Fluorescence Studies of pH-Responsive Unimolecular Micelles Formed from Amphiphilic Polysulfonates Possessing Long-Chain Alkyl Carboxyl Pendants

Shin-ichi Yusa,*,† Akihisa Sakakibara,† Tohei Yamamoto,† and Yotaro Morishima‡

Department of Applied Chemistry, Himeji Institute of Technology, 2167 Shosha,
Himeji 671-2201, Japan, and Department of Macromolecular Science, Graduate School of Science,
Osaka University, Toyonaka, Osaka 560-0043, Japan

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ABSTRACT: Reversible pH-induced changes in self-associative behavior in water of random copolymers of 50 mol % sodium 2-(acrylamido)-2-methylpropanesulfonate (NaAMPS) and 50 mol % 6-acrylamidohexanoic acid (AmH), 8-acrylamidooctanoic acid (AmO), or 11-acrylamidoundecanoic acid (AmU) were investigated by various fluorescence techniques with use of fluorescence-labeled polymers. Fluorescence intensities, lifetimes, depolarization, and quenching for the polymers labeled with naphthalene (4 mol %) indicated that all the polymers adopt an open-chain conformation at basic pHs whereas NaAMPS/AmO and NaAMPS/AmU polymers collapsed into a compact conformation upon a decrease in pH to an acidic region. No such pH-induced conformational change was found for NaAMPS/AmH polymers. Nonradiative energy transfer (NRET) for NaAMPS/AmO and NaAMPS/AmU polymers doubly labeled with naphthalene (4 mol %) and pyrene (1 mol %) indicated a strong tendency for intrapolymer hydrophobic self-association, the polymers with pendent octanoic acid and undecanoic acid groups forming unimolecular micelles (unimer micelles) at acidic pHs. The mean aggregation numbers (N_{agg}) of these alkyl carboxylic acid residues in the unimer micelles were determined to be 86 and 74, respectively. These N_{agg} values were fairly close to the numbers of the AmO and AmU units per polymer chain (85 and 69, respectively), indicative of the formation of unimer micelles. The unimer micelles were disrupted into an open-chain conformation when pH was increased to a basic pH, thus exhibiting reversible pH-induced formation and disruption behavior.

Introduction

In recent years, much attention is being devoted to self-associating water-soluble hydrophobically modified polymers in both academic and industrial communities for their scientific and practical interest.[1,2] Self-association of polymer-bound hydrophobes occurs either within a single polymer chain or between different polymer chains depending primarily on macromolecular architecture. For example, a random copolymer of sodium 2-(acrylamido)-2-methylpropanesulfonate (NaAMPS) and *N*-dodecylmethacrylamide of an equimolar composition has a strong preference for intrapolymer self-association forming unimolecular micelles (unimer micelles) with a defined size even in concentrated aqueous solutions.[3−5] A characteristic of the unimer micelle is an ability to incorporate hydrophobic small molecules into its hydrophobic microdomains, leading to “nanoencapsulation” of small molecules. This encapsulation by the unimer micelle is different from the solubilization of small molecules by conventional surfactant micelles in that the unimer micelles are much less dynamic in nature than surfactant micelles.[6−9]

Association structures and hence solution properties of some amphiphilic polyelectrolytes are sensitive to changes in external conditions such as ionic strength (added salt), shear stress, temperature, pH, and the nature of the solvent.[10−12] Such stimuli-responsive polymer systems have attracted considerable interest because of their potential ability to capture and deliver materials, which may find pharmaceutical or environmental application.[13] In an earlier paper, we reported on pH-responsive self-association in water of random copolymers of NaAMPS and 11-acrylamidoundecanoic acid (AmU) of an equimolar composition (NaAMPS/AmU).[14] At basic pHs, electrostatic repulsion between pendent undecanoate anions causes the polymer chain to adopt an open-chain conformation. At acidic pHs, on the other hand, protonated pendent undecanoic acid groups behave as hydrophobes; intrapolymer association of the pendent undecanoic acid groups causes the polymer chain to collapse into a unimer micelle, thus showing a pH-induced conformational change. The pH-induced change from an open chain to a unimer micelle and vice versa occurs rapidly (at least within 300 s) within a narrow range of pH. This pH-responsive behavior is expected to depend on the hydrophobic and hydrophilic balance of the pendent alkyl carboxyl group.

The present work focuses on the effect of the length of the alkyl chain in the pendent alkyl carboxyl group on the pH-responsive behavior. In this work, we synthesized a series of copolymers of NaAMPS and 6-acrylamidohexanoic acid (AmH), 8-acrylamidooctanoic acid (AmO), or AmU of nearly equimolar compositions with or without fluorescence labels (Chart 1). The fluorescence labeling was performed by copolymerization using naphthalene- and/or pyrene-substituted methacrylamide monomers together with NaAMPS and AmH, AmO, or AmU. For characterization of pH-dependent self-association behavior, various fluorescence techniques were employed, including fluorescence intensities, lifetimes, depolarization, quenching, and nonradi-

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[†] Himeji Institute of Technology.

[‡] Osaka University.

* To whom correspondence should be addressed. E-mail: yusa@chem.eng.himeji-tech.ac.jp.

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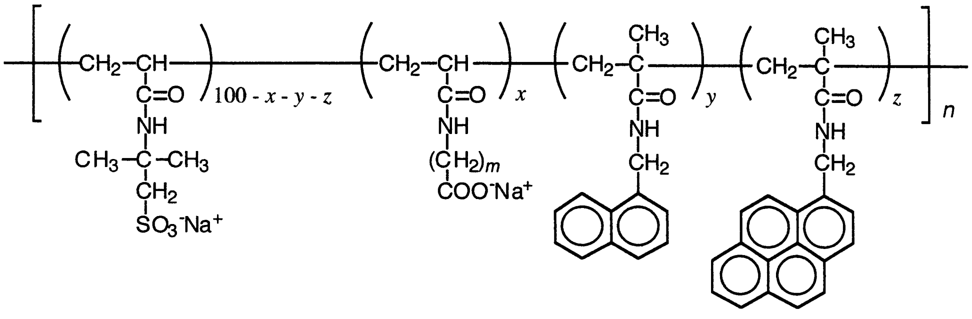
Chart 1. Structures of Polymers



NaAMPS polymers
$x = 0 \quad y = 0 \quad z = 0$
$x = 0 \quad y = 4 \quad z = 0$
$x = 0 \quad y = 0 \quad z = 1$
$x = 0 \quad y = 4 \quad z = 1$

NaAMPS/AmH polymers
$m = 5 \quad x = 50 \quad y = 0 \quad z = 0$
$m = 5 \quad x = 46 \quad y = 4 \quad z = 0$
$m = 5 \quad x = 49 \quad y = 0 \quad z = 1$
$m = 5 \quad x = 45 \quad y = 4 \quad z = 1$

NaAMPS/AmO polymers
$m = 7 \quad x = 50 \quad y = 0 \quad z = 0$
$m = 7 \quad x = 46 \quad y = 4 \quad z = 0$
$m = 7 \quad x = 49 \quad y = 0 \quad z = 1$
$m = 7 \quad x = 45 \quad y = 4 \quad z = 1$

NaAMPS/AmU polymers
$m = 10 \quad x = 50 \quad y = 0 \quad z = 0$
$m = 10 \quad x = 46 \quad y = 4 \quad z = 0$
$m = 10 \quad x = 49 \quad y = 0 \quad z = 1$
$m = 10 \quad x = 45 \quad y = 4 \quad z = 1$

ative energy transfer (NRET). A time-resolved fluorescence quenching (TRFQ) technique was used to estimate mean aggregation numbers of the alkyl carboxylic acid residues in hydrophobic microdomains.

Experimental Section

Monomers. 6-Acrylamidohexanoic acid and 11-acrylamidoundecanoic acid were synthesized according to the method reported by Shibaev.[15] Sodium 6-acrylamidohexanoate (AmH) and sodium 11-acrylamidoundecanoate (AmU) were prepared by neutralization of the respective monomers in the acid form with equimolar sodium hydroxide in methanol followed by precipitation with excess ether. *N*-(1-Pyrenylmethyl)methacrylamide (PyMAm)[8] and *N*-(1-naphthylmethyl)methacrylamide (NpMAm)[16] were prepared as reported previously.

Sodium 8-acrylamidooctanoate (AmO) was synthesized as follows: 8-Aminooctanoic acid (15.0 g, 94.2 mmol) was dissolved in an aqueous solution (1 L) of NaOH (45.0 g, 1.13 mol) together with a small amount of 2,6-di-*tert*-butylcresol. Acryloyl chloride (90.5 g, 552 mmol) was added to this solution at 0 °C over a period of 30 min. After stirring for 3 h, the reaction mixture was acidified with HCl to pH 3. Precipitates were collected by filtration and dissolved in chloroform. The solution was washed with a dilute HCl aqueous solution (pH 2−3) and then with pure water. The chloroform solution was dried over anhydrous Na_2SO_4. Chloroform was removed by evaporation, and the crude product was recrystallized from a mixture of acetone and petroleum ether. 8-Acrylamidooctanoic acid obtained was neutralized with equimolar NaOH in methanol followed by precipitation of the salt with ether; yield 18.3 g (91.1%). ^1H NMR (500 MHz, D_2O): $\delta = 1.28-1.32$ (m, 6H, CH_2), 1.52−1.58 (m, 4H, CH_2), 2.19 (t, 2H, CH_2), 3.25 (t, 2H, CH_2), 5.74−5.79 (m, 1H, vinyl CH), 6.16−6.29 (m, 2H, vinyl CH). ^{13}C NMR (DEPT) (125 MHz, D_2O): $\delta = 25.80$ (CH_2), 26.07 (CH_2), 28.25 (CH_2), 28.28 (CH_2), 28.68 (CH_2), 37.52 (CH_2), 39.48 (CH_2), 126.92 (CH_2), 130.13 (CH), 168.20 (quaternary), 183.78 (quaternary).

Reagents. 2,2′-Azobis(isobutyronitrile) (AIBN), pyrene, and 3,4′-dimethylbenzophenone (DMBP) were recrystallized from methanol. Methanol was dried over 4 Å molecular sieves and distilled. Water was purified with a Millipore Milli-Q system. Other reagents were used as received.

Preparation of Polymers. Copolymerizations of NaAMPS with AmH, AmO, or AmU were carried out in homogeneous solutions in methanol in the presence of AIBN at 60 °C for 12 h under vacuum. Copolymers obtained were purified by reprecipitation from a methanol solution into excess ether. The copolymers were then dissolved in water, and the aqueous solutions were dialyzed against a dilute NaOH aqueous solution (pH 8) for 1 week. The copolymers in the form of sodium sulfonate and sodium carboxylate were recovered by a freeze-drying technique. Copolymer compositions were determined from the intensity ratio of ^1H NMR resonance bands associated with the methylene protons in the NaAMPS unit (3.3 ppm) and the methylene protons neighboring the amide bond in the AmH, AmO, and AmU units (ca. 2.8 ppm) in D_2O at 85 °C. Terpolymers of 50 mol % NaAMPS, AmH (or AmO or AmU), and 4 mol % NpMAm (or 1 mol % PyMAm), reference copolymer of NaAMPS and 4 mol % NpMAm (or 1 mol % PyMAm), and polymers labeled doubly with naphthalene (4 mol %) and pyrene (1 mol %) (Chart 1) were prepared and purified in a manner similar to the case of the nonlabeled copolymers described above. The contents of naphthalene and pyrene labels were determined by UV absorption spectroscopy. Apparent weight-average molecular weights (M_w) for all the polymers, estimated by gel-permeation chromatography (GPC), were in the range $(2.6-9.4) \times 10^4$.

Measurements. Gel-Permeation Chromatography (GPC). GPC analysis was performed with a Tosoh HLC-8020 equipped with a Tosoh RI-8020 refractive index detector and two TSKgel *a*-M columns (Tosoh) using an *N*,*N*-dimethylformamide (DMF)/water (50/50, v/v) mixture containing 50 mM LiBr as eluent at a flow rate of 1.0 mL/min. Molecular weights of the copolymers were calibrated with sodium polystyrenesulfonate standard samples.

Absorption and Steady-State Fluorescence Spectra. UV−vis absorption spectra were recorded on a Hitachi U-3000 spectrophotometer. Steady-state fluorescence spectra were recorded on a Shimadzu RF-550 fluorescence spectrophotometer. Sample solutions containing naphthalene-labeled polymers were excited at 290 nm. For the preparation of sample solutions, polymer samples were dissolved in water containing 0.1 M NaCl, and pH of the solutions was adjusted by adding a small amount of an aqueous solution of NaOH or HCl of a proper concentration.

Time-Resolved Fluorescence. Fluorescence decay data were collected on a Horiba NAES 550 system equipped with a flash lamp filled with hydrogen. Sample solutions containing naphthalene-labeled polymers were excited at 290 nm using a band-pass filter. Naphthalene fluorescence decay was monitored at >330 nm using a cutoff filter. The decay and response functions were both measured simultaneously. The decay curves were analyzed by an ordinary deconvolution technique.

Fluorescence Depolarization. Fluorescence anisotropy (r) was measured on a Hitachi F-4500 fluorescence spectrophotometer equipped with filter polarizers. Fluorescence spectra for naphthalene labels were obtained by excitation at 290 nm. The r value was calculated from

$$r = \frac{I_{\mathrm{vv}} - I_{\mathrm{vh}}G}{I_{\mathrm{vv}} + 2I_{\mathrm{vh}}G} \tag{1}$$

where I_{vv} and I_{vh} are observed fluorescence intensities measured with parallel and perpendicular orientations to the vertically polarized exciting beam, respectively. G is the factor for instrumental correction (i.e., $G = I_{\mathrm{hv}}/I_{\mathrm{hh}}$).

Fluorescence Quenching with Thallium Ions. Small amounts of a stock solution of thallium nitrate were added to aqueous solutions of naphthalene-labeled polymers. The concentrations of the polymers were adjusted to 1.0 g/L. Fluorescence measurements were performed with excitation at 290 nm.

Nonradiative Energy Transfer (NRET). For interpolymer NRET experiments, a naphthalene-labeled polymer was mixed with the same quantity of a pyrene-labeled polymer in a 0.1 M NaCl aqueous solution. To this mixed solution was added a proper amount of aqueous NaOH or HCl while the concentration ratios of naphthalene and pyrene labels were kept constant. A mixed solution of naphthalene-labeled and pyrene-labeled polymers was excited at 290 nm. Fluorescence spectra were recorded in the wavelength range 300−550 nm. For intrapolymer NRET experiments, a 0.1 M NaCl aqueous solution of a doubly labeled polymer was excited at 290 nm, and fluorescence spectra were recorded in the wavelength range 300−550 nm. The ratio of the intensities of pyrene fluorescence to naphthalene fluorescence ($I_{\mathrm{py}}/I_{\mathrm{Np}}$), which is a measure for an NRET efficiency, was calculated from the fluorescence intensities at 338 and 394 nm for naphthalene and pyrene fluorescence, respectively.

Time-Resolved Fluorescence Quenching (TRFQ). Mean aggregation numbers (N_{agg}) of polymer-bound alkyl carboxylic acid residues were determined using pyrene probes solubilized in hydrophobic microdomains. 3,4′-Dimethylbenzophenone (DMBP) was used as quencher for pyrene fluorescence. In the absence of DMBP, pyrene fluorescence decay was single-exponential. The presence of the quencher affected the fluorescence decay which was best-fitted to the Infelta−Tachiya equation derived form fluorescence quenching in monodisperse micelles assuming the distribution of fluorescence probe molecules over the hydrophobic microdomain is frozen on the time scale of the fluorescence lifetime:[17−19]

$$I(t) = I(0)\exp\left[-\frac{t}{\tau_0} - n\{1 - \exp(-k_{\mathrm{q}}t)\}\right] \tag{2}$$

where $I(t)$ and $I(0)$ are the fluorescence intensities at time t and zero, respectively, following the excitation, τ_0 is the fluorescence lifetime of pyrene in the absence of DMBP, n is the average number of quenchers in the hydrophobic microdomain, and k_{q} is the first-order quenching constant in the microdomain. N_{agg} for the polymers is calculated from n value as[20,21]

$$N_{\mathrm{agg}} = n\,\frac{C_{\mathrm{p}}q_{\mathrm{R}}}{[\mathrm{Q}]} \tag{3}$$

where q_{R} and [Q] are the hydrophobe content of the polymer (in moles of hydrophobic groups per gram of polymer) and the molar quencher concentration, respectively. In this study, we assumed that all hydrophobic groups participate in the event of their aggregation and that all the quencher molecules are completely solubilized in hydrophobic microdomains.

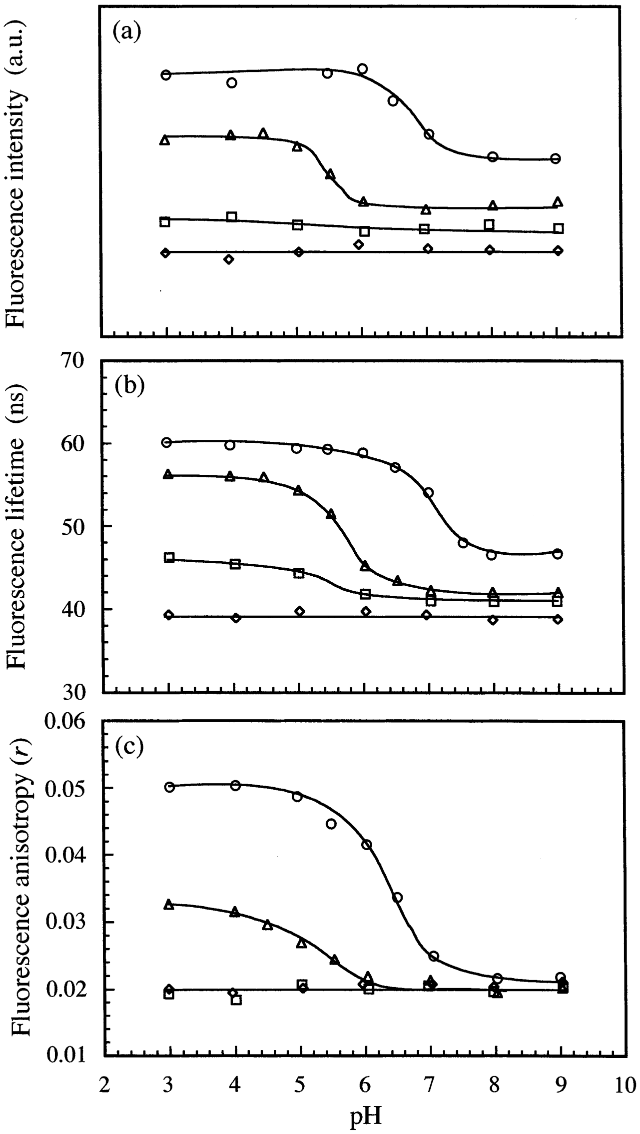


Figure 1. Plots of fluorescence intensity (a), lifetime (b), and anisotropy (r) (c) for the naphthalene-labeled NaAMPS (◇), NaAMPS/AmH (□), NaAMPS/AmO (△), and NaAMPS/AmU polymers (○) as a function of pH at $C_{\mathrm{p}} = 1.0$ g/L in 0.1 M NaCl.

Results and Discussion

Steady-State and Time-Dependent Fluorescence. It is known that the fluorescence intensity[22,23] and lifetime[8,24] of aromatic chromophores increase in less polar media. Figure 1a shows fluorescence intensities at 338 nm in steady-state fluorescence spectra for naphthalene-labeled polymers at a polymer concentration (C_{p}) of 1.0 g/L in 0.1 M NaCl aqueous solutions plotted against pH. The fluorescence intensities for the naphthalene-labeled NaAMPS (reference polymer without alkyl carboxylic acid unit) and NaAMPS/AmH polymers are practically constant independent of pH over the whole range of pH studied ($3 \le \mathrm{pH} \le 9$). In contrast, the fluorescence intensities for the naphthalene-labeled NaAMPS/AmO and NaAMPS/AmU polymers increase with decreasing pH in the ranges $6 < \mathrm{pH} < 4$ and $8 < \mathrm{pH} < 5$, respectively. When pH was decreased from 9 to 3 and subsequently increased back to 9, completely reversible changes without hysteresis in the fluorescence intensity were observed for the NaAMPS/AmO and NaAMPS/AmU polymers.

A similar dependence on pH was observed for fluorescence lifetime, as can be seen from comparison of parts a and b of Figure 1. Fluorescence decay data for the naphthalene-labeled polymers in 0.1 M NaCl aqueous solutions were best-fitted to a single-exponential function ($\chi^2 = 1.2-1.6$). Figure 1b shows fluorescence lifetimes for the naphthalene-labeled polymers plotted as a function of pH. The lifetimes for the naphthalene-labeled NaAMPS and NaAMPS/AmH polymers are almost constant over the entire range of pH investigated, whereas the lifetimes for the naphthalene-labeled NaAMPS/AmO and NaAMPS/AmU polymers increase with decreasing pH in the ranges $6 < \text{pH} < 4$ and $8 < \text{pH} < 5$, respectively. These data indicate that the pendent alkyl carboxyl groups in the NaAMPS/AmO and NaAMPS/AmU polymers are ionized at pHs above 6 and 8, respectively, and the polymer chains adopt an open-chain conformation. On the other hand, the protonated pendent alkyl carboxyl groups in the NaAMPS/AmO and NaAMPS/AmU polymers form hydrophobic microdomains at pHs below 4 and 5, respectively, where naphthalene labels are incorporated.

Since the fluorescence properties of pyrene polymers were confirmed to be similar to those of naphthalene-labeled polymers, we focused only on the naphthalene-labeled polymers in this subsection.

Fluorescence Anisotropy. When naphthalene labels are incorporated into hydrophobic microdomains formed from pendent alkyl carboxyl groups at low pHs, rotational motions of the labels may be restricted.[25,26] To obtain information about the mobility of the naphthalene labels, fluorescence anisotropy (r) was measured at an emission maximum of 338 nm at varying pHs in 0.1 M NaCl (Figure 1c). The r values for the naphthalene-labeled NaAMPS and NaAMPS/AmH polymers are almost constant over the whole range of $3 \leq \text{pH} \leq 9$, whereas the values for the naphthalene-labeled NaAMPS/AmO and NaAMPS/AmU polymers increase with decreasing pH in the ranges $6 < \text{pH} < 4$ and $8 < \text{pH} < 5$, respectively. This tendency is fairly close to those observed for the fluorescence intensity and lifetime. These observations, taken together with the results from the fluorescence intensity and lifetime, indicate that the polymer-bound octanoic and undecanoic acid residues in the respective polymers undergo self-association at acidic pHs forming hydrophobic microdomains in which naphthalene labels are incorporated, and hence the local motions of the labels are restricted. At pH 3, the value of r for the naphthalene-labeled NaAMPS/AmU polymer is larger than that for the NaAMPS/AmO polymer, suggesting more restricted motions in hydrophobic microdomains formed from undecanoic acid residues. It has been reported that the extent of motional restriction for naphthalene labels incorporated in hydrophobic microdomains formed from polymer-bound alkyl residues depends on the length of the alkyl chain.[4,5,27] From comparison of r values for naphthalene-labeled copolymers of NaAMPS with *N*-hexylmethacrylamide, *N*-dodecylmethacrylamide, and *N*-octadecylmethacrylamide in water, it has been established that the extent of the motional restriction or the tightness of hydrophobic microdomain increases with increasing the number of carbon atoms in the polymer-bound alkyl chain.[4,5,27] Thus, it could be concluded that microdomains formed under acidic conditions by self-association of alkyl carboxylic acid residues in the NaAMPS/AmU polymer are tighter than those of the NaAMPS/AmO polymer.

Fluorescence Quenching. Fluorescence of aromatic chromophores is known to be quenched by Tl^+ ions due to an external heavy-atom effect that requires a short-range interaction.[28] In general, fluorescence quenching for polyanion-bound aromatic chromophores by Tl^+ cations occurs efficiently because metal cations are electrostatically concentrated in the vicinity of polyanions.[29,30] Stern−Volmer plots, I_0/I vs Tl^+ concentration, where I_0 and I are the fluorescence intensities for a polyanion-bound aromatic chromophore in the absence and presence of Tl^+ ions, exhibit an upward curvature as a result of mixed dynamic and static quenching: dynamic quenching by atmospheric Tl^+ ions and static quenching by condensed Tl^+ ions.[31] However, when the chromophores are incorporated in hydrophobic microdomains of amphiphilic polyanions, fluorescence quenching is suppressed because the chromophores are protected from the access of Tl^+ cations. Therefore, fluorescence quenching by Tl^+ ions provides an opportunity to investigate self-association behavior of amphiphilic polyanions labeled with an aromatic chromophore.

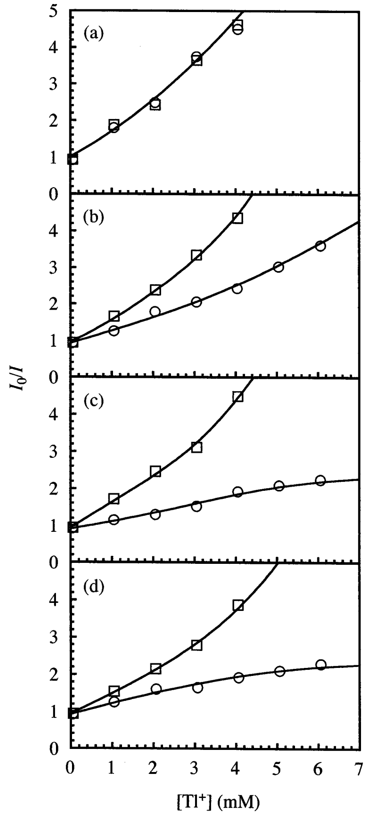


Figure 2. Stern−Volmer plots for the fluorescence quenching by thallium nitrate for the naphthalene-labeled NaAMPS (a), NaAMPS/AmH (b), NaAMPS/AmO (c), and NaAMPS/AmU polymers (d) at $C_p = 1.0$ g/L in 0.1 M NaCl at pH 3 (○) and 9 (□).

Figure 2 shows Stern−Volmer plots for naphthalene-labeled polymers in 0.1 M NaCl at pH 3 and 9 using Tl^+ as quencher. In the case of the naphthalene-labeled NaAMPS reference polymer, the Stern−Volmer plots show an upward curvature at pH 3 and 9 (Figure 2a), as expected for polyanion-bound chromopores.[31] For the reference polymer, which adopts an open-chain conformation, some Tl^+ cations are condensed on the polymer while some Tl^+ cations are in an atmospheric interaction with the polymer.[31] Therefore, the quenching occurs via a dynamic mechanism with atmospheric Tl^+ cations and also via a static mechanism with condensed Tl^+ cations, Stern−Volmer plots showing a upward curvature. The efficiency of the fluorescence quenching is independent of pH (Figure 2a) because the reference polymer is fully charged at pH 3 and 9. At pH 9, Stern–Volmer plots for the naphthalene-labeled NaAMPS/AmH, NaAMPS/AmO, and NaAMPS/AmU polymers also show an upward curvature and a high quenching efficiency similar to those for the reference polymer. Therefore, the quenching mechanism and quenching efficiency for all the NaAMPS/AmH, NaAMPS/AmO, and NaAMPS/AmU polymers at pH 9 are practically the same as those for the reference polymer. Thus, it can be concluded that the NaAMPS/AmH, NaAMPS/AmO, and NaAMPS/AmU polymers adopt an open-chain conformation at pH 9 similar to that of the reference polymer. At pH 3, the NaAMPS/AmH polymer exhibits an upward curvature although the efficiency of the quenching at pH 3 is significantly lower than that at pH 9 (Figure 2b). It should be noted that the NaAMPS/AmH polymer shows significantly different behavior in fluorescence quenching at pH 3 compared with that of the NaAMPS reference polymer although these two polymers show similar behavior in other fluorescence properties as discussed in the preceding subsections. This is because fluorescence quenching by Tl^+ is sensitive to the polymer charge density. Given that the AmH content in the NaAMPS/AmH polymer is 46 mol % (Chart 1), the charge density for this polymer at pH 3 is roughly half that of the NaAMPS reference polymer. Therefore, the Tl^+ quenching for the NaAMPS/AmH polymer at pH 3 is significantly less effective than for the reference polymer (Figure 1a,b).

The fluorescence quenching for the NaAMPS/AmO and NaAMPS/AmU polymers exhibits a downward curvature at pH 3, and the quenching efficiency is considerably low. These observations are a manifestation that the naphthalene labels in the NaAMPS/AmO and NaAMPS/AmU polymers are protected from the access of Tl^+ cations at pH 3, indicative of the incorporation of the labels in hydrophobic microdomains formed from protonated pendent alkyl carboxyl residues.

Nonradiative Energy Transfer (NRET). NRET between an energy donor and acceptor covalently attached to the polymer chain is a useful tool to investigate conformational changes based on the fact that the NRET efficiency depends on the distance between an energy donor and acceptor.[32–35] Naphthalene and pyrene are often used for a pair of an energy donor and acceptor because this pair has a reasonably large spectral overlap, and naphthalene can be selectively excited at a wavelength near 290 nm. NRET occurs when naphthalene and pyrene come close to each other within the Förster radius (R_0 = 2.86 nm for transfer from 1-methylnaphthalene to pyrene[36]). Changes in the extent of NRET can be represented by the intensity ratio of pyrene to naphthalene fluorescence (I_{Py}/I_{Np}). An increase in I_{Py}/I_{Np} indicates a decrease in the average distance between naphthalene and pyrene. We applied this technique to the present polymer systems to see whether hydrophobic self-association occurs intra- or intermolecularly. For NRET experiments, we employed polymers singly labeled with naphthalene (4 mol %), singly labeled with pyrene (1 mol %), and doubly labeled with naphthalene (4 mol %) and pyrene (1 mol %) (Chart 1). Naphthalene labels were excited at 290 nm, and fluorescence emissions from the naphthalene and pyrene labels were monitored at 338 and 394 nm, respectively.

In an aqueous mixture of singly labeled polymers, each labeled with naphthalene or pyrene, each polymer chain may exist separately at high pHs because of interpolymer electrostatic repulsions. If self-association of polymer-bound alkyl carboxyl residues occurs within the same polymer chain at low pHs, the polymer would form a unimer micelle, and naphthalene and pyrene labels would be incorporated in different unimer micelles. In such a circumstance, NRET from naphthalene to pyrene labels is unlikely to occur. On the other hand, if the polymer-bound alkyl carboxyl groups associate between different polymer chains, NRET from naphthalene to pyrene labels may occur. In Figure 3a, I_{Py}/I_{Np} ratios for 0.1 M NaCl aqueous solutions of a mixture of naphthalene-labeled and pyrene-labeled polymers, each possessing the same pendent alkyl carboxyl groups, are plotted as a function of pH. I_{Py}/I_{Np} ratios for the singly labeled polymers are small and virtually constant independent of pH. This indicates that the average distances between the naphthalene and pyrene labels for all the polymer mixtures are not close enough for NRET to occur at any pHs. Therefore, it may be concluded that all the polymers exist as a single molecular state at all pHs. Taken together with the observation that hydrophobic microdomains are formed in the case of the NaAMPS/AmO and NaAMPS/AmU polymers at acidic pHs, as discussed in the previous subsection, the NaAMPS/AmO and NaAMPS/AmU polymers may exist as unimer micelles at low pHs due to intrapolymer self-association of the pendent alkyl carboxyl groups.

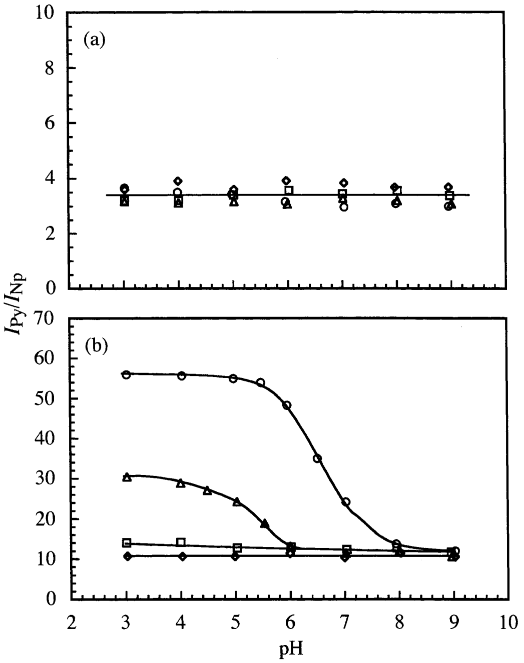


Figure 3. Intensity ratio for fluorescence from pyrene and naphthalene labels (I_{Py}/I_{Np}) as a function of pH for mixed solutions of naphthalene and pyrene singly labeled polymers (a) and for solutions of naphthalene and pyrene doubly labeled polymers (b) at C_p = 1.0 g/L in 0.1 M NaCl with excitation at 290 nm: NaAMPS (◇), NaAMPS/AmH (□), NaAMPS/AmO (△), and NaAMPS/AmU polymers (○).

If self-association of the pendent alkyl carboxyl groups takes place completely within the same polymer chain, there should be a chance for a certain fraction of naphthalene and pyrene labels in the doubly labeled polymers to come close to each other within the Förster radius, and thereby an increased I_{Py}/I_{Np} ratio may be observed. In Figure 3b are plotted I_{Py}/I_{Np} ratios for the doubly labeled polymers as a function of pH in 0.1 M NaCl aqueous solutions. In the case of the doubly labeled NaAMPS and NaAMPS/AmH polymers, I_{Py}/I_{Np} ratios are almost constant over the whole range of pH studied. In contrast, in the case of the NaAMPS/AmO and NaAMPS/AmU polymers, I_{Py}/I_{Np} ratios increase significantly with decreasing pH in the pH ranges $6 < \text{pH} < 4$ and $8 < \text{pH} < 5$, respectively. These observations indicate that a decrease in the polymer size and hence a decrease in the naphthalene/pyrene separation occur with decreasing pH in these pH regimes due to intrapolymer hydrophobic self-association of the protonated alkyl carboxyl residues. This tendency is consistent with those observed for the fluorescence intensities and lifetimes (Figure 1a,b).

Aggregation Number (N_{agg}) of the Polymer-Bound Hydrophobes. If the self-association of the pendent alkyl carboxyl groups occurs completely within the same polymer chain, the number of the AmO or AmU units per polymer chain must be equal to N_{agg} for the NaAMPS/AmO and NaAMPS/AmU polymers at low pHs. We determined N_{agg} of the pendent alkyl carboxyl groups in the nonlabeled polymers (Chart 1) by a TRFQ technique in conjunction with the Infelta's[18] and Tachiya's[19] kinetic models using pyrene and DMBP as a fluorescence probe and quencher, respectively.[37,38] TRFQ studies were performed with solutions containing a constant concentration of molecular pyrene ([pyrene] $= 2.2 \times 10^{-7}$ M) with varied concentrations of the polymers and DMBP. The concentrations of molecular pyrene were calculated from the absorbance at 334 nm using $\epsilon_{334} = 5.4 \times 10^4\ \text{M}^{-1}\ \text{cm}^{-1}$.[39] The pyrene concentration is sufficiently low so that excimer formation can be neglected. In the absence of polymer, a 2.2×10^{-7} M pyrene solution in 0.1 M NaCl at pH 3 shows a single-exponential fluorescence decay with a lifetime of 133 ns. In the presence of the NaAMPS/AmO and NaAMPS/AmU polymers, 0.1 M NaCl aqueous solutions containing 2.2×10^{-7} M pyrene at pH 3 exhibited single-exponential decays with lifetimes of 284 and 348 ns, respectively. These observations indicate that all pyrene molecules were solubilized in hydrophobic microdomains formed by the polymer-bound alkyl carboxyl groups, and no pyrene molecules remain in the bulk water phase.

Figure 4a shows examples of pyrene fluorescence decay data for five different quencher concentrations in the presence of the NaAMPS/AmU polymer at $C_p = 10$ g/L in 0.1 M NaCl aqueous solutions at pH 3. In the absence of the quencher, a 2.2×10^{-7} M pyrene solution in the presence of 10 g/L NaAMPS/AmU polymer shows a single-exponential fluorescence decay with a lifetime of 348 ns. From the best fit of the fluorescence decay data to eq 2, all the parameters in eq 2 can be determined. A N_{agg} value can be calculated from the slope of the linear plot shown in Figure 4b based on eq 3 along with the hydrophobe content (q_R) in the polymer. Thus, we obtained $N_{agg} = 74$ for the NaAMPS/AmU polymer in 0.1 M NaCl at pH 3. Similarly, we obtained $N_{agg} = 86$ for the NaAMPS/AmO polymer in 0.1 M NaCl at pH 3.

From the contents of the AmO and AmU units in the polymers and the number-average molecular weights, the numbers of the AmO and AmU units per polymer chain can be roughly calculated to be 85 and 69, respectively. These numbers are quite close to the N_{agg} values of 86 and 74 for the NaAMPS/AmO and NaAMPS/AmU polymers, respectively. Therefore, we conclude that unimer micelles are formed from the NaAMPS/AmO and NaAMPS/AmU polymers at low pHs due to completely intramolecular hydrophobic associations.

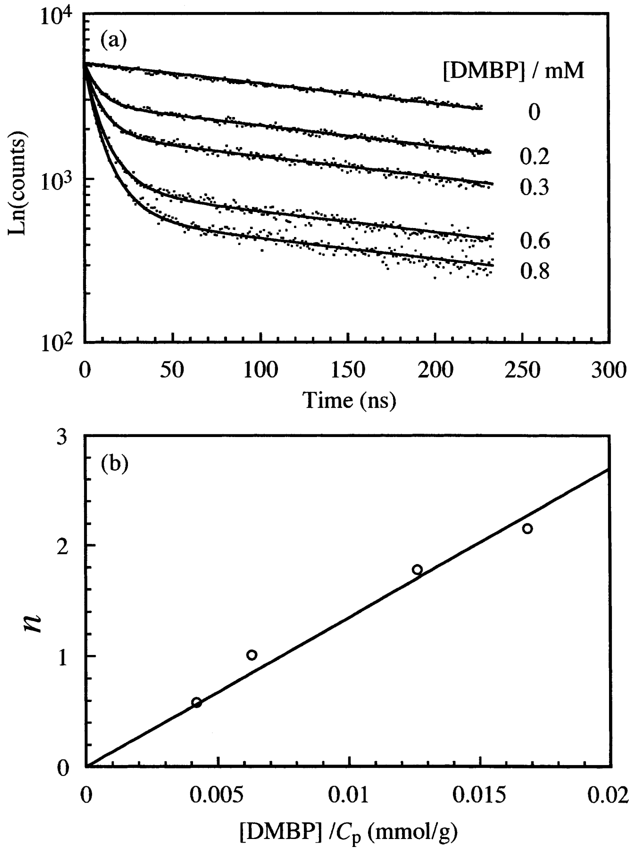


Figure 4. (a) Comparison of fluorescence decay curves for pyrene (2.2×10^{-7} M) solubilized in 0.1 M NaCl aqueous solutions of the NaAMPS/AmU copolymer at $C_p = 10$ g/L in the absence and presence of various concentrations of DMBP at pH 3. The best fit curves from use of a single-exponential and Infelta–Tachiya functions are indicated in the figure. (b) Plot of the fitting parameter n as a function of the ratio of DMBP concentration to the polymer concentration for solutions of the pyrene solubilized in the NaAMPS/AmU copolymer in 0.1 M NaCl at $C_p = 10$ g/L.

Conclusions

Self-association behavior in 0.1 M NaCl aqueous solutions of NaAMPS (50 mol %) copolymers with AmH, AmO, or AmU were investigated as a function of pH by various fluorescence techniques using fluorescence-labeled polymers. The fluorescence intensities, lifetimes, depolarization, and quenching for the naphthalene-labeled NaAMPS/AmO and NaAMPS/AmU polymers indicated that polymer-bound octanoic acid and undecanoic acid residues formed hydrophobic microdomains under acidic conditions, and polymer chains adopt a compact conformation. In contrast, the naphthalene-labeled NaAMPS/AmH polymer adopted an open conformation over the range of pH investigated ($3 \leq \text{pH} \leq 9$). Intrapolymer NRET from naphthalene to pyrene in the NaAMPS/AmO and NaAMPS/AmU polymers doubly labeled with naphthalene and pyrene indicated a strong tendency for intramolecular hydrophobic self-association to form unimer micelles at acidic pHs. N_{agg} values of the pendent octanoic acid and undecanoic acid residues in these unimer micelles under acidic pHs were estimated to be 86 and 74, respectively. These N_{agg} values were fairly close to the number of the AmO and AmU units per polymer chain, supporting that the NaAMPS/AmO and NaAMPS/AmU polymers formed unimer micelles at low pHs. These unimer micelles were easily disrupted into an open-chain conformation when pH was increased to basic pHs.

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