Macromolecules **1998,** *31,* 109−115

Synthesis and Solution Properties of Fluorescently Labeled Amphiphilic (*N*-alkylacrylamide) Oligomers

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Received July 22, 1997; Revised Manuscript Received November 12, 1997

ABSTRACT: Amphiphilic poly(*N*-alkylacrylamide)s substituted at one chain end with the hydrophobic moiety *N*-*n*-octadecyl-[*N*-4-(1-pyrenyl)butyl] were prepared by end-group modification of carboxyl-terminated oligomers of *N*-isopropylacrylamide (NIPAM), *N*-(3-methoxypropyl)acrylamide (MPAM), and *N*,*N*-bis(2-methoxyethyl)acrylamide (BMEAM). The oligomers, M_n 2000−10 000, were prepared by free-radical polymerization in the presence of mercaptopropionic acid acting as a chain-transfer agent. The oligomers were characterized by potentiometric titration of the end groups, ^{1}H NMR spectroscopy, and UV absorption measurements. Quasi-elastic light-scattering measurements and fluorescence experiments revealed the existence of oligomeric micelles, consisting of a hydrophobic core hosting pyrene aggregates captured within the octadecyl chain assembly, and a corona of solvated poly(*N*-alkylacrylamide) chains. The oligomeric PNIPAM micelles interact readily with hydrophobically modified PNIPAM, as evidenced by the following photophysical observations: (1) disappearance of pyrene excimer emission in favor of pyrene monomer emission and (2) relief of the pyrene/pyrene self-quenching prevalent within the intact micellar cores.

Introduction

Water-soluble polymers are added to many commercial products, such as paints, inks, cosmetic formulations, foodstuffs, coating fluids, pharmaceutical preparations, and drug delivery vehicles.[1] In most applications the polymers act as viscosity and rheology modifiers or as encapsulating agents. Amphiphilic copolymers, which contain hydrophilic and hydrophobic segments in the same molecule, are particularly effective in these applications. Their macroscopic properties can be traced to the existence in solution of organized hydrophobic microdomains created by the interactions between hydrophobic segments. The chemical structure of the polymers is a determining factor in directing the formation of hydrophobic assemblies, either within a single macromolecule (unimer) or among several chains. Key structural factors are, on the one hand, the chemical composition of the copolymer, especially the structure and the amount of the hydrophobic group, and, on the other hand, the architecture of the macromolecule, namely whether the hydrophobic groups are attached at random along the chain or appended to one or both chain ends.

Aqueous solutions of poly-(*N*-isopropylacrylamide) (PNIPAM) are well-known to undergo a thermally reversible phase transition: the polymer is very soluble in cold water, but it separates from solutions heated to 32 °C,[2] a temperature usually considered as a lower critical solution temperature (LCST). Detailed investigations of this phase transition have established that at the LCST the PNIPAM chains undergo a collapse from hydrated extended coils to hydrophobic globules, which aggregate and form a separate phase.[3] Several types of hydrophobic modifications of poly-(*N*-isopropylacrylamide) have been reported recently. Ringsdorf et al.[4] and Schild and Tirrell[5] prepared a spectrum of randomly modified HM-PNIPAM, where the hydrophobic substituents are alkyl groups of various chain length, from decyl to octadecyl. Both studies yielded clear evidence for the existence of interpolymeric micelles, which persisted in solutions of polymer concentration as low as 10 ppm. We also prepared HM-PNIPAMs that carry two octadecyl groups at one chain end.[6] The polymers were prepared by free-radical polymerization of *N*-isopropylacrylamide (NIPAM) using a lipophilic initiator, 4,4′-azobis(4-cyano-*N*,*N*-dioctadecyl)pentamide.[7]

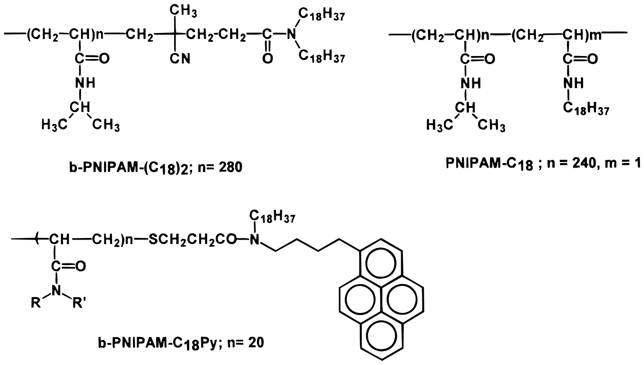

Figure 1. Chemical structure and composition of the polymers studied.

We report here the synthesis and solution properties of oligomers of NIPAM, *N*-(3-methoxypropyl)acrylamide (MPAM), and *N*,*N*-bis(2-methoxyethyl)acrylamide (BMEAM) carrying at one chain end the hydrophobic moiety *N*-*n*-octadecyl-*N*-[4-(1-pyrenyl)butyl] (Figure 1). This hydrophobic substituent was designed to permit a study of the oligomer solution properties by fluorescence spectroscopy. In this moiety, the chromophore and the hydrophobic substituent are attached covalently to the same amide nitrogen of the end group. The close spatial proximity between chromophore and octadecyl chain ensures that the fluorescent dye is located selectively within the hydrophobic microdomains created by the long alkyl substituents. This fluorescent moiety was used in previous studies of high-molecular-weight HM-PNIPAMs, linked either randomly along the polymer backbone[4,8] or to one chain end.[9]

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S0024-9297(97)01111-X CCC: $15.00

The overall objective of the present study was to develop methods to examine the interactions of hydrophobically modified *N*-alkylacrylamide oligomers with organized assemblies, from simple surfactant micelles to polymeric micelles, and ultimately liposomes. In this contribution, we describe the preparation of oligomers of various lengths, starting from carboxylated oligomers obtained by free-radical polymerization of the *N*-alkylacrylamides in the presence of mercaptopropionic acid used as a chain-transfer agent.[10,11] We report measurements of steady-state fluorescence spectroscopy and dynamic light scattering. We investigate the influence of temperature on the photophysical properties of the oligomers. We examine the interactions of *b*-PNIPAM-C_{18}Py with a randomly modified polymer, PNIPAM-C_{18}, and the end-modified *b*-PNIPAM-$(C_{18})_2$ (Figure 1). These studies bring new insights to the exchange amongst polymer micelles of different morphologies but analogous chemical composition.

Experimental Section

Materials. Water was purified with a NANOpure deionizing system. Reagent-grade solvents were used in all syntheses. Tetrahydrofuran was distilled over sodium before use. *N*-Isopropylacrylamide was a gift from Kohjin Co. Japan. It was purified by recrystallization from toluene/hexane (1/1 v/v). All other reagents were obtained from Aldrich Chemical Corporation. Monomers were distilled prior to use. AIBN was recrystallized from MeOH. Sephacryl S-300 and Sephadex G-10 were obtained from Pharmacia Biotech Corporation. *N*-[4-(1−pyrenyl)butyl]-*N*-*n*-octadecylamine was prepared as previously described.[4] The HM-PNIPAM samples substituted with octadecyl chains were prepared as described previously.[4,6]

Syntheses. (A) Monomer Preparation. (i) N-(3-Methoxypropyl)acrylamide (MPAM). A solution of acryloyl chloride (12.9 mL, 0.15 mol) in toluene (25 mL) was added dropwise over 3 h to a solution of *N*-(3-methoxypropyl)amine (12.20 mL, 0.1 mol) and triethylamine (20.91 mL, 0.15 mol) in dry toluene (250 mL) kept at 0 °C under nitrogen. At the end of the addition the mixture was kept at 4 °C overnight. The resulting mixture was filtered to remove $NEt_3 \cdot HCl$. The filtrate was stripped of solvent. The oily residue was purified by vacuum distillation to yield *N*-(3-methoxypropyl)acrylamide: yield, 51%; ^1H NMR ($CDCl_3$) δ 6.70 (s, 1H, NH), 6.13 (dd, 1H, $J = 1.9, 17.0$ Hz), 6.05 (dd, 1H, $J = 17.0, 10$ Hz), 5.49 (dd, 1H, $J = 1.9, 10.0$ Hz), 3.37 (t, 2H), 3.32 (q, 2H), 3.23 (s, 3H), 1.71 (m, 2H) ppm.

(ii) *N,N*-Bis(2-methoxyethyl)acrylamide (BMEAM). The procedure used for the preparation of MPAM was applied, yielding BMEAM as an oil purified by vacuum distillation at 115 °C (mm Hg): yield, 88%; ^1H NMR ($CDCl_3$) δ 6.55 (dd, 1H, $J = 10.4, 16.7$ Hz), 6.20 (dd, $J = 2.1, 16.7$ Hz), 5.54 (dd, 1H, $J = 2.1, 10.45$ Hz), 3.51 (t, 2 H), 3.45 (t, 2H), 3.40 (t, 2 H), 3.21 (s, 6H) ppm.

(B) Homopolymer Preparations. The monomer (0.01 mol) and AIBN (0.1 mmol) were dissolved in dry dioxane. The solution was purged with N_2 for 10 min. It was heated to 60 °C for 20 h. The polymers were recovered by precipitation in either hexane (PMEAM) or diethyl ether (PNIPAM, PMPAM). They were purified by repeated precipitation from THF into diethyl ether and dried in vacuo (25 °C) overnight.

(C) Oligomer Preparations. (i) General Procedure for the Preparation of Carboxyl-Terminated Oligomers. The molar ratios of monomer/initiator/mercaptopropionic acid employed are indicated in Table 1. A solution of the monomer (1−10 g) and mercaptopropionic acid in methanol was purged with nitrogen for 15 min. AIBN (recrystallized from MeOH) was added at once, and the solution was heated to 60 °C under nitrogen for 20 h. The solution was cooled to room temperature. The polymer was precipitated into diethyl ether (PNIPAM, PMPAM) or hexanes (PBMEAM) and purified by repeated reprecipitation from THF into diethyl ether.

Table 1. Preparation and Characteristics of the Carboxyl-Terminated Oligomers

polymer	[M]/[I]/[S][a]	M_n	DP_n	LCST (°C)	LCST[b] (°C) (ref)
PNIPAM-COOH	100:1:8	2 200	19.3	33.3	31 (31)
	100:1:4	3 500	30.8	33.7	
	100:1:2	14 600	128.4	34.3	
	100:1:1	25 000	219.8	32.6	
PMPAM-COOH	100:1:8	1 900	13.3	>60	>60 (44.5)
PBMEAM-COOH	100:1:8	2 000	10.7	49	49.5 (41.5)

[a] Molar ratio of monomer [M], chain transfer agent [S], and initiator [I]. [b] LCST of polymer with no carboxyl end group (reference value in parentheses).

(ii) Preparation of *b*-PNIPAM-C_{18}Py. A solution of PNIPAM-COOH (0.46 g, 2×10^{-4} mol of COOH), *N*-hydroxysuccinimide (0.035 g, 3×10^{-4} mol), dicyclohexylcarboximide (DCC, 0.13 g, 6×10^{-4} mol), and (dimethylamino)pyridine (0.05 g, 2×10^{-4} mol) in dry THF (10 mL) was stirred under nitrogen for 24 h at room temperature. The mixture was filtered. The activated polymer was precipitated into dry diethyl ether, separated by filtration, and dried in vacuo (25 °C) for 2 h. The material was then dissolved in a minimal amount of dry THF (5 mL). *N*-[4-(1-Pyrenyl)butyl]-*N*-*n*-octadecylamine was added to the solution in 50% molar excess, relative to the polymer end group. The mixture was stirred at room temperature in the dark for a period of up to 2 weeks. Isopropylamine was added to the mixture to quench any unreacted succinimide group. The mixture was stirred for an additional 2 h and filtered. The polymer was isolated from the filtrate by precipitation into diethyl ether. It was purified by size exclusion chromatography over Sephacryl S-300 eluted with tris-buffered saline (50 mM Tris, 150 mM NaCl, pH 7.4). The material eluting first (UV_{340nm}-active) was isolated. The collected fractions were desalted on Sephadex G-25 columns and freeze-dried.

(iii) Preparation of *b*-PMPAM-C_{18}Py. This polymer was prepared from PMPAM-COOH and purified following the procedure described for *b*-PNIPAM-C_{18}Py.

(iv) Preparation of *b*-PBMEAM-C_{18}Py. This polymer was prepared from PMEAM-COOH and purified as described for *b*-PNIPAM-C_{18}Py, except that hexane was used as the nonsolvent for polymer precipitation.

Instrumentation. Proton NMR spectra were recorded on Brucker AC spectrometers operating at a frequency of either 200 or 500 MHz. IR spectra were run on a Bio-Rad FTS-40 FTIR spectrometer. A Tanager automated titrator (Tanager Scientific Systems Inc.) equipped with a dual pH meter and a Titrimeter model 8901 and the TANA 58-2 software provided by the manufacturer was used to perform titrations under the following conditions: pH range, 3−11; titrant volume, 0.2−0.005 mL; time between additions of titrant and recording measurements, 600 s; response time, 2 s. Four replicate measurements were performed. Size exclusion chromatography was performed on a Gradifac system (Pharmacia Biotech equipped with a XK 16 × 70 column and a UV-1 detector). UV absorbance was monitored at 250 nm. Unless otherwise stated Tris-buffered saline was used as the eluant. The flow rate was set at 0.5 mL min^{-1}. UV spectra were recorded with a Hewlett-Packard 8452 photodiode array spectrometer equipped with a Hewlett-Packard 89090A temperature controller and operated under the HP Chemstation software. Micelle sizes were determined using a Model BI9000AT instrument (Brookhaven Instrument Corp.) equipped with a Lexel Argon laser ($\lambda = 514.5$ nm) with the scattering angle set at 90°. Data analysis was performed with the CONTIN software provided by the manufacturer.

Titrations. A solution of a polymer aliquot (ca. 0.1 mg) in aqueous sodium nitrate (19.8 mL, 0.01 M) was brought to pH 3 by addition of aqueous HCl (0.2 mL, 0.1 N). The solution was titrated in the absence of CO_2 at 25 °C with aqueous NaOH (0.1 M). Titration of a blank solution was performed under identical conditions to determine the amount of titrant required to neutralize excess HCl.

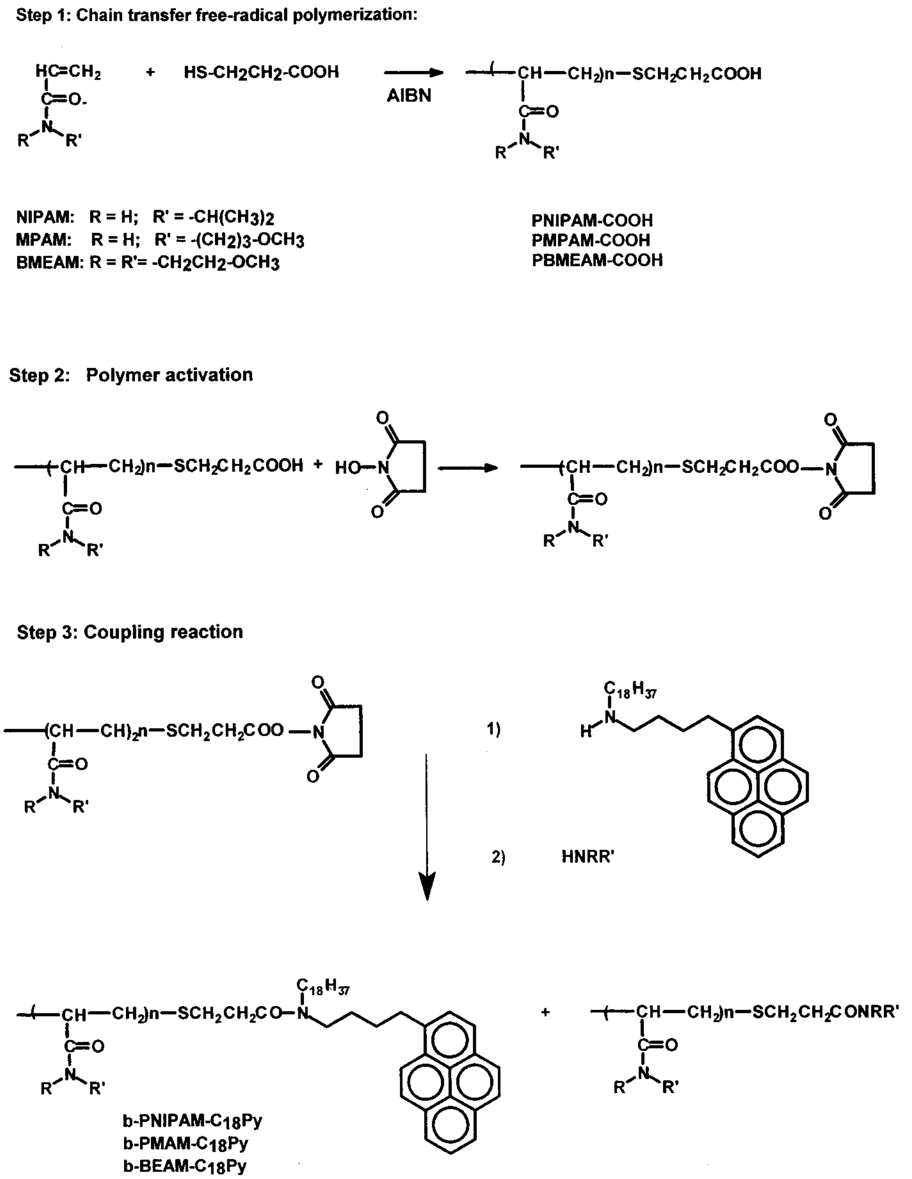

Figure 2. Synthetic scheme for the preparation of the hydrophobically-modified oligomeric poly(*N*-alkylacrylamides).

Fluorescence Measurements. Fluorescence spectra were recorded on a SPEX Fluorolog 212 spectrometer equipped with a DM3000F data system. The temperature of the water-jacketed cell holder was controlled with a Neslab circulating bath. The temperature of the fluid was measured with a thermocouple immersed in the sample. Excitation spectra were recorded in the ratio mode. Emission spectra were not corrected. They were recorded at an excitation wavelength of 346 nm (aqueous solutions) or 342 nm (methanolic solutions). Excitation and emission slit widths were set at 1.5 mm.

Solutions for Spectroscopic Analysis. Samples were prepared by dilution of stock solutions of the polymers (0.5 g L^{-1}) kept at 5 °C overnight to ensure complete dissolution of the polymers. Aliquots of the stock solutions were diluted to the desired concentration. They were kept at room temperature for at least 2 h prior to spectroscopic measurements. Solutions in water were not degassed. Solutions in methanol were degassed by a 1-min vigorous bubbling of methanol-saturated argon through the solutions. For the experiments on mixed micelle interactions, stock solutions of *b*-PNIPAM-(C_{18})Py, *b*-PNIPAM-$(C_{18})_2$, and PNIPAM-C_{18} were prepared by allowing the polymer to dissolve in water for 1 day before diluting to a known volume. Aqueous solutions of *b*-PNIPAM-(C_{18})Py (0.03 g L^{-1}) were prepared from the stock solution (0.3 g L^{-1}). Increasing amounts of stock solutions of *b*-PNIPAM-$(C_{18})_2$ or PNIPAM-C_{18} were added to the *b*-PNIPAM-(C_{18})Py-containing solutions, while keeping the pyrene concentration constant. The sample solutions were kept at room temperature in the dark until they equilibrated (about 1 day) prior to fluorescence measurements.

Determinations of the Spectroscopic Parameters. The pyrene excimer to monomer emission intensity ratio (I_E/I_M) was calculated as the ratio of the intensity at 480 nm to that at 378 nm. Quantum yields were calculated by integration of peak areas of corrected spectra in wavenumber units, using quinine sulfate in 1 N H_2SO_4 ($\Phi = 0.60$, $\lambda_{exc} = 290$ nm, 25 °C).[12] Beer's law corrections were applied for optical density changes at the excitation wavelength.

Results and Discussion

Synthesis, Purification, and Characterization of the Oligomers. Carboxyl terminated oligomers of end-labeled poly(*N*-alkylacrylamides) were prepared by free-radical polymerization of an *N*-alkylacrylamide in the presence of mercaptopropionic acid acting as a chain transfer agent (Figure 2, step 1). This method yields oligomers of a predetermined degree of polymerization (DP_n).[13] Thus we obtained carboxyl-terminated oligomers of *N*-isopropylacrylamide (NIPAM), *N*-(3-methoxypropyl)acrylamide (MPAM), and *N*,*N*-bis(2-methoxyethyl)acrylamide (BMEAM). Number-average molecular weights (M_n) of the oligomers were determined by an acid−base titration of the terminal carboxyl group. They ranged from 2000 to 25 000, depending on the monomer/initiator/chain-transfer agent molar ratio (Table 1).

Quantitative attachment of the hydrophobic end group to the oligomers proved to be difficult to achieve. Several types of coupling reactions were investigated in order to find a preparation method that would best overcome two difficulties associated with this step, namely, the low reactivity of a secondary amine in condensation reactions with carboxylic acids and the well-documented sluggishness of polymer analog reactions.[14] Best yields were obtained when the carboxylated oligomers were converted first to the corresponding *N*-hydroxysuccinimido (NHS) derivatives and purified by reprecipitation under anhydrous conditions (Figure 2, step 2). The success of the polymer activation was ascertained from an analysis of the ^{1}H NMR spectra of NHS-terminated polymers. Indicative of the conversion is the appearance of a multiplet at 2.8 ppm assigned to the succinimide methylene protons. Coupling of [4-(1-pyrenyl)butyl]-*N*-*n*-octadecylamine to the activated oligomers was performed at room temperature under anhydrous conditions. Excess amine, relative to the carboxyl end groups, and reaction times of up to 2 weeks were required in order to achieve acceptable degrees of conversion. The progress of the reaction was monitored by ^{1}H NMR spectroscopy, focusing on the disappearance of the multiplet at 2.8 ppm ascribed to the succinimide methylene protons. At the end of the reaction, the unreacted *N*-hydroxysuccinimide groups were converted to terminal *N*-alkylamides by treatment with the corresponding amine (see step 3 in Figure 2). The polymers were isolated by precipitation from THF solutions into diethyl ether or hexanes and subjected to three consecutive reprecipitations in order to separate residual [4-(1-pyrenyl)butyl]-*N*-*n*-octadecylamine from the oligomer.

Conversion yields, based on the respective carboxyl-terminated polymers, were determined by UV analysis of polymer solutions in THF with *N*-[4-(1-pyrenyl)butyl]-*N*-*n*-octadecylacrylamide (ϵ_{341}(THF) = 38 000) as the reference compound. In all cases the isolated materials contained only approximately 20−40% by weight of labeled polymers. The major contaminant was the non-fluorescing amide-terminated oligomer that formed upon quenching of the coupling reaction with the corresponding amine. Thus, PNIPAM-C_{18}Py of degree of polymerization DP_n contains some amount of PNIPAM terminated with the corresponding *N*-alkylamide group. Separation of a significant fraction of unlabeled oligomer was achieved by aqueous size exclusion chromatography. The polymer samples were fractionated into two bands: (i) a UV_{340nm}-active fraction eluting within the void volume of the column that contained all the pyrene-labeled polymer and (ii) a slower moving non-UV_{340nm}-absorbing fraction eluting next, with a retention time identical to that of the corresponding unmodified oligomer. A significant increase in pyrene content was noted in the polymer isolated from the fastest moving fraction; however, UV analysis indicated that each sample still contained some unlabeled polymer, suspected to coelute with the polymeric micelles. Further purification of the polymers was attempted but could not be achieved.

The composition of the purified polymers was confirmed by ^{1}H NMR analysis of $CDCl_3$ solutions of the polymers. In the case of *b*-PNIPAM-C_{18}Py the ratio of isopropyl groups to *n*-octadecyl groups was calculated from the area of the singlet at 3.97 ppm, due to the resonance of the C-2 proton of the isopropyl groups, and the area of the triplet centered at 0.85 ppm, attributed to the terminal methyl protons of the octadecyl chain. For *b*-PMPAM-C_{18}Py and *b*-PBMEAM-C_{18}Py the signals at 3.3 and 3.4 ppm, respectively, were used, together with the 0.85 ppm triplet. This ratio and the known number degree of polymerization of each polymer gave a measure of the purity level of each sample. Data obtained from ^{1}H NMR spectra analysis corroborate the UV analysis, within the experimental uncertainty of each method.

Solution Properties of the *N*-Alkylacrylamide Oligomers in Water. All oligomers were soluble in water at, and below, room temperature. Optically clear solutions were prepared with no difficulty for oligomer concentrations as high as 5 g L^{-1}. Aqueous solutions of the *N*-methoxypropylacrylamide oligomers could be heated up to 60 °C with no apparent phase transition. This seemed surprising, since Ito[15] had reported a cloud point of 44.5 °C for this polymer. To confirm our finding, we prepared a high molecular weight sample of PMAM by free-radical polymerization in the absence of chain transfer agent. In our hands, this polymer also failed to exhibit a cloud point in aqueous solutions of concentration as high as 1.0 g L^{-1}. The measured cloud points of the other oligomers are listed in Table 1, together with reported and measured values of the corresponding polymers obtained by polymerizations in the absence of chain-transfer agent. The cloud points of the carboxyl-terminated oligomer were slightly higher than those of high-molecular-weight samples, presumably as a consequence of the presence of the hydrophilic terminal carboxyl group, which increases the hydrophilicity of the polymer.[16] For the hydrophobically modified samples, the cloud points were the same as those of the parent polymers.[17] Similar trends were observed in previous studies of hydrophobically modified PNIPAM of higher molecular weight, where the hydrophobic end group was either a di-octadecyl moiety[6] or the octadecyl/pyrenyl substituent used in the present study.[9] This observation suggests that the hydrophobic groups are not exposed to the aqueous environment but rather aggregate in a micellar structure consisting of an alkyl-rich core surrounded by PNIPAM chains.

The formation of micellar structures was confirmed by quasi-elastic light scattering (QELS) measurements carried out at 25 °C with solutions of the hydrophobically-modified oligomers (0.01 g L^{-1}). Micelle sizes, reported as effective diameters determined by a cumulative analysis of the data, range from 20 to 30 nm (Table 2). The size of the micelles is dictated by the action of two competing forces: (a) the hydrophobic interactions experienced by the alkyl/pyrenyl groups in water, which tend to sequester them in the aggregated core of the micelles, and (b) the excluded volume repulsion between the polymer chains, which limits the aggregate size. We note that the *b*-PNIPAM-Py micelles are slightly larger than those formed by the other two oligomers. The larger degree of polymerization of this sample may account for this observation. A similar trend can be seen if one compares the micelle size of *b*-PNIPAM-Py (30 nm) to that (70 nm) of micelles of the end-labeled *b*-PNIPAM$(C_{18})_2$ of higher molecular weight (Table 2).[9] The chemical structure of the hydrophobic tail also may play a role in controlling the size of the polymeric micelles. This effect could be assessed through

Table 2. Physical Properties of the Hydrophobically Modified Poly(*N*-alkylacrylamides)

polymer	NIPAM/ C_{18}/Py[a]	M_n	DP	LCST (°C)	D[d] (nm)	ref
b-PNIPAM-C_{18}Py	20:1:1	2 300	19.3[b]	31.5	30 ± 1	this work
b-PMPAM-C_{18}Py	14:1:1	1 900	13.3		20 ± 2	this work
b-PBMEAM-C_{18}Py	11:1:1	2 000	10.7	44.0	25 ± 2	this work
PNIPAM-C_{18}	240:1:0			30.3	38 ± 1	4
b-PNIPAM-$(C_{18})_2$	140:1:0	25 000	280[c]	32.0	72 ± 5	6

[a] Molar ratio determined by UV and/or ^{1}H NMR. [b] Determined by titration of carboxyl-terminated precursor (see text). [c] Determined by ^{1}H NMR spectroscopy. [d] Effective diameter, measured at 25 °C in water.

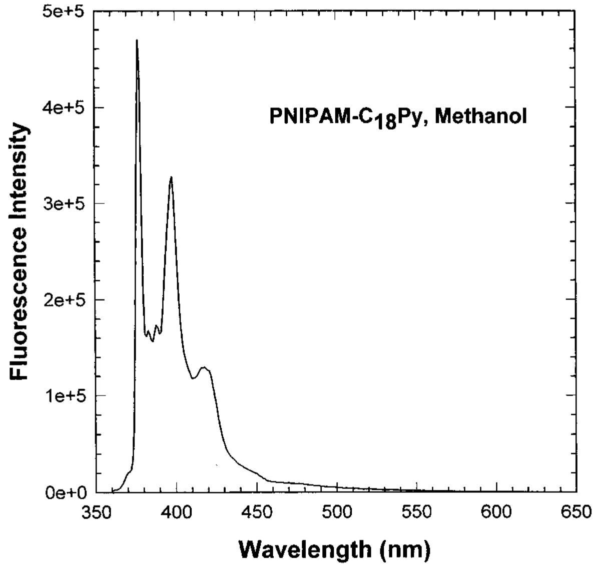

Figure 3. Fluorescence spectrum of pyrene-labeled poly(*N*-isopropylacrylamide) (b-PNIPAM-C_{18}Py) in methanol; polymer concentration, 0.01 g L^{-1}; $\lambda_{exc} = 342$ nm.

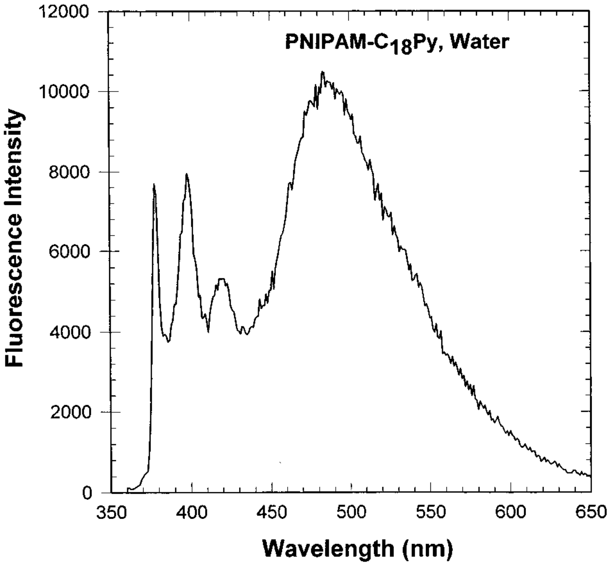

Figure 4. Fluorescence spectrum of pyrene-labeled poly(*N*-isopropylacrylamide) (b-PNIPAM-C_{18}Py); polymer concentration, 0.01 g L^{-1}; $\lambda_{exc} = 346$ nm.

a study of oligomers of similar degree of polymerization but with different end groups.

Fluorescence Spectroscopy. The fluorescence of the oligomers in a good solvent, such as methanol, reflects the well-known photophysical properties of the pyrene chromophore. It consists of two contributions: a strong well-resolved emission due to spatially isolated excited pyrene chromophores (intensity I_M, pyrene 'monomer' emission) with the [0, 0] band at 378 nm and a weak featureless emission centered at 490 nm, attributed to pyrene excimer emission (intensity I_E) (Figure 3). Oligomers dissolved in water possess markedly different photophysical properties (Figure 4). Two key features of their emission in water are particularly revealing. First, the overall fluorescence intensity is unusually weak, as confirmed by a determination of the emission quantum yield. The values were $\Phi_{monomer} = 0.004$ and $\Phi_{excimer} = 0.043$, for pyrene monomer and pyrene excimer emission, respectively. The pyrene monomer quantum yield is about two orders of magnitude lower than typical quantum yields of 1-pyrenyl groups.[18] The second key feature of the emission of the oligomer concerns the relative contributions of pyrene monomer and pyrene excimer to the overall fluorescence. The emission is dominated by a broad signal centered at 478 nm attributed to pyrene excimer emission. A much weaker contribution of isolated excited pyrene ('monomer' emission) is also detected, with the [0, 0] band at 378 nm. The fact that the excimer emission is so strong, relative to the pyrene monomer emission, implies that the pyrene-emitting groups are in close spatial proximity, presumably within the hydrophobic core of the micellar aggregates. The low emission quantum yields also vouch for the existence of tightly packed pyrene-rich microdomains, which favor the formation of nonemissive pyrene/pyrene aggregates. The micellar or aggregated structures do not exist in good solvents for the polymer, as evidenced for example by the extremely weak contribution of excimer emission to the fluorescence of the oligomers in methanol (Figure 3). In this situation, oligomer chains are isolated from each other. Pyrene excimer emission can occur only as a result of diffusion of oligomers toward each other and the encounter of an electronically excited pyrene and a pyrene in the ground state.

Concentration Studies. In solutions of hydrophobically modified polymers there exists usually a concentration below which the polymers do not aggregate but form unimolecular polymer micelles. The emission of pyrene-labeled polymers can yield this concentration, since, in very dilute solutions where only unimers exist, pyrene excimers can no longer form. We prepared a series of increasingly dilute solutions of the oligomers in aqueous buffer, ranging in concentration from 10^{-7} mol L^{-1} (5×10^{-4} g L^{-1}) to 5×10^{-5} mol L^{-1} (1×10^{-2} g L^{-1}). For solutions of *b*-PMAM-C_{18}Py and *b*-PNMEAM-C_{18}Py, a small decrease in the excimer emission intensity relative to the monomer emission intensity was detected in solutions of concentration lower than approximately 10^{-6} mol L^{-1}. In the case of *b*-PNIPAM-C_{18}Py, the ratio of excimer to monomer emission intensity remained constant over the entire concentration range probed.[19] These data suggest that oligomeric aggregates exist already in solutions where the oligomer concentrations are still high enough for reliable fluorescence measurements.

Temperature Effects. The pyrene emission exhibited only a weak dependence on temperature, when

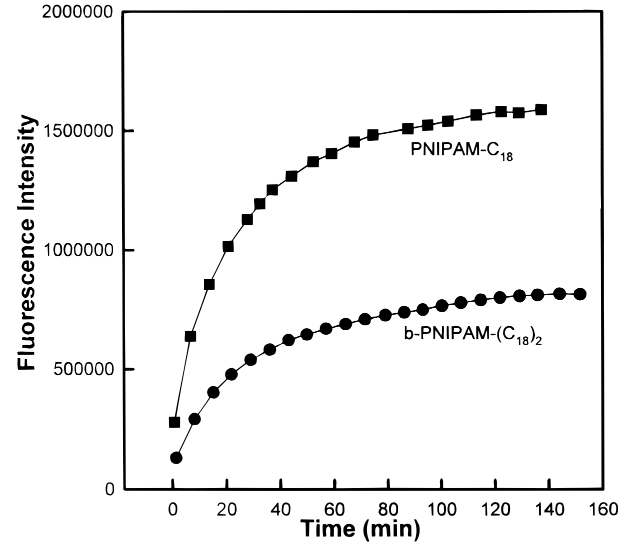

Figure 5. Plot of the changes in the fluorescence intensity at $\lambda = 378$ nm (monomer emission) of an aqueous solution of PNIPAM-C_{18}Py (0.03 g L^{-1}) as a function of time after the addition of b-PNIPAM-$(C_{18})_2$ (0.5 g L^{-1}, $[C_{18}H_{37}] = 5 \times 10^{-5}$ mol L^{-1}) and PNIPAM-C_{18} (1.0 g L^{-1}, $[C_{18}H_{37}] = 5.8 \times 10^{-5}$ mol L^{-1}); $\lambda_{exc} = 344$ nm.

solutions of the pyrene-labeled oligomers in water were heated through their cloud points. For solutions of *b*-PNIPAM-C_{18}Py and *b*-PBMEAM-C_{18}Py, the ratio I_E/I_M underwent a slight decrease over a narrow temperature range corresponding to the macroscopic phase separation (32 and 44 °C, respectively). The increase in temperature causes the collapse of the polymer chains onto the hydrophobic core of the micelles, without significant changes in the assembly of the pyrenyl/octadecyl groups. Solutions of *b*-PMPAM-C_{18}Py, which do not exhibit macroscopic phase separation in water (see above), were also monitored by fluorescence as a function of temperature, from 25 to 60 °C. Their spectra remained unaltered.

Interactions of Hydrophobically Modified PNIPAM with *b*-PNIPAM-C_{18}Py Micelles. (A) Case of the End-Modified Polymer, *b*-PNIPAM-$(C_{18})_2$. The pyrene emission of *b*-PNIPAM-C_{18}Py (0.03 g L^{-1}, 1.5×10^{-5} mol L^{-1}) was monitored as a function of increasing amounts of *b*-PNIPAM-$(C_{18})_2$. A sharp increase in pyrene monomer emission takes place when the unlabeled polymer concentration exceeds 1×10^{-3} g L^{-1}, signaling the onset of mixing of the polymeric micelles. When the *b*-PNIPAM-$(C_{18})_2$ concentration reaches 1.0 g L^{-1}, the monomer emission intensity assumes a maximum value which is not affected significantly by further addition of unlabeled polymer. It is interesting to note that the total excimer emission intensity is not affected significantly by the addition of *b*-PNIPAM-$(C_{18})_2$. However the fluorescence quantum yields of both pyrene monomer and excimer emissions increase dramatically in mixed micelles, compared to those recorded from solutions of *b*-PNIPAM-C_{18}Py alone. For example in a solution consisting of PNIPAM-C_{18}Py (0.03 g L^{-1}) and *b*-PNIPAM-$(C_{18})_2$ (1.0 g L^{-1}), $\Phi_{monomer} = 0.356$ and $\Phi_{excimer} = 0.120$, for the pyrene monomer and excimer emissions, respectively. These changes indicate a disruption of the pyrene/pyrene aggregates present in the oligomeric micelle cores in the presence of the hydrophobically modified PNIPAM. In parallel we conducted a series of control experiments, where unmodified PNIPAM (M_v 200 000) was added to a solution of *b*-PNIPAM-C_{18}Py. The emission of the oligomer remained constant in the presence of PNIPAM of concentration as high as 1 g L^{-1}.

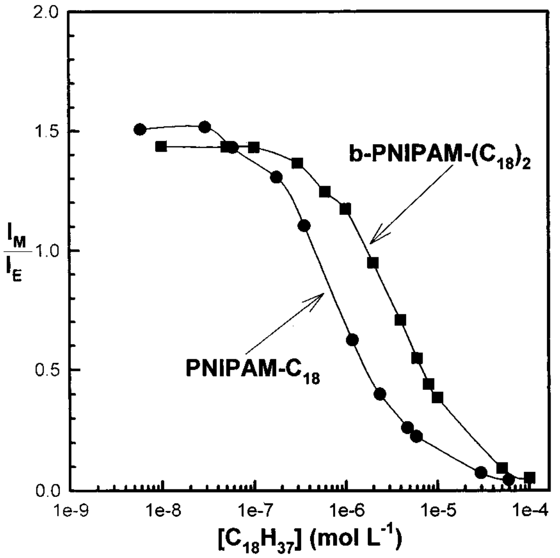

Figure 6. Plot of the changes in the ratio I_E/I_M for an aqueous solution of b-PNIPAM-C_{18}Py (0.03 g L^{-1}) as a function of the concentration of b-PNIPAM-$(C_{18})_2$ and PNIPAM-C_{18} (logarithmic scale) expressed as a function of the octadecyl group concentration (mol L^{-1}, logarithmic scale); temperature, 25 °C; $\lambda_{exc} = 344$ nm.

The data presented were obtained from mixed solutions pre-equilibrated at room temperature for 28 h. Time-dependent studies were carried out as well, to assess the equilibration rate. A known amount of *b*-PNIPAM-$(C_{18})_2$ (0.5 g L^{-1}) was added to a solution of *b*-PNIPAM-C_{18}Py (0.03 g L^{-1}) and the monomer emission intensity was monitored as a function of time (Figure 5). We determined that equilibrium was attained after approximately 1 h. The fluorescence of the sample remained unaltered for over 2 weeks. The effect of temperature was monitored on pre-equilibrated samples (*b*-PNIPAM-C_{18}Py, 0.03 g L^{-1}; *b*-PNIPAM-$(C_{18})_2$, 1.0 g L^{-1}) as well. Mixed solutions were heated from 25 to 35 °C and subsequently cooled to room temperature. A small increase in pyrene monomer emission intensity was detected at 32 °C, a temperature slightly above the LCST of the polymers. When the sample was cooled, the pyrene emission was identical to that of the initial mixture.

(B) Case of the Randomly Modified Polymer, PNIPAM-C_{18}. The effect of added PNIPAM-C_{18} of increasing concentration on the emission of PNIPAM-C_{18}Py (0.03 g L^{-1}) was monitored under conditions identical to those described in the case of the block copolymer PNIPAM-$(C_{18})_2$ described previously.[6] The same overall trends were observed. In order to compare the two systems, the data will be discussed in terms of octadecyl chain concentration rather than polymer concentration. The changes in the ratio I_E/I_M for the emission of *b*-PNIPAM-C_{18}Py in the presence of increasing unlabeled polymers plotted as a function of the $[C_{18}H_{37}]$ concentration are depicted in Figure 6. We note that the ratio I_E/I_M undergoes a sharp transition as a function of added HM-PNIPAM's, the transition occurring at lower octadecyl chain concentration in the case of the randomly modified polymer. In both cases the micellar assemblies formed by the Py-labeled oligomers are destroyed according to an interaction mechanism only modestly sensitive to the structure of the HM-PNIPAM added (see Figure 7).

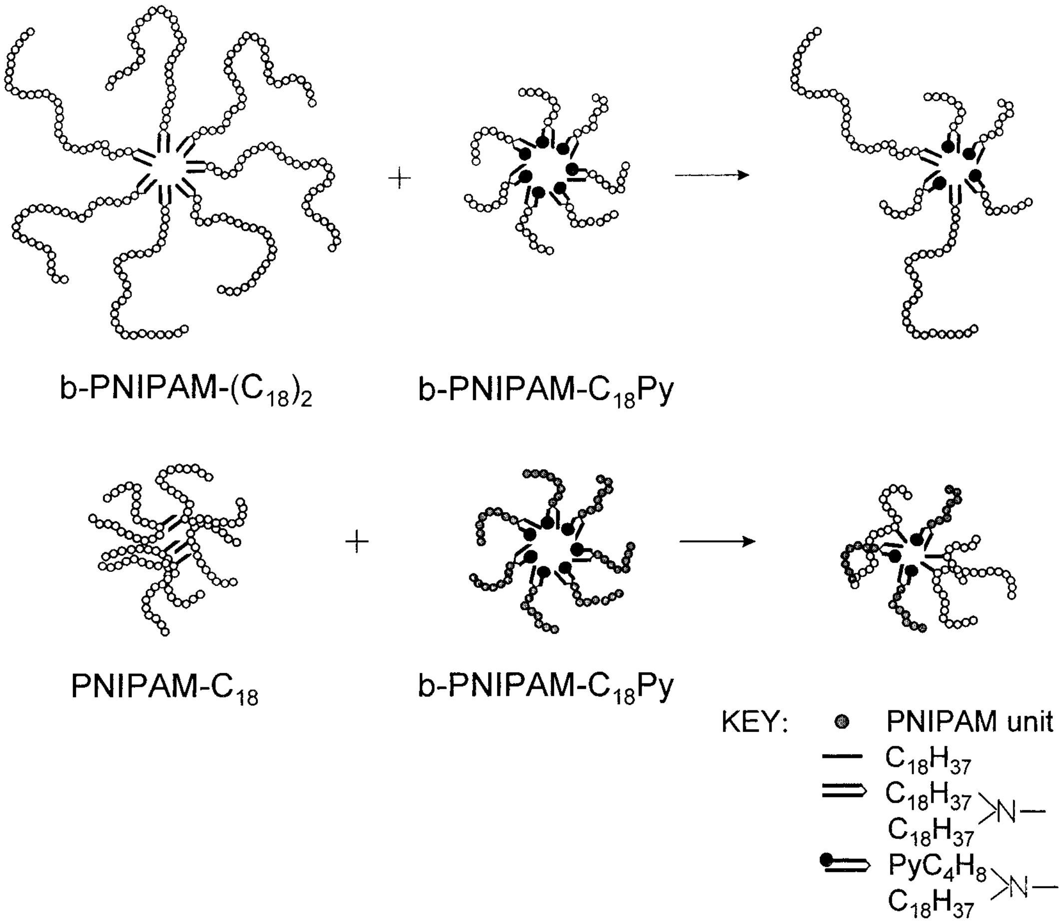

Figure 7. Schematic representation of the interactions between micelles of the pyrene-labeled oligomers and the assemblies formed by end-substituted HM-PNIPAM (top) and randomly-grafted HM-PNIPAM (bottom).

Conclusions

End-labeled oligomeric poly(*N*-alkylacrylamide)s form micelles in water, with a rigid core of octadecyl chains and pyrene groups and a diffuse corona of solvated PNIPAM chains. From the changes with temperature of the pyrene emission, we conclude that at the LCST the *N*-alkylacrylamide chains collapse onto the hydrophobic core, without profound disruption of the core. The oligomeric micelles of PNIPAM C_{18}Py are not destroyed by added PNIPAM homopolymer. In contrast they form mixed micelles with hydrophobically modified PNIPAM carrying octadecyl chains either at one chain end or randomly along the polymer backbone. In the mixed micelles, the pyrene substituents are incorporated within the octadecyl chains assembly, thus alleviating the thermodynamically unfavorable pyrene–pyrene interactions occurring in the original oligomeric micelles.

Acknowledgment. Financial support of the Natural Sciences and Engineering Research Council of Canada, of the Medical Research Council of Canada, and of the Ontario Center for Materials Research is gratefully acknowledged.

References and Notes

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