A Proton-Transfer Probe of a Polymer−Water Interface. 2-Naphthol-Labeled Poly(isopropyl)acrylamide

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Abstract: Phase transitions in poly(*N*-isopropylacrylamide) (**PNIPAM**) covalently labeled with a 2-naphthol were examined by steady-state and time-resolved spectroscopy. 2-Naphthol undergoes excited-state proton transfer (ESPT) at a rate that is dependent upon water concentration in aqueous solvent systems. Upon conversion to the phase-separated state in naphthol-labeled **PNIPAM**, the efficiency of excited-state proton transfer diminished, consistent with formation of a globular phase in which exposure of the probe molecules to the water phase was reduced.

Introduction

Polyacrylamides exhibit unusual phase properties. Poly(*N*-isopropylacrylamide) (**PNIPAM**) is soluble in cold water but undergoes a reversible phase separation upon heating, exhibiting a lower critical solution temperature (LCST) of 31−32 °C. This property of **PNIPAM** was first reported by Heskins and Guillet.[1] Phenomena occurring at the LCST of aqueous polymer solutions of **PNIPAM** have been observed by a wide variety of experimental techniques, including calorimetry,[1] viscometry,[1] turbidimetry,[1] and light scattering.[2] The most extensive and most elegant of these are the studies of Françoise Winnik,[3] in which pyrene chromophores were covalently attached to the **PNIPAM** backbone, and the effect of the environment on the rate of formation of excimers was probed. Use of pyrene excimer emission necessarily examines intra- or interpolymer interactions in which the role of the solvent water is described in an indirect manner. In this paper, we describe the application of steady-state and time-resolved fluorescence measurements to the study of **PNIPAM** solution properties below and above its LCST through the use of polymers not with an excimer label but with a proton-transfer label, i.e., 2-naphthol. Such a label provides a direct probe of the polymer/water interface and allows us to draw more detailed conclusions about the structure of the polymer below and above the LCST.

Hydroxyarenes such as 2-naphthol and its derivatives undergo a significant increase in acidity, a Förster shift, upon photoexcitation. In the presence of a suitable proton acceptor, most commonly water, a rapid proton transfer occurs. This process is manifested by the appearance of two fluorescence bands due to emission from excited naphthol and naphtholate forms, respectively, and by a characteristic time dependence of the emission intensity on the nanosecond time scale. The rate of the proton transfer exhibits a nonlinear dependence upon water concentration in mixed aqueous solvent systems, a phenomenon that has been studied heavily in the groups of Robinson,[4] Shizuka,[5] Kelley,[6] and Agmon and Huppert.[7] Robinson and co-workers, in particular, have attributed the nonlinear dependence to the requirement for the formation of water clusters in the proton transfer step. Although this interpretation has been challenged by Huppert, Agmon, and co-workers,[8] at least in the low-water regime the difference in proton transfer efficiency is related to the diminished cluster stability when the last water molecule is replaced by methanol. Regardless of explanation, however, it is clear that water concentration is a significant variable in the proton transfer efficiency of 2-naphthol, and thus excited-state proton transfer provides a useful probe for the aqueous environment at polymer−water interfaces. A naphthol label has been used in the study of a protein, although homogeneous solutions were used.[9] The particular case of **PNIPAM**, however, provides a unique opportunity for examining that interface as a polymer undergoes phase separation above the LCST.

Experimental Section

Materials. 6-Methoxy-2-naphthonitrile, triethylamine, acryloyl chloride, pyridinium hydrochloride, and *N*-isopropylacrylamide were purchased from Aldrich Chemical Co.; *N*-hydroxysuccinimide was purchased from TCI America; and 2,2′-azobis(2-methylpropionitrile) (**AIBN**) was purchased from Alfa.

6-(Acetamidomethyl)-2-methoxynaphthalene (2).[10] A 3.25 g (17.8 mmol) portion of 6-methoxy-2-naphthonitrile and approximately 1.5 g of Raney-nickel catalyst were mixed in acetic anhydride, hydrogen was

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added at an initial pressure of 32 psi, and the mixture was shaken at room temperature for 22 h. The catalyst was filtered and washed several times with methanol, the solvent was removed, and a white material was recovered. The acetic anhydride filtrate was quenched with methanol, and after evaporation of the resulting methyl acetate, an oily material was obtained. Precipitation of the desired compound was accomplished by adding water to the mixture. The white solid obtained was filtered, washed with water, and recrystallized from acetone−water to yield 3.44 g (15.0 mmol, 84.5%) of **2**: mp 162.5−163 °C, ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 1.883 (s, 3H), 3.851 (s, 3H), 4.359 (d, 2H, J = 5.37 Hz), 7.139 (dd, 1H, J = 2.50, 8.95 Hz), 7.285 (d, 1H, J = 2.50 Hz), 7.354 (dd, 1H, J = 1.65, 8.40 Hz), 7.652 (br s, 1H, $\Delta\nu_{1/2}$ = 1.65 Hz), 7.766 (d, 1H, J = 8.40 Hz), 7.776 (d, 1H, J = 8.95 Hz), 8.395 (t, 1H, J = 5.37 Hz); MS *m/e* 229 (M^+, base), 186 (79.6), 171 (46.6), 128 (22.58). Anal. Calcd for $C_{14}H_{15}NO_2$: 229.1103. Found: 229.1095.

6-(Aminomethyl)-2-methoxynaphthalene (3).[11] A 2.29 g (10.0 mmol) portion of 6-(acetamidomethyl)-2-methoxynaphthalene was mixed with 100 mL of Claisen's alkali (35.2 g of KOH and 25.2 mL of water diluted to 100 mL with MeOH). The mixture was refluxed for 18 h and allowed to cool to room temperature. After evaporation of solvent, the remaining oily white solid was filtered and washed several times with water to yield 1.81 g (9.7 mmol, 96% yield) of colorless crystals: mp 108−109 °C; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 1.789 (t, 2H, J = 7.07 Hz), 3.807 (d, 2H, J = 7.07 Hz), 3.846 (s, 3H), 7.119 (dd, 1H, J = 2.59, 8.95 Hz), 7.269 (d, 1H, J = 2.59 Hz), 7.438 (dd, 1H, J = 1.74, 8.44 Hz), 7.714 (br s, 1H, $\Delta\nu_{1/2}$ = 1.74 Hz), 7.739 (d, 1H, J = 8.44 Hz), 7.748 (d, 1H, J = 8.95 Hz); MS *m/e* 186 (M^+ − 1, 49.3), 171 (base), 128 (30.0), 115 (21.4), 69 (40.0). Anal. Calcd for $C_{12}H_{13}NO$: 187.0997. Found: 187.0992.

6-(Aminomethyl)-2-naphthol (6AMN2). A mixture of 1.50 g (6.98 mmol) of 6-(aminomethyl)-2-methoxynaphthalene and 12.0 g of pyridine hydrochloride was flushed with dry nitrogen for 20 min and heated to 220 °C. The mixture was heated at reflux for 20 min under nitrogen and poured into ice-water. The solution was neutralized with 10% NaOH, saturated with solid NaCl, filtered, and washed with water. The mother liquor was extracted with BuOH (3 × 30 mL), and the combined organic fractions were washed with a saturated solution of NaCl and extracted with 10% NaOH (4 × 25 mL). The aqueous layer was neutralized and saturated with solid NaCl to yield additional solid. The combined materials (0.97 g, 5.6 mmol, 70% yield) were recrystallized from methanol−water to yield the desired compound: mp 221−223 °C dec; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 4.089 (s, 2H), 7.139−7.104 (m, 2H, J = 2.38, 9.37 Hz), 7.481 (dd, 1H, J = 1.71, 8.48 Hz), 7.758−7.707 (m, 2H), 7.831 (br s, 1H), 8.301(br s, 2H), 9.900 (br s, 1H); MS *m/e* 173 (M^+, 94.0), 172 (base), 145 (32.5), 127 (28.4), 115 (22.5). Anal. Calcd for $C_{11}H_{11}ON$: 173.0840641. Found: 173.084045.

6-(Acetamidomethyl)-2-naphthol (6AAN2). To a mixture of 0.49 g (2.80 mmol) of 6-(aminomethyl)-2-naphthol (**6AMN2**) and 0.40 mL (2.87 mmol) of triethylamine was added, in an ice bath, 2.00 mL of acetic anhydride dropwise. The mixture was stirred at room temperature for 30 min and at reflux for 1 h and allowed to cool to room temperature, MeOH was added, and the resulting methyl acetate was evaporated under vacuum. The residue was diluted with water, and the resulting precipitate was filtered and washed with water. By ^{1}H-NMR and mass spectroscopy the solid was identified as 6-(acetamidomethyl)-2-naphthyl acetate. This material was treated with concd HCl (2.0 mL) in MeOH (15 mL) for 30 min and allowed to cool to room temperature. Water was added, and the resulting precipitate was filtered, washed with water, and dried under vacuum: mp 213−214 °C dec; ^{1}H-NMR acetone-d_6, 300 MHz) δ 1.980 (s, 3H), 4.480 (s, 2H), 7.121 (dd, 1H, J = 2.44, 8.79 Hz), 7.166 (d, 1H, J = 2.44 Hz), 7.357 (dd, 1H, J = 1.83, 8.52 Hz), 7.645 (d, 1H, J = 8.52 Hz), 7.672 (br s, 1H, $\Delta\nu_{1/2}$ = 1.83 Hz), 7.730 (d, 1H, J = 8.79 Hz); MS *m/e* 215 (M^+, 90.5), 172 (base), 157 (42.3), 145 (23.2), 127 (23.2), 115 (21.1). Anal. Calcd for $C_{13}H_{13}O_2N$: 215.0946. Found: 215.0950.

Poly[*N*-isopropylacrylamide-*co*-*N*-(acryloxy)succinimide] (PNIPAM/NASI).[3] *N*-Isopropylacrylamide (5.0 g, 44.0 mmol) and *N*-(acryloxy)succinimide (0.15 g, 0.88 mmol) were dissolved in *tert*-butyl alcohol (30 mL) at 70 °C under argon. 2,2-Azobis(2-methylpropionitrile) (30 mg) in 2-methyl-2-propanol (1 mL) was added to the solution. The mixture was stirred at 70 °C for 20 h and cooled to ambient temperature and the solvent evaporated. The polymer was purified by successive precipitations from tetrahydrofuran solution with hexane and from methanol solution with anhydrous diethyl ether.

Preparation A, 200:1 (PNIPAM/N2/220). To 6.45 mg (0.037 mmol) of 6-(aminomethyl)-2-naphthol dissolved in 5 mL of THF and 2 mL of DMF was added 0.5645 g (0.096 mmol of active sites) of **PNIPAM/NASI**. The solution was stirred at room temperature in the dark for 24 h, and 0.15 mL (1.76 mmol) of isopropylamine was added; stirring was continued for 2 h. The reaction mixture was poured into hexane. The solvent was decanted and the polymer dissolved in MeOH. Anhydrous ethyl ether was added, the precipitate filtered, and precipitation of the filtrate was repeated. After each precipitation, the absorption spectra of the polymer remained unchanged, indicating that all the unreacted label had been removed.

Preparation B, 400:1 (PNIPAM/N2/440). Preparation A was followed except that 3.10 mg (0.018 mmol) of 6-(aminomethyl)-2-naphthol and 0.30 mL (3.52 mmol) of isopropylamine were used.

Fluorescence Measurements. Steady-state fluorescence spectra were recorded on a SPEX Fluorolog 112X spectrofluorometer equipped with a DM3000F data system. Sample fluorescence was collected at right angles from the excitation beam with entrance and exit slits of 1.5 mm. All spectra were corrected for the wavelength dependence of the photomultiplier response. Concentrations were in the range of 10^{-4} to 10^{-5} M. The excitation wavelength was set at 291 nm. The temperature of the water-jacketed cell holder was controlled with a Frigomix 1497 circulating bath. The temperature of the sample fluid was estimated using a calibration curve obtained by measuring the temperature of a water sample as a function of the temperature in the circulating bath, using the identical time−temperature program used for the actual experiments. The time/temperature data were fit to a multiple linear using least-squares regression, and the derived coefficients were used to correct the temperature observed in the circulation bath.

Time-based scans were recorded on the same instrument using an excitation wavelength of 291 nm. The solutions were heated to an initial temperature of 47 °C, and the fluorescence emission intensities were recorded every 60 s while the temperature in the bath was allowed to fall to room temperature. With the aid of the calibration curve, the time scale was transformed to a temperature scale. The fluorescence emission intensity was then plotted against this corrected temperature.

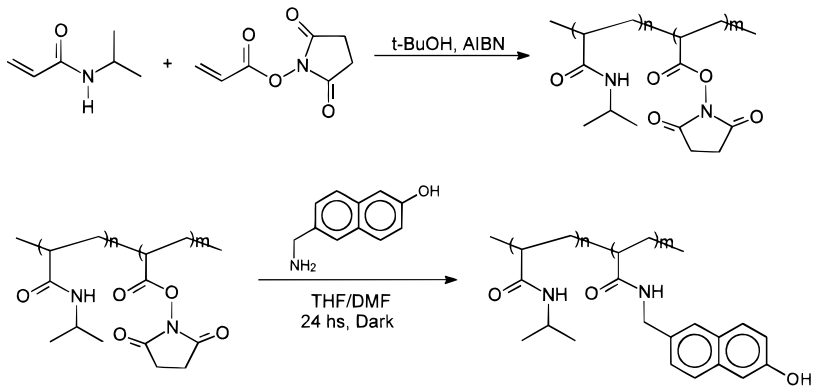
Excited-state lifetimes were measured with a time-correlated single photon counting fluorimeter consisting of a Photochemical Research Associates flashlamp and optics. For stray light minimization, during the monitoring of the sample fluorescence at high temperatures, a 336 nm Hoya cutoff filter for neutral emission and a 400 nm Corion cutoff filter for anion emission were employed. The electronics end consisted of Ortec components coupled to an Ortec multichannel analyzer plug-in board in an IBM AT computer, which monitored pulses from a cooled Hamamatsu R928 photomultiplier. Fluorescence lifetimes were determined by iterative deconvolution employing Photon Technology International software using a lamp scattering profile and the experimental emission data. Label concentrations were between 10^{-4} and 10^{-5} M. Samples were purged with argon or nitrogen for 5 min prior to data acquisition.

Cloud points of aqueous solutions of **PNIPAM** and **6AMN2**-labeled **PNIPAM** were determined by spectrophotometric detection of the changes in turbidity in solutions upon cooling, at 500 nm. The turbidity is equal to the absorbance of the solution at a wavelength for which the sample does not absorb light (**6AMN2** has no absorption at 500 nm). Time-based scans were recorded using an excitation wavelength of 500 nm. The solutions were heated to an initial temperature of *ca.* 49 °C, and the changes in turbidity were recorded every 60 s while the temperature in the bath was allowed to fall to room temperature. With the aid of the calibration curve, the time scale was transformed into a temperature scale. The points of sudden disappearance of turbidity on cooling were determined graphically. Measurements were performed at least twice on each sample, and the results were averaged; values were reproducible within ±0.2 °C.

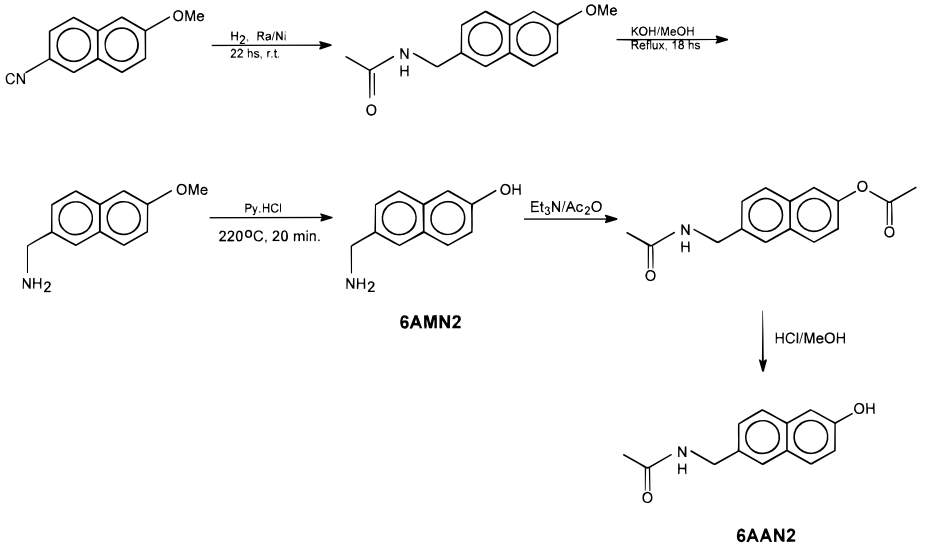
Samples for Spectroscopic Analysis. Labeled polymer solutions were prepared at room temperature. They were allowed to stand in

(11) Mozingo, R. *Organic Syntheses;* Wiley: New York, 1955; Collect. Vol. Vol. III, p 181.

Scheme 1. Synthetic Scheme for the Preparation of **6AMN2**-Labeled **PNIPAM**



Scheme 2. Synthetic Scheme for the Preparation of **6AMN2** and **6AAN2**



the dark for 24 h before being diluted to a known volume. In each sample the amount of **6AMN2** incorporated into the polymer was calculated from UV absorption data of polymer solution in methanol, using 6-(acetamidomethyl)-2-naphthol (**6AAN2**, $\lambda_{max} = 334$ nm, $\epsilon = 2278$ L mol^{-1} cm^{-1}) as a reference compound and assuming that the molar absorptivity of reference and polymer naphthol chromophore were identical.

Gel Permeation Chromatography. GPC was performed on a Waters Maxima 820 instrument, equipped with a Waters 410 DRI detector and a Maxima 820 data analysis system. A UBONDAPAK column, 30 cm in length and 3.9 cm in diameter, with a particle size of 10 μm and packing C18, packing type silica/C18, functionality C18 was used. HPLC grade THF was used as the eluent at a flow rate of 1.0 mL min^{-1}, the duration of the experiment was 40.0 min, and the concentration of the samples was *ca.* 10.0 mg mL^{-1} in all cases.

Results

The labeled polymers were prepared by reaction of 6-(aminomethyl)-2-naphthol with a copolymer of *N*-isopropylacrylamide and *N*-(acryloxy)succinimide according to the method of Hoffman[12] (Scheme 1), while **6AMN2** and **6AAN2** were prepared as depicted on Scheme 2. By varying the initial ratio of the amine to the *N*-(acryloxy)succinimide groups of the copolymer, two **PNIPAM** samples incorporating amounts of **6AMN2** ranging from a monomer ratio of 400:1 to 200:1 were prepared. These are represented as **PNIPAM/N2/X**, where **X** represents the approximate ratio of unlabelled to labelled monomer. For a noncovalently attached standard, the functionally equivalent 6-(acetamidomethyl)-2-naphthol (**6AAN2**) was prepared by reaction of **6AMN2** with acetic anhydride, followed by hydrolysis of the ester linkage in the resulting naphthyl acetate. The degree of incorporation of **6AMN2** in the polymer was determined spectrophotometrically, using **6AAN2** as a standard and assuming identical molar absorptivities. By this criterion, **PNIPAM/N2/220** contained 4×10^{-5} mol of label per gram of polymer, corresponding to a monomer ratio of 1 label unit every 219 **NIPAM** units, while **PNIPAM/N2/440** contained 2×10^{-5} mol per gram of polymer, corresponding to a ratio of 1:440.

(12) Cole, C. A.; Schreiner, S. M.; Priest, J. H.; Monji, N.; Hoffman, A. S. *ACS Symp. Ser*. **1987**, No. 350, 245.

Table 1. Molecular Weight and Critical Temperature Data for Labeled PNIPAM

polymer	M_n[b]	M_w[b]	LCST (°C)
PNIPAM	158 091	1 143 381	31.8[a]
PNIPAM/N2/220	98 739	602 293	31.6 ± 0.4
PNIPAM/N2/440	127 014	800 368	31.8 ± 0.4

[a] Ringsdorf, H.; Venzmer, J.; and Winnik, F. M. *Macromolecules* **1991**, *24*, 1678. [b] By GPC (relative to polystyrene).

Molecular weights and molecular weight distributions were calculated from GPC measurements run in THF and calibrated with polystyrene standards (see Table 1).

Cloud points of **PNIPAM/N2/X** were determined by the spectrophotometric method. The LCST of the labeled polymers is reported on Table 1.

Fluorescence Measurements. The emission spectra of **PNIPAM/N2/220** in methanol and in water at room temperature are shown in Figure 1. In methanolic solution, the polymer

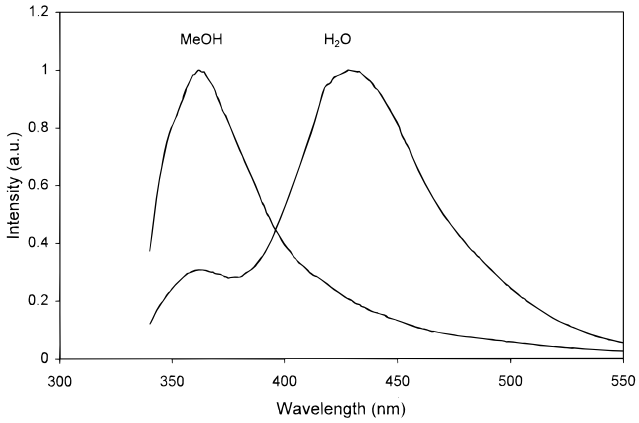


Figure 1. Emission spectra of **PNIPAM/N2/220** in H_2O and in MeOH at room temperature, $\lambda_{ex} = 333$ nm.

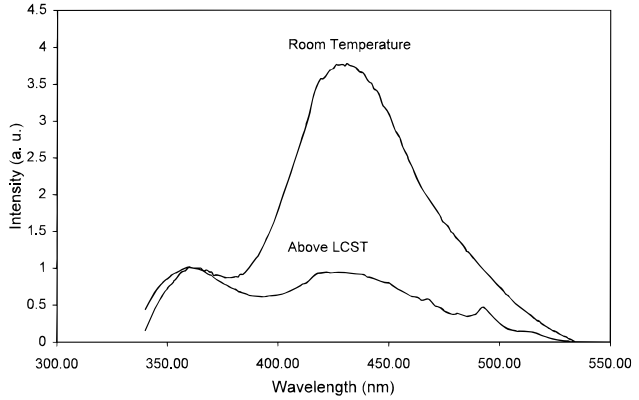


Figure 2. Normalized emission spectra of **PNINAM/N2/440** in water at room temperature and above the LCST (approximate temperature 40° C), $\lambda_{ex} = 291$ nm.

shows an emission due to neutral 2-naphthol (intensity I_n) with a maximum at 361 nm; no anion emission is detected. In water, a strong emission centered at 431 nm (intensity I_a, "anion" emission) is observed.

The room-temperature emission spectra in water of polymers **PNIPAM/N2/220** and **PNIPAM/N2/440** (Figure 2) show a strong anion emission for both samples. Above the LCST (Figure 2), the ratio of intensities is reduced dramatically for the labeled polymers.

When the anion to neutral emission ratios I_a/I_n were plotted vs temperature, an inflection point was observed for both polymers, as well as for the probe molecule **6AAN2** in aqueous solvent containing identical concentrations of **PNIPAM** (see Figure 3). This inflection point occurred at *ca.* 31 °C, corresponding to the published LCST of **PNIPAM**.[1] LCST determination by turbidimetry showed that the presence of the 2-naphthol label did not change this value appreciably (see Table 1). The same experiment carried out in D_2O revealed a reduced intensity ratio, consistent with the known solvent isotope effect for excited-state proton transfer. However, there was no observable change in the LCST (see Figure 4).

Time-correlated single-photon counting was used to analyze the decay of the neutral emission at 363 nm and the decay of the anion emission at 431 nm for all polymers studied, as well as for **6AAN2** in aqueous solutions of **PNIPAM**. With few exceptions, both neutral and anion decays gave excellent statistics using a sum of two exponentials (Table 2). Also reported in Table 2 are the fractional populations undergoing decay by each channel. The neutral decays above the LCST gave somewhat inferior correlations, but in all cases the χ^2 was below 2.000.

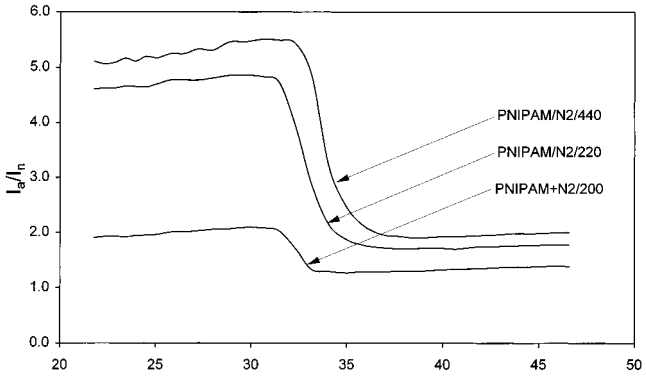


Figure 3. Temperature dependence of the ratio of anion to neutral fluorescence emission intensity for **6AMN2**-labeled polymers in water, $\lambda_{ex} = 291$ nm.

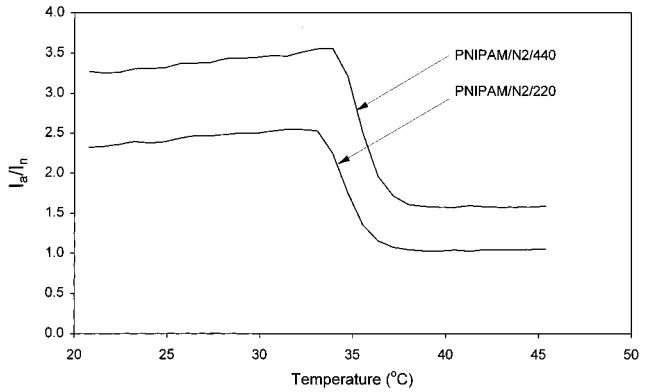


Figure 4. Temperature dependence of the ratio of anion to neutral fluorescence emission intensity for **6AMN2**-labeled **PNIPAM** in D_2O solutions, $\lambda_{ex} = 291$ nm.

Discussion

The use of spectroscopic probes to study polymer structure is based upon the concept that the probe does not significantly change the polymer thermodynamics. Of course, all such studies suffer from a Maxwell's demon problem. That is, does the probe molecule change the very properties that one wishes to investigate? The use of fluorescence as the spectroscopic probe offers some advantages, including both its toleration of solution turbidity and its sensitivity at relatively low fluorophore concentrations, i.e., concentrations for which the perturbation is expected to be minimized. For instance, using the emission intensity ratio I_a/I_n instead of the absolute intensity allows for correction of light-scattering effects. The use of varying probe/monomer ratios provides a further check on artifacts produced by polymer substitution. We first consider the fluorescence of **6AAN2** in water and in aqueous **PNIPAM** solutions. In water, the room-temperature fluorescence of **6AAN2** consists largely of the anion fluorescence typical of a system undergoing rapid excited-state proton transfer, with a ratio of anion to neutral emission of 2.5:1. In the presence of 2.0 g/L of **PNIPAM**, the intensity ratio I_a/I_n decreases to 1.9:1, indicative of a partial sequestration of **6AAN2** in the polymer. Upon heating past the LCST, I_a/I_n decreases to 1.3:1. If we use the simplest two-phase model, and further assume that fully sequestered 2-naphthol exhibits only neutral emission as indicated by the absence of anion emission in pure carboxamide solvents, we can estimate that, at room temperature, 79.3% of **6AAN2** exists in the aqueous environment or within water pools within the condensed phase, while above the LCST, the amount of **6AAN2** in direct contact with water is reduced to 48.1%. In the case of the labeled polymers in water, however, the intensity ratio I_a/I_n is *ca.* 5 at room temperature and decreases to *ca.* 2 upon heating

Table 2. Iterative Deconvolution of Naphthol Emission Data

experiment	T (°C)	A_1	τ_1	F_1	A_2	τ_2	F_2	A_3	τ_3	F_3	χ^2
PNIPAM/N2/220											
neutral (361 nm)	25	0.913	3.882	0.802	0.087	10.106	0.198				1.102
	30	0.715	2.500	0.490	0.285	6.538	0.510				1.124
	35	0.745	1.758	0.390	0.255	8.021	0.610				1.218
	40	0.775	1.552	0.394	0.225	8.223	0.606				1.197
anion (431 nm)	25	−0.473	2.814	0.093	1.000	12.942	0.907				1.082
	30	−0.542	2.526	0.097	1.000	12.756	0.903				1.119
	35	−0.466	0.527	0.020	1.000	11.929	0.980				1.201
	40	−0.236	0.476	0.009	1.000	11.736	0.991				1.155
PNIPAM/N2/440											
neutral (361 nm)	25	0.824	2.443	0.606	0.176	7.426	0.394				1.181
	30	0.880	2.700	0.712	0.120	8.056	0.288				1.136
	35	0.891	1.668	0.606	0.109	8.922	0.394				1.124
	40	0.907	0.978	0.539	0.093	8.159	0.461				1.593
anion (431 nm)	25	−0.558	1.017	0.044	1.000	12.472	0.956				1.267
	30	−0.525	0.781	0.034	1.000	11.621	0.966				1.390
	35	−0.833	2.558	0.265	0.248	12.872	0.396	0.752	3.625	0.339	1.131
	40	−0.407	0.099	0.005	0.578	11.820	0.843	0.422	2.909	0.152	1.164
6AAN2+**PNIPAM** (1:200)											
neutral (361 nm)	25	0.725	3.789	0.590	0.275	6.934	0.410				1.327
	30	0.774	3.535	0.633	0.226	7.001	0.367				1.324
	35	0.761	1.666	0.428	0.239	7.087	0.572				1.493
	40	0.798	1.253	0.424	0.202	6.727	0.576				1.359
anion (431 nm)	25	−0.596	4.244	0.169	1.000	12.441	0.831				1.543
	30	−0.583	4.213	0.169	1.000	12.062	0.831				1.529
	35	−0.681	4.258	0.198	1.000	11.752	0.802				1.462
	40	−0.643	4.354	0.196	1.000	11.470	0.804				1.271

above the LCST, while in D_2O, I_a/I_n is *ca.* 3.5 at room temperature and *ca.* 2 above the LCST. A covalently attached 2-naphthol would be expected to orient itself to minimize hydrophilic interactions and maximize hydrophobic interactions with the polymer.

In organic solvents **PNIPAM** is believed to behave as a flexible coil, while there is evidence[2,13] that in water the polymer chains behave as isolated rigid rods. This stiffening of the chain is attributed to hydrogen bonding between water molecules and the amide groups of the polymer. Near the LCST, Heskins and Guillet[1] suggest that **PNIPAM** behaves as flexible coils undergoing extensive interpolymeric association. What is most surprising is that the model compound 6-acetamido-2-naphthol itself shows diminished anion intensity, i.e., less efficient proton transfer, relative to the labeled polymer. This suggests that **PNIPAM** is able to sequester the mobile naphthol more efficiently than the covalently attached probe, which by virtue of its reduced conformational flexibility may be brought into greater contact with the aqueous environment. In the case of **PNIPAM/N2/220** and **PNIPAM/N2/440**, the proton transfer is reduced but not completely eliminated, suggesting that water molecules may be enclosed as the polymer coils upon itself, perhaps assuming a micelle-like structure. Alternatively, a nonrandom displacement of probe molecules, or an orientation of the probe molecules to avoid the hydrophobic environment, may contribute to the presence of residual proton transfer. It is interesting to note that the deuterium isotope effect appears to be more important in the low-temperature regime than above the LCST, thus suggesting that at low temperature the naphthol groups are more exposed to the water environment due to the rodlike structure of the polymer backbone.

Finally, any analysis of polymer dynamics based upon steady-state fluorescence data, particularly when those data are expressed as ratios of fluorescence intensities, is subject to misinterpretation, since fluorescence intensities are a ratio of spectroscopic rates. In the case of anion emission, moreover, the spectroscopic rates include both an appearance rate constant and a decay rate constant. Thus, a decrease in I_a/I_n may reflect (1) a decrease in the neutral decay rate, (2) a decrease in the anion formation rate, of (3) an increase in the anion decay rate. All of this further assumes that the fluorescence decay rate remains constant. The existence of a strong solvent deuterium isotope effect for the emission ratio certainly indicates that proton transfer is a critical factor in determining the partition between hydrophobic and hydrophilic environments for the photoexcited naphthol. Thus, the conclusions drawn here must be validated by an appeal to the decay rate constants themselves from time-correlated single-photon counting data.

The effects of environment on the decay of the neutral can be manifested in two ways as the temperature increases past the LCST. One way is simply a decrease in the proton transfer rate. The other is an increase in the partition ratio between hydrophobic and hydrophilic sites. Of course, the dynamics of polymer motions bringing the covalent probe into proximity with aqueous solvent will also be convoluted into the microscopic decay rates. Thus, the use of simple exponentials in the analysis of the decay rates is, at best, a crude approximation. Nevertheless, both neutral and anion decay processes gave excellent statistics using a sum of two exponentials (Table 2).[14] n the case of anion decay, one exponential term represents the formation, i.e., proton transfer, rate constant $1/\tau_1$, while the second represents the anion decay rate $1/\tau_2$. The latter is virtually independent of the polymer or temperature. In the case of neutral emission, two lifetimes are calculated. The short lifetime τ_1 is associated with naphthol undergoing proton transfer while the long lifetime $1/\tau_2$ represents sequestered naphthol. Using this analysis, the effect of increasing temperature past the LCST is both to increase the population of naphthols in incompetent sites for proton transfer, as measured by the increase in fractional population of the long-lived component F_2, and to increase the rate of proton transfer $1/\tau_1$ in the competent sites (Table 2).

(13) Fujishige, S. *Polym. J. (Tokyo)* **1987**, *19*, 297.

(14) For consistency, the emission rates are reported in τ, i.e., 1/k.

Conclusions

Steady-state and time-resolved fluorescence spectroscopy involving excited-state proton transfer has been used to probe the solution behavior of PNIPAM in water below and above the LCST. The evidence collected supports the idea that at room temperature **PNIPAM** assumes an extended conformation that still allows considerable coiling and sequestering of hydrophobic sites. When heated above its LCST, the polymer assumes a more highly coiled conformation, leading to globular aggregates, which minimize hydrophilic interactions and retard proton-transfer. In any event, the use of excited-state proton transfer constitutes a further means for studying such polymer−water interactions.

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