Solution Properties of Hydrophobically-Modified Phosphorylcholine-Based Polymers in Water and in the Presence of Surfactants

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The photophysical properties of a fluorescently labeled amphiphilic polybetaine have been investigated by steady state and time-resolved fluorescence spectroscopy. The copolymer consists of *N*-isopropylacrylamide and *N*-phosphorylcholine-*N*′-ethylenedioxybis(ethyl)acrylamide units in ∼1/1 molar ratio, as well as 5 mol % of *N*-[(1-pyrenyl)-4-butyl]-*N*-*n*-(octadecyl)acrylamide. In water, individual copolymer chains associate in multichain aggregates held together by hydrophobic interactions between the hydrocarbon chains and by ion pair formation between the phosphorylcholine groups. By monitoring the changes in the ratio of the pyrene excimer emission intensity (I_E) to the pyrene monomer emission intensity (I_M), we established (1) that the polymer assemblies are disrupted by the addition of divalent salts, such as $CaCl_2$ and (2) that interactions take place between the polymer and anionic, cationic, zwitterionic, or neutral surfactants. The mechanism of binding is discussed in terms of surfactant charge and chain length and compared to the association of surfactant to a copolymer of *N*-isopropylacrylamide and *N*-phosphorylcholine-*N*′-ethylenedioxybis(ethyl)acrylamide devoid of hydrophobic substituents.

Introduction

Polyzwitterions, which are polymers carrying both cationic and anionic charges, have found numerous practical applications, such as ion exchangers, chelators of trace metals in drinking water, additives in sewage treatment fluids, and components of shampoos and conditioners, to name but a few.[1−3] Beyond their practical importance, polyzwitterions in the bulk or in solution offer a fertile ground for fundamental studies. In particular, the solutions of polyzwitterions often exhibit the so-called "anti-polyelectrolyte effect". Unlike polyelectrolytes, which tend to adopt a shrunken coil conformation in the presence of added electrolytes,[4] polyzwitterions in water often undergo chain expansion with increasing ionic strength. This effect is prevalent in the case of polybetaines, defined as polyzwitterions in which the anionic and cationic moieties belong to the same monomer unit. The cationic unit of polybetaines is usually a quaternary ammonium group. Typical anionic moieties are carboxylates (carboxybetaines),[5] sulfonates (sulfobetaines),[6] phosphates/phosphinates (phosphobetaines).[7] Given the biological importance of phosphobetaines, such as phosphorylcholine (PC) and its derivatives, there has been an intensive effort over the past decades to prepare synthetic PC-polymers.[8,9] Many biomedical devices, including surgical stents[10] and extended wear contact lenses,[11] have been designed with PC-polymer biomaterials, taking advantage of the remarkable resistance to protein adhesion of their surface. PC-polymers have found applications also in the form of hydrogels[12] for oral drug delivery,[13] nanospheres for systemic drug delivery,[14] or as additives in cosmetic formulations.[15] The latter applications exploit the biocompatibility of PC-polymers, the pH-sensitivity of PC-polymer/polyacrylate networks, and the exceptional ability of PC-polymer to bind water molecules.[16]

We recently reported a preparation of PC-based amphiphilic polymers by a route involving the attachment of PC-groups via reductive amination of phosphorylcholine glyceraldehyde by primary amine groups linked to preformed hydrophobically modified copolymers.[17,18] Examples of PC-polymers prepared by this method include random copolymers of *N*-isopropylacrylamide (NIPAM), *N*-(phosphorylcholine)-*N*′-ethylenedioxybis-(ethyl)acrylamide, and an acrylamide carrying a hydrophobic group, such as *n*-octadecyl, 1*H*, 1*H*-perfluoro-*n*-octyl, or *N*-[(1-pyrenyl)-4-butyl]-*N*-*n*-octadecyl. All polymers were soluble in water. An investigation, by ^{1}H NMR spectroscopy, dynamic light scattering, surface tensiometry, and fluorescence spectroscopy of the properties of these polymers in water, revealed that they form micellar structures consisting of hydrophobic clusters insulated from the aqueous environment by a shell of hydrated PC-units.[18] We also examined by isothermal titration calorimetry and fluorescence probe studies the interactions of the phosphorylcholine polymer PNIPAM-PC (Figure 1) with various surfactants.[19] The study indicated that PNIPAM-PC interacts with anionic surfactants well below the surfactants critical micelle concentration (cmc), but that neutral, cationic, or zwitterionic surfactants do not bind to the polymers in the pre-micellar concentration domain. As only negatively charged surfactants were shown to bind to PNIPAM-PC, we concluded that the surfactant/PC-polymer interactions are controlled by electrostatic interactions between the *N*,*N*,*N*-trimethylammonium groups on the polymer and the surfactant headgroups.

In the course of the analysis of fluorescence data gathered in a study of the pyrene-labeled polymer PNIPAM-PC-C_{18}Py (Figure 1) in water, we noted that the emission of pyrene which, as described in the first section of this paper, consists of contributions from pyrene monomer and excimer, was unusually weak. This observation was attributed to the occurrence of

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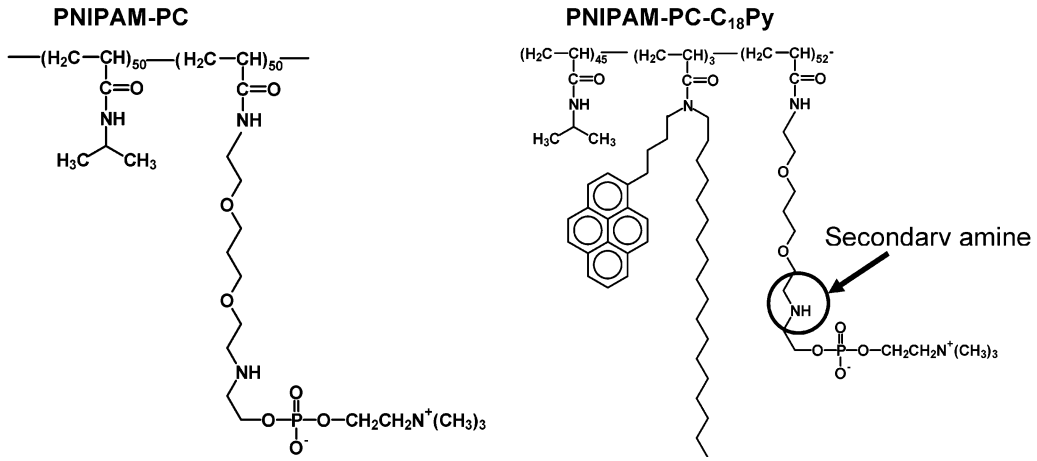

Figure 1. Chemical structure of the polymers used in this study. The arrow points to the amine group acting as a quencher of pyrene fluorescence.

intrapolymeric quenching of excited pyrene by the secondary amine groups which serve as linkers of the PC groups to the polymer backbone (Figure 1). As nearly 50 mol % of the polymer units bear secondary amines, there is a high local concentration of quenchers near the pyrene groups, at least when the polymer adopts a collapsed-coil conformation.

In the present study, we monitor how various external stimuli affect polymer conformation and interpolymeric association by exploiting two photophysical tools, (i) the relative intensity of pyrene excimer and monomer emissions and (ii) the intrapolymeric quenching of the pyrene emission. We review briefly the photophysics of PNIPAM-PC-C_{18}Py in water. Then, using data gathered from steady state and time-dependent fluorescence spectroscopy measurements, we describe how the solution properties of PNIPAM-PC-C_{18}Py are affected by changes in pH and by the presence of two types of electrolytes, a monovalent salt (NaCl) and a divalent cation ($CaCl_2$). Finally we monitor the interactions of anionic, cationic, neutral, and amphoteric surfactants with PNIPAM-PC-C_{18}Py. Because the fluorescent dye is linked to the polymer in close proximity to the octadecyl chains, the fluorescence studies will give us information on the changes perceived by the polymer and on the importance of the hydrophobic interactions between surfactant and polymer. As such, they will complement our previous study, focused primarily on the fate of the surfactants and on their interactions with the betaine units. They will yield information on the binding mechanism of surfactants to amphiphilic polybetaines and on the structure of the mixed micelles that form upon addition of the surfactants to aqueous polybetaine solutions.

Experimental Section

Materials. Water was deionized using a Milli Q water purification system (Millipore). Sodium *n*-hexadecyl sulfate (SHS) was obtained from Lancaster Chemicals. *N*-*n*-Hexadecyl-*N*,*N*,*N*-trimethylammonium chloride (HTAC) was purchased from Tokyo Kasei Chemicals. Sodium *N*-*n*-hexadecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate (HDAMPS) was obtained from Sigma Chemicals Corporation. *n*-Hexadecylmonohepta(ethyleneglycol) ($C_{16}H_{33}EO_7$, Nikkol BC-4SY) was purchased from Nikko Chemicals. Diethanolamine was purchased from Aldrich Chemical Corp. It was purified by distillation prior to use. 1-Hexadecanoyl-2-hydroxy-sn-glycero-3-phosphocholine (HL-PC) was purchased from Avanti Chemicals. The polymer PNIPAM-PC-C_{18}Py was prepared as reported previously,[18] and has the following composition: NIPAM (45 mol %), phosphorylcholine groups (52.0 mol %), (Pybutyl)-$C_{18}H_{37}$ (3 mol %).

Fluorescence Measurements. Steady-state fluorescence spectra were measured with a Fluorolog Tau-3 spectrometer (Jobin-Yvon Horiba) equipped with a GRAMS/32 (Galactic Ind) data analysis system. Temperature control of the samples was achieved using a water-jacketed cell holder connected to a Neslab circulating bath. All measurements were carried out at 25 °C. The slits were set at 0.5 mm (excitation) and 0.25 mm (emission). The excitation wavelength was 346 nm. Solutions were not degassed. The pyrene excimer-to-monomer ratio (I_E/I_M) was calculated by taking the ratio of the intensity (peak height) at 476 nm (I_E) to the intensity at 376 nm (I_M).

Fluorescence lifetimes were measured on a Fluorolog-Tau-3 multifrequency phase modulation fluorimeter (Jobin-Yvon Horiba Inc). The excitation light from a 450 W xenon lamp was modulated with a Pockels cell. Phase and modulation values were determined relative to a glycogen aqueous solution. The excitation wavelength was set at 346 nm. Pyrene monomer and excimer emissions were monitored at 376 and 476 nm, respectively. The frequency of the analyzing light was chosen in the range of 0.1−100 MHz. Data were analyzed with the Datamax Spectroscopy software based on GRAMS/32 from Galactic Ind. Data were fit to a multiexponential decay law, where a_i and τ_i are the preexponential factors and the lifetime of the i th component, respectively. The goodness of the fit was determined by the χ^2 value ($\chi^2 < 1.1$) and examination of the residuals. The preexponential factors a_i are related to the observed fractional intensity contribution f_i by the relation $f_i = a_i\tau_i / \sum_j a_j\tau_j$. The average lifetime $\langle\tau\rangle$ was calculated from $\langle\tau\rangle = \sum a_i\tau_i^2 / \sum a_i\tau_i$.

Fluorescence Quenching. Assuming that quenching takes place by a dynamic mechanism, the ratio of the fluorescence intensity in the absence (I_0) and in the presence (I) of quencher is equal to the ratio of lifetimes in the absence (τ_o) and presence (τ) of quencher. These ratios are related to the Stern−Volmer constant K_{SV} and quencher Q concentration by (eq 1), where k_q is the quenching rate constant[20]

$$\frac{I_o}{I} = \frac{\tau_o}{\tau} = 1 + K_{SV}[Q] = 1 + k_q\tau[Q] \qquad (1)$$

Solution Preparation. Samples for analysis were prepared from polymer stock solutions (5.0 or 0.5 g L^{-1}) kept at room temperature for 24 h. Solutions of increasing salt (NaCl or $CaCl_2$) concentration were obtained by addition of concentrated salt solution aliquots to a polymer stock solution (0.5 g L^{-1}). Solutions were kept in the dark at room temperature for 12 h prior to measurements. For studies of the pH effects, solutions

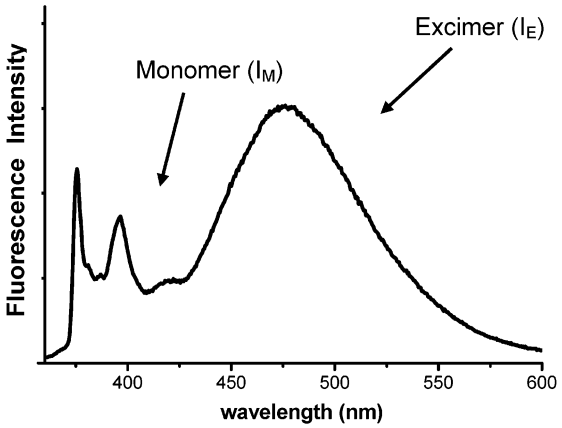

Figure 2. Fluorescence spectrum of a solution in water of PNIPAM-PC-C_{18}Py (polymer concentration: 0.01 g L^{-1}, temperature: 25 °C, $\lambda_{exc} = 346$ nm).

were prepared in citrate buffers of various pH values. Mixed polymer/surfactant solutions were obtained by adding aliquots of concentrated surfactant solutions to a solution of PNIPAM-PC-C_{18}Py (0.01 g L^{-1}). They were kept in the dark at room temperature for 12 h prior to measurements. For quenching experiments, small volumes of diethanolamine were added to a saturated pyrene solution in water ([Py] $\sim 7 \times 10^{-7}$ mol L^{-1}).

Results and Discussion

Spectroscopy of the Labeled Polymer in Water: A Brief Review. The steady-state fluorescence spectrum of a dilute aqueous solution of PNIPAM-PC-C_{18}Py (0.01 g L^{-1}) consists of two contributions: a well-resolved emission with the (0,0) band located at 376 nm due to locally isolated excited pyrene (pyrene monomer emission, intensity I_M) and a strong, broad, and featureless emission centered at 478 nm attributed to the emission of pyrene excimer (intensity I_E) (Figure 2). The fact that pyrene excimer emission is so strong, even though the pyrene solution concentration is low ($\sim 10^{-6}$ mol L^{-1}), implies that the pyrene groups are in close spatial proximity. From a combination of steady-state and time-resolved measurements (see below), it was ascertained that the excimer forms via dynamic encounter of an excited pyrene and a ground-state pyrene,[21] with no evidence of pyrene aggregation in the polymer solution.[22]

Time-dependent fluorescence data were obtained for the pyrene monomer and excimer emissions. To interpret the monomer decay, it was necessary to use multiexponential functions. Thus, the pyrene monomer decay of PNIPAM-PC-C_{18}Py in water was fitted to a double exponential decay law (Table 1), with an average lifetime $\langle \tau_M \rangle = 31.3$ ns. This lifetime is significantly shorter than typical values recorded for aqueous solutions of pyrene-labeled hydrophobically modified poly-(*N*-isopropylacrylamides) ($\langle \tau_M \rangle \approx 60$ ns, aerated solution).[23] This reduction of fluorophore lifetime was attributed to the intrapolymeric quenching of excited pyrene by the secondary amine group present on each phosphorylcholine-bearing monomer unit (Figure 1). It is known that amines act as powerful quenchers of pyrene fluorescence.[20] For example, diethanolamine, a model secondary amine, quenches the fluorescence of pyrene in water with a Stern−Volmer constant of ~ 2 M^{-1} L (data not shown).

To confirm this hypothesis, we monitored the emission of PNIPAM-PC-C_{18}Py solutions as a function of pH from 2.5 to 10.0. The pyrene monomer emission was weak in strongly basic solutions and gradually increased as the solutions became more acidic. It reached a constant value for solutions of pH $\leq$ 5.5.

TABLE 1: Lifetimes Recovered from the Fit of the Fluorescence Decays for the Pyrene Monomer and Excimer Emissions of PNIPAM-PC-C_{18}Py in Aqueous Solution and in the Presence of Salts

	monomer		excimer	
solution	τ (ns)	fraction	τ (ns)	fraction
water	34.4 ± 0.2	0.62	4.0 ± 0.2	−0.05
	8.2 ± 0.2	0.37	63.4 ± 0.2	1.00
	$\langle\tau\rangle$ = 31.3 ± 0.2			
NaCl (0.1 M)	34.8 ± 0.2	0.66	6.2 ± 0.2	−0.10
	6.7 ± 0.2	0.34	63.4 ± 0.2	0.90
	$\langle\tau\rangle$ = 32.3 ± 0.2		25.3 ± 0.2	0.10
			$\langle\tau\rangle$ = 61.9 ± 0.2	
NaCl (1.0 M)	37.4 ± 0.2	0.66	9.9 ± 0.2	−0.10
	7.5 ± 0.2	0.34	64.0 ± 0.2	0.90
	$\langle\tau\rangle$ = 34.6 ± 0.2		25.0 ± 0.2	0.10
			$\langle\tau\rangle$ = 62.4 ± 0.2	
NaCl (3.3 M)	52.6 ± 0.2	0.65	9.7 ± 0.2	−0.10
	13.7 ± 0.2	0.35	64.8 ± 0.2	0.90
	$\langle\tau\rangle$ = 47.8 ± 0.2		20.6 ± 0.2	0.10
			$\langle\tau\rangle$ = 63.3 ± 0.2	
$CaCl_2$ (0.1 M)	42.4 ± 0.2	0.63	26.5 ± 0.2	−0.06
	7.8 ± 0.2	0.37	63.3 ± 0.2	0.90
	$\langle\tau\rangle$ = 38.1 ± 0.2		33.3 ± 0.2	0.10
			$\langle\tau\rangle$ = 61.6 ± 0.2	
$CaCl_2$ (1.0 M)	49.1 ± 0.2	0.77	11.2 ± 0.2	−0.10
	6.0 ± 0.2	0.23	64.1 ± 0.2	0.90
	$\langle\tau\rangle$ = 47.6 ± 0.2		10.0 ± 0.2	0.10
			$\langle\tau\rangle$ = 63.2 ± 0.2	
$CaCl_2$ (5.0 M)	97.5 ± 0.2	0.56	27.5 ± 0.2	−0.50
	66.3 ± 0.2	0.12	66.3 ± 0.2	0.30
	21.2 ± 0.2	0.32	26.7 ± 0.2	0.70
	$\langle\tau\rangle$ 86.5 ± 0.2		$\langle\tau\rangle$ = 47.1 ± 0.2	

TABLE 2: Lifetimes Recovered from the Fit of the Fluorescence Decays for the Pyrene Monomer and Excimer Emissions of PNIPAM-PC-C_{18}Py in Aqueous Solution of Various pH

	monomer		excimer	
solution	τ (ns)	fraction	τ (ns)	fraction
pH 2.5	76.0 ± 0.2	0.58	2.5 ± 0.2	−0.04
	15.0 ± 0.2	0.42	71.5 ± 0.2	1.0
	$\langle\tau\rangle$ = 69.2 ± 0.2			
pH 7.0	64.2 ± 0.2	0.53	15.0 ± 0.2	−0.1
	16.7 ± 0.2	0.47	65.1 ± 0.2	1.0
	$\langle\tau\rangle$ = 56.6 ± 0.2			
pH 10.0	34.1 ± 0.2	0.59	9.0 ± 0.2	−0.05
	9.4 ± 0.2	0.41	61.3 ± 0.2	1.0
	$\langle\tau\rangle$ = 30.6 ± 0.2			

The increases in emission intensity in acidic solutions correspond to an increase of the Py monomer lifetime (Table 2). Polymer-bound amines are expected to be protonated in acidic solutions. Quaternary ammonium groups are notoriously ineffective quenchers of fluorescence. Thus, the increase in pyrene monomer emission intensity and lifetime in solutions of low pH once again reflects the lessening of the quenching effect. The polymer takes up a net positive charge, which is expected to trigger some chain expansion. The pH-triggered changes in polymer conformation remain modest, as the pyrene excimer emission intensity stays constant at all pH values, implying persistence of the hydrophobic domains.

The pyrene excimer time-dependent profile, recorded in neutral polymer solutions, exhibited a growing-in component (τ 4 ns) and a decay profile fitted to a single-exponential function with a lifetime of 63.4 ns. These data are consistent with

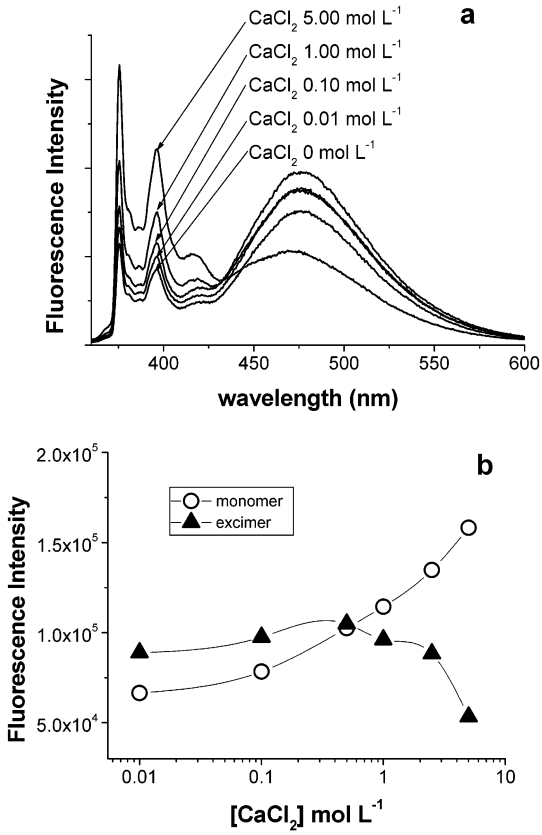

Figure 3. Fluorescence spectra of a solution in water of PNIPAM-PC-C_{18}Py containing increasing amounts of $CaCl_2$ (a) and plot of the changes of I_E (full triangle) and I_M (open circle) as a function of $CaCl_2$ concentration (b) (temperature, 25 °C; λ_{exc} = 346 nm; polymer concentration, 0.01 g L^{-1}).

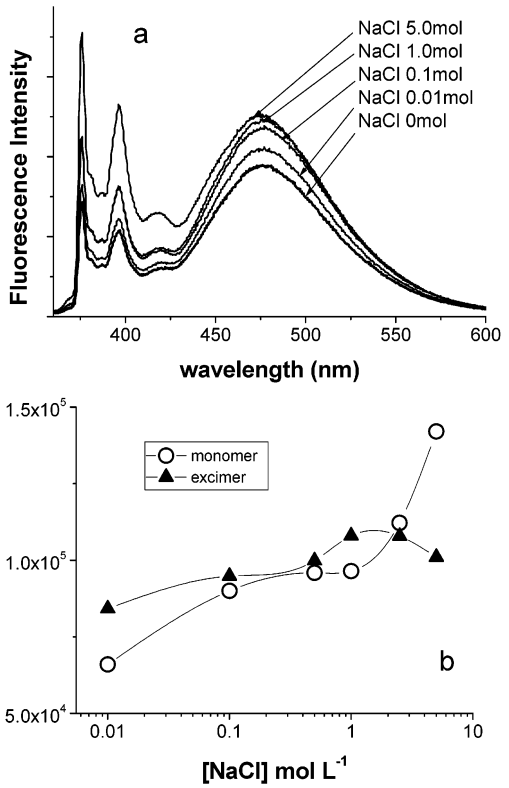

Figure 4. Fluorescence spectra of a solution in water of PNIPAM-PC-C_{18}Py containing increasing amounts of NaCl (a) and plot of the changes of I_E (full triangle) and I_M (open circle) as a function of NaCl concentration (b) (temperature, 25 °C; λ_{exc} = 346 nm; polymer concentration, 0.01 g L^{-1}).

dynamic excimer formation process. We note that the excimer growing-in time is short, compared to values ($\tau \sim 24$ ns) recorded for solutions of pyrene-labeled PNIPAM samples carrying similar amounts of *N*-*n*-octadecyl-*N*−(1-pyrenyl)-4-butyl groups.[23] The short time required to produce pyrene excimer emission upon excitation of pyrene signals that PNIPAM-PC-C_{18}Py in water adopts a conformation such that the pyrene substituents are in close proximity within the hydrophobic domains.

Ionic Strength Effects. Many polymeric betaines are insoluble in pure water due to the formation of intra- and interchain ion pairs that create an ionically cross-linked network. They become soluble, however, upon addition of electrolytes. The solubilization process reflects the penetration of the electrolytes in the ionic network, screening the net electrostatic attraction among polymer chains,[24] and triggering chain expansion.[25] The ionic strength at solubilization depends on the nature of the salt, broadly following the Hoffmeister lyotropic series,[26] and, for a given salt, it varies as a function of the chemical structure of the betaine, ranging from millimolar values, as in the case of sulfobetaines,[27] to molar concentrations. Other macroscopic properties of polybetaine solutions, such as their intrinsic viscosity, are also affected by the addition of salts.[28]

In this study of the solution properties of hydrophobically modified polybetaines, we took advantage of the fluorescent label to monitor the changes in inter- and intrapolymeric association. Emission spectra of PNIPAM-PC-C_{18}Py in aqueous solutions of increasing salt concentration were measured using either a divalent cation ($CaCl_2$) or a monovalent cation (NaCl). The effects are illustrated in Figure 3a, which presents emission spectra of PNIPAM-PC-C_{18}Py in water and in $CaCl_2$ solutions ranging in salt concentration from 0.01 to 5.0 M. Also presented (Figure 3b) are the changes with [$CaCl_2$] of the intensities of the two emissive species. Upon addition of $CaCl_2$ to solutions of PNIPAM-PC-C_{18}Py, the monomer emission intensity increases weakly for 0.01 M < [$CaCl_2$] < 0.5 M and raises sharply for [$CaCl_2$] > 1 M. The excimer emission remains nearly constant for [$CaCl_2$] < 1 M, before decreasing abruptly upon further salt addition. The concomitant drop in excimer emission intensity and increase in monomer emission intensity signal the salt-induced disruption of the hydrophobic microdomains. The increase in monomer emission from PNIPAM-PC-C_{18}Py solutions of salt concentration for which the excimer intensity remains unaffected is attributed to a relief of Py* quenching by the polymer-bound amine groups, since the excimer emission intensity is not affected in solutions of such low salt. It corresponds to the expansion of the polymer chains, the "antipolyelectrolyte effect" commonly detected in polybetaine solutions. Note that the disruption of the hydrophobic microdomains, detected by a decrease in excimer emission, requires higher salt concentrations. This conclusion was corroborated by the observed increase of the Py* monomer lifetime. (Table 1).

Next, we investigated the effect of increasing concentration of a monovalent salt, NaCl, on the photophysics of PNIPAM-PC-C_{18}Py. The results of the study are shown in Figure 4, which displays polymer emission spectra from solutions in water and in salt solutions containing from 0.01 to 5 M NaCl (Figure 4a) and the salt-driven changes in the intensities I_E and I_M of the excimer and monomer intensities, respectively (Figure 4b). Addition of NaCl to a PNIPAM-PC-C_{18}Py solution results in an increase of *both* monomer and excimer emission intensities, with a stronger increase of the monomer emission. Even in a

TABLE 3: Important Surfactant Concentrations in Their Interactions with PNIPAM-PC-C_{18}Py[a]

surfactant	polymer	surfactant cmc (mol L^{-1})	ref	surfactant/polymer c_{onset} (mol L^{-1})	cac (mol L^{-1})
SHS	PNIPAM-PC-C_{18}Py	5.8×10^{-4}	34	$2.3 \pm 0.2 \times 10^{-7}$	$2.3 \pm 0.2 \times 10^{-6}$
SHS	PNIPAM-PC	5.8×10^{-4}	34	$4.0 \pm 0.1 \times 10^{-6}$	$2.8 \pm 0.1 \times 10^{-5}$
SDS	PNIPAM-PC-C_{18}Py	8.2×10^{-3}	35	$7.4 \pm 0.2 \times 10^{-7}$	$1.2 \pm 0.1 \times 10^{-5}$
HTAC	PNIPAM-PC-C_{18}Py	1.9×10^{-3}	36	$5.6 \pm 0.1 \times 10^{-5}$	$7.5 \pm 0.1 \times 10^{-4}$
HDAMPS	PNIPAM-PC-C_{18}Py	4.4×10^{-5}	19	$5.6 \pm 0.2 \times 10^{-7}$	$6.9 \pm 0.2 \times 10^{-6}$
$C_{16}H_{33}EO_7$	PNIPAM-PC-C_{18}Py	5.2×10^{-5}	31	$1.3 \pm 0.1 \times 10^{-7}$	$1.1 \pm 0.2 \times 10^{-6}$
HL-PC	PNIPAM-PC-C_{18}Py	2.5×10^{-5}	37	$1.2 \pm 0.2 \times 10^{-6}$	$5.0 \pm 0.2 \times 10^{-6}$

[a] Polymer concentration, 0.1 g L^{-1}.

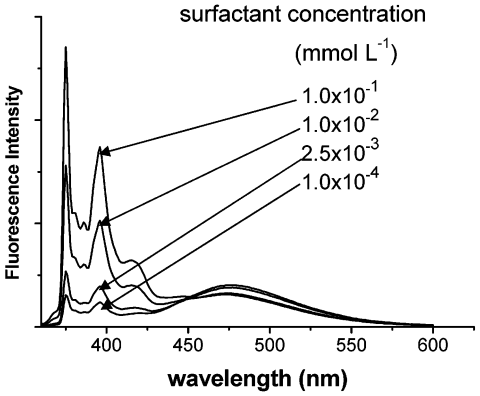

Figure 5. Fluorescence spectra of a solution in water of PNIPAM-PC-C_{18}Py containing increasing amounts of SHS (temperature, 25 °C; $\lambda_{exc} = 346$ nm, polymer concentration, 0.01 g L^{-1}).

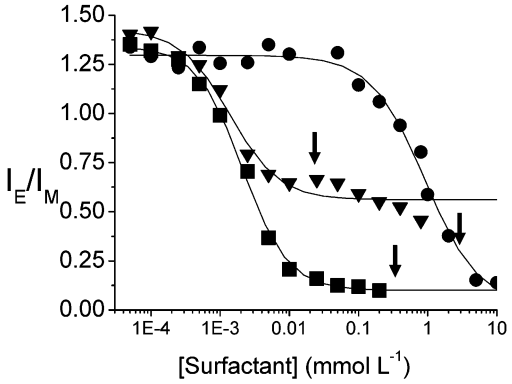

Figure 6. Plots of the changes of the ratio I_E/I_M of the intensities of pyrene excimer and monomer emissions as a function of surfactant concentration in solutions of PNIPAM-PC-C_{18}Py in water: SHS (full square), HTAC (full circle), $C_{16}H_{33}EO_7$ (inverted full triangle). Polymer concentration, 0.01 g L^{-1}. The arrows indicate the surfactant concentration corresponding to the cmc.

solution containing 8.0 M NaCl, the excimer remains strong, implying that, unlike the divalent cation Ca^{2+}, the monovalent Na^+ does not disrupt effectively the hydrophobic microdomains. The overall emission intensity enhancement and the gradual lengthening of the Py* lifetime with increasing [NaCl] reflect a mild expansion of the chains polymer with concomitant increase of the separation distance between pyrene and the polymer-bound amines, as observed also in solution of low $CaCl_2$ concentration (Figure 3).

Surfactants/PNIPAM-PC-C_{18}Py Interactions. The effect of the addition of sodium hexadecyl sulfate (SHS) to an aqueous solution of PNIPAM-PC-C_{18}Py on the fluorescence of the pyrene label is illustrated in Figure 5, where we show the fluorescence spectra of the polymer in water and in the presence of SHS (1×10^{-7} mol L^{-1} < [SHS] <1×10^{-4} mol L^{-1}) of concentration lower than its cmc (5.8×10^{-4} mol L^{-1}).[19,29] The pyrene monomer emission is significantly enhanced in the presence of SHS, and the relative contribution of the excimer emission is much reduced, even though, in absolute terms, in the most concentrated surfactant solution, the excimer emission intensity decreases by less than 20% of its value in the absence of surfactant. The increase in pyrene monomer emission intensity is accompanied by a lengthening of its lifetime from $\langle\tau\rangle = 31.3$ ns in water to $\langle\tau\rangle = 97.2$ ns in a solution containing 1×10^{-4} mol L^{-1} SHS. This increase in lifetime indicates that the chromophore is shielded from quenching by neighboring amines, presumably as it becomes entrapped within surfactant clusters bound to the polymer.

In Figure 6 we plot the changes of the ratio I_E/I_M of pyrene excimer to monomer emission intensities for PNIPAM-PC-C_{18}-Py solutions as a function of the concentration of an anionic surfactant, SHS, a cationic surfactant, HTAC, and a neutral surfactant, $C_{16}H_{33}EO_7$. The addition of surfactant is only sensed by the polymer above a surfactant concentration characteristic of each surfactant (c_{onset}, Table 3) for which the pyrene monomer emission increases. This concentration, obtained graphically from the intersection of two straight lines, the horizontal line with an almost constant value of the ratio I_E/I_M, and a line approximating the steep downward section of the sigmoid curve, is well below the cmc value of each surfactant (Table 3). It corresponds to the starting point of the growth of surfactant clusters along the polymer chain. The inflection point of the sigmoid curves is taken as a measure of the critical aggregation concentrations (cac) of the polymer/surfactant systems. This value is often used to characterize the cooperative binding of surfactants onto polymer chains.[30] The addition of SDS to aqueous PNIPAM-PC-C_{18}Py had the same qualitative effect as described in the case of SHS (Table 3): the ratio I_E/I_M decreased

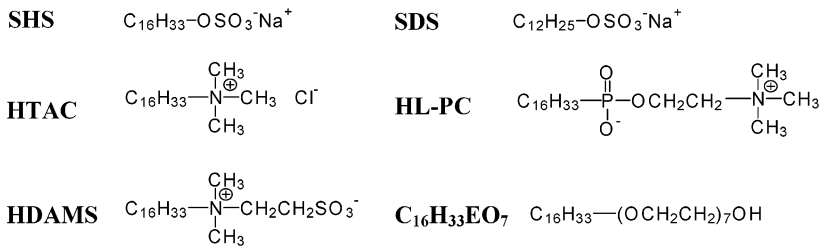

Figure 7. Structure of the surfactants used in the study.

sharply for SDS concentrations lower than the surfactant cmc, but the changes in I_E/I_M occurred over a wider surfactant concentration domain (1×10^{-6}mol L^{-1} < [SDS] < 6×10^{-4} mol L^{-1}) than in the case of SHS, implying a lesser degree of cooperativity of the interaction mechanism.

We carried out a series of experiments with other surfactants which all have a *n*-hexadecyl tail, but carry different headgroups. Thus, we used a cationic surfactant, *N*-*n*-hexadecyl-*N*,*N*,*N*-trimethylammonium chloride (HTAC), a neutral surfactant, hepta(ethyleneglycol) mono-*n*-hexadecyl ether ($C_{16}H_{33}EO_7$), and two zwitterionic surfactants, *N*-*n*-hexadecyl-*N*-dimethyl-3-ammonio-1-propanesulfonate (HDAMPS) and 1-hexadecanoyl lisophosphocholine (HL-PC) (Figure 7). Recall that in our study of the interactions of surfactants with PNIPAM-PC, we failed to detect interactions between the polymers and any of the surfactants listed above.[19] In the present study, each surfactant was added in increasing amounts to a solution of PNIPAM-PC-C_{18}Py, and the changes in pyrene monomer and excimer emissions were monitored. The addition of the cationic and of the zwitterionic surfactants to solutions of PNIPAM-PC-C_{18}Py resulted in a sharp decrease of I_E/I_M, as illustrated (Figure 6) in the case of the cationic surfactant, with c_{onset} and cac values lower than the respective surfactant cmc values (Table 3). Addition of the neutral surfactant also triggered a decrease of I_E/I_M as the surfactant reached a concentration close to its cmc, but the excimer contribution to the polymer emission remained high, even when the surfactant concentration exceeds the cmc by several orders of magnitude (Figure 6). This observation may be taken as an indication that mixed $C_{16}H_{33}$-EO_7/polymer clusters entrap several polymer hydrophobic substituents, unlike the cationic or zwitterionic surfactants, a fact attributable to the larger size of the $C_{16}H_{33}$-EO_7 micelles, compared to the size of the anionic and cationic surfactant micelles.[31]

Conclusions

Several aspects of this work are important as they increase our understanding of the solution properties of polymeric betaines in water, in salt solutions, and in the presence of detergents. By monitoring the photophysics of the polymer in solution of various ionic strengths, we confirmed that a change in ionic strength brings about only minor modifications in the expansion of a polybetaines if the added salt is monovalent,[28] but it triggers a noticeable change in polymer conformation and aggregation if the added salt is divalent. More importantly, the works demonstrate that surfactants bind to hydrophobically modified PNIPAM-PC, independently of the charge of their headgroup. Complexes are formed via a modestly cooperative mechanism yielding soluble complexes. The behavior is similar to that observed with neutral amphiphilic polymers, such as HM-PNIPAM[32] and hydroxypropylcellulose,[33] but radically differs from the comportment of the unmodified copolymer PNIPAM-PC. In the latter case, the interactions are driven by electrostatic interactions, whereas in the case of PNIPAM-PC-C_{18}Py, it is the hydrophobic substituent that steers complex formation.

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