Interaction of Pyrene-Labeled Hydrophobically Modified Polyelectrolytes with Oppositely Charged Mixed Micelles Studied by Fluorescence Quenching

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Pyrene-labeled hydrophobically modified polyanions were prepared by terpolymerization of sodium 2-(acrylamido)-2-methylpropanesulfonate, N-dodecylmethacrylamide (2.5–7.5 mol %), and N-(1-pyrenylmethyl) methacrylamide (1 mol %). Dynamic interactions of these polymers with mixed micelles of n-dodecyl hexa(oxyethylene) glycol monoether (C₁₂E₈), cetyltrimethylammonium chloride (CTAC), and cetylpyridinium chloride (CPC) (quencher for pyrene fluorescence) were monitored by fluorescence quenching. The charge on the micelle was varied systematically by varying the mole fraction of CTAC (Y) in the mixed micelle. All quenching experiments were performed at \( Y < Y_p \), where \( Y_p \) is a critical \( Y \) at which the polymer–micelle systems undergo macroscopic phase separation. A kinetic model was developed to estimate the binding constant \( (K) \) (where \( K = k_1/k_{-1} \)), association rate constant \( (k_1) \), and lifetime of bound micelle on the polymer (residence time) \( (1/k_{-1}) \) from steady-state and time-dependent fluorescence-quenching data. This analysis led to the following results: \( K \) increases with \( Y \) because both \( k_1 \) and \( 1/k_{-1} \) increase with \( Y \), \( k_1 \) being more dependent on \( Y \) than \( 1/k_{-1} \). \( K \) markedly increases with increasing concentration of dodecyl groups in the polymers, even at small \( Y \), indicating a strong enhancement of polymer–micelle interactions by hydrophobic interactions. This large increase in the binding constant arises mainly from a large increase in the residence time with increase in the hydrophobe content in the polymers, \( 1/k_{-1} \) being much more dependent on the hydrophobe content than \( k_1 \). The results indicate that complex formation results from hydrophobic interactions between dodecyl groups and the micelle superimposed on the effect of electrostatic force.

Introduction

Polyelectrolytes interact strongly with oppositely charged mixed micelles of ionic/nonionic surfactants, normally leading to phase separation. These coacervation or precipitation phenomena are related to the phase separation observed for mixtures of oppositely charged polyelectrolytes and arise from the electrostatic attraction of the two macroions, leading to the displacement of microions and the concomitant loss of hydration. However, just as polyelectrolytes of low or highly asymmetric linear charge densities may form soluble complexes, polyelectrolyte–micelle complexes may form stable, equilibrium molecular species if the electrostatic attractions are attenuated by proper adjustment of the polyion linear charge density \( (\bar{\xi}) \), the micelle surface charge density \( (\sigma) \), or the ionic strength \( (I) \). Under these conditions, soluble complexes with dimensions between 1 and 10 times those of the polyelectrolyte may be formed.

There are two distinct reasons for interest in such systems. First, these soluble polymer–micelle complexes may be investigated by a huge range of experimental methods, including turbidimetry, dynamic and static light scattering, viscometry, electrophotonic light scattering, microcalorimetry, dye solubilization, and equilibrium dialysis. Such studies provide information about the way in which some organized structures can arise in purely synthetic systems by a combination of electrostatic and hydrophobic interactions.

Second, despite their dynamic nature, micelles can be viewed as models for small charged colloidal particles, inasmuch as their interaction with polyelectrolytes is controlled by micelle charge density and geometry. Thus, the interaction of micelles with oppositely charged polyelectrolytes strongly resembles the interaction of those same polyelectrolytes with other particles of similar size and charge, for example, proteins and dendrimers. In all these cases, complex formation occurs when \( \sigma \) reaches an adequate level, and the magnitude of this value varies directly with \( I \) and inversely with \( \bar{\xi} \). The appearance of the complexed state is sufficiently abrupt to enable the identification of a critical surface charge density \( (\sigma_{crit}) \) so that the foregoing observations may be expressed as

\[
\sigma_{crit} \approx \bar{\xi}^{-1}k^a
\]

where \( k \) is the Debye–Hückel parameter. The observation of such phase-transition-like behavior is consistent with theoretical predictions for the interaction of polyelectrolytes with oppositely charged flat, cylindrical, or spherical surfaces and with more recent simulations. These results are also relevant to efforts directed toward analytical solutions for the electrostatics of DNA–protein association.

Despite the range of studies applied to such systems, little is known about the dynamics of the polyelectrolyte–micelle interaction. The simulations noted above suggest that transient interactions take place prior to the appearance of a “bound”
colloid state ("prior to" in the sense of "at a higher ionic strength", but "at a lower colloid surface charge density" would be equivalent); however, no experimental verification of such predictions exists. Fluorescence techniques offer, in principle, considerable insight into such questions. 26,27 Although fluorescence probes have been frequently used to study polymer−surfactant interactions, 28 the goal of such investigations has been the determination of the aggregation number of bound (i.e., versus free) micelles. A different application of fluorescence to polyelectrolyte−colloid interactions was presented in our investigation of the fluorescence behavior of a pyrene-tagged polyanion arising from its photophysical interaction with tryptophan residues in lysozyme. 29 More recently, we used a similar polymer in conjunction with quencher-carrying mixed micelles to characterize the microscopic polyelectrolyte−micelle phase transition. 26,27 The intensity of the polyelectrolyte−micelle interaction in such systems may be modulated in two ways: by controlling the ratio of cationic to nonionic surfactants in the micelle (i.e., α) and by controlling the ionic strength (i.e., κ). The enhancement of quenching upon increase in α or decrease in κ was investigated by steady-state and time-dependent fluorescence spectroscopy to provide insight into the dynamics of polyelectrolyte−micelle association. 26,27

The behavior of fluorescence-labeled polyelectrolytes may be perturbed by the physicochemical properties of the label. Thus, turbidimetric, light scattering, and fluorescence studies revealed that the interaction of pyrene-labeled poly(sodium 2-(acrylamido)-2-methylpropanesulfonate) (PAMPS) with mixed micelles of n-dodecyl hexa(oxyethylene) glycol monoether (C12EO6) and cetyltrimethylammonium chloride (CTAC), although predominantly driven by electrostatic forces, occurred preferentially with pyrene sites. 26 Thus, we observed a conjoint effect of hydrophobic and electrostatic interactions on the polyion−micelle interaction. 26,27

One aspect of the present work is the resolution of electrostatic and hydrophobic contributions to the binding of micelles to polyelectrolytes. As noted, fluorescence-quenching studies can yield substantial insight into the thermodynamics and kinetics of polyelectrolyte−micelle association. However, since the binding that is studied takes place at the fluorophore itself, its hydrophobic contribution is difficult to isolate. To control hydrophobic contributions in a more systematic way, we have incorporated variable amounts of n-dodecyl side chains into pyrene-labeled PAMPS (Scheme 1). Since the pyrene groups in PyPAMPS (Scheme 1) have a rather modest hydrophobic interaction with micelles, penetrating only into the ethylene oxide corona, 26,27 it is useful to observe the influence of a more effective hydrophobe.

The growing interest in hydrophobically modified water-soluble polymers 30 encompasses their interactions with surfactants 31−33 and proteins. 34 A fundamental consideration is the competition between intrapolymer association (intramolecular micellization) and intermolecular hydrophobic interactions (between polymer hydrophobes and the cosolute). Intrapolymer micelles could provide a solubilizing environment for the cosolute. On the other hand, intramolecular micellization could make polymer hydrophobes less available for interaction with another molecule. In the present case, polymer-bound pyrene may be used as a probe to monitor the dependence of polymer−micelle interaction on the polymer hydrophobe concentration and so to distinguish between these two scenarios.

In this work, we employed pyrene-labeled hydrophobically modified PAMPS (PyDodPAMPS shown in Scheme 1) and C12EO6/CTAC mixed micelles in which cetylpyridinium chloride (CPC) was solubilized. Polymer−micelle interactions were monitored by steady-state and time-dependent fluorescence quenching. A kinetic model, based on an association equilibrium, allowed us to estimate the binding constant, residence time, and association rate constant from quenching data.

Experimental Section

Materials. Pyrene-labeled hydrophobically modified polyamions were prepared by terpolymerization of sodium 2-(acrylamido)-2-methylpropanesulfonate (AMPS), N-dodecylmethacrylamide (DodMAM), and N-(1-pyrenylmethyl)methacrylamide (PyMAM) according to a method reported previously. 35 The contents of dodecyl groups in the terpolymers were 2.5, 5, and 7.5 mol %. A copolymer of AMPS and PyMAM was also prepared as previously described. 35 The content of pyrene units in the co- and terpolymers was 1 mol %, which was determined by UV absorbance at 343 nm.

C12EO6 (Nikko Chemical) was used without further purification. CTAC and CPC (both form Wako Pure Chemicals) were recrystallized twice from methanol. Milli-Q water was used for fluorescence measurements and turbidimetric titration.

Fluorescence. Steady-state fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer with excitation at 343 nm. To prepare a CPC-bearing C12EO6 micelle stock solution, a mixture of 0.05 g/L polymer, a mixture of 0.5 mM CPC, 30 mM C12EO6, and a predetermined concentration of NaCl was stirred overnight.

For "type I" fluorescence titration, 26,27 a solution of 50 mM CPC in PyDodPAMPS (PyDodPAMPS shown in Scheme 1) and C12EO6/CTAC mixed micelles in which cetylpyridinium chloride (CPC) was solubilized. Polymer−micelle interactions were monitored by steady-state and time-dependent fluorescence quenching. A kinetic model, based on an association equilibrium, allowed us to estimate the binding constant, residence time, and association rate constant from quenching data.

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For "type I" fluorescence titration, 26,27 a solution of 50 mM CTAC in a predetermined concentration of NaCl was added to a mixture of 0.05 g/L polymer and the CPC-solubilized C12EO6 stock solutions at a constant ionic strength.

Fluorescence decays were measured by a time-correlated single-photon-counting technique using a Horiba NAES 550 system. Decay curves were analyzed by a conventional deconvolution technique. Sample solutions were the same as those used for the steady-state fluorescence measurements.

Turbidimetric Titration. Turbidimetric titrations were carried out at 420 nm with a JASCO V-520 spectrophotometer with a 1-cm path-length quartz cuvette. Type I turbidimetric titrations were performed at 25 ± 1 °C by adding a solution of 50 mM CTAC to a mixture of 0.05 g/L polymer, 30 mM C12EO6, and 0.5 mM CPC at a constant ionic strength.

The turbidity values were corrected by subtracting the turbidity of a polymer-
free blank. The blank-corrected turbidity (100% transmission) was plotted as a function of $Y$, the mole fraction of the cationic surfactant in the mixed micelle, defined as $Y = ([\text{CTAC}] + [\text{CPC}])/([\text{CTAC}] + [\text{CPC}] + [\text{C}_{12}\text{E}_6])$.

### Quasielastic Light Scattering (QELS)

QELS was carried out at a scattering angle of $\theta = 90^\circ$ with an Otsuka Electronics Photol DLS-7000 light-scattering spectrometer equipped with a 75-mW Ar laser. All QELS measurements were performed at 25 °C. Sample solutions were filtered with a 0.2-µm membrane filter prior to measurement. Correlation functions were analyzed by a histogram method and used to determine the diffusion coefficient ($D$) of samples. $D$ was converted into the hydrodynamic radius ($R_\text{h}$) using the Stokes–Einstein equation, $R_\text{h} = k_b T/(6\pi \eta D)$, where $k_b$ is the Boltzmann constant, $T$ is the absolute temperature, and $\eta$ is the solvent viscosity.

### Results

#### Fluorescence Quenching by Free CPC

Pyrene fluorescence is known to be quenched by CPC. Figure 1 compares Stern–Volmer plots for fluorescence quenching of pyrene-labeled polyAMPs (PyPAMPS) (Scheme 1) by CPC in the absence and presence of varying concentrations of $\text{C}_{12}\text{E}_6$ at $I = 0.2$. Here, $I_0$ and $I$ are the steady-state fluorescence intensities in the absence and presence of CPC, respectively. In the absence of $\text{C}_{12}\text{E}_6$, the quenching with CPC is highly efficient. Time-dependent fluorescence quenching shows no decrease in fluorescence lifetime with an increase in the CPC concentration but merely a decrease in the fluorescence peak intensity (peak count in a single-photon-counting measurement of fluorescence decays). This indicates that CPC, a cationic quencher, binds closely to pyrene sites in the polyanion and that the quenching occurs extremely rapidly, compared to the fluorescence lifetime, in a static mechanism. In fact, the steady-state-quenching data follow the Perrin kinetics as shown in Figure 2. However, in the presence of micellar $\text{C}_{12}\text{E}_6$ (cmc for $\text{C}_{12}\text{E}_6$ is 0.06 mM at 20 °C), the quenching is remarkably suppressed (Figures 1 and 2). This indicates that CPC molecules are solubilized in $\text{C}_{12}\text{E}_6$ micelles, and direct interaction with PyPAMPS is prevented. As the concentration of $\text{C}_{12}\text{E}_6$ decreases, the molar ratio CPC/$\text{C}_{12}\text{E}_6$ increases and the positive charge density of the CPC-carrying micelle increases, allowing the micelle to interact electrostatically with the polyanion. Thus, significant quenching is observed for $[\text{CPC}] = 0.8$ mM in the presence of 10 mM $\text{C}_{12}\text{E}_6$, corresponding to a cationic mole fraction of 0.075, consistent with the mole fraction of cationic surfactant in the mixed micelle ($Y$) required for substantial quenching in the CTAC/$\text{C}_{12}\text{E}_6$ system (see below).

#### Fluorescence Quenching with CPC-Carrying Micelles

To study the interactions of pyrene-labeled polymers with CPC-carrying $\text{C}_{12}\text{E}_6$/CTAC mixed micelles, we employed constant concentrations of 0.5 mM CPC and 30 mM $\text{C}_{12}\text{E}_6$. At these conditions practically all CPC molecules are solubilized in the micelles (Figure 1), giving an average number of CPC per micelle of about 5, assuming the aggregation number of $\text{C}_{12}\text{E}_6$ to be $3 \times 10^2$. The micelle charge density can be continuously increased by a “type I” titration, which involves the addition of CTAC to CPC-carrying $\text{C}_{12}\text{E}_6$ micelles. Figure 3 compares fluorescence spectra of PyPAMPS and PyDODPAMPS with various dodecyl contents (Scheme 1) in the presence of 30 mM $\text{C}_{12}\text{E}_6$ at $I = 0.2$ with and without 0.5 mM CPC at varying $Y$, where $Y = ([\text{CTAC}] + [\text{CPC}] + [\text{C}_{12}\text{E}_6])$. Under these conditions, the contribution of CPC to $Y$ is 0.016. Fluorescence of PyPAMPS is only slightly quenched at $Y < 0.04$, owing to dynamic quenching arising from collisional encounters of CPC-carrying micelles with pyrene sites in PyPAMPS, as indicated by single-exponential decays of pyrene fluorescence at $Y \leq 0.04$ (Figure 4). These observations, taken together with the results in Figure 1, indicate that essentially all CPC molecules are incorporated into the micelles and that no free CPC remains in the bulk-water phase. At $Y \approx 0.05$, however, fluorescence quenching of PyPAMPS begins to increase significantly with $Y$ and fluorescence decay becomes a double-exponential with a shorter lifetime component on the order of 50 ns (Figure 4). This value corresponds to a critical $Y$ value ($Y_*$), corresponding to the critical micelle charge density $\sigma_{\text{crit}}$ in eq 1, at which soluble polymer–micelle complexes are formed. Fluorescence of PyPAMPS is strongly quenched in the region $Y > Y_*$, exhibiting double-exponential decays, up to the occurrence of macroscopic phase separation at $Y = Y_\text{p}$. At $Y \leq 0.04$, PyPAMPS and micelles encounter each other by collisions and the residence time of the micelles in the collision complex may be much shorter than the lifetime of pyrene fluorescence. At $Y \geq 0.05$, however, an association complex can form in which the micelle residence time may be comparable to or longer than the lifetime of pyrene fluorescence, as will be discussed later in detail.

In the case of hydrophobically modified polymers, by contrast, fluorescence is strongly quenched and the fluorescence decays are double-exponential even at $Y = 0.02$. With an increase in the dodecyl content in PyDODPAMPS, the regime of the strong quenching is observed at smaller $Y$ values. The extent of quenching at $Y = 0.02$ increases with increasing dodecyl content in PyDODPAMPS. The quenching is increased by an increase in $Y$, but the effect of $Y$ on the quenching becomes less when the dodecyl content is increased.

Figure 5 compares type I turbidimetric titration and fluorescence-quenching data for PyPAMPS and PyDODPAMPS at $I = 0.2$. Here, $I_0$ and $I$ are the steady-state fluorescence intensities in the absence and presence of CPC, respectively. In the absence of $\text{C}_{12}\text{E}_6$, the quenching with CPC is highly efficient. Time-dependent fluorescence quenching shows no decrease in fluorescence lifetime with an increase in the CPC concentration but merely a decrease in the fluorescence peak intensity (peak count in a single-photon-counting measurement of fluorescence decays). This indicates that CPC, a cationic quencher, binds closely to pyrene sites in the polyanion and that the quenching occurs extremely rapidly, compared to the fluorescence lifetime, in a static mechanism. In fact, the steady-state-quenching data follow the Perrin kinetics as shown in Figure 2. However, in the presence of micellar $\text{C}_{12}\text{E}_6$ (cmc for $\text{C}_{12}\text{E}_6$ is 0.06 mM at 20 °C), the quenching is remarkably suppressed (Figures 1 and 2). This indicates that CPC molecules are solubilized in $\text{C}_{12}\text{E}_6$ micelles, and direct interaction with PyPAMPS is prevented. As the concentration of $\text{C}_{12}\text{E}_6$ decreases, the molar ratio CPC/$\text{C}_{12}\text{E}_6$ increases and the positive charge density of the CPC-carrying micelle increases, allowing the micelle to interact electrostatically with the polyanion. Thus, significant quenching is observed for $[\text{CPC}] = 0.8$ mM in the presence of 10 mM $\text{C}_{12}\text{E}_6$, corresponding to a cationic mole fraction of 0.075, consistent with the mole fraction of cationic surfactant in the mixed micelle ($Y$) required for substantial quenching in the CTAC/$\text{C}_{12}\text{E}_6$ system (see below).
As can be seen in Figure 5a, macroscopic phase separation occurs near \(Y = 0.15\); PyDodPAMPS exhibits \(Y_p\) slightly lower than that of PyPAMPS. In Figure 5b, the normalized fluorescence intensities, \(I/I_0\), are plotted as a function of \(Y\) at \(I_0 = 0.2\), where \(I\) is the fluorescence intensity for the pyrene-labeled polymers in the presence of CPC-carrying C12E6/CTAC micelles at varying \(Y\), and \(I_0\) is the fluorescence intensity in the presence of CPC-free micelles at \(Y = 0\). Strong quenching occurs at \(Y < Y_p\), which provides evidence of binding between the pyrene-labeled polymers and CPC-carrying micelles at \(Y < Y_p\). For dodecyl-containing polymers, strong quenching occurs at \(Y = 0\), and no \(Y_c\) is recognized. The extent of quenching at \(Y \approx 0\) increases with increasing dodecyl content and the quenching is increased by an increase in \(Y\), the quenching being less dependent on \(Y\) at higher dodecyl contents. These observations indicate that polymer–micelle association can take place solely through hydrophobic interactions of the polymer-bound dodecyl groups with micelles at \(Y = 0\). However, polymer–micelle association is enhanced by conjoint electrostatic interactions, which are more significant for polymers with lower dodecyl contents.

**Kinetic Analysis**

To quantitatively interpret the fluorescence-quenching data, we propose a simple kinetic model based on an association equilibrium for pyrene-labeled polymers and CPC-carrying C12E6/CTAC mixed micelles (Scheme 2). In Scheme 2, \(P\) denotes the pyrene site in the polymer, \(M\) the quencher-carrying mixed micelle, \(PM\) the complex between \(P\) and \(M\), \(k_1\) and \(k_{-1}\) the association and dissociation rate constants, respectively, \(\tau_0\) the fluorescence lifetime of pyrene in the absence of the
quencher, and $k_q$ the first-order quenching rate constant within the complex. At equilibrium, the concentration of the complex is given by

$$[\text{PM}] = K [P] [M]$$  \hspace{1cm} (2)

where $K$ is the association equilibrium constant (binding constant), that is, $K = k_1/k_{-1}$.

When the system is irradiated with UV light at equilibrium, both free (uncomplexed) and complexed pyrene chromophores are photoexcited. Fluorescence quenching occurs within the complex, but photoexcited free pyrene ($P^*$) can encounter quencher-carrying micelles within its lifetime and can form a complex ($P^*\text{M}$) with the rate constant $k_1$.

Assuming that the rate of deactivation of singlet-excited pyrene in the complex is much faster than the rate of dissociation of the complex, that is, $\tau_{0-1} + k_q \gg k_{-1}$, one can derive the rate equations for $[P^*]$ and $[P^*\text{M}]$ under transient conditions with excitation at time $t = 0$ by a light pulse of negligible duration:

$$\frac{d[P^*]}{dt} = -(\tau_0^{-1} + k_q) [P^*] [M]$$  \hspace{1cm} (3)

$$\frac{d[P^*\text{M}]}{dt} = -(\tau_0^{-1} + k_q) [P^*\text{M}] + k_1 [P^*] [M]$$  \hspace{1cm} (4)

When eqs 3 and 4 are solved and the initial condition $[P^*] = [P^*]_{t=0}$ at $t = 0$ and $[P^*\text{M}] = [P^*\text{M}]_{t=0}$ at $t = 0$ is applied, the total concentrations of the photoexcited free and complexed pyrene sites at time $t$ are given by

$$[P^*] + [P^*\text{M}] = A \exp(-t/\tau_1) + B \exp(-t/\tau_2)$$  \hspace{1cm} (5)

where

$$A = [P^*\text{M}]_{t=0} (1 - k_{-1}/(k_q - k_1[M]))$$  \hspace{1cm} (6)

$$B = [P^*]_{t=0} (k_q/(k_q - k_1[M]))$$  \hspace{1cm} (7)

$$(1/\tau_1) = (1/\tau_0) + k_q$$  \hspace{1cm} (8)

and

$$(1/\tau_2) = (1/\tau_0) + k_1[M]$$  \hspace{1cm} (9)

On the other hand, under steady-state conditions, the rate equations are given by

$$\frac{d[P^*]}{dt} = \left\{ [P]/([P] + [PM]) \right\} I_a - (\tau_0^{-1} + k_1[M][P^*])$$  \hspace{1cm} (10)

$$\frac{d[P^*\text{M}]}{dt} = \left\{ [PM]/([P] + [PM]) \right\} I_a + k_1[M][P^*] - (\tau_0^{-1} + k_q) [P^*\text{M}]$$  \hspace{1cm} (11)

where $I_a$ is the rate of light absorption. Under steady-state conditions, $d[P^*]/dt = 0$ and $d[P^*\text{M}]/dt = 0$. Thus, the total steady-state concentrations of excited pyrenes are

$$[P^*] = [P^*\text{M}] = \left\{ [P]/([P] + [PM]) \right\} I_a (1 + K[M]) (\tau_0^{-1} + k_1[M]) + k_q / (\tau_0^{-1} + k_1[M] (\tau^{-1} + k_q))$$  \hspace{1cm} (12)

The ratio of fluorescence quantum efficiencies in the presence and absence of the quencher-carrying micelle is given by

$$\Phi/\Phi_0 = \frac{\tau_1/\tau_0 + (\tau_2/\tau_0)(1 - \tau_1/\tau_0)}{1/[1 + K[M]]}$$  \hspace{1cm} (13)

By knowing the micelle concentration $[M]$, one can calculate the binding constant $K$ from steady-state fluorescence data ($\Phi/\Phi_0$) and fluorescence-decay data ($\tau_0$, $\tau_1$, and $\tau_2$) via eq 13. The association rate constant $k_1$ and quenching rate constant $k_q$ can be calculated from fluorescence-decay data by using eqs 9 and 8, respectively. From $K$ and $k_1$, one can calculate the residence time ($1/k_{-1}$) of the micelle in the complex.

Applying the kinetic model to the steady-state and time-dependent fluorescence data in Figures 3 and 4, we calculated the association rate constants, residence times, and binding constants at $I = 0.2$. Results are plotted as a function of $Y$ in Figure 6. For PyPAMPS the binding constant $K$ increases by about 1 order of magnitude from $2 \times 10^4$ to $3.5 \times 10^5$ M$^{-1}$ when $Y$ is increased from 0.05 to 0.11. The increase in $K$ with increasing $Y$ can arise from both the increase in the association rate constant $k_1$ (Figure 6a) and the residence time $1/k_{-1}$ (Figure 6b), but the effect of $Y$ on $k_1$ is more than twice its effect on $1/k_{-1}$. We conclude that fluorescence quenching for PyPAMPS at $Y < 0.04$ is due to collisional encounters, on the basis of the fact that fluorescence decays are single-exponential and that the steady-state fluorescence quenching is due to a decrease in the lifetime of fluorescence. It is to be noted that the polymer–micelle association model (Scheme 2) is only valid for cases where $Y \geq 0.05$.

The binding constant depends strongly on the hydrophobe content in PyDodPAMPS. Even when the dodecyl content is as low as 2.5 mol %, $K$ values are more than 1 order of magnitude larger than those for PyPAMPS. As the dodecyl content is increased to 7.5 mol %, $K$ increases by 2 orders of magnitude. This large increase in $K$ is mainly due to a large increase in $1/k_{-1}$ with an increase in the dodecyl content (Figure 6b). The residence times for PyPAMPS
weights of PyPAMPS and PyDodPAMPS are on the order of 5
water and from 0.2 to 0.3 dL/g in 0.1 M NaCl (molecular
radii \( R_0 \)).

When the dodecyl
are on the order of microseconds, whereas the residence times
for PyDodPAMPS (2.5 mol % dodecyl) are about 1 order of
magnitude longer than those of PyPAMPS. When the dodecyl
content is increased to 7.5 mol %, the residence time increases
to about 100 \( \mu s \), while the association rate constant \( k_1 \)
shows a more modest increase (Figure 6a). Both the residence time
and binding constant also depend, to a lesser degree, on the charge
density of the mixed micelle, indicating the influence of
electrostatic interactions.

**Discussion**

The ratios of the third to first vibrational fine structure \( I_3/ I_1 \) \(^{41}\) in the fluorescence spectra of PyPAMPS and PyDod-
PAMPS in aqueous solution range from 0.59 to 0.61 regardless
diodecyl content, indicating that pyrene labels are in contact with
the aqueous phase without hydrophobic association with
dodecyl groups.\(^{42}\) It was previously reported that random copolymers of AMPS and DodMAm formed unimolecular micelles in dilute aqueous solution, owing to intrapolymer association of dodecyl groups, when the dodecyl content exceeded ca. 30 mol %.\(^{42}\) However, PyDodPAMPS employed in the present study adopts a more or less extended random coil conformation in aqueous solution, depending on ionic strength. The reduced viscosities of PyPAMPS and PyDod-
PAMPS at 0.1 g/dL (30 °C) range from 2 to 2.4 dL/g in pure
water and from 0.2 to 0.3 dL/g in 0.1 M NaCl (molecular
weights of PyPAMPS and PyDodPAMPS are on the order of 5
\( \times 10^4 \)), independent of the dodecyl content. The hydrodynamic
radii \( R_h \) of PyPAMPS and PyDodPAMPS (7.5 mol % dodecyl
concentration) in 0.1 M NaCl determined by dynamic light
scattering are 7.50 and 7.55 nm, respectively. However, when
the dodecyl content is increased to 10 mol %, \( R_h \) slightly
decreases to 7.30 nm, while it significantly decreases to 5.35
nm when the dodecyl concentration is further increased to 20
mol %, arising from intrapolymer hydrophobic association of
dodecyl groups. Therefore, it can be concluded that there is
no intrapolymer hydrophobic association of dodecyl groups in
PyDodPAMPS in the present study and that all hydrophobes in
the polymers are available to interact with micelles; that is, each
dodecyl group, and pyrene label as well, in PyDodPAMPS can
interact with micelles upon encounter.

The size of C\(_{12}E_6/CTAC \) micelles in the absence of polymers
was measured by dynamic light scattering. Over the range of
compositions of interest (0.05 < Y < 0.25) the apparent \( R_h \) was
7.5 ± 0.2 nm, independent of Y. The extent to which the micelle
aggregation number (\( N \)) may change upon binding to polymer
is the subject of some debate. For the binding of dodecyltri-
methylammonium bromide (DTAB) to sodium polystyrene-
sulfonate, Almgren et al.\(^{43}\) found a 50% drop in \( N \). On the
other hand, Chu and Thomas\(^{44}\) found an increase in \( N \) when
decyltrimethylammonium bromide (DeTAB) binds to poly-
(methacrylic acid), while Thalberg et al.\(^{45}\) reported no change
in \( N \) when either DeTAB or DTAB binds to hyaluronic acid.
Brackman and Engberts\(^{46}\) reported a decrease in \( N \) when
hexadecyltrimethylammonium salicylate micelles bind to the
nonionic polymers poly(propylene oxide) or poly(methyl vinyl
ether), but they found no change in \( N \) when the polymer was
poly(ethylene oxide) or poly(vinylpyrrolidone). In the kinetic
analysis of the fluorescence data in the present study, we assume
that the size of C\(_{12}E_6/CTAC \) micelles does not change
appropriately upon binding to the polymers.

As we previously reported,\(^{35}\) the distribution of AMPS,
dodecyl, and pyrenyl units in the polymer chain of PyDod-
PAMPS is completely random. Accordingly, in the case of
PyPAMPS with 2.5 mol % dodecyl content, for example, each
pyrene label should have a closest-neighboring dodecyl group
within 20 AMPS units apart (ca. 6 nm apart if the chain is fully
extended). These considerations, taken together with the size
of a C\(_{12}E_6 \) micelle (a hydrodynamic diameter of ca. 15 nm),
lead to the conceptual model for PyDodPAMPS–micelle
association, depicted in Figure 7. When the micelle encounters
a polymer, it may first associate with either a dodecyl or pyrene
site (step 1 in Figure 7) and a “primary” complex may be
formed. In step 1 in Figure 7, the dodecyl group that associates
first with the micelle is not necessarily the one closest to a
pyrene label. Since the diameter of the micelle (ca. 15 nm) is
larger than the average distance between dodecyl and pyrene
sites (maximally ca. 6 nm in the case of 2.5 mol % dodecyl
concentration), neighboring pyrene and dodecyl groups may also
associate with a micelle simultaneously (not illustrated in Figure
7). In the primary complex, free (uncomplexed) dodecyl or
pyrene sites (maximally ca. 6 nm in the case of 2.5 mol % dodecyl
concentration), neighboring pyrene and dodecyl groups may also
lead to an equilibrium complex in which a number of dodecyl
groups and pyrene labels penetrate into a micelle. These processes
should occur in a cooperative manner with rates much faster
than the rate of step 1 because of relatively favorable entropy.
Since the rate-determining step for complex formation is step
1, the association rate constant \( k_1 \) may correspond to step 1.
Thus, the finding that \( k_1 \) increases with an increase in the
dodecyl content is reasonable because the number of binding
sites in step 1 increases with dodecyl content (the number of
dodecyl sites per pyrene site is larger in polymers with higher
dodecyl contents).

**Figure 6.** Association rate constant (a), residence time (b), and binding constant (c) as a function of \( Y \) for PyPAMPS and PyDodPAMPS at \( I = 0.2 \).
In the equilibrium complex, dodecyl groups may act as "hydrophobic anchors", holding pyrene labels tightly in the micellar phase. This situation may lead to a large increase in the residence time $1/k_{-1}$. Because the micelle is large relative to the spacing between dodecyl sites, a number of dodecyl groups can associate with a micelle for the polymers with higher dodecyl contents. Thus, the observed large increase in $1/k_{-1}$ with dodecyl content may be rationalized by an increase in the number of dodecyl anchors per pyrene label.

In the kinetic model, we assume a simple single-step equilibrium (Scheme 2). As discussed above, the association process can be represented by a single rate-determining step (step 1). On the other hand, dissociation is likely to be a multistep process, as illustrated in Figure 7. $1/k_{-1}$ represents the residence time of pyrene labels in the complex because we monitor only the quenching of pyrene fluorescence. Pyrene labels may dissociate from the equilibrium complex via reverse step 2. However, if forward step 2 occurs with a much faster rate than the rate of pyrene fluorescence (the rate constant for forward step 2 $\gg 1/r_{0}$), dissociated singlet-excited pyrene labels, if any, should rapidly reenter the micelle far before they deactivate. This consideration may be reasonable because relaxation times for segment motions are on the order of submiano- or nanoseconds, which is much shorter than the lifetime of pyrene fluorescence. Therefore, the observed $1/k_{-1}$ represents the residence time of the micelle in the polymer−micelle complex. In other words, $k_{-1}$ represents the rate constant for reverse step 1 in Figure 7.

The model in Figure 7 only embodies hydrophobic associations. However, it is clear that the primary and equilibrium complexes are additionally stabilized by electrostatic interactions with an increase in $Y$, thus contributing to an increase in $1/k_{-1}$ as well as $k_{1}$ with increasing $Y$.

**Conclusions**

Dynamic interactions of PyPAMPS and PyDodPAMPS with mixed micelles of C$_{12}$E$_{6}$/CTAC/CPC were studied by fluorescence quenching. The binding constant ($K$), residence time ($1/k_{-1}$), and association rate constant ($k_{1}$) for the polymer−micelle interaction at varying mole fractions of CTAC ($Y$) in the mixed micelle were estimated by applying a kinetic model to steady-state and time-dependent fluorescence-quenching data. $K$, $k_{1}$, and $1/k_{-1}$ increase with increasing $Y$; $k_{1}$ is more dependent on $Y$ than $1/k_{-1}$. Dodecyl groups in PyDodPAMPS strongly enhance the polymer−micelle interaction, leading to very large $K$ and $1/k_{-1}$ even at small $Y$. Thus, the polymer−micelle association results from hydrophobic interactions between dodecyl groups and micelles with or without the conjoint action of electrostatic force.

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**References and Notes**

(1) For reviews, see the following. (a) Tsuchida, E.; Abe, K. In Interactions between Macromolecules in Solution and Intermacromolecular Complexes, Advances in Polymer Science 45; Springer-Verlag: Berlin, 1982. (b) Smid, J.; Fish, D. Encyclopedia of Polymer Science and Technology; Wiley-Interscience: New York, 1988; Vol. 11, p 720.


(28) See, for example, the following. (a) Abuin, E. B.; Scaiano, J. C. Am. Chem. Soc. 1984, 106, 6274. (b) Almgren, M.; Hansson, P.; Mukhtar, E.; van Stam J. Langmuir 1992, 8, 2405. (c) Winnik, F. M.; Winnik, M.


(40) Lianos, P.; Zana, R. J. Colloid Interface Sci. 1981, 84, 100.


