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Conformational Behavior of a Series of *N*-Isopropylacrylamide−Bromostyrene Statistical Copolymers via Phosphorescence Measurements

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ABSTRACT: This paper reports on the first observation of phosphorescence emission in a thermoresponsive polymer in aqueous media. Random copolymerization of *N*-isopropylacrylamide, NIPAM, with bromostyrene, BrSTY, promotes efficient intersystem crossing into the triplet manifold, via the heavy atom effect, in an acenaphthylene label allowing generation of emission on a millisecond time scale. Luminescence techniques such as fluorescence time-resolved anisotropy, excited-state lifetime, and pyrene solubilization experiments have proven useful in probing the effects of heavy atom modification upon the thermoresponsive behavior of PNIPAM: BrSTY alters the hydrophobic/hydrophilic balance and serves to decrease the lower critical solution temperature, LCST, of the resultant polymer. The LCST is marked by a change in conformation of the macromolecule form an expanded water-swollen structure to a compact globule. The globular form of the BrSTY-modified polymer samples is so protective that phosphorescence emission is sustainable in aqueous solution at temperatures in excess of 40 °C.

Introduction

The ability of "smart" polymers to expand and contract in response to an external stimulus (temperature or pH, for example) has fueled intense interest in such systems over a number of years.[1−13] Fluorescence spectroscopic measurements have featured prominently within these investigations as a consequence of the sensitivity of the technique: luminescence spectroscopy allows examination of isolated polymer chains in solution and consequently permits probing of the response at the *molecular* level.

Poly(*N*-isopropylacrylamide), PNIPAM, is an example of a smart polymer whose response is triggered thermally:[4,5,8,9,11] PNIPAM changes conformation from an expanded water swollen structure to a compact globule at 32 °C, the lower critical solution temperature, LCST.[14,15] Under semidilute conditions, a turbid solution forms which rapidly clears again upon cooling below the critical temperature. Time-resolved anisotropy measurements[11] and nonradiative energy transfer experiments[4] have confirmed that LCST behavior in linear PNIPAM is governed by a two-stage mechanism. The first step involves intramolecular coil collapse which is followed by intermolecular aggregation between collapsed coils.

Linear PNIPAM can solubilize[11,16] low molar mass organic material in its compact form which is subsequently released into the aqueous phase upon decreasing the temperature below the LCST. The ability to expand and contract on demand has led to suggestions that PNIPAM could form the basis of a carrier system for use in industrial and medical applications.[15] Consequently, much research activity has been focused on manipulation of the conformational switch away from 32 °C to cover a wide range including the physiological temperature of 37 °C.[17−25]

Previously, we have modified PNIPAM via simple free radical[20,23] and graft copolymerization[26,27] to allow control of the conformational switch of the polymer to any desired temperature between 4 and 100 °C. In this paper, as part of an ongoing program, we present results of copolymerization of NIPAM with a comonomer, bromostyrene (BrSTY), and the first observation of phosphorescence in a thermally responsive polymer in aqueous solution: BrSTY was chosen as the chemical modifier, since triplet emission should be stimulated, via the heavy atom effect, in a suitably labeled polymer sample.[28]

We have a twofold interest in generating phosphorescence in aqueous media: (i) phosphorescence provides an alternative and unique method of probing the conformational behavior of NIPAM-based systems; (ii) generation of triplet species is attractive in terms of promoting photochemistry in certain solubilized guests. Changing the temperature to generate the expanded conformation of the polymer should release the photoproducts to the aqueous phase, allowing facile collection.

Results and Discussion

Determination of the Lower Critical Solution Temperature (LCST) via Optical Density Measurements. Five acenaphthylene (ACE)-labeled *N*-isopropylacrylamide−bromostyrene copolymer samples [ACE-NIPAM-BrSTY] were prepared, via free radical polymerization, with increasing amounts of heavy atom modifier. An ACE-labeled homopolymer (ACE-PNIPAM) was also synthesized to act as a reference. (All luminescent polymer samples typically contained ca. 0.5 mol % ACE.) See Table 1 for the BrSTY contents and molecular weight data. Further details regarding synthetic procedures and instrumentation used in this work can be found in the Supporting Information.

The lower critical solution temperature (LCST) for each of the current samples in aqueous solution was estimated from the increase in optical density (at λ = 500 nm) which accompanies phase separation of the polymer. The resultant

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Table 1. Physical Characteristics of the NIPAM-Based Polymers

sample	$\bar{M}_n$ (kg mol^{-1})	polydispersity	BrSTY content (mol %)	LCST[a] (°C)
ACE-PNIPAM	42.0	1.50		32
NIPAM-BrSTY(6.3)-ACE	32.3	2.12	6.3	20
NIPAM-BrSTY(11.0)-ACE	30.3	2.12	11.0	11
NIPAM-BrSTY(15.6)-ACE	48.5	2.44	15.6	b
NIPAM-BrSTY(30.2)-ACE	45.5	2.41	30.2	b
NIPAM-BrSTY(39.5)-ACE	40.2	2.23	39.5	b

[a] Estimated from optical density (OD) measurements (λ = 500 nm) as the temperature of onset of the increase in OD which accompanies phase separation of the polymer (here, at 10^{-2} wt %). [b] Below freezing point of water.

Table 2. Bimolecular Quenching Constants for NIPAM-Based Polymers in Methanol and Aqueous Solutions

polymer	MeOH (25 °C) k_q ($\times 10^9$) (M^{-1} s^{-1})	aqueous (5 °C) k_q ($\times 10^9$) (M^{-1} s^{-1})	aqueous (15 °C) k_q ($\times 10^9$) (M^{-1} s^{-1})	aqueous (25 °C) k_q ($\times 10^9$) (M^{-1} s^{-1})
NIPAM-BrSTY(6.3)-ACE	7.90	2.80	1.20	0.41
NIPAM-BrSTY(11.0)-ACE	8.30	1.70	0.88	0.68
NIPAM-BrSTY(15.6)-ACE	9.00			
NIPAM-BrSTY(30.2)-ACE	8.00			
NIPAM-BrSTY(39.5)-ACE	7.90			

LCSTs are also listed in Table 1. Critical behavior in PNIPAM is governed by the amphiphilic nature of the polymer: clearly, increasing the BrSTY content alters the hydrophobic to hydrophilic balance within the macromolecule and serves to shift the LCST to lower temperature. Such behavior is in accordance with theory[29] and has been observed previously by experiment in other NIPAM-based systems in our laboratories.[20,23] Further examination of Table 1 reveals that when the BrSTY content in the sample exceeds ca. 15 mol %, insolubility in aqueous solution results: under these conditions the hydrophobic to hydrophilic balance is such that the LCST lies below the freezing point of water.

Fluorescence Quenching Measurements. Over a number of years fluorescence quenching measurements have proven useful in investigation of the conformational behavior of stimuli-responsive polymers in aqueous solution (see, for example, refs 1, 10, 11, 20, 23, and 30−33 and references therein).

Dynamic collisional deactivation of a fluorescent species, F*, by a quencher, Q, can be described by the Stern−Volmer relationship

$$\tau_f^\circ/\tau_f = 1 + k_q \tau_f^\circ [\mathrm{Q}] \qquad (1)$$

where τ_f is the excited-state lifetime at some concentration of quencher, [Q], τ_f° is that in the absence of Q, and k_q is the bimolecular quenching constant. Consequently, k_q can be considered as a measure of the efficiency of deactivation of F* by Q. Use of a fluorescently labeled polymer sample can provide information concerning the degree of access of Q to the excited state and, ultimately, the "compactness" of the chain.[1,10,11,20,23,30−33]

In order to probe the conformational state of each of the current ACE-labeled copolymer samples, Stern−Volmer quenching experiments were carried out in methanol and water, respectively, using CH_3NO_2 as a quencher. The fluorescence decays were complex irrespective of temperature or CH_3NO_2 concentration for each sample studied. This appears to be a general feature in amphiphilic systems,[11,20,23] and the problems associated with analyses and treatment of the resultant decay data for use in the Stern−Volmer equation have been covered elsewhere.[20] Consequently, discussion is limited here to the main points of the procedure: the transient emission from each labeled sample at each CH_3NO_2 concentration and temperature was analyzed in terms of a triple-exponential function as in eq 2

$$I(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) + A_3 \exp(-t/\tau_3) \qquad (2)$$

where A_i represents the amount of each component (τ_i) present. This provided the best fit to the decay data as judged by statistical parameters such as χ^2 (χ^2 should be close to unity for a good fit) and the distribution of residuals. In the current work χ^2 values were 1.5 or less and the residuals randomly distributed around zero. Subsequent treatment of the data with eq 3

$$\langle \tau \rangle = \frac{\sum I_i \tau_i^2}{\sum I_i \tau_i} \qquad (3)$$

allows estimation of the *weighted* average excited-state fluorescence lifetime, $\langle \tau_f \rangle$. $\langle \tau_f \rangle$ is subsequently used with the corresponding value of [Q], via eq 1, in derivation of k_q.

Since linear Stern−Volmer plots were obtained for the various labeled polymer samples at each temperature and in each solvent, this provides confidence that the mathematical treatment of the data is justified. The resultant k_q values determined in both methanol and aqueous media at various temperatures are listed in Table 2. When dissolved in methanol at 25 °C, k_q varies between 8×10^9 and 9×10^9 M^{-1} s^{-1} for each of the BrSTY-modified NIPAM samples. This is close to that expected for diffusion-controlled quenching and indicates that the fluorescence from the ACE label is readily and efficiently deactivated by CH_3NO_2. These data imply that each of the heavy atom modified polymers adopt an open chain conformation in methanol at 25 °C.

Further consideration of the data in Table 2 reveals that a value of k_q of 2.8×10^9 M^{-1} s^{-1} results when NIPAM-BrSTY(6.3)-ACE is dispersed in aqueous solution and quenched with CH_3NO_2 at 5 °C. Since the copolymer is below its LCST at this temperature (see Table 1), an expanded coil conformation would be expected under these conditions. It would therefore not be unreasonable to observe a quenching efficiency close to that obtained in methanol at 25 °C. If allowance is made for the different solvent viscosities of methanol at 25 °C (η = 0.547 cP) and water at 5 °C (η = 1.519 cP), quenching of NIPAM-BrSTY(6.3)-ACE in the two solvents does indeed appear to be comparable: water is 3 times the viscosity of methanol, and the resultant k_q value in aqueous solution is 1/3 that obtained in methanol.

Further examination of the data in Table 2 reveals that increasing the BrSTY content from 6.3 to 11 mol %, as in NIPAM-BrSTY(11)-ACE, reduces the k_q value to 1.7×10^9 M^{-1} s^{-1} in aqueous solution at 5 °C. Since the LCST for this copolymer is 11 °C (see Table 1), a quenching efficiency comparable to that observed for NIPAM-BrSTY(6.3)-ACE at 5 °C would be anticipated. The lower than expected k_q value may be an early indication that intramolecular hydrophobic cavity formation occurs between the BrSTY units in NIPAM-BrSTY(11)-ACE under these conditions in order to minimize contact with the aqueous phase. Sequestration of the ACE label within the BrSTY intramolecular aggregates will, in turn, provide a degree of protection from CH_3NO_2, resulting in a reduction in quenching efficiency. (Such behavior has been observed previously in styrene NIPAM copolymers below the LCST, for example.[20])

At 15 °C in aqueous solution, a slight reduction in the value of k_q is apparent for NIPAM-BrSTY(6.3)-ACE when compared to that at 5 °C. This indicates that access to the label is reduced at higher temperature: since the LCST for this sample is 20 °C, this may be indicative that a gradual collapse in the conformation occurs over a broad temperature range. At 25 °C, on the other hand, a significant reduction in k_q for NIPAM-BrSTY(6.3)-ACE is clearly evident in Table 2 when compared to that at 5 °C. Presumably, at temperatures beyond its LCST, the polymer adopts a compact conformation, which protects the excited state of the ACE label and reduces the efficiency of quenching by CH_3NO_2.

For NIPAM-BrSTY(11.0)-ACE at temperatures in excess of its LCST similar trends in the bimolecular quenching data to that for NIPAM-BrSTY(6.3)-ACE are apparent: k_q varies between 7.0×10^8 and 9.0×10^8 M^{-1} s^{-1} for the higher BrSTY content sample (see Table 2), which reflects collapse of the polymer chain into a compact structure at higher temperatures.

However, the current quenching data should be viewed with the knowledge that, in general, in water-soluble systems there is potential for partitioning of the quencher between the aqueous phase and the hydrophobic regions of the polymer coil:[26,34] if the local concentration of Q in proximity to the label is different than that of the nominal bulk concentration added to the solution, then this will influence the resultant k_q as estimated from eq 1. Conceivably, the reduction in quenching efficiency observed for the BrSTY-modified NIPAM polymer solutions may not be due to any chain contraction but may merely reflect a change in quencher partitioning between the two phases. In an effort to resolve this issue, the conformational behavior of the heavy atom modified polymers was examined *directly* using time-resolved anisotropy experiments as discussed in the next section.

Time-Resolved Anisotropy Measurements (TRAMS). Since the theory behind TRAMS and the various methods of data analysis have been extensively discussed elsewhere,[35] only the main points will be covered here. Photoselection of a proportion of molecules can be achieved in a randomly oriented distribution of fluorophores by using pulsed, polarized excitation. The resultant intensities, measured in planes parallel [$i_{\parallel}(t)$] and perpendicular [$i_{\perp}(t)$] to the plane of polarization of the radiation incident upon the sample, is related to the time-dependent fluorescence anisotropy, $r(t)$, via the following equation

$$r(t) = \frac{i_{\parallel}(t) - i_{\perp}(t)}{i_{\parallel}(t) + 2Gi_{\perp}(t)} = \frac{d(t)}{s(t)} \qquad (4)$$

where $d(t)$ and $s(t)$ are the difference and sum functions, respectively. G is a factor designed[36] to correct for differences in transmission and detection efficiencies in the determination of $i_{\parallel}(t)$ and $i_{\perp}(t)$. G factors of unity resulted in experiments involving the current instrument. (G was estimated by comparison of the emission intensities analyzed in planes parallel and perpendicular to the plane of *horizontally* polarized excitation.)

If a single relaxation process is operative in the system, then $r(t)$ can be modeled by a monoexponential function as in eq 5

$$r(t) = r_0 \exp(-t/\tau_c) \qquad (5)$$

where τ_c, the correlation time, characterizes the motion under study and r_0 is the intrinsic anisotropy. The ACE label, through its mode of attachment, can only monitor segmental motion of the macromolecular backbone: τ_c consequently reflects this motion in the current labeled samples.

There are several analytical procedures which can be used in derivation of relaxation information from time-resolved anisotropy data, which have been extensively discussed elsewhere.[35] We favor use of impulse reconvolution[37] in derivation of τ_c, since the perturbing effects of the pulse profile can be removed from the data. Impulse reconvolution[37] involves analysis of $s(t)$ by a statistically adequate model function. The "best fit" to $s(t)$ is represented by an impulse response function. Combination of an appropriate model for the anisotropy decay (eq 5, for example) with the impulse response function allows analysis of $d(t)$ via an iterative least-squares reconvolution procedure. This fit is optimized by varying the fitting parameters which allows derivation of relaxation data (such as τ_c) for the process under investigation.

In accordance with previous observations in thermally responsive systems,[11,20,23] the fluorescence anisotropy decays for ACE-PNIPAM and from each of the ACE-labeled, BrSTY-modified NIPAM polymers dispersed in aqueous solution were complex at all temperatures studied. For example, χ^2 values ranged from ca. 1.4 at low temperatures to ca. 2.0 above the LCST for ACE-PNIPAM. (χ^2 should be close to unity for a good fit.) Such behavior is a general feature of stimuli-responsive polymers and is considered to reflect the heterogeneity of local environments extant within the interiors of both the expanded and compact conformations in aqueous media.[11,20,23,28,38−40]

Introduction of a second exponential term into the function as in

$$r(t) = r_1 \exp(-t/\tau_{c1}) + r_2 \exp(-t/\tau_{c2}) \qquad (6)$$

resulted in an improvement in the fitting statistics (χ^2 was approximately unity across the temperature range for ACE-PNIPAM, for example). However, no real trend in the resultant relaxation parameters was apparent: τ_{c1} was ca. 2 ns and τ_{c2} was ca. 10 ns at temperatures below the conformational transition. Above the LCST, τ_{c1} remained constant at ca. 4 ns while τ_{c2} varied between 200 and 700 ns. A "string of pearls" model has recently been proposed for PNIPAM[41,42] where a series of coiled globules are linked by more flexible water-swollen segments below the LCST. Conceivably, the two correlation times derived from the current homopolymer data may reflect aspects of this model. Introduction of hydrophobic modifiers in the form of the BrSTY units increases the complexity. Furthermore, it is also likely that we are monitoring longer range motions of the chain (such as end over end motion) in addition to shorter range intramolecular aggregation between hydrophobic units in these thermoresponsive systems. Consequently, it is difficult to resolve, with confidence, at least three correlation times from the current anisotropy data. Such complexities will be exacerbated above the LCST when PNIPAM and the BrSTY copolymers adopt the globular conformation.

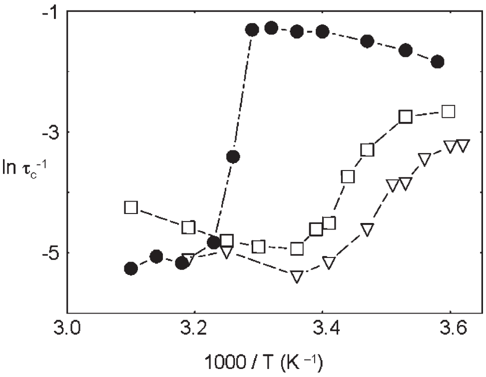

Figure 1. Arrhenius representation of the rate of macromolecular motion (ln τ_c^{-1}) in aqueous solution for ACE-NIPAM (●), BrSTY-(6.3)-NIPAM-ACE (□), and BrSTY(11.0)-NIPAM-ACE (▽). All samples contained 10^{-2} wt % in polymer; $\lambda_{ex} = 290$ nm; $\lambda_{em} = 340$ nm.

These problems in complexity can be overcome in TRAMS by use of a single-exponential function of the form of eq 5 to monitor an "average" segmental relaxation. This then allowed a relative comparison of the thermal response, via the chain dynamics, of the current copolymer series. τ_c was derived in this way for the heavy atom modified polymer samples which were soluble in water [NIPAM-BrSTY(6.3)-ACE and NIPAM-BrSTY(11.0)-ACE)] over a range of temperatures. The relaxation data for each BrSTY-based copolymer sample is shown in Arrhenius form in Figure 1, where ln τ_c^{-1} measures the *average* rate of macromolecular motion. (An Arrhenius representation simply allows better resolution of the thermal response of the current polymer samples.) Also shown, for comparison, are data for an ACE-labeled PNIPAM sample.

The conformational transition of ACE-PNIPAM is clearly apparent in Figure 1: ln τ_c^{-1} decreases in value at 32 °C (the onset of phase separation in more concentrated samples) from that representative of a fast-moving flexible coil to a compact globule above the LCST. Below 32 °C the rate of macromolecular motion displays a linear Arrhenius-type response: the higher the temperature, then the greater the segmental mobility of the polymer backbone. Such behavior is in accord with a previous study.[11]

Further examination of Figure 1 reveals that copolymerization with BrSTY results in a reduction in the magnitude of the transition. This appears to be a general feature in modification of PNIPAM as monitored by fluorescence anisotropy experiments[20,23] and other techniques such as calorimetry.[17,18] Presumably this reduction in intensity reflects intramolecular aggregate formation between BrSTY units within each sample to form hydrophobic domains below the LCST to minimize contact with the aqueous phase: clearly these partially coiled structures will have a lesser mobility than ACE-PNIPAM in its expanded conformation and is reflected by a decrease in the magnitude of the discontinuity in the ln τ_c^{-1} vs reciprocal temperature plot. Such intramolecular cavity formation has been observed previously in styrene–NIPAM copolymers via TRAMS and pyrene solubilzation experiments.[20] The current anisotropy data support the conclusions derived from quenching experiments in the previous section that intramolecular cavity formation between BrSTY units occur below the LCST for NIPAM-BrSTY(11.0)-ACE. Closer examination of the relaxation data for NIPAM-BrSTY(6.3)-ACE above the conformational transition reveals that the label becomes more mobile when compared to that of ACE-PNIPAM. This perhaps indicates that a more open, flexible, and water-expanded structure exists for NIPAM-BrSTY(6.3)-ACE at temperatures in excess of 40 °C.

Further consideration of Figure 1 reveals that increasing the content of BrSTY in the copolymer serves to shift the conformational transition to lower temperature. In addition, modification broadens the transition region itself: over a 12 °C range for NIPAM-BrSTY(6.3)-ACE and 21 °C for NIPAM-BrSTY(11.0)-ACE. These observations reflect the change, induced by copolymerization, in the hydrophobic to hydrophilic balance within the polymer, which governs criticality. Such behavior has been predicted by theory[29] and observed previously by anisotropy experiments in our laboratories.[20,23] The onset temperature, from cloud point measurements, for phase separation for NIPAM-BrSTY-(11.0)-ACE and NIPAM-BrSTY(6.3)-ACE are 20 and 11 °C, respectively (see Table 1) which can be compared to 21 and 14 °C as the temperatures which mark the onset of the conformational transition via TRAMS (see Figure 1). Clearly, the change in conformation of the macromolecular backbone corresponds to the LCST in these systems.

Pyrene Solubilization Experiments. The sensitivity of the vibrational fine structure of the emission spectrum[1,11,20,27,30,39,43,44] and the excited-state lifetime[1,11,20,23,27,30,39,44] of pyrene to changes in the probe environment has been exploited over a number of years by polymer chemists interested in the conformational behavior of water-soluble polymers. In this way, uptake of the probe into the compact structure or release into the aqueous phase upon expansion of the chain has been demonstrated via the fluorescence properties of pyrene.[1,11,20,23,27,30,39,43,44]

In order to monitor the thermoresponsive behavior in aqueous solution, decays of the fluorescence of pyrene dispersed (10^{-6} M) within the heavy atom modified NIPAM polymers were collected over a range of temperatures using an excitation wavelength of 335 nm and analysis at 380 nm. In the current work, modeling of the decay by a triple-exponential function of the form of eq 2 provided a statistically adequate description of the data. This behavior is similar to the excited-state decay kinetics of other probe/smart polymer aqueous dispersions and has been previously attributed to the broad distribution of solubilization sites accessible to the pyrene in these heterogeneous systems.[1,11,20,23,27,30,39,44] In order to observe trends in the transient data of pyrene when dispersed in the current NIPAM-based polymers in aqueous solution, an average excited-state lifetime, $\langle \tau_f \rangle$, was subsequently calculated via eq 3. $\langle \tau_f \rangle$ is plotted as a function of temperature in Figure 2. $\langle \tau_f \rangle$ of pyrene dispersed in ACE-PNIPAM was also derived, across the temperature range, to act as a reference. From examination of the lifetime data in Figure 2, the onset of the conformational transition of ACE-PNIPAM is clearly evident at ca. 32 °C: below this temperature a short $\langle \tau_f \rangle$ is apparent (ca. 130 ns) which is similar to that of pyrene in water.[39] Above the LCST, $\langle \tau_f \rangle$ increases to ca. 180 ns, which is characteristic of the probe in more hydrophobic media and is similar to that observed in a previous study of pyrene dispersed in PNIPAM.[11] Clearly, the compact globular structure of ACE-PNIPAM is capable of encapsulating pyrene. Furthermore, reversible release and uptake of the probe by ACE-PNIPAM upon varying the temperature of the system can be demonstrated, via the average excited-state lifetime.

Further consideration of the data in Figure 2 reveals that modification with BrSTY serves to shift the conformational switch to lower temperature and reduces the magnitude of the transition. Such behavior has been observed previously from pyrene solubilization experiments following hydrophobic modification of PNIPAM with styrene.[20] At low temperature, $\langle \tau_f \rangle$ of pyrene when dispersed in NIPAM-BrSTY(6.3)-ACE varies from ca. 175 ns at 5 °C to ca. 160

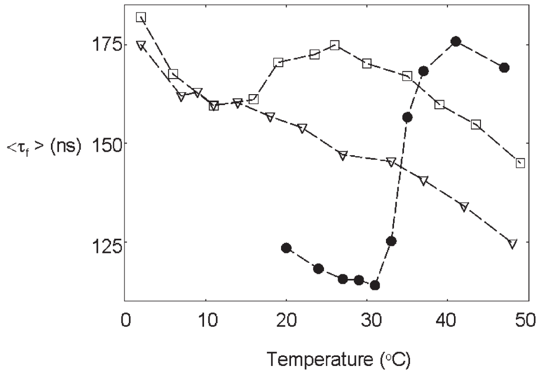

Figure 2. Mean lifetime data, $\langle \tau_f \rangle$, as a function of temperature for pyrene (10^{-6} M) dispersed in ACE-PNIPAM (●), BrSTY(6.3)-NIPAM-ACE (□), and BrSTY(11.0)-NIPAM-ACE (▽). All samples contained 10^{-2} wt % in polymer; λ_{ex} = 335 nm; λ_{em} = 380 nm.

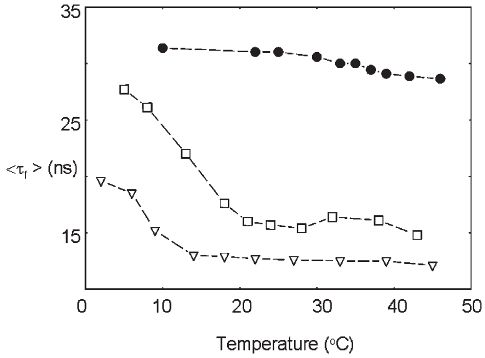

Figure 3. Mean lifetime data, $\langle \tau_f \rangle$, as a function of temperature for ACE-PNIPAM (●), BrSTY(6.3)-NIPAM-ACE (□), and BrSTY(11.0)-NIPAM-ACE (▽). All samples contained 10^{-2} wt % in polymer; λ_{ex} = 290 nm; λ_{em} = 340 nm.

ns at 16 °C (cf. Figure 2). This is much greater than that of the probe in water (ca. 132 ns[39]) and implies the existence of solubilizing pockets within the heavy atom modified sample below its LCST. Further consideration of Figure 2 reveals that a broad transition is apparent in the $\langle \tau_f \rangle$ data, the onset of which occurs at ca. 16 °C for NIPAM-BrSTY(6.3)-ACE (τ_f increases from ca. 160 ns at 16 °C to ca. 175 ns at 25 °C). The onset temperature of the discontinuity is very close to that of the LCST as sensed by cloud point measurements (20 °C, see Table 1) and presumably reflects encapsulation of the pyrene into the collapsed, compact form of the polymer. Above the transition, $\langle \tau_f \rangle$ for the probe/NIPAM-BrSTY-(6.3)-ACE dispersion never attains the level of pyrene in ACE-PNIPAM (cf. Figure 2). This could be a consequence of two effects: (i) The interior of the collapsed structure may be more water-swollen than that of PNIPAM in its compact conformation. This appears to be in agreement with the data from the time-resolved anisotropy experiments discussed in the previous section. (ii) It could be that the modified polymer serves to quench the lifetime of the probe through the heavy atom effect which would lead to a reduction in the apparent $\langle \tau_f \rangle$ of the pyrene.

These effects are exaggerated when the $\langle \tau_f \rangle$ data for the pyrene/NIPAM-BrSTY(11.0)-ACE dispersions are considered: the lifetime decreases monotonously from 5 °C up to 50 °C (cf. Figure 2) and never attains the level of that of NIPAM-BrSTY(6.3)-ACE above temperatures of 20 °C. Such would be expected if the heavy atom effect *is* operative in this system in population of the triplet manifold: the more bromine in the sample, then the more the $\langle \tau_f \rangle$ of pyrene should be quenched.

Between 5 and 15 °C, the average lifetime of the probe dispersed in NIPAM-BrSTY(11)-ACE is superior to that of pyrene in ACE-PNIPAM. Presumably, intramolecular cavity formation occurs which act as solubilizing pockets for the probe. This provides corroborative evidence for the occurrence of hydrophobic domains as sensed by the fluorescence quenching experiments discussed earlier. However, it could be that these cavities are sufficiently fluid and expanded enough such that they do not effectively quench the singlet state of the probe.

Phosphorescence Measurements. Historically, it has long since been recognized that the experimental conditions, which promote intense phosphorescence from an analyte, involve use of a low-temperature glass[45] [i.e., an organic solvent (or solvent mixture) that sets to form a glass at −196 °C without crystallizing] or glassy synthetic materials such as polymers.[46] In the latter context, subsequent investigations have proven that there is a direct link between the physical transitions within the macromolecular bulk phase and the intensity of phosphorescence observed.[47−52] In order to promote room temperature stabilized phosphorescence (RTSP) in aqueous media, a sufficiency of triplet species must be created. Furthermore, the concentration of adventitious quenchers (such as oxygen) should be such that bimolecular quenching of the triplet states is reduced to a negligible level. This can simply be achieved by purging the solution with nitrogen to remove oxygen. In order to provide a protective environment for the triplet states and generate RTSP, researchers have used guest molecules solubilized in sodium dodecyl sulfate micelles[53,54] and methacrylic acid-based polymers[28,55,56] in which intersystem crossing (ISC) is enhanced using the external heavy atom effect through replacement of a population of sodium ions by species such as Tl^+. In an extension of this theme, the internal heavy atom effect (i.e., an aromatic species containing an intrinsic heavy atom) has been used to promote phosphorescence from probes dispersed in and covalently attached to polyelectrolytes in order to probe the pH-dependent behavior.[28,57] If triplet emission can be successfully generated in the current heavy atom modified thermoresponsive polymers, this would provide a novel and unique means of probing the conformational behavior of such materials. Furthermore, invaluable information concerning the "protective nature" of the globular structures created in these systems at temperatures in excess of their respective LCST may be derived.

Prior to phosphorescence measurements on the water-soluble BrSTY-NIPAM samples, $\langle \tau_f \rangle$ of the ACE label using an excitation wavelength of 290 nm and analysis at 340 nm was determined (via eqs 2 and 3) as a function of temperature: since the triplet state is created at the expense of the singlet, evidence for efficient ISC should be apparent as a reduction in the naphthyl excited-state fluorescence lifetime.[28] Examination of the data in Figure 3 reveals that for ACE-PNIAPM no real change in the singlet excited-state lifetime is discernible: $\langle \tau_f \rangle$ varies between 31.3 ns at low temperature to 28.8 ns above 32 °C. However, a more dramatic thermal response is evident upon examination of the $\langle \tau_f \rangle$ data for NIPAM-BrSTY(6.3)-ACE: the fluorescence lifetime decreases monotonously from 28 ns at 5 °C to 16 ns at 21 °C where a plateau is reached. Interestingly, this latter temperature is similar to that of the LCST as sensed by cloud point measurements (i.e., 20 °C; see Table 1). Clearly, quenching of the singlet state through the heavy atom effect allows information regarding the nature of the conformational switch to be derived. At 5 °C the singlet state is longer lived than that above 20 °C. Such would be expected in the

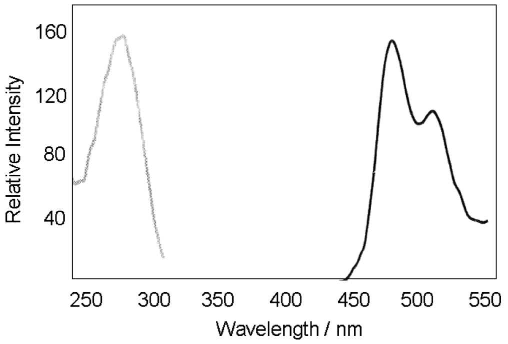

Figure 4. Phosphorescence excitation and emission spectra from BrSTY(6.3)-NIPAM-ACE (10^{-2} wt %) at 25 °C in aqueous solution (N_2-saturated; λ_{em} = 490 nm; λ_{ex} = 290 nm).

expanded conformation where there is less opportunity for diffusive bromine−ACE collisional encounters. As the temperature is raised from 10 °C through to 20 °C, $\langle\tau_f\rangle$ decreases as the chain begins to collapse. However, from the data shown in Figure 3, it is temping to conclude that the conformational collapse is not a sudden event but occurs gradually over a broad temperature range. This behavior is in agreement with the time-resolved anisotropy data discussed earlier which sense the switch over a 12 °C range for NIPAM-BrSTY(6.3)-ACE and a 21 °C range for NIPAM-BrSTY(11.0)-ACE. This is also in accord with an independent fluorescence energy transfer investigation of PNIPAM which revealed a gradual collapse of the polymer chain prior to the LCST.[4] Further consideration of the data in Figure 3 reveals that at low temperature $\langle\tau_f\rangle$ for NIPAM-BrSTY-(6.3)-ACE is always inferior to that of ACE-PNIPAM. This provides strong evidence for the existence of intramolecular hydrophobic cavities in NIPAM-BrSTY(6.3)-ACE below its LCST: aggregation of the BrSTY units would serve to contract the chain and increase the chance of collisional deactivation of the ACE labels through the heavy atom effect. τ_f would consequently decrease as the triplet state is promoted at the expense of the singlet.

At temperatures beyond the LCST of NIPAM-BrSTY-(6.3)-ACE, the fluorescence lifetime reaches a plateau as the polymer adopts its compact conformation. In this state, the maximum degree of coiling (and quenching) would be expected: $\langle\tau_f\rangle$ is ca. 16 ns, which is much less than that of ACE-PNIPAM above its LCST ($\langle\tau_f\rangle$ is ca. 28 ns). The heavy-atom modification strategy would appear to be successful in that a relatively high degree of quenching of the excited state occurs in the compact conformation. It remains to be seen whether NIPAM-BrSTY(6.3)-ACE affords sufficient protection for the triplet states produced to allow observation of phosphorescence emission in aqueous media.

Examination of $\langle\tau_f\rangle$ as a function of temperature for NIPAM-BrSTY(11.0)-ACE (see Figure 3) reveals the same general trend as that for NIPAM-BrSTY(6.3)-ACE: the fluorescence lifetime decreases gradually at low temperature until a plateau is reached at ca. 12 °C (the LCST for this sample is 11 °C from cloud point measurements, see Table 1). Furthermore, $\langle\tau_f\rangle$ is less than that in NIPAM-BrSTY(6.3)-ACE at all temperatures studied. Such would be expected if the reduction in fluorescence lifetime *is* due to the heavy atom effect: since the BrSTY content is greater in NIPAM-BrSTY(11.0)-ACE, the degree of quenching of $\langle\tau_f\rangle$ would also be superior to that in NIPAM-BrSTY(6.3)-ACE.

The three polymer samples were subsequently purged of oxygen by bubbling nitrogen through the solutions for 20 min. No phosphorescence was evident form ACE-PNIPAM at any temperature accessed between 5 and 55 °C. However, emission characteristic of triplet naphthalene was discernible for both ACE-NIPAM-BrSTY(6.3)-ACE and BrSTY(11.0)-ACE. This is gratifying since one of the potential uses of polymers of this kind could be as analytical media for the production of phosphorescence in analytes. Figure 4 shows example phosphorescence excitation and emission spectra from ACE-NIPAM-BrSTY-(6.3)-ACE at 25 °C in aqueous media. It was difficult, however, to make any quantitative phosphorescence measurements since large fluctuations in intensity resulted from the phase-separated polymers at higher temperatures. More reliable measurements, however, could be made using the phosphorescence lifetime, τ_p.

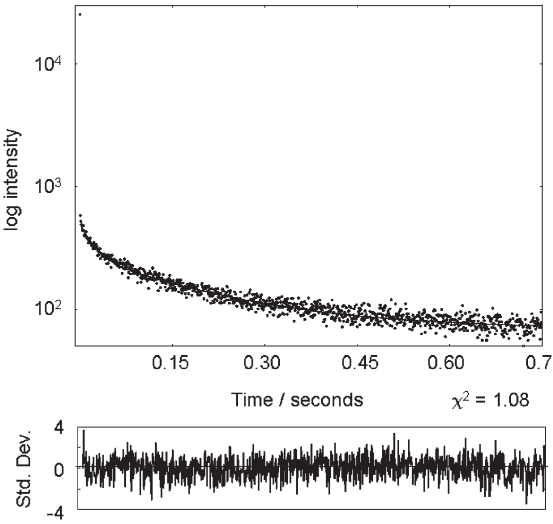

Figure 5. Phosphorescence decay of BrSTY(6.3)-NIPAM-ACE (10^{-2} wt %) at 33 °C in aqueous solution, "best fit" to a double-exponential model and resultant residuals. (λ_{ex} = 290 nm; λ_{em} = 490 nm; N_2-saturated).

The time-resolved triplet emission was complex at all temperatures accessed for both of the current bromine-modified polymer samples in aqueous media: a minimum of two exponential terms (as in eq 7) was required for an adequate fit on statistical grounds.

$$I_p(t) = I_{p1}\exp(-t/\tau_{p1}) + I_{p2}\exp(-t/\tau_{p2}) \qquad (7)$$

Figure 5 shows an example fit to the phosphorescence decay of NIPAM-BrSTY(6.3)-ACE at 33 °C with λ_{ex} = 290 nm and λ_{em} = 490 nm. The low value of χ^2 (χ^2 should be unity for a good fit) and the random distribution of residuals provide statistical confidence in the model. The lifetimes derived as a consequence of this form of analysis were 20.4 and 188 ms, respectively. The longer-lived component is closely related to that of an ACE labeled poly(acrylic acid) (ACE-PAA) film (ca. 720 ms) at 380 °C, which is near the glass transition temperature. (In the glass state of ACE-PAA the concentration of quenchers such as oxygen is reduced such that a long-lived emission results.) Quite remarkably, the longer-lived component for the ACE-NIPAM-BrSTY(6.3) copolymer in aqueous media compares favorably to that in the ACE-PAA film: the triplet state of ACE attached to the heavy atom modified NIPAM sample in solution is only ca. 4 times less than that in the bulk state of PAA. Presumably in the compact conformation of ACE-NIPAM-BrSTY(6.3), sufficient protection for the triplet state of ACE exists from the quenching effects of the aqueous phase, which results in a long-lived phosphorescence.

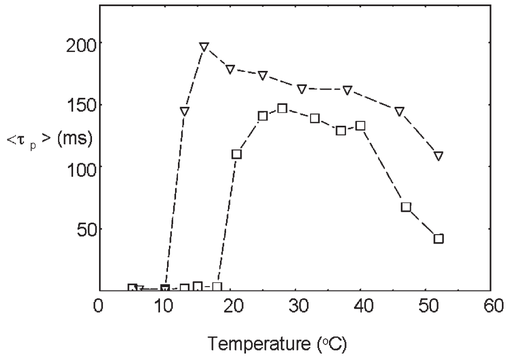

Figure 6. Mean phosphorescence lifetime data, $\langle\tau_p\rangle$, as a function of temperature, for BrSTY(6.3)-NIPAM-ACE (□) and BrSTY(11.0)-NIPAM-ACE (▽). All samples contained 10^{-2} wt % in polymer (λ_{ex} = 290 nm; λ_{em} = 490 nm; N_2-saturated).

The fact that a multiexponential model is required to describe the transient phosphorescence emission of the heavy atom modified NIPAM polymers is perhaps not surprising: such complexity in decay behavior has been observed previously[28] for ACE-labeled BrSTY−methacrylic acid polymers and may be a consequence of the heterogeneity of sites available to the triplet states in aqueous media.

In order for information concerning the conformational behavior to be obtained for both ACE-NIPAM-BrSTY(6.3) and ACE-NIPAM-BrSTY(11.0), the decay data for each sample were treated with eq 3 to allow characterization of an average phosphorescence lifetime, $\langle\tau_p\rangle$, across the entire temperature range. $\langle\tau_p\rangle$ data are shown, as a function of temperature, in Figure 6. Even a cursory inspection of the triplet lifetimes in Figure 6 reveals that both thermally responsive heavy atom modified polymers are capable of sustaining long-lived emission over a wide temperature range. What is particularly impressive is the fact that significant levels of phosphorescence can be observed in the current heavy atom modified systems in fluid media at temperatures in excess of 40 °C. Production of phosphorescence from analytes normally involves use of a low-temperature glass[45] or glassy synthetic materials such as polymers.[46−52] In aqueous media at room temperature, on the other hand, triplet emission can be observed form micellar solutions[53,54] or water-soluble polymers[28,32,55−57] which form highly protective compact globular structures. The fact that the current BrSTY-modified samples produce phosphorescence at 50 °C opens up the possibility for applications which require use in water at high temperature.

Further examination of Figure 6 reveals that the onset of longer-lived phosphorescence emission occurs at 21 °C for NIPAM-BrSTY(6.3)-ACE and at 13 °C for BrSTY(11.0)-ACE. These temperatures are close to that of the LCST for each sample as sensed by cloud point measurements [NIPAM-BrSTY(6.3)-ACE, 20 °C; BrSTY(11.0)-ACE, 11 °C]. Such would be expected if the LCST corresponds to the onset of the conformational transition: presumably in the compact conformation of each heavy atom modified polymer, sufficient protection for the triplet states exists from the quenching effects of the aqueous phase which results in a long-lived phosphorescence. Furthermore, the phosphorescence lifetime for NIPAM-BrSTY(11.0)-ACE is superior to that of NIPAM-BrSTY(6.3)-ACE at each temperature accessed. This is also to be expected. The higher the heavy atom content, then the greater the intensity of phosphorescence produced until the loading is sufficiently high to self-quench the triplet states.[32] Presumably, with the current set of polymers, this critical bromine concentration has not been reached. (Further heavy atom modification reduces the LCST to such an extent that the polymer is rendered insoluble in aqueous media; see Table 1.)

Clearly, the phosphorescence lifetime data represent an alternative approach for investigation of the conformational behavior of thermally responsive polymers in aqueous solution providing valuable information concerning the protective nature of the globular form. Observation of phosphorescence opens up potential applications for NIPAM-based heavy atom modified materials: the fact that triplet states are readily generated and sustained in such polymers could be used to induce photochemical reactions in sequestered guest molecules, for example. Changing the temperature below the LCST will release the photoproducts to the aqueous phase, allowing facile separation and collection. Furthermore, the fact that long-lived triplet emission can be sustained in aqueous media at high temperatures in responsive polymers could be of importance in sensing applications. Studies of such systems continue in our laboratories.

Conclusions

1. Luminescence techniques have proven useful in probing the effects of heavy atom modification upon the thermoresponsive behavior of PNIPAM.
2. Random copolymierzation of NIPAM with bromostyrene alters the hydrophobic/hydrophilic balance and serves to change the LCST of the resultant thermoresponsive polymer as revealed by fluorescence time-resolved anisotropy as well as excited-state lifetime and pyrene solubilization experiments.
3. Generation of phosphorescence emission, via the heavy atom effect, is possible in the bromine-containing samples and provides a novel and unique means of probing the conformational behavior. The globular form of the bromostyrene-modified polymer samples is so protective that phosphorescence emission is observable even at 50 °C in aqueous solution. This has implications for carrying out enhanced photochemistry in such media, in generation of phosphorescence in analytes at high temperatures, or for sensing applications.

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Supporting Information Available: Further experimental details regarding the synthetic procedures and instrumentation used in this work. This material is available free of charge via the Internet at http://pubs.acs.org.

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