*-PNIPAM are similar to each other within the $3 \times 10^9 - 10^{11}$ s^{-1} ω range. However, $\Delta\varepsilon'$ and $\Delta\varepsilon''$ for the *l*-PNIPAM solution are significantly smaller than those for the *c*-PNIPAM solution for $\omega < 10^9$ s^{-1}.

The ratio of the relaxation strength of bulk water to that of pure water ($\Phi = \varepsilon_w/\varepsilon_w{}^p = 0.59$ for the solution shown in Figure 2) is related to the hydration value, *m*, by the equation $\Phi = (1 - \phi)/(1 - \phi/2) - 10^{-3}m\bar{V}_w c$, where ϕ is the volume fraction of PNIPAMs ($\phi = 10^{-3}\bar{V}_p c$) and $\bar{V}_p$ and $\bar{V}_w$ are the partial molar volumes of the NIPAM repeating unit and of water, respectively. Under these conditions, we obtain hydration numbers of ~12 for *c*-PNIPAM in water below 30 °C (<$LCST^C$) and of ~13 for the *l*-PNIPAM in water below 25 °C (<$LCST^L$), irrespective of the *c* values examined, as shown in Figure 3. The *m* values in both solutions decrease rapidly as the solution temperature approaches the LCST values, as observed previously for solutions of a PNIPAM sample of high molecular weight.[4] We reported earlier that the transition enthalpy for *c*-PNIPAMs is slightly lower than the transition enthalpy recorded for *l*-PNIPAMs (by 1.2−1.5 kJ mol^{-1} depending on their molecular weights).[11] This observation is in good agreement with the fact that the *m* value is lower for *c*-PNIPAM, compared to *l*-PNIPAM, since the enthalpy of transition corresponds to the heat evolved upon release of bound water into bulk. It is interesting to note that the *m* value per NIPAM unit in the *l*-PNIPAM with azido and alkyne end groups is slightly larger than the *m* value determined under the same conditions for solutions of linear PNIPAMs prepared by using standard free radical polymerization conditions.[4] The differences may reflect the role played by end groups in controlling the hydration of PNIPAM.

The fast, large relaxation observed in the $\Delta\varepsilon'$ and $\Delta\varepsilon''$ curves in the range of $10^{10} < \omega < 10^{11}$ s^{-1} (Figure 2) can be described by a Debye-type relaxation mode with a relaxation time $\tau_{ex} = 22$ ps at 24.6 °C. This mode was assigned previously to the exchange process of the water molecules bound to the PNIPAM chains with bulk water.[4] For the solutions investigated here, the activation energy, E^*_{ex}, for this relaxation process was evaluated to be 25 kJ mol^{-1}, a value similar to that recorded for solutions of high molecular weight PNIPAM (of $M_w = 300 \times 10^3$ g mol^{-1}). In all cases, the activation energy is slightly larger than that of the rotational relaxation of pure water ($E^*_w{}^p = E^*_w$, ca. 19 kJ mol^{-1}), therefore, a trend believed to reflect the additional energy necessary to break hydrogen bonds linking the water molecules to the NIPAM units.[4] The magnitude of the relaxation strength per concentration unit of the hydrated water molecules in the exchange process, $\varepsilon_{ex}(cm)^{-1}$, is approximately the same for solutions of *c*-NIPAM and *l*-PNIPAM (Figure 4), and it is identical to that recorded previously for high molecular weight PNIPAM. This observation suggests that the topology and molecular weight of the PNIPAM chain do not affect the dynamics of the exchange process of the water

of hydration to bulk water. Although the source dipoles originate from water molecules in both the exchange and the rotational relaxation process of bulk water, the value of $\varepsilon_{ex}(mc)^{-1} = 1.5$ M^{-1} is larger than that of bulk water, $10^{-3}\varepsilon_w\bar{V}_w$, by ca. 0.2 M^{-1}. Such a discrepancy between the values has been reported in aqueous systems possessing τ_{ex} longer than τ_w.[4]

The slow, broad relaxation process found in the $\Delta\varepsilon'$ and $\Delta\varepsilon''$ curves for ω lower than 10^9 s^{-1} can be described by the summation of three sets of Debye-type relaxation modes with times of $\tau_{seg1} = 0.9$, $\tau_{seg2} = 6.5$, and $\tau_{seg3} = 50$ ns at 24.6 °C for the *c*-PNIPAM, irrespectively of concentration (Figure 2). This relaxation process has been assigned to the rotational relaxation modes of PNIPAM segments bearing amide groups with relatively large dipoles.[4] Correlation times (τ_c) of ^{13}C nuclei evaluated from longitudinal NMR relaxation times (T_1) revealed that the average relaxation time of the segment mode for *c*-PNIPAM was ca. 36 ns, using the relationship $\bar{\tau}_{seg} = 3\tau_c$,[4,15] and longer than that for *l*-PNIPAM, as shown in the Supporting Information. Each dielectric relaxation time in the segment mode decreases with increasing temperature via the same activation energy, E^*_{seg}, as that of bulk water molecules, E^*_w. The relative ratios of the relaxation strengths and also times between each Debye-type relaxation mode found in the segment modes were well kept constant, since the shape of the frequency dependence of the segment modes was almost independent of *T* and *c*. Since the concentrations of the *l*-PNIPAM solution were higher than the overlap concentration, estimated to be ~1.1 M (~111 g L^{-1}) at 25 °C from the reciprocal of its calculated intrinsic viscosity,[16] the *l*-PNIPAM chains are barely in contact in the solution of highest concentration ($c = 1.2$ M). Because cyclic polymers have slightly smaller molecular dimensions and, consequently, higher overlap concentrations than those of linear polymers of similar molecular weights, the *c*-PNIPAM chains scarcely enter in contact with each other even at the highest concentrations used in this study. The distribution of relaxation times of the slow relaxation process, τ_{seg1}, τ_{seg2}, and τ_{seg3}, found in the range lower than 10^9 s^{-1} for the *l*-PNIPAM solution (Figure 2) is in good agreement with those observed for solutions of different concentrations and also with those reported for solutions of linear PNIPAM[4] with different molecular weights and several polymer concentrations (including those lower than the overlap concentration) when the solution temperature was 25 °C. These observations strongly suggest that the distribution of relaxation times of the slow relaxation process assigned to the segment mode is not significantly influenced by the polymer concentration (and the onset of slight overlapping between polymer chains); i.e., the segment mode corresponds to local molecular motions of the polymer chains. This leads us to suggest that the data obtained in this study for *c*-PNIPAM solutions essentially provide information on the dynamic features of the hydration of isolated single *c*-PNIPAM chains in aqueous solution.

The concentration-normalized total relaxation strength of the segmental mode ($\varepsilon_{seg}c^{-1} = (\varepsilon_{seg1} + \varepsilon_{seg2} + \varepsilon_{seg3})c^{-1}$) is independent of *T* in solutions of *l*-PNIPAM and *c*-PNIPAM well below their LCSTs, as seen in Figure 4. However, the $\varepsilon_{seg}c^{-1}$ value for *c*-PNIPAM is more than twice as large as that of *l*-PNIPAM (ca. 15 M^{-1} vs 6 M^{-1}). The latter value is in good agreement with the $\varepsilon_{seg}c^{-1}$ value reported earlier for aqueous solutions of high and low molecular weight linear PNIPAMs without the couplers and even for solutions of the monomer, NIPAM.[4] Therefore, the large $\varepsilon_{seg}c^{-1}$ measured in *c*-PNIPAM solutions must be related to topological constraints imposed by the cyclization. The increase of the average relaxation times

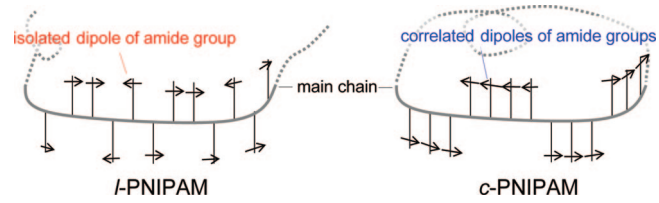

Figure 5. Schematic depiction of differences in coopratividies in segments between *c*-PNIPAM and *l*-PNIPAM.

for *c*-PNIPAM simply reflects the absence of the fast moving chain end. However, if the relaxation modes were simply shifted toward the longer times region for the cyclic polymer solutions due to, for example, a self-concentration effect[17] stronger than for linear polymer solutions, one would not observe the increase in the total magnitude of $\varepsilon_{seg}c^{-1}$ found experimentally (cf. Figure 2). We attribute the increase in the $\varepsilon_{seg}c^{-1}$ value for *c*-PNIPAM to an enhanced cooperativity in the motions of the monomer units, such as the formation of transient correlated dipoles of amide groups in the *c*-PNIPAM chain, which are formed by parallel connection in a head-to-tail fashion, as schematically depicted in Figure 5. The average number of amide groups correlated in molecular motions per segment is slightly greater in *c*-PNIPAM than that in *l*-PNIPAM due to the lack of fast, random molecular motions of chain ends. This additional cooperativity found only in *c*-PNIPAM slightly increases the average size of a segment and is likely to be implicated in the slight increase of the LCST of *c*-PNIPAM, compared to that of *l*-PNIPAM, for solutions in the concentration range probed by the DR measurements described here. The change in the segment size of PNIPAMs in aqueous solution depending on the chain topology and due to the correlation in molecular motions of monomers via dipolar interaction is possibly related to some hydration and dehydration characteristics which are capable of governing the value of LCST as described above. However, it should be noted that the change in the segment size hardly influences the τ_{ex} value; i.e., the exchange process of the water molecules hydrated onto the PNIPAM chains with bulk water is a much faster molecular event than the segment mode.

According to experimental and theoretical comparative studies[18−20] of the solution properties of cyclic and linear polystyrenes (*c*-PS and *l*-PS), *c*-PSs experience an additional repulsive intermolecular interaction, called "topological interaction", as a consequence of its cyclic topology. Hence, *c*-PS is more soluble, and the upper critical solution temperature (UCST, which is identical to the theta (Θ) temperature at an infinitely high molecular weight) of *c*-PS is lower than that of *l*-PS in cyclohexane. In the case of PNIPAM aqueous solutions, such topological interactions may also enhance the solubility of *c*-PNIPAM compared to a *l*-PNIPAM of identical molecular weight. Consequently, the LCST of aqueous *c*-PNIPAM solutions is expected to increase slightly compared to solutions of the linear counterpart, due to the topological interactions. As seen in Figure 3, the relative values of the experimental LCSTs follow the $LCST^L < LCST^C$ expected by analogy with the situation of *c*-PS and *l*-PS in cyclohexane. The effect of the cyclic topology on the LCST of PNIPAM in water warrants further studies, which are beyond the scope for this study. The chemical structures of the chain couplers used to make cyclic PNIPAMs are also implicated in the control of the value of LCST in aqueous solutions. For combinations of *c*-PNIPAMs and *l*-PNIPAMs[12,13] obtained with a chain coupler different from that used in this study,[11] the order of $LCST^C$ (~27 °C) < $LCST^L$ (~34 °C) is opposite to the order observed in this study. Such

a reversal in the order of the LCSTs is possibly caused by a difference in hydrophobicity of the chain couplers.

Several factors need to be taken into account in order to elaborate a precise theoretical thermodynamic understanding of the phenomena related to the LCST of cyclic PNIPAMs in aqueous solution. Previous reports have highlighted effects intrinsic to the cyclic topology, such as the intermolecular topological interactions and the absence of end groups, as well as effects related to the synthetic sequence employed and the chemical structure of the linker groups. Our study has revealed the occurrence of an additional cooperativity in the molecular motions of the monomer units of *c*-PNIPAM compared to *l*-PNIPAM.

Experimental Methods

A detailed description of the DR measurements has been reported elsewhere.[4] The instrumentation consists of an RF LCR meter (Agilent Technologies, 4287A) equipped with a homemade electrode cell and a dielectric material probe system (Hewlett-Packard, 85070B). The $\bar{V}_p$ values for the *c*-PNIPAM and *l*-PNIPAM were evaluated via density measurements of aqueous solutions of the PNIPAMs at each T using a DMA5000 densitometer (Anton Paar, Graz, Austria). T_1 ^{13}C NMR measurements for D_2O solutions of the *c*-PNIPAM and *l*-PNIPAM were performed using JEOL EX-270 (resonance frequency for ^{13}C: 67.80 MHz) and JEOL Lambda-500 (126.7 MHz) spectrometers at 25 °C via an inversion recovery pulse sequence under the deuterium lock mode. The T_1 values obtained at different resonance frequencies provided the correlation time, τ_c.[15] This τ_c value was subsequently converted to the rotational relaxation time with the same physical meaning of dielectric average relaxation times of the segment mode using the expression $\bar{\tau}_{seg} = 3\tau_c$.

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Supporting Information Available: Synthesis and characterization of the *c*-PNIPAM and *l*-PNIPAM and correlation and longitudinal ^{13}C NMR relaxation times for D_2O solutions of the *c*-PNIPAM and *l*-PNIPAM. This material is available free of charge via the Internet at http://pubs.acs.org.

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