Quenching of Fluorescence from Pyrene-Labeled Poly(*N*-isopropylacrylamide) Solutions Heated above Their Lower Critical Solution Temperature

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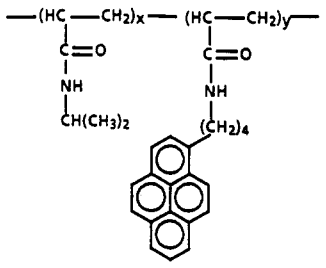
ABSTRACT: Fluorescence quenching experiments were carried out on aqueous solutions of pyrene-labeled poly(*N*-isopropylacrylamide)s in dilute solution and in the presence of unlabeled poly(*N*-isopropylacrylamide), below and above their lower critical solution temperature (LCST). The quenchers of fluorescence were two water-soluble compounds: ethylpyridinium bromide (EPB) and nitromethane (CH_3NO_2). Above the LCST the pyrene labels experience a remarkable protection against quenching by EPB and CH_3NO_2. Below the LCST the fluorescence of pyrene is quenched, but the simple Stern–Volmer model is not obeyed.

Many water-soluble polymers undergo phase separation when their aqueous solutions are heated.[1] Classic examples include cellulose ethers,[2] such as (hydroxypropyl)cellulose and poly(*N*-alkylacrylamide)s, such as poly(*N*-isopropylacrylamide) (PNIPAM).[3] Not much is known at the molecular level about the local environment of the polymer phase above the lower critical solution temperature (LCST). One approach to obtaining this kind of information is through fluorescence quenching studies of polymers labeled to a small extent with a fluorescent dye. Described here is the unusual behavior, above the LCST, of two samples of pyrene-labeled poly(*N*-isopropylacrylamide) (PNIPAM/Py). The two samples have the same molecular weight, but they differ in the number of labels attached to the polymer backbone (see structure). One sample, PNIPAM/Py/20, has on average one



PNIPAM/Py/200 : x/y = 200
PNIPAM/Py/20 : x/y = 20

pyrene group per 20 monomer units, and the other, PNIPAM/Py/200, has on average one pyrene group per 200 monomer units.[4] At ambient temperature the polymers show a pyrene monomer emission due to excited isolated pyrenes and an excimer emission. This emission originates mostly from preformed pyrene aggregates and not from excited-state complexes resulting from the encounter of isolated excited pyrenes.[4] Such ground-state pyrene aggregates have been reported to occur in aqueous solutions of other labeled water-soluble polymers, such as Py-labeled (hydroxypropyl)cellulose[5] and poly(ethylene oxide).[6] The excimer emission is greatly reduced in PNIPAM/Py solutions heated above their LCST. This paper describes that these polymers exhibit a remarkably different response to quenchers below and above the LCST of their solutions.

Table I
Quenching Data for PNIPAM/Py/20 and PNIPAM/Py/200 in Methanol at 25 °C

polymer		K_{SV}, mol^{-1} L	
		EPB	CH_3NO_2
PNIPAM/Py/20[a]	monomer	1250 ± 20	880 ± 30
	excimer	674 ± 23	430 ± 10
PNIPAM/Py/200[b]	monomer	1050 ± 20	730 ± 20
	excimer	1100 ± 43	650 ± 30

[a] 5 ppm, 25 °C. [b] 60 ppm, 25 °C.

Two different kinds of experiments were carried out. In one, dilute solutions of the labeled PNIPAM/Py in water were examined above and below the LCST. These experiments are sensitive to the specific behavior of the labeled chains. In the second type of experiments, traces of labeled chains were mixed with a larger quantity of unlabeled PNIPAM. In these experiments the PNIPAM/Py serve as probes to convey information about phase separation at the LCST of PNIPAM itself. Quenching experiments were performed on solutions of PNIPAM/Py/20 (5 ppm) and PNIPAM/Py/200 (60 ppm) in methanol and in water and in aqueous solutions of PNIPAM (5 g L^{-1}) to which PNIPAM/Py/20 (5 ppm) or PNIPAM/Py/200 (25 ppm) were added as polymeric probes.

Two quenchers of pyrene fluorescence were employed: nitromethane (CH_3NO_2), a water-soluble neutral compound, and ethyl pyridinium bromide (EPB), a cationic water-soluble material. Both species quench pyrene fluorescence at a diffusion-controlled rate by an electron-transfer mechanism. The results are reported in terms of the Stern–Volmer model.[7] In this treatment the fluorescence intensities I_0 and I in the absence and in the presence of a quencher, respectively, are related to the quencher concentration [Q] by eq 1, where K_{SV} is the Stern–Volmer quenching constant, k_q is the bimolecular quenching constant, and τ_0 is the lifetime of the fluorophore in the absence of quencher:

$$I_0/I = 1 + K_{SV}[Q] = 1 + k_q\tau_0[Q] \quad (1)$$

With both quenchers linear Stern–Volmer plots were obtained for quenching of pyrene excimer and monomer emissions in methanolic solutions of PNIPAM/Py/20 and PNIPAM/Py/200.[8] Stern–Volmer parameters are reported in Table I.

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Table II
Quenching Data for PNIPAM/Py/20 and PNIPAM/Py/200 in Water[a]

		solns of dilute labeled polymer				solns of labeled polymer and PNIPAM			
		PNIPAM/Py/20[b]		PNIPAM/Py/200[c]		PNIPAM/Py/20[d]		PNIPAM/Py/200[e]	
quencher	T, °C	K_{SVa}, mol^{-1} L	f_a	K_{SVa}, mol^{-1} L	f_a	K_{SVa}, mol^{-1} L	f_a	K_{SVa}, mol^{-1} L	f_a
EPB	25	203 ± 14	0.93 ± 0.02	230 ± 20	0.78 ± 0.01	290 ± 20	0.87 ± 0.02	240 ± 10	0.85 ± 0.01
	33		0		0		0		0
CH_3NO_2	25	490 ± 50	0.75 ± 0.01	377 ± 20	0.86 ± 0.02	490 ± 30	0.73 ± 0.01	340 ± 20	0.86 ± 0.02
	33	500 ± 60	0.62 ± 0.02		0		0		0

[a] f_a, estimated fraction of accessible chromophores (monomer emission); K_{SVa}, Stern–Volmer quenching constant for the accessible chromophores (pyrene monomer emission); the values were estimated by applying eq 2 to the experimental data. [b] 5 ppm. [c] 60 ppm. [d] PNIPAM/Py/20 (5 ppm), PNIPAM (5 g L^{-1}). [e] PNIPAM/Py/200 (25 ppm), PNIPAM (5 g L^{-1}).

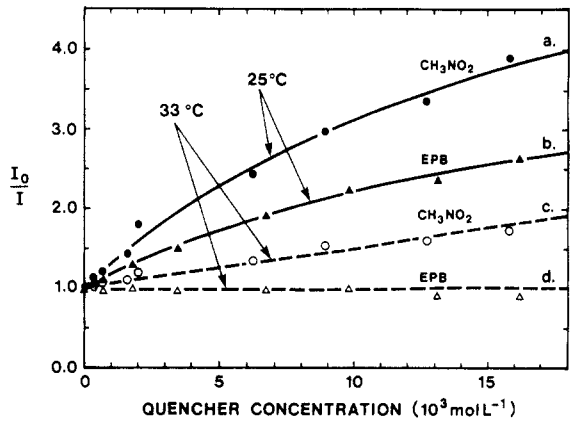

Figure 1. Ratio I_0/I of pyrene monomer emission intensity in the absence and in the presence of a quencher in aqueous solutions of PNIPAM/Py/200 (60 ppm) as a function of quencher concentration: (a) nitromethane, 25 °C; (b) EPB, 25 °C; (c) nitromethane, 33 °C; (d) EPB, 33 °C.

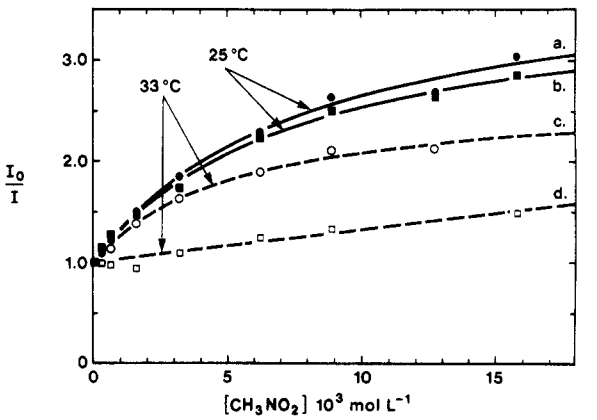

Figure 2. Ratio I_0/I of pyrene monomer emission intensity in the absence and in the presence of nitromethane for aqueous solutions of PNIPAM/Py/20 (5 ppm) as a function of nitromethane concentration: (a) 25 °C, with added PNIPAM (5 g L^{-1}) (full square); (b) 25 °C, without PNIPAM (full circle); (c) 33 °C, without PNIPAM; (d) 33 °C, with PNIPAM (5 g L^{-1}).

Aqueous solutions of PNIPAM undergo phase separation when heated to 32 °C.[3] In order to examine the changes that occur at the LCST, experiments were carried out at 25 and 33 °C. At 25 °C, in dilute solutions of the labeled polymers the pyrene monomer emission is quenched by EPB and nitromethane. However, the ratio I_0/I does not vary linearly with quencher concentration: it increases rapidly at low quencher concentration and only slightly for quencher concentrations higher than 1 × 10^{-2} M (Figure 1a,b). Quenching of pyrene excimers was inefficient in both cases. More than 70% of the total excimer fluorescence remained unquenched at quencher concentrations as high as 1.5 × 10^{-2} M, for which there is less than 10% residual excimer from solutions of the polymers in methanol. At 33 °C, pyrene monomer emission was still quenched by nitromethane, although less efficiently than at 25 °C (Figure 1c). By contrast, EPB did not quench pyrene emission at all. Neither the monomer (Figure 1d) nor the excimer emissions were affected by this quencher.

In samples where the labeled polymers were employed as probes in PNIPAM, at 33 °C, the pyrene emission was quenched neither by EPB nor by CH_3NO_2. At 25 °C the large amounts of unlabeled PNIPAM did not affect the fluorescence properties of the polymers. The emission spectra and quenching behaviors of the labeled polymers were identical with those of dilute labeled polymer solutions (Figure 2). In summary, at 25 °C both EPB and nitromethane quench pyrene emission from PNIPAM/Py in water, but the simple Stern–Volmer model is not followed; above the LCST, EPB is an ineffective quencher of pyrene emission from PNIPAM/Py in water and in PNIPAM-containing samples. While nitromethane still shows some quenching effect in the absence of PNIPAM, its effectiveness is strongly reduced. These observations are diagnostic of a difference in quencher concentration in the bulk of the solution and in the polymer microenvironment.[9]

The picture of the polymer-rich phase above the LCST put forward by Heskins and Guillet in their description of the PNIPAM/water system is that of an assembly of large aggregates of flexible coiled chains.[3] A hydrophobic environment is created from which water and aqueous solutes should to a large extent be excluded. Under these circumstances hydrophobic chromophores incorporated in the polymer-rich phase should be protected against quenching from water-soluble quenchers. This is observed when the labeled polymers are incorporated into an unlabeled PNIPAM-rich phase.

Remarkably, in dilute PNIPAM/Py solutions above the LCST the pyrene emission is still quenched to a certain extent by CH_3NO_2. A fraction of the chromophores must remain accessible to quenchers restricted mostly to the water phase. This fraction can be estimated by applying a modified Stern–Volmer model derived for systems in which there are heterogeneities in quencher and in chromophore concentrations and diffusion coefficients.[10] This model assumes that only a fraction f_a of the fluorophores is readily quenchable, while a fraction $(1 - f_a)$ is protected against quenching. The fraction of accessible chromophores and the Stern–Volmer quenching constant K_{SVa} of this accessible fraction can be obtained from eq 2. This relationship was used to generate from

$$\frac{I_0}{I} = \left[1 - \frac{f_a K_{SVa}[Q]}{1 + K_{SVa}[Q]}\right]^{-1} \quad (2)$$

the experimental data the curves drawn in Figures 1 and 2. In all cases the quality of the fit from eq 2 improved

on the fits to a simple Stern–Volmer model. Estimated values of K_{SV} and f_a are listed in Table II. Interesting features of the PNIPAM/Py–water system emerge from a comparison of the estimated values of f_a for different quenchers and polymers.

At room temperature, in dilute PNIPAM/Py solutions the pyrene groups protected against quenching (25% or less of the total population) must be located in a hydrophobic microenvironment. One process that would create such microdomains is interchain association. It has been shown by light scattering and viscosity measurements that aggregation takes place in aqueous PNIPAM solutions below their LCST.[3,11] This tendency toward aggregation is expected to be enhanced in solutions of labeled polymers due to the presence of the hydrophobic chromophores. Above the LCST the fraction of pyrenes accessible to quenchers is greatly reduced in all cases but one, that of PNIPAM/Py/20 and CH_3NO_2. In this system only ca. 40% of the pyrene population is protected against quenching. As a result of the high degree of labeling in PNIPAM/Py/20, it is possible that the process of heat-induced coiling of the chains leads to aggregates where some pyrene groups remain located close to the surface, thus quenchable by nitromethane. The fact that no quenching is observed with EPB may be accounted for by a decreased solubility of this ionic compound in the water/PNIPAM/Py/20 interphase.[12]

This paper reports the first application of fluorescence quenching techniques to the study of the heat-induced phase separation of aqueous polymer solutions. It conveys information at the molecular level about the structure of the polymer-rich phase that separates above the LCST. It shows that this phase is extremely hydrophobic in nature, whether it originates from solutions containing large amounts of PNIPAM or from dilute solutions of lightly labeled polymers. When the degree of labeling of the polymer is high, the solution properties of the polymer in water below and above the LCST are affected noticeably by the presence of the hydrophobic labels.

References and Notes

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(8) Fluorescence spectra were measured with a SPEX Fluorolog 212 spectrometer equipped with a DM3000F data system. The temperature of the water-jacketted cell holder was controlled with a Neslab circulating bath. The temperature of the sample fluid was measured with a thermocouple immersed in the fluid. The excitation wavelength was 330 nm. The monomer emission intensities (I_M) were calculated as the half-sum of the emission intensities at 376 and 396 nm. Since the fine structure of the 1-alkyl-substituted pyrene emission is not affected to any significant extent by the polarity experienced by the chromophore, this evaluation method of I_M is justified. Solutions in methanol were degassed by vigorous bubbling with methanol-saturated argon. Solutions in water were not degassed. Solutions containing PNIPAM/Py/20 (5 ppm) and PNIPAM (5 g L^{-1}) were prepared 24 h before the addition of quencher. For measurements above the LCST, aqueous solutions of the labeled polymers containing a given quencher concentration were heated to 33 °C in the spectrometer cell holder. They were kept at this temperature for 30 min. After this time their fluorescence spectrum was recorded at 33 °C.

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(12) The process of heat-induced coiling of PNIPAM/Py in dilute solutions is phenomenologically analogous to the pH-induced coiling of pyrene-labeled poly(methacrylic acid) studied by Chu and Thomas[13] and polyacrylic acid studied by Arora and Turro.[14] These polyacids exist in an extended form at high pH as a result of electrostatic repulsion between the negative charges on the polymer backbone. They undergo chain coiling as the pH is decreased. When the polymers adopt a coiled form, the pyrene labels are protected against neutral quenchers.

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Registry No. PNIPAM, 25189-55-3; EPB, 1906-79-2; CH_3NO_2, 75-52-5.