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Interaction of a pyrene-labeled cholesterol-bearing polyanion with surfactant micelles studied by fluorescence quenching

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Abstract

The dissociation of multipolymer aggregates of a cholesterol-bearing polyanion by surfactant micelles in water and the formation of polymer–micelle complexes were investigated using fluorescence techniques. Interchain aggregates of cholesterol residues in a random terpolymer of sodium 2-(acrylamido)-2-methylpropanesulfonate (94 mol%), cholesteryl 6-methacryloyloxyhexanoate (5 mol%), and *N*-(1-pyrenylmethyl)methacrylamide (1 mol%) are completely disrupted by the addition of nonionic micelles of *n*-dodecyl hexa(oxyethylene) glycol monoether ($C_{12}E_6$) resulting in polymer–micelle complexes. Fluorescence of pyrene labels in the terpolymer indicated that $C_{12}E_6$ micelles bind predominantly to cholesterol sites while the micelle interacts only weakly with pyrene sites. When a small amount of *n*-hexadecyltrimethylammonium chloride (CTAC), a cationic surfactant, was added to the $C_{12}E_6$ micelle, positively charged $C_{12}E_6$/CTAC mixed micelles bind to sulfonate sites in the terpolymer. This additional electrostatic interaction strengthens the binding of the micelle to pyrene sites as well as cholesterol sites. The pyrene–micelle interaction thus increased was investigated by steady-state and time-dependent fluorescence quenching techniques using *n*-hexadecylpyridinium chloride, a quencher, added to the $C_{12}E_6$/CTAC mixed micelle. Fluorescence quenching data for varying micelle charge densities (i.e. the mole fraction of CTAC (Y) in the mixed micelle) were analyzed using a kinetic model. Results made it clear that the residence time of the mixed micelle on the pyrene site increases with increasing Y. A long residence time of ca. 19 μs was observed at $Y = 0.09$ as a result of an interplay of interaction of the micelle charge with the polymer charge and hydrophobic interaction of the micelle with mainly cholesterol sites. © 2002 Published by Elsevier Science Ltd.

Keywords: Cholesterol-bearing polyanion; Polymer–micelle complexes; Fluorescence quenching

1. Introduction

With increasing interest in hydrophobically modified water-soluble polymers in recent years, their interactions with surfactants are a growing area of research both in academics and in industry [1–3]. In our previous work on the self-association of the random terpolymer of sodium 2-(acrylamido)-2-methylpropanesulfonate (NaAMPS) (94 mol%), cholesteryl 6-methacryloyloxyhexanoate (Chol-C_5-MA) (5 mol%), and *N*-(1-pyrenylmethyl)methacrylamide (1-PyMAm) (1 mol%) (Chart 1), the polymer-bound cholesterol residues were found to show a strong tendency for interpolymer self-association [4,5]. Results of quasielastic light scattering (QELS) and fluorescence studies revealed that the polymer formed multipolymer aggregates of a 'closed' type in 0.1 M NaCl aqueous solutions where hydrophobic microdomains of cholesterols are surrounded by loops of polyanion segments [4,5]. The hydrodynamic radius (R_h) of the aggregate was independent of the polymer concentration (C_p) over a wide range ($0.1 \leq C_p \leq 10$ g l^{-1}) [4]. When nonionic micelles of *n*-dodecyl hexa(oxyethylene) glycol monoether ($C_{12}E_6$) were added to 0.1 M NaCl aqueous solutions of the polymer, the multipolymer aggregates were disrupted into polymer–micelle complexes whose hydrodynamic size was much smaller than that of the multipolymer aggregate [5]. These findings suggest that cholesterol–micelle association is preferential over cholesterol–cholesterol self-association and that the formation of the polymer–micelle complex is totally due to the binding of $C_{12}E_6$ micelles to individual cholesterol sites when the micelle exists in excess.

In the present work, the disruption of multipolymer cholesterol aggregates of the same NaAMPS/Chol-C_5-MA/1-PyMAm terpolymer (Chart 1) by surfactant micelles

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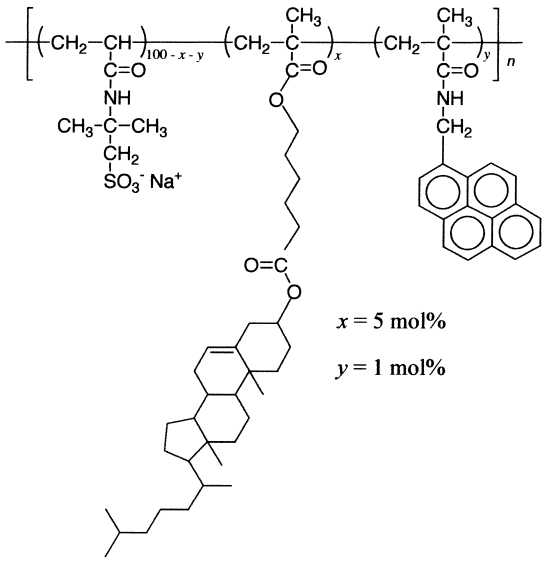

NaAMPS/Chol-C_5-MA/1-PyMAm Terpolymer

Chart 1.

and the formation of polymer–micelle complexes were investigated in detail focusing on the effect of additional electrostatic interaction on the polymer–micelle association using mixed micelles of $C_{12}E_6$ and *n*-hexadecyltrimethylammonium chloride (CTAC). The strength of the polyanion–micelle interaction was systematically varied by changing the micelle charge density (i.e. the molar ratio of the cationic and nonionic surfactants in the mixed micelle).

Recently, we have established a fluorescence technique based on the combination of steady-state and time-dependent fluorescence spectroscopy to investigate the dynamic nature of polyelectrolyte–micelle complexes in aqueous solutions [6,7]. This technique uses a pyrene-labeled polyelectrolyte in conjunction with quencher-carrying mixed micelles. An enhancement of fluorescence quenching arises from interactions between a pyrene-labeled polyanion and quencher-carrying mixed micelles upon increase in micelle charge. In the present work, we applied this fluorescence technique using pyrene labels in the terpolymer in combination with *n*-hexadecylpyridinium chloride (CPC), a quencher for pyrene fluorescence, added to the $C_{12}E_6$/CTAC mixed micelle in small amount. For estimation of the residence time of the mixed micelle on pyrene sites in the polymer, we employed a kinetic model proposed previously [6].

2. Experimental section

2.1. Materials

The pyrene-labeled cholesterol-bearing polyanion (Chart 1), the terpolymer of NaAMPS, Chol-C_5-MA, and 1-PyMAm, is the same as that used in our previous work [4]. The contents of the Chol-C_5-MA unit and the pyrene label are 5 and 1 mol%, respectively [4]. $C_{12}E_6$ (Nikko Chemical) was used without further purification. CTAC and CPC (both from Wako Pure Chemicals) were recrystallized twice from methanol. Milli-Q water was used for all the measurements.

2.2. Quasielastic light scattering

QELS measurements were carried out at a fixed scattering angle of 90° with an Otsuka Electronics Photal DLS-7000DL light scattering spectrometer equipped with a 75.0 mW Ar laser lamp. An ALV-5000E digital multi-τ correlator was employed for data collection. All QELS measurements were performed at 25 ± 1 °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 diffusion coefficients (D) of samples. D was converted into the hydrodynamic radius (R_h) using the Stokes–Einstein equation, $R_h = k_B T/(6\pi\eta D)$, where k_B is the Boltzmann constant, T is the absolute temperature, and η is the solvent viscosity. A 50 mM CTAC solution was added to a mixture of 1.5 g l^{-1} polymer and 10 mM $C_{12}E_6$ at [NaCl] = 0.2 M. The mole fraction of the cationic surfactant in the mixed micelle is defined as Y, where $Y = [\text{CTAC}]/([\text{CTAC}] + [C_{12}E_6])$.

2.3. Turbidimetric titration

Turbidimetric titration [8–11] was performed on a JASCO V-520 spectrophotometer at 25 ± 1 °C by adding an aqueous solution of 50 mM CTAC to a 0.2 M NaCl aqueous solution containing 0.05 g l^{-1} polymer and 30 mM $C_{12}E_6$ in a 1 cm path length quartz cuvette. All transmittance ($\%T$) values were corrected by subtracting the turbidity of a polymer-free blank. The blank-corrected turbidity was represented as $(100 - \%T)$ at 420 nm.

2.4. Fluorescence

Steady-state fluorescence spectra for pyrene labels were recorded on a Hitachi F-4500 fluorescence spectrophotometer with excitation at 343 nm at 25 ± 1 °C. To prepare a CPC-containing $C_{12}E_6$ micelle stock solution, a mixture of predetermined concentrations of CPC, $C_{12}E_6$, and NaCl was stirred overnight. For fluorescence quenching measurements at varying Y [6,7], varying amounts of 50 mM CTAC solution was added to a mixture of 0.05 g l^{-1} polymer, 30 mM $C_{12}E_6$, and 0.5 mM CPC at [NaCl] = 0.2 M. The mole fraction of the cationic surfactant in the mixed micelle is defined as Y, where $Y = ([\text{CTAC}] + [\text{CPC}])/([\text{CTAC}] + [\text{CPC}] + [C_{12}E_6])$.

Fluorescence decays were measured by a time-correlated single-photon counting technique using a Horiba NAES 550 system equipped with a flash lamp filled with hydrogen.

Decay curves were analyzed using a conventional deconvolution technique. Sample solutions were the same as those used for the steady-state fluorescence measurements.

To study the interactions between the polymer and CPC-carrying $C_{12}E_6$/CTAC mixed micelles, we employed constant concentrations of 0.5 mM CPC and 30 mM $C_{12}E_6$. Under these conditions, practically all CPC molecules were solubilized in the micelles giving an average number of CPC per micelle of about 5 assuming an aggregation number of 3×10^2 for $C_{12}E_6$ micelle [6,12].

3. Results and discussion

As reported previously, the NaAMPS/Chol-C_5-MA/1-PyMAm terpolymer forms interpolymer aggregates of $R_h \approx 50$ nm [5]. Each aggregate is composed of roughly 50 polymer chains, where ca. 175 cholesterol residues constitute about 10 microdomains, each microdomain consisting of 17–19 cholesterol groups. Upon addition of $C_{12}E_6$ micelles, the aggregates of cholesterol residues dissociate into individual cholesterol species, if not completely, to associate with $C_{12}E_6$ micelles, thus forming polymer–micelle complexes of $R_h \approx 10$ nm [5].

When a solution of CTAC, a cationic surfactant, is added to a solution of the polymer–micelle complex, all CTAC molecules are incorporated into $C_{12}E_6$ micelles [10], and hence additional electrostatic interaction occurs between positively charged $C_{12}E_6$/CTAC mixed micelles and negatively charged sulfonate sites in the terpolymer. Fig. 1 shows distributions of relaxation times in QELS for solutions of the terpolymer and $C_{12}E_6$/CTAC mixed micelles of varying mole fractions of CTAC (i.e. Y). A relaxation time distribution for a micelle-free solution is also presented for comparison. Upon addition of $C_{12}E_6$ micelles ($Y = 0$), the distribution peak top shifts toward shorter relaxation times, corresponding to a decrease in R_h from ca. 50 to 10 nm. When CTAC was added to $Y = 0.02$ or higher, the distribution peak top shifts slightly toward longer relaxation times. At $Y = 0.09$, for example, R_h at the peak top corresponds to ca. 13 nm. These observations indicate that the size of the polymer–micelle complex increases with increasing Y because the number of micelles bound to a polymer chain increases with increasing electrostatic interaction between the terpolymer and micelle [13].

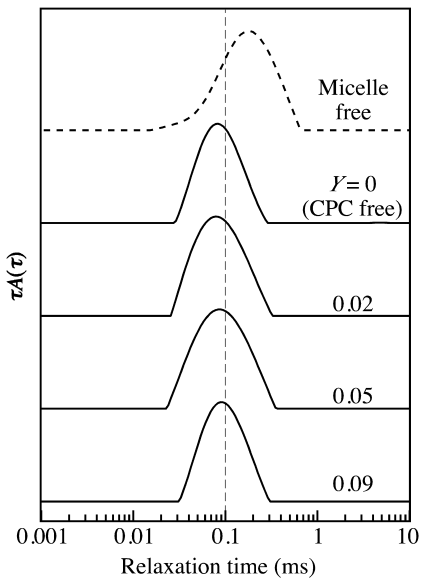

Fig. 1. Distributions of the relaxation times in QELS for different values of Y: $C_p = 1.5$ g l^{-1}, [$C_{12}E_6$] = 10 mM, and [NaCl] = 0.2 M. A distribution for the terpolymer in the absence of micelle is shown for comparison.

As Y was increased further beyond 0.13, solutions became turbid due to macroscopic phase separation (Fig. 2). Generally, the interaction of polyelectrolytes with oppositely charged surfactant micelles are so strong that irreversible macroscopic phase separation occurs [14,15]. However, polyelectrolyte–micelle complexes are soluble in water when the charge of the micelle is sufficiently low [9,11]. In the present case of the cholesterol-bearing polyanion with $C_{12}E_6$/CTAC mixed micelles in 0.2 M NaCl aqueous solutions, the solution is optically clear when $Y < 0.13$ but it becomes abruptly turbid when Y is increased to 0.14, a critical micelle mole fraction of CTAC (Y_p) at which macroscopic phase separation starts to occur (Fig. 2). Therefore, in the present study, we varied Y only in the range $0 \leq Y \leq 0.09$ where polymer–micelle complexes are soluble in water.

The intensity ratio of the third to first vibronic fine structure (I_3/I_1) in pyrene fluorescence spectra is sensitive to the micropolarity of local environments where pyrene exists [16]. It is reported that the I_3/I_1 ratio for pyrene labels of an NaAMPS/1-PyMAm (1 mol%) copolymer in a 0.2 M NaCl aqueous solution in the presence of $C_{12}E_6$/CTAC mixed micelles is 0.60 at $Y < 0.05$ but the I_3/I_1 ratio decreases with increasing Y reaching a minimum value of ca. 0.5 near $Y = Y_p$ [7,13]. Fig. 3 shows the I_3/I_1 ratio for pyrene labels in the NaAMPS/Chol-C_5-MA/1-PyMAm terpolymer in a 0.2 M NaCl aqueous solution in the presence of $C_{12}E_6$/CTAC mixed micelles plotted as a function of Y. Under conditions for this fluorescence measurement, where the

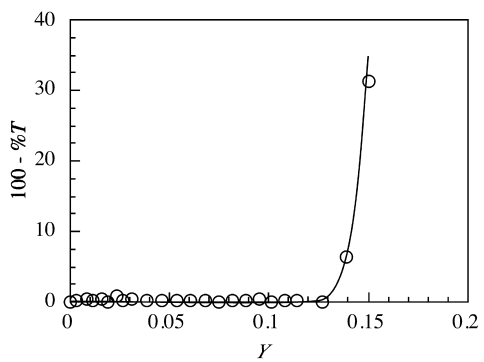

Fig. 2. Turbidity as a function of Y: $C_p = 0.05$ g l^{-1}, [$C_{12}E_6$] = 30 mM, [CPC] = 0.5 mM, and [NaCl] = 0.2 M.

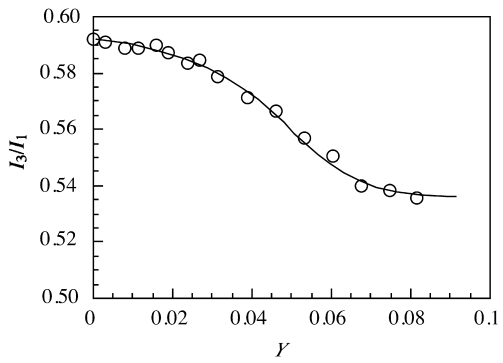

Fig. 3. I_3/I_1 ratio for pyrene labels in the terpolymer as a function of Y in the absence of CPC: $C_p = 0.05$ g l^{-1}, [$C_{12}E_6$] = 30 mM, and [NaCl] = 0.2 M.

micelle is in large excess (ca. 1500 times) over the cholesterol residue in molar concentration ($C_p = 0.05$ g l^{-1}, [$C_{12}E_6$] = 30 mM ($Y = 0$)), all cholesterol residues are expected to be bound to $C_{12}E_6$ micelles in the polymer–micelle complex [5]. The I_3/I_1 ratio of ca. 0.59 at $Y = 0$ in Fig. 3 suggests that essentially all pyrene labels are exposed to the aqueous phase and not bound to micelles. The I_3/I_1 ratio, however, decreases with increasing Y, reaching a smaller value of ca. 0.54 near $Y = Y_p$. These results suggest that pyrene labels in the cholesterol-bearing polymer are bound to $C_{12}E_6$/CTAC mixed micelles only when electrostatic interactions occur between the polymer charge and mixed micelles.

Pyrene fluorescence is known to be quenched by CPC [17]. Fig. 4 shows fluorescence spectra for pyrene labels in the cholesterol-bearing polymer in a 0.2 M NaCl aqueous solution in the presence of 30 mM $C_{12}E_6$ with and without 0.5 mM CPC at varying Y. The fluorescence intensity strongly decreases in the presence of the CPC-carrying mixed micelles, suggesting that pyrene labels are bound to the mixed micelle when the polymer and micelles interact electrostatically. Furthermore, the fluorescence intensity decreases with increasing Y, indicating that the electrostatic

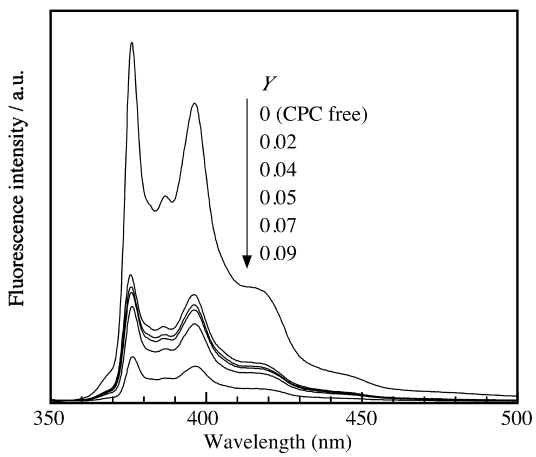

Fig. 4. Steady-state fluorescence spectra for the polymer in the presence of $C_{12}E_6$ micelles ($Y = 0$) and CPC-carrying $C_{12}E_6$/CTAC mixed micelles at varying Y: $C_p = 0.05$ g l^{-1}, [$C_{12}E_6$] = 30 mM, [CPC] = 0.5 mM, and [NaCl] = 0.2 M.

Table 1
Ratios of I/I_0 in steady-state fluorescence spectra and decay parameters for fluorescence of pyrene labels in the absence and presence of micelles of varying Y

Micelle	Y	I/I_0	τ_i (ns)/α_i[a]	
None			187/0.82	346/0.18
$C_{12}E_6$	0.00		187/0.95	279/0.05
$C_{12}E_6$/CTAC	0.09		187/0.03	212/0.97
$C_{12}E_6$/CTAC/CPC	0.02	0.35	56/0.23	176/0.77
$C_{12}E_6$/CTAC/CPC	0.04	0.33	56/0.38	173/0.62
$C_{12}E_6$/CTAC/CPC	0.05	0.31	54/0.43	170/0.57
$C_{12}E_6$/CTAC/CPC	0.07	0.28	52/0.53	169/0.47
$C_{12}E_6$/CTAC/CPC	0.09	0.27	54/0.56	159/0.44

[a] Fitting function: $I(t) = I(0)\sum\alpha_i \exp(-t/\tau_i)$.

interaction enhances the binding of micelles to pyrene sites [6]. In Table 1 are listed the normalized fluorescence intensity ratios, I/I_0, where I is the fluorescence intensity in the presence of CPC-carrying $C_{12}E_6$/CTAC mixed micelles at varying Y, and I_0 is the fluorescence intensity in the presence of CPC-free $C_{12}E_6$ micelles at $Y = 0$. It is important to note that even at $Y = 0.02$, fluorescence is already quenched to an I/I_0 ratio of 0.35. The I/I_0 ratio further decreases to 0.27 at $Y = 0.09$ due to an increased electrostatic interaction between the polyanion and the micelle. These observations clearly indicate that interactions between pyrene labels and mixed micelles exist even at very low Y. Hence, the fluorescence quenching was found to be much more sensitive to such interactions than the I_3/I_1 ratio and QELS.

Fig. 5 compares fluorescence decay profiles for the terpolymer in the absence and presence of micelles in 0.2 M NaCl aqueous solutions. The terpolymer itself (in the

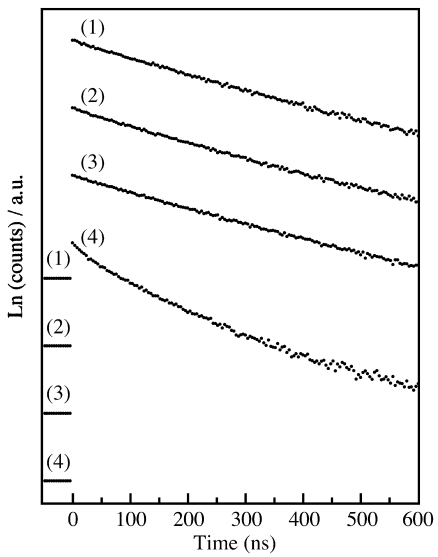

Fig. 5. Fluorescence decays for the terpolymer in the absence of micelles (1) and in the presence of $C_{12}E_6$ micelles ($Y = 0$) (2), $C_{12}E_6$/CTAC mixed micelles with $Y = 0.09$ (3), and CPC-carrying $C_{12}E_6$/CTAC mixed micelles with $Y = 0.09$ (4): $C_p = 0.05$ g l^{-1}, [$C_{12}E_6$] = 30 mM, [CPC] = 0.5 mM, and [NaCl] = 0.2 M.

absence of micelles) shows a double-exponential decay with fluorescence lifetimes of 187 ns ($\alpha_1 = 0.82$) and 346 ns ($\alpha_2 = 0.18$) (Table 1). The shorter lifetime of 187 ns is close to a lifetime of 185 ns reported for the NaAMPS/1-PyMAm (1 mol%) copolymer observed in a 0.2 M NaCl aqueous solution [6]. Therefore, it should be reasonable to consider that ca. 82% of pyrene labels in the cholesterol-bearing terpolymer are exposed to the aqueous phase and ca. 18% of pyrene labels are incorporated into hydrophobic microdomains formed by cholesterol residues.

Since the I_3/I_1 ratio indicated that essentially all pyrene labels were exposed to the aqueous phase in the presence of $C_{12}E_6$ micelles ($Y = 0$), we attempted to fit fluorescence decay data to a single-exponential function with a lifetime constrained to 187 ns. The fits were found to be reasonably good but a best-fit was obtained with a double-exponential function with lifetimes of 187 ns ($\alpha_1 = 0.95$) (constrained) and 279 ns ($\alpha_2 = 0.05$). The presence of a small fraction of a longer lifetime component is indicative of a weak interaction of pyrene sites with $C_{12}E_6$ micelles at $Y = 0$. If the residence time of the micelle-bound to a pyrene site is comparable to or shorter than the fluorescence lifetime of pyrene, fluorescence decay should be a single-exponential with a lifetime slightly longer than that for pyrene exposed to the aqueous phase. In fact, this is not the case. Therefore, the residence time of the micelle at the pyrene site should be sufficiently longer than the fluorescence lifetime of pyrene. Thus, it is inferred that only ca. 5% of pyrene labels are bound to the $C_{12}E_6$ micelles at $Y = 0$. It is reasonable to consider that this small fraction of micelle-bound pyrene labels does not reflect on the I_3/I_1 ratio (Fig. 3) simply because the I_3/I_1 ratio is less sensitive than fluorescence decay.

In the presence of $C_{12}E_6$/CTAC mixed micelles of $Y = 0.09$ (CPC-free), fluorescence decay data were best-fitted to a double-exponential function with lifetimes of 187 ns ($\alpha_1 = 0.03$) (constrained) and 212 ns ($\alpha_2 = 0.97$). A very small fraction of the 187 ns component was still observed together with a lifetime significantly longer than that observed for a long lifetime component at $Y = 0$ (Fig. 5 and Table 1). The decay data at $Y = 0.09$ indicate that almost all pyrene sites are in association with $C_{12}E_6$/CTAC mixed micelles while a very small fraction of the pyrene exists in the bulk aqueous phase.

Upon addition of CPC-carrying $C_{12}E_6$/CTAC mixed micelles, efficient quenching of pyrene fluorescence occurs, as can be seen in Fig. 5 for $Y = 0.09$. The decay data were best-fitted to a double-exponential function. In Table 1 are listed the lifetimes and fractions for the fast and slow decay components observed at varying Y. The short lifetime values are more or less the same, ranging 52–56 ns, independent of Y while the long lifetime decreases gradually with increasing Y. However, the fraction of the short lifetime component increases significantly with increasing Y while that of the long lifetime component decreases.

We previously proposed a kinetic model to quantitatively explain fluorescence quenching for polymer-bound fluorescence labels by quencher-carrying micelles [6]. This kinetic model assumes an association equilibrium for the binding of the micelle to the label at $Y < Y_p$. Appling this kinetic model, the association equilibrium can be expressed as

$$\mathrm{Py} \underset{k_{-1}}{\overset{k_1[\mathrm{M}]}{\rightleftharpoons}} \mathrm{Py{\cdot}M} \tag{1}$$

where Py denotes the pyrene label in the terpolymer, M the CPC-carrying $C_{12}E_6$/CTAC mixed micelle, Py·M the pyrene–micelle associate (complex), and k_1 and k_{-1} the association and dissociation rate constants, respectively. When the equilibrium system is irradiated with UV light, pyrene labels in both free and micelle-bound states are photoexcited (Eqs. (2) and (3), respectively).

$$\mathrm{Py} \underset{\tau_0^{-1}}{\overset{h\nu}{\rightleftharpoons}} \mathrm{Py^*} \tag{2}$$

$$\mathrm{Py{\bullet}M} \underset{\tau_0^{-1}+k_q}{\overset{h\nu}{\rightleftharpoons}} \mathrm{Py^*{\bullet}M} \tag{3}$$

Photoexcited free and micelle-bound pyrene labels deactivate with rate constants represented in Eqs. (2) and (3), where τ_0 is the fluorescence lifetime of free pyrene in the bulk aqueous phase and k_q the first-order rate constant for fluorescence quenching within the pyrene–micelle associate. As confirmed experimentally in an earlier work [6], all CPC molecules are completely solubilized in $C_{12}E_6$/CTAC mixed micelles. Thus, the only quenching pass for singlet-excited free pyrene is to encounter with a CPC-carrying $C_{12}E_6$/CTAC mixed micelle to associate (Eq. (4)).

$$\mathrm{Py^*} \underset{k_{-1}}{\overset{k_1[\mathrm{M}]}{\rightleftharpoons}} \mathrm{Py^*{\cdot}M} \tag{4}$$

Here, we assume that the rate of deactivation of photoexcited pyrene in the pyrene–micelle associate is much faster than the rate of dissociation of the micelle from the pyrene site, that is $\tau_0^{-1} + k_q \gg k_{-1}$.

According to this kinetic model, the total concentrations of the free and micelle-bound pyrene at time t are given by a double-exponential function with lifetimes τ_1 and τ_2 when the system is photoexcited by a light pulse at time $t = 0$ [6]. The reciprocals of these lifetimes are given by Eqs. (5) and (6):

$$(1/\tau_1) = (1/\tau_0) + k_q \tag{5}$$

$$(1/\tau_2) = (1/\tau_0) + k_1[\mathrm{M}] \tag{6}$$

On the other hand, the ratio of fluorescence quantum efficiencies in the presence and absence of the quencher-carrying micelle (Φ/Φ_0) is given by [6]

$$\Phi/\Phi_0 = \tau_1/\tau_0 + (\tau_2/\tau_0)(1 - \tau_1/\tau_0)\{1/(1 + K[\mathrm{M}])\} \tag{7}$$

On the basis of steady-state fluorescence data (Φ/Φ_0) and fluorescence decay data (τ_0, τ_1, and τ_2), one can calculate $k_1[\mathrm{M}]$ and $K[\mathrm{M}]$ from Eqs. (6) and (7), respectively, from

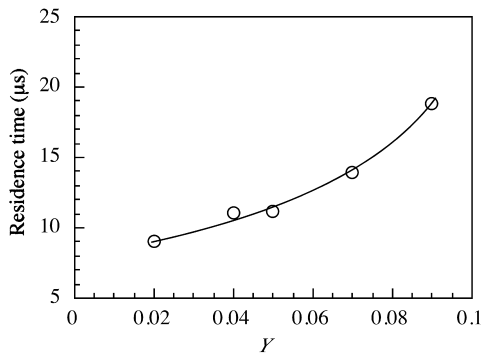

Fig. 6. Residence times as a function of Y: $C_p = 0.05\ g\ l^{-1}$, $[C_{12}E_6] = 30$ mM, [CPC] = 0.5 mM, and [NaCl] = 0.2 M.

which one can calculate the residence time ($1/k_{-1} = K[M]/k_1[M]$) of the micelle in the pyrene–micelle associate. We calculated the values of $1/k_{-1}$ using data listed in Table 1 at varying Y. The residence time depends on Y, as can be seen in Fig. 6. For example, the residence time is ca. 9 μs at $Y = 0.02$ but it increases to ca. 19 μs when Y is increased to 0.09.

In the case of the interaction of the NaAMPS/1-PyMAm (1 mol%) copolymer with CPC-carrying $C_{12}E_6$/CTAC mixed micelles in 0.2 M NaCl aqueous solutions, residence times were estimated to be 1–2 μs, as reported previously [6]. The residence time for the present pyrene-labeled cholesterol-bearing polyanion is about 10 times longer than those for the cholesterol-free polyanion. The residence time of the micelle on the pyrene site of the polymer is governed by an interplay of the electrostatic and hydrophobic interactions of the micelle with polymer charge and cholesterol sites, respectively.

Fig. 7 shows a conceptual model for the complex formation between the pyrene-labeled cholesterol-bearing polyanion and $C_{12}E_6$ micelles with and without CTAC. In the absence of CTAC, polymer–micelle complexes are formed only through the binding of micelles to cholesterol sites, although a weak dynamic interaction between the micelle and pyrene labels exists. Upon addition of CTAC to $C_{12}E_6$ micelles, the mixed micelle interacts electrostatically with polyanion charges. Thus, an interplay of hydrophobic and electrostatic interactions between the micelle and polymer strengthens the polymer–micelle interaction. In the polymer complex with $C_{12}E_6$ micelles ($Y = 0$), the micelles are bound to cholesterol sites whereas pyrene sites are mostly exposed to the aqueous phase. In the complex with $C_{12}E_6$/CTAC mixed micelles ($Y > 0$), however, pyrene sites are incorporated into micelles because of the additional electrostatic interaction conjoint with hydrophobic interaction.

4. Conclusions

Multipolymer aggregates of a pyrene-labeled cholesterol-bearing polyanion were shown to be completely disrupted into polymer–micelle complexes when $C_{12}E_6$ micelles are added to aqueous solutions of the polymer. Steady-state and time-dependent fluorescence of pyrene labels suggested that the nonionic $C_{12}E_6$ micelles bind

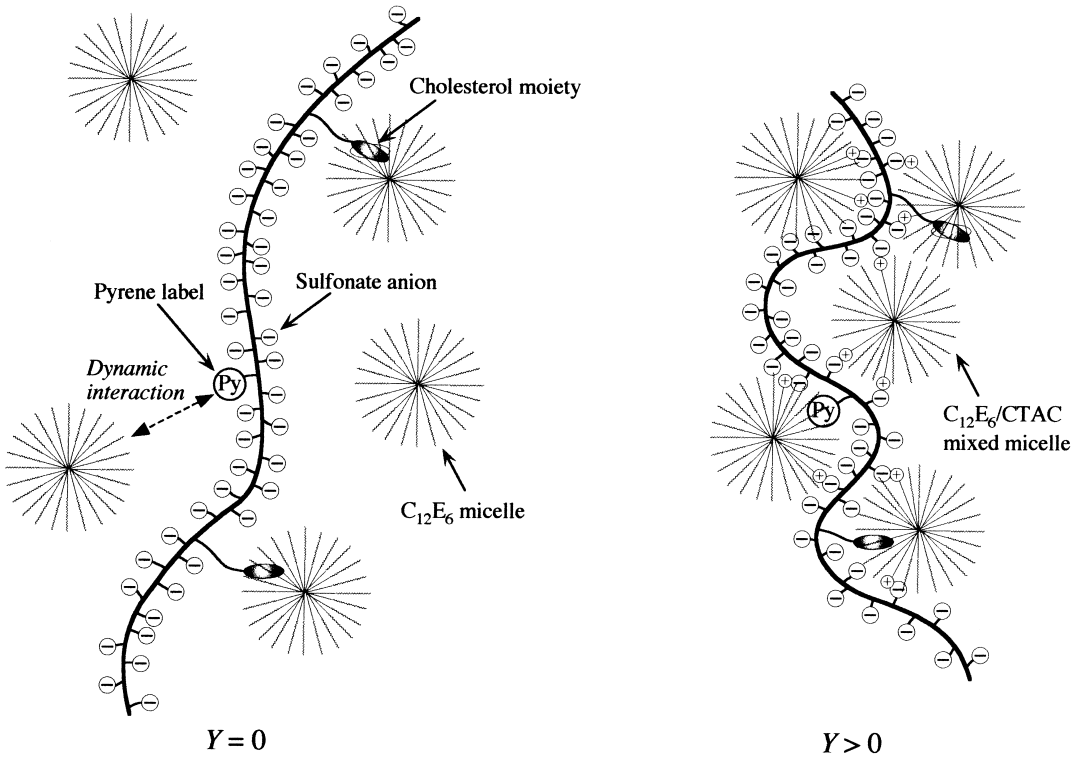

Fig. 7. Conceptual illustration of a hypothetical model for polymer–micelle complexes.

predominantly to cholesterol sites while pyrene sites weakly interact with the nonionic micelle. When mixed micelles of $C_{12}E_6$/CTAC were added to a polymer solution, the positively charged mixed micelle binds to polymer charges in addition to cholesterol sites, leading to a strong enhancement of the binding of the micelle to pyrene sites. Interactions between pyrene labels and $C_{12}E_6$/CTAC mixed micelles were investigated by steady-state and time-dependent fluorescence quenching techniques using CPC, a quencher, added to the $C_{12}E_6$/CTAC mixed micelle. Fluorescence quenching data were analyzed using a kinetic model proposed previously. The residence time of the mixed micelle on the pyrene site in the polymer was estimated as a function of the mole fraction of CTAC (Y). It was found that the residence time of the mixed micelle on the pyrene site increases with increasing Y. A long residence time of ca. 19 μs was observed at $Y = 0.09$ as a result of an interplay of hydrophobic interaction of the micelle with cholesterol sites and electrostatic interaction of micelle charge with polymer charge.

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