Self-Association in Water of Copolymers of Sodium 2-(Acrylamido)-2-methylpropanesulfonate and *N*-Oleylmethacrylamide Characterized by Fluorescence Quenching

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ABSTRACT: Associative properties of copolymers of sodium 2-(acrylamido)-2-methylpropanesulfonate (AMPS) and *N*-oleylmethacrylamide (OleMAM) with varying OleMAM content (f_{Ole}) in aqueous solutions were characterized by fluorescence quenching using naphthalene-labeled polymers. Fluorescence emitted from naphthalene labels was quenched when the labels were incorporated in hydrophobic microdomains formed from oleyl groups. It was thus possible to monitor the formation of hydrophobic microdomains and hence the self-association behavior of polymers *via* fluorescence quenching. The self-association of oleyl groups occurred progressively from $f_{\text{Ole}} = 3$ to 20 mol%, as in the case of copolymers of AMPS and *N*-octadecylmethacrylamide, a methacrylamide *N*-substituted with a saturated alkyl chain of the same length as the oleyl group. Time-dependent fluorescence and absorption spectral data suggested that the *cis* double bonds in the oleyl groups form a cluster when the polymer-bound oleyl groups associate to form hydrophobic microdomains. Quasielastic light scattering data indicated that the AMPS-OleMAM copolymers had a stronger tendency for intrapolymer association than copolymers of AMPS and *N*-octadecylmethacrylamide.

KEY WORDS Amphiphilic Polyelectrolytes / Self-Association / Hydrophobic Association / Unimolecular Micelles / Sodium 2-(Acrylamido)-2-methylpropanesulfonate / *N*-Oleylmethacrylamide / Fluorescence Quenching /

Water-soluble amphiphilic copolymers of hydrophilic and hydrophobic monomers are used in many commercial products such as paints and coatings, inks, cosmetics, and foodstuff.^{1–6} Water-based polymer systems are attracting interest, in recent years, for potential substitution of conventional organic-based polymer systems for environmental safety reasons. In many applications, associative properties of amphiphilic polymers are utilized as viscosity and rheology modifiers of aqueous fluids.

Hydrophobically-modified polyelectrolytes were extensively studied in the past decades.^{7–25} A characteristic of hydrophobic self-associations in hydrophobically-modified polyelectrolytes is that hydrophobic association competes with electrostatic repulsion, showing a marked tendency that intra- and/or interpolymer hydrophobe association depends strongly on macromolecular architectures. The type of hydrophobes covalently attached to the polymer is an important structural factor of self-association properties of hydrophobically-modified polyelectrolytes in aqueous solution.^{14,17,18} Polymer-bound hydrophobes extensively studied so far include saturated aliphatic hydrocarbons such as hexyl,^{8,13,19} dodecyl,^{13–22,25} octadecyl,^{13,23} cyclododecyl,^{16–18} and adamantyl groups,^{16,17} and aromatic hydrocarbons such as phenyl,²⁵ naphthyl,¹¹ phenanthryl,^{20,26} and pyrenyl groups.²⁰ However, to our knowledge, no papers have been published on the association of amphiphilic polyelectrolytes bearing unsaturated hydrocarbon chains.

The association behavior of surfactant molecules with unsaturated hydrocarbon chains is quite different from that of surfactants with saturated hydrocarbons. *N,N*-

Dimethyldodecylamineoxide forms spherical micelles with a hydrodynamic mean diameter of 6–7 nm at ionic strength 0.05–0.20,²⁷ whereas *N,N*-dimethyloleylamineoxide forms long rodlike micelles (*e.g.*, 575 nm in contour length²⁸) at wide ranges of surfactant concentrations (3–190 mM) and ionic strengths (0–2.0).^{28,29} Sodium hexadecanesulfonate shows a critical micelle concentration (cmc) of 1.37 mM (at 40°C) while sodium 2-hexadecenesulfonate shows a cmc of 0.61 mM.³⁰ Krafft temperature for the former is 57°C in water and for the latter is 36°C.³⁰

Rheological properties of polymethacrylates substituted with saturated and unsaturated hydrocarbons are significantly different.³¹ Amorphous polyoleylmethacrylate exhibits main relaxation that corresponds to a glass transition.³¹ In contrast, main relaxation for polyoctadecylmethacrylate is similar to that of polyoleylmethacrylate only above the melting point, whereas below the melting point, the main relaxation is completely suppressed by side-chain crystallization.³¹ This is because octadecyl pendants of polyoctadecylmethacrylate are crystalline, whereas oleyl pendants of polyoleylmethacrylate are not crystalline due to the *cis* double bond in the alkyl sequence.

Thus, associative properties in water of amphiphilic polyelectrolytes hydrophobically-modified with saturated and unsaturated hydrocarbon chains of the same number of carbon atoms may differ.

Self-associative behavior and hence the formation of hydrophobic microdomains of amphiphilic polymers in water can be characterized by various fluorescence techniques using fluorescence-labeled polymers based on different photophysical and photochemical principles, *e.g.*, change in vibronic structures in steady-state fluores-

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cence spectra and in fluorescence lifetimes depending on micropolarity,^{11–16} change in fluorescence depolarization depending on microviscosity,^{12,13,22,26} protection of fluorescence labels in hydrophobic microdomains from bulk quenchers,^{15–17} and intrapolymer nonradiative energy transfer (NRET) between a donor and acceptor labeled on the same polymer chain.^{15,23}

In a separate experiment, the fluorescence of naphthalene was quenched by oleyl alcohol, an unsaturated fatty alcohol, in methanol. We thus incorporated oleyl groups into a polyelectrolyte together with a small number of naphthalene labels expecting that self-association behavior may be characterized *via* quenching of naphthalene fluorescence by oleyl groups. We prepared terpolymers of sodium 2-(acrylamido)-2-methylpropanesulfonate (AMPS), *N*-oleylmethacrylamide (OleMAM), and *N*-(1-naphthylmethyl)methacrylamide (1NpMAM) (Chart 1), and fluorescence quenching for the naphthalene label by oleyl groups was investigated in aqueous solutions as a function of OleMAM content (f_{Ole}). In addition to fluorescence measurement, quasielastic light scattering (QELS) was measured for characterization of the association behavior of AMPS–OleMAM copolymers. Results were compared with those reported on the copolymer of AMPS and *N*-octadecylmethacrylamide (C₁₈MAM), a copolymer possessing saturated alkyl chains of the same number of carbon atoms as the oleyl group.¹³

EXPERIMENTAL

Monomers

N-Oleilmethacrylamide (OleMAM) was synthesized as follows: To a solution of 5.0 g (19 mmol) of oleylamine (Sigma-Aldrich) in 80 mL of benzene was added 5.0 mL (36 mmol) of triethylamine with vigorous stirring. To this stirred mixture was added dropwise 3.0 mL (31 mmol) of methacryloyl chloride (Tokyo Chemical Industry) in 20 mL of benzene over a period of 10 min under cooling in an ice bath. After stirring for 24 h at room temperature, the reaction mixture was filtered to remove triethylamine hydrochloride. The filtrate was successively washed with aqueous 1 M HCl, 1 M NaHCO₃, Milli-Q, and saturated NaCl solutions. The organic layer was dried over Na₂SO₄ for 2 h. The volatiles were removed under reduced pressure to give a crude product which was purified by recrystallization at 4°C in 70.3% yield; bp 140–141°C (0.7 mmHg); ¹H NMR (CDCl₃): δ 0.9 (t, 3H), 1.3–1.5 (m, 24H), 2.0 (m, 7H), 3.3 (q, 2H), 5.3 (m, 1H), 5.5 (m 2H), 5.6 (s, 1H), and 5.9 (s, 1H).

Anal. Calcd for C₂₂H₄₁NO: C, 78.74%; H, 12.31%; N, 4.17%.

Found: C, 77.42%; H, 12.20%; N, 4.09%.

N-(1-Naphthylmethyl)methacrylamide (1NpMAM) was synthesized as reported previously.¹¹ 2-(Acrylamido)-2-methylpropanesulfonic acid (AMPS) was purchased from Wako Pure Chemicals and used without further purification. 2,2'-Azobis(isobutyronitrile) (AIBN) (Wako Pure Chemicals) was recrystallized twice from ethanol prior to use.

Copolymers

Naphthalene-labeled copolymers were prepared by terpolymerization of AMPS, OleMAM (0, 3, 10, 20, 30, or

R: CH₃(CH₂)₇CH=CH(CH₂)₇CH₂–

$x = f_{\text{Ole}} = 0 - 35 \text{ mol } \%$

Chart 1.

35 mol%), and 1NpMAM (1 mol%) in the presence of 0.1 mol% (based on the total monomers) of AIBN in *N,N*-dimethylformamide (DMF) in a glass ampule, which was outgassed by six freeze–pump–thaw cycles on a vacuum line before sealing. The sealed ampule was immersed in a water bath thermostated at 60°C for 12 h. The reaction mixture was poured into excess diethyl ether to precipitate polymers, that were then purified by three reprecipitations from methanol into diethyl ether and dissolved in a dilute aqueous NaOH solution. The alkaline solution was dialyzed against pure water for a week and finally lyophilized. Compositions of the naphthalene-labeled polymers were determined by elemental analysis (N/C ratios) and UV absorption spectroscopy.

Other Materials

Oleyl alcohol (Tokyo Chemical Industry) was distilled under reduced pressure prior to use. Naphthalene, methanol (HPLC grade), and NaCl (all from Wako Pure Chemicals) were used without further purification. Water for measurements was purified on a Milli-Q system.

Measurements

Absorption spectra were recorded with a JASCO V-550 spectrophotometer. Steady-state fluorescence spectra were measured on a Hitachi F-4500 fluorescence spectrophotometer using a 1-cm path length quartz cuvette with excitation at 290 nm.

Fluorescence decay was measured by a time-correlated single-photon counting technique using a Horiba NAES-550 system equipped with a flash lamp filled with H₂. Sample solutions were excited at 290 nm and monitored with cutoff filter (HOYA UV32) placed between the sample and detector. The decay and response functions were measured simultaneously. Observed decay curves were analyzed by a conventional deconvolution technique.

Fluorescence anisotropy (r) was measured on a Hitachi F-4500 fluorescence spectrophotometer equipped with filter polarizers. Fluorescence spectra for naphthalene-labeled polymers were obtained at 25°C by excitation at 290 nm, and the fluorescence intensity at 325 nm was employed for the estimation of r . Excitation and emission slit widths were maintained at 10.0 nm. r was calculated from

$$r = (I_{vv} - gI_{vh}) / (I_{vv} + 2gI_{vh}) \quad (1)$$

where I_{vv} and I_{vh} are the fluorescence intensities measured with parallel and perpendicular orientations of the excitation and emission polarizers, respectively. g is the factor for the instrumental correction (i.e., $g = I_{hv}/I_{hh}$).

QELS data were obtained at $25 \pm 0.1^\circ\text{C}$ using an Otsuka Electronics Photol DLS-7000 light scattering spectrometer equipped with an Ar laser lamp (60 mW at 488 nm) and detector optics. An ALV-5000E digital multiple- τ correlator (Langen-GmbH) was used for data collection. The autocorrelation function was measured at varying angles from 50° – 130° . Sample solutions were filtered with a $0.2 \mu\text{m}$ pore size membrane filter prior to measurement. The observed intensity autocorrelation function, $g^{(2)}(t)$, is related to normalized electric-field autocorrelation function, $g^{(1)}(t)$, by the Siegert relation,

$$g^{(2)}(t) = B [1 + \beta |g^{(1)}(t)|^2] \quad (2)$$

where β is a constant parameter for an optical system and B is a baseline term. Laplace inversion of the correlation curves was performed using a constrained regularization program REPES³² to obtain relaxation time distribution, $\tau A(\tau)$, according to

$$g^{(1)}(t) = \int \tau A(\tau) \exp(-t/\tau) d\ln \tau \quad (3)$$

where τ is the relaxation time. The hydrodynamic radius (R_h) was calculated from the measured translational diffusion coefficient using the Stokes–Einstein equation. Details of QELS measurement and data analysis were reported elsewhere.¹³

For all measurements, sample solutions were prepared as follows: Solid polymer samples (recovered by a freeze-drying technique from aqueous solutions of the polymers) were dissolved in pure water above 90°C with vigorous stirring for about 15 min. After cooling the solutions to room temperature, a predetermined amount of NaCl was added to the polymer solutions, if necessary, to adjust ionic strength.

RESULTS AND DISCUSSION

Quenching of Naphthalene Fluorescence by Oleyl Alcohol in Methanol

Figure 1 shows Stern–Volmer plots for the steady-state fluorescence intensity and fluorescence lifetime for quenching of naphthalene fluorescence by oleyl alcohol in methanol. If the fluorescence quenching is completely dynamic in nature, the Stern–Volmer equation is expressed as

$$I_0/I = \tau_0/\tau = 1 + K_{sv}[Q] \quad (4)$$

where I and I_0 are the steady-state fluorescence intensities in the presence and absence of a quencher, respectively, τ and τ_0 are the fluorescence lifetimes in the presence and absence of the quencher, respectively, $[Q]$ is the concentration of the quencher, and K_{sv} is the Stern–Volmer constant. Fluorescence decay of naphthalene in the presence of oleyl alcohol in methanol was not single-exponential but best-fitted to a double-expo-

Figure 1. Stern–Volmer plots for fluorescence quenching for naphthalene molecules by oleyl alcohol in methanol at 25°C : Concentration of naphthalene; 1 mM.

Table I. Best-fit parameters for fluorescence decay for naphthalene molecules at varying concentrations of oleyl alcohol measured in methanol^a

[Oleyl Alcohol] (mM)	$\tau(\text{ns})/\alpha$	$\langle \tau \rangle^b(\text{ns})$	χ^2
0	15.3 / 1.0	—	1.26
10	15.2 / 0.942	3.5 / 0.058	14.5
20	15.2 / 0.942	2.1 / 0.058	14.4
50	14.9 / 0.893	3.4 / 0.107	13.7
100	14.6 / 0.829	3.0 / 0.171	12.6
200	13.7 / 0.701	2.7 / 0.299	10.4

^a Fitting function; $I(t) = \sum \alpha_i \exp(-t/\tau_i)$, where τ_i is the fluorescence lifetime of the i th component and α_i is its fraction. ^b Average lifetime defined by $\langle \tau \rangle = \sum \alpha_i \tau_i$.

nential function (Table I). Thus, average lifetime values were used for τ_0/τ plots in Figure 1. As seen in Figure 1, plots for I_0/I and τ_0/τ do not superimpose on each other, i.e., τ_0/τ are smaller than those of I_0/I at all quencher concentrations, suggesting a mix of dynamic and static quenching mechanisms.³³ An apparent K_{sv} estimated from the slope of the I_0/I plot in Figure 1 is 5.5 M^{-1} . Using a natural (unperturbed) lifetime for naphthalene under the same conditions ($\tau_0 = 15.3 \text{ ns}$ (Table I)), an apparent second-order rate constant for dynamic quenching was calculated as $k_q = 3.6 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$, which appears to be one order of magnitude smaller than the diffusion-limiting value.^{34,35} As seen from Table I, the longer lifetime and its fraction decrease with increasing quencher concentration while the fraction of the shorter lifetime increases with quencher concentration. These steady-state and time-dependent fluorescence data suggest that naphthalene and oleyl alcohol associate with each other in methanol, leading to fluorescence quenching in static nature in addition to ordinary collisional quenching. At present, the quenching mechanism is not well understood but from the fact that there is no spectral overlap between absorption spectra of oleyl alcohol in methanol and fluorescence spectra of naphthalene (see later subsection), energy transfer from singlet-excited naphthalene to oleyl groups is ruled out. Thus, a plausible mechanism may be uphill electron transfer from excited naphthalene to oleyl groups.

Figure 2. Fluorescence spectra for naphthalene labels in the polymers with varying f_{Ole} (a) in pure water, (b) in 0.05 M NaCl aqueous solutions, and (c) in methanol at 25°C: Polymer concentration; 1 g L⁻¹.

Figure 3. Normalized fluorescence intensity for naphthalene labels in the polymers plotted as a function of f_{Ole} at 25°C: Polymer concentration; 1 g L⁻¹.

Steady-State Fluorescence Spectra for the Polymers

Figure 2 compares steady-state fluorescence spectra for naphthalene labels in the AMPS-OleMAM copolymers with varying f_{Ole} in pure water, in 0.05 M NaCl aqueous solution, and in methanol. In all cases, fluorescence intensity decreases significantly with increasing f_{Ole} . This decrease in the fluorescence intensity is more significant in water than in methanol. The fluorescence intensities normalized with the intensity at $f_{\text{Ole}} = 0$ mol% are plotted as a function of f_{Ole} in Figure 3. In aqueous solutions, the fluorescence intensity decreases markedly with increasing f_{Ole} in the range $3 < f_{\text{Ole}} < 20$ mol%, exhibiting saturation at $f_{\text{Ole}} > 30$ mol%. In methanol solution, decrease in the fluorescence intensity with increasing f_{Ole} in the range $3 < f_{\text{Ole}} < 20$ mol% is much more gradual than in aqueous solution. This decrease in the fluorescence intensity with increasing f_{Ole} is obviously due to increase in the number of oleyl groups in the vicinity of naphthalene labels on the same polymer chain. In aqueous solution, more naphthalene labels would be incorporated into hydrophobic microdomains formed by oleyl groups as f_{Ole} increases, resulting in more effective quenching in water than in methanol. Assuming quenching efficiency in water to reflect the extent of intrapolymer self-association of oleyl groups, plots in Figure 3 indicate intrapolymer hydrophobic association of oleyl groups to proceed considerably in the range $3 < f_{\text{Ole}}$

Figure 4. Fluorescence decay profiles for naphthalene labels in the polymers with varying f_{Ole} in 0.05 M NaCl aqueous solutions at 25°C. The decay plots are presented in the figure with their baselines vertically shifted for ease of comparison.

< 20 mol% in pure water and in 0.05 M NaCl aqueous solutions. The range of hydrophobe content where hydrophobic association progressively occurs agrees fairly well with the case of AMPS-C₁₈MAM copolymers reported previously.¹³

Fluorescence Lifetimes for Naphthalene Labels in the Polymers

For many aromatic chromophores, fluorescence lifetime is longer in less polar media.¹⁶ Thus the polarity of microenvironments around a fluorescence label is reflected in fluorescence lifetime. Fluorescence decay for naphthalene labels was measured in methanol and aqueous media with and without added salt. Figure 4 shows decay data for varying f_{Ole} observed in 0.05 M NaCl aqueous solutions. An appreciable fraction of the fluorescence decay becomes faster with increasing f_{Ole} ,

Table II. Best-fit parameters for fluorescence decays for naphthalene labels in AMPS-OleMAM copolymers with varying OleMAM contents (f_{Ole}) measured in pure water^a

f_{Ole} (mol%)	τ (ns) / α	$\langle \tau \rangle^b$ (ns)	χ^2
0	35.6 / 1.0	—	1.30
3	36.8 / 1.0	—	1.50
10	40.6 / 0.901 10.4 / 0.099	37.6	1.54
20	44.5 / 0.533 16.6 / 0.333 3.4 / 0.134	29.7	1.01
30	46.6 / 0.344 17.1 / 0.384 3.8 / 0.272	23.6	1.06
35	47.0 / 0.332 17.2 / 0.388 4.0 / 0.280	23.4	1.21

^a Fitting function; $I(t) = \sum \alpha_i \exp(-t/\tau_i)$, where τ_i is the fluorescence lifetime of the i th component and α_i is its fraction. ^b Average lifetime defined by $\langle \tau \rangle = \sum \alpha_i \tau_i$.

Table III. Best-fit parameters for fluorescence decay for naphthalene labels in AMPS-OleMAM copolymers with varying OleMAM contents (f_{Ole}) measured in 0.05 M NaCl aqueous solutions^a

f_{Ole} (mol%)	τ (ns) / α	$\langle \tau \rangle^b$ (ns)	χ^2
0	35.7 / 1.0	—	1.22
3	39.1 / 1.0	—	1.40
10	44.8 / 0.792 12.8 / 0.208	38.1	1.55
20	47.5 / 0.415 17.6 / 0.395 3.4 / 0.190	27.3	1.12
30	49.9 / 0.243 15.8 / 0.414 4.1 / 0.344	20.1	1.56
35	51.3 / 0.231 16.9 / 0.415 4.1 / 0.354	20.3	1.67

^a Fitting function; $I(t) = \sum \alpha_i \exp(-t/\tau_i)$, where τ_i is the fluorescence lifetime of the i th component and α_i is its fraction. ^b Average lifetime defined by $\langle \tau \rangle = \sum \alpha_i \tau_i$.

but there is a significant fraction of a long-lived component whose lifetime increases with f_{Ole} . Fluorescence decay data for the copolymer with $f_{\text{Ole}} = 3$ mol% in pure water and in 0.05 M NaCl aqueous solutions were best-fitted to a single-exponential function, whereas decay data could be fitted to a double- or triple-exponential function when $f_{\text{Ole}} = 10$ –35 mol%. The best-fit parameters for the copolymers with varying f_{Ole} in pure water and in 0.05 M NaCl are listed in Tables II and III, respectively. Decay data in methanol were best-fitted to a double-exponential function except for $f_{\text{Ole}} = 0$ mol% (data not shown). Multi-exponential decay for the copolymers suggest complicated mechanisms for fluorescence quenching. This is particularly true for polymers with higher f_{Ole} in aqueous solution.

Average fluorescence lifetimes for the copolymers in methanol and in aqueous solutions with and without added 0.05 M NaCl are plotted as a function of f_{Ole} in Figure 5 (for $f_{\text{Ole}} = 0$ and 3 mol%, lifetimes for single-exponential decay are plotted). In the case of $f_{\text{Ole}} = 0$ mol%, fluorescence lifetime for naphthalene labels in methanol is shorter than that in aqueous solution with and without added salt, due to the nature of naphthalene fluorophores; *i.e.*, we confirmed, in a separate experiment, that the fluorescence lifetimes of molecular naphthalene measured in methanol and in pure water are 15 and 36 ns, respectively. Figure 5 shows that average lifetime increases slightly with increasing f_{Ole} up to 3 mol% in 0.05 M NaCl and up to 10 mol% in pure water. When f_{Ole} is further increased, lifetime decreases with increasing f_{Ole} , exhibiting saturation at $f_{\text{Ole}} > 30$ mol%. Fluorescence lifetimes in pure water are slightly longer than in 0.05 M NaCl aqueous solution at $f_{\text{Ole}} > 10$ mol%, suggesting that naphthalene labels are more quenched in 0.05 M NaCl than in pure water.

Figure 5. Plots of the average lifetime, $\langle \tau \rangle$, for naphthalene labels as a function of f_{Ole} at 25°C: Polymer concentration; 1 g L⁻¹.

Although fluorescence decay in aqueous solutions is complicated, lifetime for the longest-lived component increases systematically with increasing f_{Ole} (Tables II and III). This longest-lived component should be due to fluorophores that are intact from quenching by oleyl groups. But a question arises as to why some naphthalene fluorophores can escape from quenching even though incorporated in oleyl domains. It is reasonable to consider that the quencher site is the carbon-carbon double bond located in the center of the oleyl group. If the oleyl domain is formed by association of oleyl groups with completely random orientation, all naphthalene fluorophores incorporated in the oleyl domain should equally undergo quenching. However, the presence of a significant fraction of the unquenched fluorophore component suggests that oleyl double bond sites form clusters in the domain, and some naphthalene fluorophores located at a distance from the cluster in the domain may escape from quenching.

Figures 6a and 6b compare absorption spectra of oleyl alcohol in methanol and in water, respectively. The spectra in water markedly different from those in methanol in that the absorption band in water had a long tail toward longer wavelengths. Absorption spectra of the polymers with varying f_{Ole} in 0.05 M NaCl aqueous solutions are shown in Figure 7. With increasing f_{Ole} , the absorption band becomes broader on the longer wavelength side. This is fairly similar to that observed for oleyl alcohol in water presented in Figure 6b. Thus, oleyl double bonds should form clusters in hydrophobic microdomains and naphthalene labels incorporated in the hydrophobic microdomains sufficiently far apart from the double bond cluster may be responsible for the longest-lived component in fluorescence decay.

The increase in fluorescence lifetime with increasing f_{Ole} indicates the incorporation of labels into hydrophobic microdomains formed by oleyl groups. Figure 8 shows the longest lifetime (τ_3) (Tables II and III) for naphthalene labels in the AMPS-OleMAM copolymers observed in aqueous media plotted as a function of hydrophobe content (f_{Ole}). Values of τ_3 continue to increase with increasing f_{Ole} up to 35 mol% although at a slower rate at $f_{\text{Ole}} > 20$ mol%. The tendency observed for AMPS-OleMAM copolymers is similar to that for AMPS-C₁₈MAM copolymers reported earlier¹³ (Figure 8). The depend-

Figure 6. Absorption spectra of oleyl alcohol in methanol (a) and water (b) at 25°C. Concentrations of oleyl alcohol are 1 mM (1), 0.5 mM (2), and 0.1 mM (3). Absorbance at 400 nm was adjusted to zero for all solutions.

ence of the extent of hydrophobic association on the hydrophobe content for the AMPS-OleMAM copolymer may thus be similar to that for the AMPS- C_{18} MAM copolymer, independent of the presence of a double bond in the hydrophobe.

The observed changes in fluorescence lifetime for naphthalene labels with increasing f_{Ole} may be explained as follows. As reported in earlier papers,^{13,15} fluorescence lifetime for naphthalene labels becomes significantly longer as the label is incorporated in hydrophobic microdomains formed by associations of polymer-bound hydrophobes. For AMPS- C_{18} MAM copolymers, increase in fluorescence lifetime was observed even at very low C_{18} MAM content of 5 mol% (Figure 8), indicating the labels to start to experience hydrophobic microenvironment even at this low hydrophobe content. There seems to be two competing effects on fluorescence lifetime in the case of the AMPS-OleMAM copolymers, *i.e.*, hydrophobic microenvironmental and quenching effects, the former leading to increase in fluorescence lifetime and the latter, to decrease in lifetime. When f_{Ole} is sufficiently low, fluorescence lifetime becomes longer with increasing f_{Ole} because the microenvironmental effect is dominant over the quenching effect. However, when f_{Ole} is increased beyond a certain level, the quenching effect dominates over the microenvironmental effect, leading to large increase in quenching with increasing f_{Ole} . The quenching occurs more when salt is added because chain compaction is enhanced by salt.

Figure 7. Normalized absorption spectra of solutions of the polymers with varying f_{Ole} in 0.05 M NaCl aqueous solutions at 25°C: Polymer concentration; 1 g L⁻¹.

Figure 8. Fluorescence lifetime as a function of hydrophobe content for the longest-lived component of naphthalene labels in the AMPS-OleMAM copolymers (●,▲) and for the naphthalene labels in the AMPS- C_{18} MAM copolymers (○,△) in water (▲,△) and in 0.05 M NaCl (●,○) at 25°C: Polymer concentration; 1 g L⁻¹.

Fluorescence Depolarization

The incorporation of naphthalene labels in hydrophobic microdomains formed by oleyl groups was indicated by fluorescence depolarization data, due to the fact that rotational motion of naphthalene labels is restricted when the label is incorporated in a hydrophobic microdomain formed by polymer-bound oleyl groups. Figure 9 shows fluorescence anisotropy (r) plotted as a function of hydrophobe content for AMPS-OleMAM and AMPS- C_{18} MAM copolymers in 0.05 M NaCl aqueous solutions at 25°C. For the AMPS-OleMAM copolymers, fluorescence depolarization arises from naphthalene fluorophores that escaped from quenching by oleyl groups. Namely, naphthalene labels in hydrophobic microdomains at a certain distance from the double bond site of the oleyl group may possibly emit fluorescence. If the lifetime of a fluorophore is not too short compared to rotational correlation time for the fluorophore, fluorescence depolarization may be observed. As seen in Figure 9, r increases with hydrophobe content (f_{Ole}). This indicates fluorescence depolarization to slow down when naphthalene fluorophores are incorporated in hydrophobic microdomains.

The data in Figure 9 indicate that motion of naphtha-

Figure 9. Anisotropy, r , in fluorescence depolarization in 0.05 M NaCl aqueous solutions at 25°C plotted as a function of hydrophobe content for the AMPS-OleMAM and AMPS-C₁₈MAM copolymers: Polymer concentration; 1 g L⁻¹.

lene labels is more restricted with increasing f_{Ole} , in some contrast to fluorescence lifetime (Figures 5 and 8). Fluorescence lifetime reflects the micropolarity of hydrophobic microdomains formed by oleyl groups whereas the fluorescence anisotropy reflects the microviscosity (*i.e.*, rigidity) of hydrophobic microdomains. A comparison of Figure 9 with Figures 5 and 8 suggests that micropolarity is not necessarily parallel microviscosity. At $f_{\text{Ole}} = 10$ mol%, the formation of hydrophobic microdomains is indicated by significant increase in fluorescence lifetime (Figure 8) but increase in anisotropy from $f_{\text{Ole}} = 0$ to 10 mol% is very small (Figure 9). At $f_{\text{Ole}} > 20$ mol%, saturation for micropolarity is indicated by fluorescence lifetime (Figure 8) whereas significant increase in microviscosity is indicated by fluorescence anisotropy (Figure 9).

Values of r for AMPS-OleMAM copolymers were slightly larger than for AMPS-C₁₈MAM copolymers reported earlier¹³ (Figure 9). This suggests that rotational motion of naphthalene labels in the AMPS-OleMAM copolymers is more restricted than in AMPS-C₁₈MAM copolymers at the same hydrophobe content. Double bonds in oleyl groups may thus form clusters, forming a tighter hydrophobic domain than for AMPS-C₁₈MAM copolymers.

Quasielastic Light Scattering (QELS)

We measured relaxation time distribution at varying scattering angles (50°–130°), and the relaxation rate (Γ) (*i.e.*, reciprocal of relaxation time (τ)) at each peak top was plotted against q^2 , giving a straight line passing through the origin. From the translational diffusion coefficient (D) estimated from the slope of the plot, apparent hydrodynamic radii (R_h) were calculated from the Einstein-Stokes equation. The distributions of the relaxation times in QELS and R_h for the polymers with varying hydrophobe contents at a polymer concentration of 1 g L⁻¹ in 0.05 M NaCl observed at a scattering angle of 90° at 25°C are shown in Figure 10.

In our earlier studies on AMPS copolymers with a methacrylamide *N*-substituted with an alkyl chain such as hexyl (C₆), dodecyl (C₁₂), or octadecyl (C₁₈) group,^{13,15,21} relaxation time distribution was bimodal at hydrophobe content lower than 10 mol%. A fast mode ob-

Figure 10. Distributions of the relaxation times in QELS measured at $\theta = 90^\circ$ or 1 g L⁻¹ polymer solutions containing 0.05 M NaCl at 25°C.

served was attributed to unimers (single molecular state) and a slow mode to multipolymer aggregates. The apparently bimodal distribution in Figure 10 for the copolymer with $f_{\text{Ole}} = 3$ mol% is indication of the presence of interpolymer aggregates. From monodisperse distribution and relatively small R_h for copolymers with f_{Ole} from 10 to 30 mol%, it appears that polymers exist as unimolecular micelles in this f_{Ole} range. Comparing R_h for AMPS-OleMAM copolymers with those for AMPS-C₁₈MAM copolymers, hydrodynamic size for the copolymers with unsaturated C₁₈ chains is much smaller than for copolymers with saturated C₁₈ chains. R_h for the AMPS-OleMAM copolymer with $f_{\text{Ole}} = 35$ mol% is 7.6 nm while R_h for the AMPS-C₁₈MAM with the same hydrophobe content is *ca.* 15 nm.

We reported that copolymers of AMPS and methacrylamides *N*-substituted with alkyl groups show intrapolymer hydrophobic association yielding unimolecular micelles.^{15,22} However, these copolymers undergo interpolymer as well as intrapolymer association at hydrophobe contents higher than a certain level. Hydrophobe content at which interpolymer association starts to occur depends on the length of the alkyl chain. In the case of the AMPS-C₁₈MAM copolymer of a 30 mol% hydrophobe content, the polymers undergo mostly intrapolymer association but some polymer chains undergo interpolymer association and hence R_h is larger than expected for unimolecular micelles.

For AMPS-OleMAM copolymer with $f_{\text{Ole}} = 30$ mol%, R_h seems to be that expected for a unimolecular micelle. A double bond in the C₁₈ chain may thus affect self-association behavior of the polymer-bound hydrophobes so that intrapolymer hydrophobic association occurs more easily.

CONCLUSION

Naphthalene fluorescence is quenched by oleyl alco-

hol, and thus we characterized the self-associative behavior in water of AMPS-OleMAM copolymers with varying OleMAM content *via* fluorescence quenching of naphthalene labeled on the copolymer. Quenching occurred more when naphthalene labels were incorporated in hydrophobic microdomains formed by oleyl groups. Thus, the formation of hydrophobic microdomains can be monitored by steady-state and time-dependent fluorescence measurements. The self-association of oleyl groups occurred progressively from $f_{\text{ole}} = 3$ to 20 mol% to form hydrophobic microdomains, suggesting that the *cis* double bond in the oleyl group forms a cluster when oleyl groups associate to form microdomains. AMPS-OleMAM copolymers showed greater intrapolymer hydrophobic association than the copolymers of AMPS and *N*-octadecylmethacrylamide, a methacrylamide *N*-substituted with a saturated alkyl chain of the same length as the oleyl group.

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