Characterization of Unimolecular Micelles of Random Copolymers of Sodium 2-(Acrylamido)-2-methylpropanesulfonate and Methacrylamides Bearing Bulky Hydrophobic Substituents

Yotaro Morishima,* Shigeki Nomura, Takahiro Ikeda, Michiko Seki, and Mikiharu Kamachi*

Department of Macromolecular Science, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan

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ABSTRACT: The self-organization of random copolymers of sodium 2-(acrylamido)-2-methylpropanesulfonate and methacrylamides bearing bulky hydrophobic groups, such as lauryl (LA), cyclododecyl (CD), and 1-adamantyl (AD) groups, was investigated by fluorescence, NMR relaxation, FTIR, light scattering, and small-angle X-ray scattering techniques. In aqueous solution, the hydrophobes in these polymers form clusters as a result of either intramolecular or intermolecular self-association. Studies of nonradiative energy transfer between polymers labeled with naphthalene and pyrene revealed that the polymers having the CD and AD groups formed unimolecular micelles (unimers) in a wide range of concentrations up to several weight percent, whereas the polymer having the LA groups could exist as a unimer only in a much lower concentration range (<ca. 0.2 wt %); the CD and AD residues had a strong tendency for intramolecular self-association, whereas the LA residues tended to associate intermolecularly. The pyrene labels are encapsulated within the clusters of the hydrophobes and protected from the aqueous phase as indicated by the suppression of fluorescence quenching by thallium ions. The unimers of the CD- and AD-containing polymers are extremely compact; e.g., the CD-containing polymer with $M_w = 5.1 \times 10^5$ forms a unimer with a mean hydrodynamic radius of 5.5 nm. Motions of the CD groups in their clusters are much more restricted than those of the LA groups as indicated by ^{1}H-NMR relaxation times. FTIR suggests that hydrogen bonding between the spacer amide bonds may be contributing to reinforcement of the compact unimer structure.

Introduction

In recent years, there has been a growing interest in self-organization phenomena of amphiphilic polymers in aqueous solution. This is, in part, because of their biological relevance and, in part, because of their potential importance in a variety of applications such as paints, coatings, drugs, and personal care goods.

Over the past decade, various amphiphilic polyelectrolytes were functionalized with various chromophores.[1–8] The functionalization with chromophores is of interest because of the following two main reasons: (1) photochemical processes can be greatly modulated owing to microenvironmental effects, and (2) the chromophores can act as "reporters" to provide useful information on the conformational and dynamic properties of the polymers.

Recently, we reported on the photophysical and photochemical behavior of pyrene,[9] azobenzene,[10,11] and zinc tetraphenylporphyrin[12,13] covalently incorporated into copolymers of sodium 2-(acrylamido)-2-methylpropanesulfonate (AMPS) and methacrylamido monomers carrying bulky hydrophobic groups such as lauryl (LA), cyclododecyl (CD), 1-adamantyl (AD), and 1-naphthyl (1-Np) groups. In aqueous solution, the hydrophobic chromophores are buried in the hydrophobic microdomains of the amphiphilic polysulfonates in which the chromophores are forced to experience nonpolar microenvironments and protected from the aqueous phase. Since the dynamic motions of the chromophores are restricted in the hydrophobic microdomains, chromophore–chromophore interactions are essentially be prohibited. We have established that these microenvironmental effects bring about large modifications of the photophysical and photochemical properties of the chromophores encapsulated in the hydrophobic microdomains.[9–15] However, an important question that remains unanswered is whether such hydrophobic microdomains are formed as a result of intramolecular self-organization or intermolecular aggregation of the hydrophobic groups.

In the present study, we attempt to answer this question by focusing on the associative behavior of the random copolymers of AMPS and methacrylamido monomers bearing LA, CD, and AD substituents in aqueous solution.

In highly dilute aqueous solutions, the hydrophobic association may occur within a polymer molecule, and thereby a unimolecular micelle (unimer) may be formed. However, as the concentration is increased, multimolecular aggregates may be formed due to intermolecular hydrophobic association. Various types of amphiphilic polyelectrolytes have been synthesized so far, which include random copolymers,[5–7,9–19] block copolymers,[20–23] and alternating copolymers.[24–30] Recently, McCormick et al.[31] showed that the sequence distribution of charged and hydrophobic units along the polymer chain was an important structural factor to determine whether the hydrophobic self-association was an intra- or intermolecular event and that the block sequence of hydrophobic units had a tendency for intermolecular association, whereas random sequence tended to associate intramolecularly.[31]

For characterization of the self-organization of the amphiphilic AMPS copolymers and the nature of thus formed hydrophobic microdomains, we employed fluorescence, ^{1}H-NMR relaxation, FTIR, static light scattering (SLS), dynamic light scattering (DLS), and small-angle X-ray scattering (SAXS) techniques. For the fluorescence studies, the polymers were labeled with a small amount of pyrenyl (Py) or 2-naphthyl (2-Np)

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Chart 1

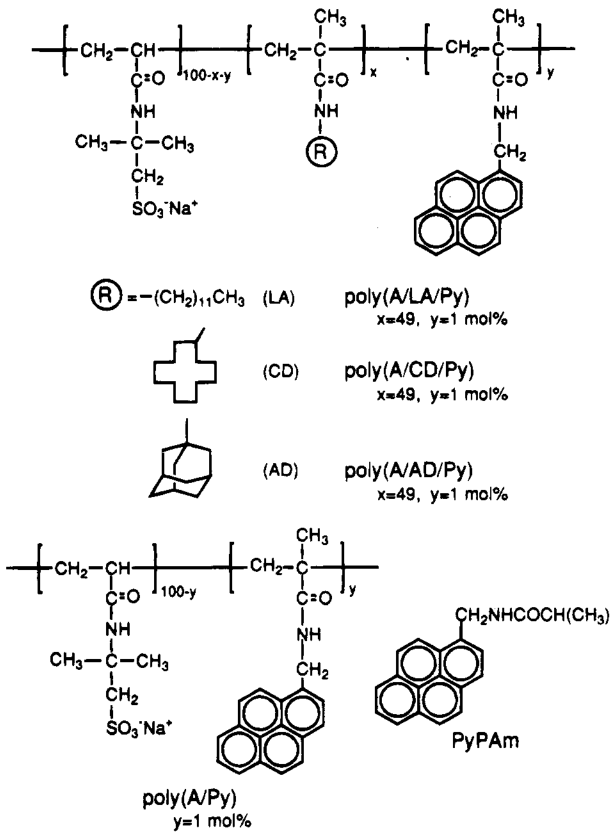
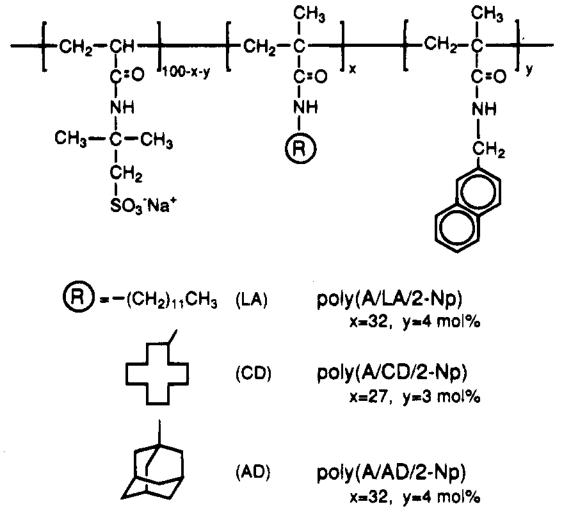


Chart 2



moieties by a terpolymerization technique employing the corresponding monomers (Charts 1 and 2).

Experimental Section

Monomers. *N*-Laurylmethacrylamide (LAMAm),[18] *N*-cyclododecylmethacrylamide (CDMAm), *N*-(1-adamantyl)methacrylamide (ADMAm), and *N*-(1-pyrenylmethyl)methacrylamide (1PyMAm) were prepared as reported previously.[9] *N*-(2-Naphthylmethyl)methacrylamide (2NpMAm) was prepared in a manner analogous to the synthesis of *N*-(1-naphthylmethyl)-methacrylamide.[19] 2-(Acrylamido)-2-methylpropanesulfonic acid (AMPS) was used as received from Nitto Chemical Industry Co.

Polymers. The terpolymer of 50 mol % AMPS, 49 mol % LAMAm (or CDMAm or ADMAm), and 1 mol % 1PyMAm (Chart 1) and the reference copolymer of 99 mol % AMPS and 1 mol % 1PyMAm (Chart 1) were prepared as reported previously.[9] The terpolymer of AMPS, 32 mol % LAMAm (or ADMAm), and 4 mol % 2NpMAm and the terpolymer of AMPS, 27 mol % CDMAm, and 3 mol % 2NpMAm (Chart 2) were prepared by free-radical terpolymerization initiated by 2,2′-azobis(isobutyronitrile) (AIBN) in a manner analogous to the synthesis of the corresponding pyrene-labeled terpolymers.[9] The terpolymerizations were carried out in glass ampules containing monomers and AIBN (0.5 mol % based on the total monomers) in *N*,*N*-dimethylformamide (DMF) solution. The ampules were outgassed by several freeze–pump–thaw cycles on a vacuum line. The sealed ampules were immersed in a water bath thermostated at 60 °C for 12 h. The reaction mixtures were poured into a large excess of ether to precipitate the resulting polymers. The polymers were further purified by three reprecipitations from methanol into ether and then dissolved in dilute aqueous NaOH. The alkaline solutions were dialyzed against pure water for a week and finally lyophilized. The compositions of the terpolymers were determined by N/C and S/C ratios and absorption spectra. The copolymer of AMPS and 42 mol % LAMAm (or 37 mol % CDMAm or 36 mol % ADMAm) (Chart 3) was prepared in an analogous manner.

Other Materials. Analytical grade thallium nitrate was used without further purification. Water was doubly distilled and deionized by passing through an ion-exchange column.

Measurements. a. Nonradiative Energy Transfer. A 0.014 wt % aqueous solution of poly(A/LA/Py) was mixed with the same volume of a 0.014 wt % aqueous solution of poly(A/LA/2-Np). A 10 wt % aqueous solution of poly(A/LA) was added to the above solution of the mixture of poly(A/LA/Py) and poly(A/La/2-Np) such that the concentrations of total polymers varied in the range 0.014–7 wt %. The solutions of varying concentrations of the total polymers were subjected to fluorescence measurements. Fluorescence spectra were recorded on a Shimadzu RF-02A spectrofluorometer with excitation at 290 nm at room temperature. The same procedures were followed for the CD- and AD-containing polymer systems. The same experiments were also performed with methanol solutions of the LA-, CD-, and AD-containing polymer systems in place of aqueous solutions. All sample solutions were allowed to stand under ambient conditions for a few hours and deaerated by bubbling with Ar gas for 30 min immediately before the fluorescence measurements. We confirmed that there was no effect of aging of the sample solutions, in both water and methanol, on fluorescence spectra.

b. Fluorescence Quenching with Thallium Nitrate. Small amounts of a stock solution of thallium nitrate were added to aqueous solutions of the Py-labeled polymers, and the solutions were deaerated by bubbling with Ar gas for 30 min. The concentration of the polymers was adjusted to 0.01 wt %. Fluorescence measurements were performed with excitation at 345 nm.

c. ^{1}H-NMR. Proton NMR spectra were obtained with a JEOL EX-270 spectrometer operating at 270 MHz for protons using a deuterium lock at a constant temperature of 40 °C during the whole run. NMR tubes containing 1 wt % D_2O solutions of the terpolymers were outgassed on a vacuum line and flame-sealed prior to the measurements. The resonance due to impure water in the D_2O solutions was decoupled. Proton spin–lattice relaxation times (T_1) were determined by using a simple inversion–recovery technique with a $180°-\tau-90°$ pulse sequence.[32–34] Proton spin–spin relaxation times (T_2) were determined by using the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequences $\{90°_x\tau(180°_y2\tau)_n\}$.[35]

d. FTIR. IR spectra were taken as KBr pellets on a Jasco FT/IR-8300 spectrophotometer. The KBr pellets were prepared with polymer samples recovered from their aqueous solutions by freeze-drying techniques.

e. Light Scattering. Static light scattering (SLS) and dynamic light scattering (DLS) data were obtained with an Otsuka Electronics Photoal DLS-700 light scattering spectrophotometer. Sample solutions containing polymers and 0.1 M NaCl in water were repeatedly filtered through 0.2-μm filters. For SLS measurements, values of d*n*/d*c* (refractive index increment against concentration) were determined with an Otsuka DRM-1020 differential refractometer. The concentrations of the polymers in the sample solutions for the SLS measurements were in the range 1.25–5.0 mg/mL.

f. Small-Angle X-ray Scattering (SAXS). SAXS patterns for the aqueous and methanol solutions of the polymers (ca. 10 wt %) were recorded with a MAC Science DIP1000 X-ray diffraction system. Cu Kα radiation with a wavelength of 0.154 nm was used. The SAXS results were corrected for absorption and background scattering.

Results and Discussion

Terpolymers with Pyrene Labels. We previously reported that the copolymerizations of AMPS–LAMAm, AMPS–CDMAm, and AMPS–ADMAm were "ideal copolymerization" systems which yield copolymer compositions equal to monomer feed compositions and completely random distributions of the monomer units along the polymer chains.[9] The contents of the hydrophobic groups in the Py-labeled polymers, poly(A/LA/Py), poly(A/CD/Py), and poly(A/AD/Py) (Chart 1), are sufficiently high for the hydrophobic association to occur in aqueous solution prevailing over electrostatic repulsions between the AMPS segments. The Py residues labeled on the polymers are incorporated into the hydrophobic microdomains of the LA, CD, and AD groups in these terpolymers.[9]

The Py labels show only monomeric fluorescence because the loading amount of the Py labels is as low as 1 mol %. It is known that the ratio of the third to the first vibronic bands (I_3/I_1) in pyrene fluorescence spectra depends on the polarity in microenvironments where pyrene exists.[36] I_3/I_1 values being larger in less polar media. We confirmed that this applies to the model compound, PyPAm (Chart 1), which has a substituent group similar to the Py labels in the terpolymers and the reference copolymer poly(A/Py) (Chart 1).[37,38] The I_3/I_1 ratio for PyPAm is 0.59, reflecting the polarity of water, and poly(A/Py) shows the same value in water.[38] This is because the reference copolymer assumes an extended conformation in pure water, and the Py labels are exposed to the aqueous phase. In contrast, the I_3/I_1 values for poly(A/LA/Py), poly(A/CD/Py), and poly(A/AD/Py) are 0.80, 0.83, and 0.76, respectively,[9] all larger than that for the reference copolymer and the model compound. This is an indication that the Py labels are buried within the hydrophobic microdomains in these polymers.

Nonradiative Energy Transfer between Polymers. In general, hydrophobic microdomains are formed in amphiphilic polyelectrolytes by either intramolecular or intermolecular self-association of hydrophobes. Since the distribution of the monomer units is completely random in the present polymers, a tendency for intramolecular association may be expected.[31]

Nonradiative energy transfer between polymers tagged with an energy donor and an energy acceptor on separate polymer molecules provides a sensitive tool to probe into intermolecular associative interactions.[39–41] Naphthalene and pyrene labels have been successfully used as a singlet energy donor and acceptor, respectively, because they have a large spectral overlap, and the former can be almost selectively excited at wavelengths near 290 nm.

We applied this technique to the present polymer systems to see whether the hydrophobic self-association is an intra- or intermolecular event. For this particular experiment we also prepared LA-, CD-, and AD-containing polymers labeled with 2-naphthyl (2-Np) groups (Chart 2). If the self-association of the hydrophobic groups is a completely intramolecular event, the terpolymers would form unimers in aqueous solution, and the fluorescence labels, both the pyrene and naphthalene moieties, would be confined within the hydrophobic microdomains in the unimers. Therefore, in an aqueous solution of a mixture of a 2-Np-labeled polymer and a Py-labeled polymer both having the same aliphatic hydrophobic groups, the 2-Np and Py labels would completely be separated from each other in the individual unimers. Moreover, each unimer should exist separately in the aqueous solution because of electrostatic repulsion; the exterior of the unimers is negatively charged. In this circumstance, the energy transfer from singlet-excited 2-Np labels in one unimer to Py labels in another unimer should be unlikely to occur. On the other hand, if the hydrophobic groups associate intermolecularly, there should be a chance for a certain fraction of the 2-Np and Py labels to come close to each other within the Förster radius (R_0 = 2.86 nm for transfer from 2-methylnaphthalene to pyrene[42]), and thereby fluorescence from the Py label should be observed due to energy transfer when the 2-Np label is excited.

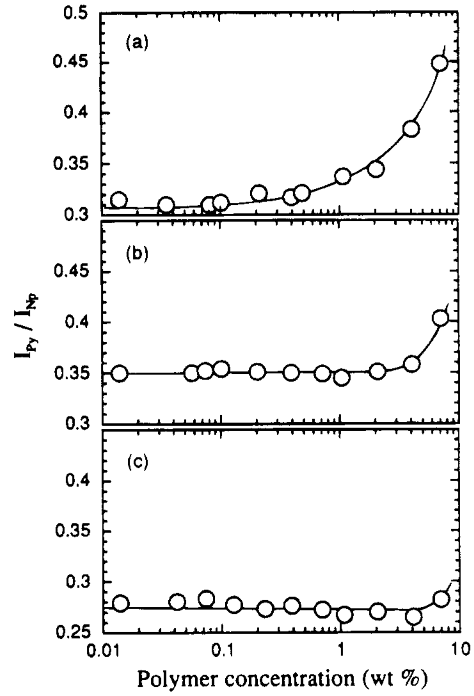
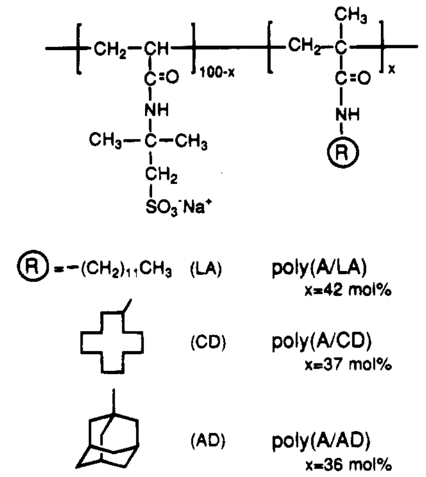


Figure 1. Ratio of the intensities of fluorescence from the Py and 2-Np labels (I_{Py}/I_{Np}) as a function of the concentration of the total polymers in aqueous solutions of the mixtures of the Py-labeled and 2-Np-labeled polymers: (a) poly(A/LA/Py) and poly(A/LA/2-Np); (b) poly(A/CD/Py) and poly(A/CD/2-Np); (c) poly(A/AD/Py) and poly(A/AD/2-Np). The total polymer concentrations were increased by adding the nonlabeled polymers having the same hydrophobic groups (see the Experimental Section).

Aqueous solutions of the mixture of the 2-Np-labeled polymer and the Py-labeled polymer, each having the same hydrophobic groups, were irradiated at 290 nm, and fluorescences from the 2-Np and Py labels were monitored at 340 and 395 nm, respectively. In Figure 1 are plotted the ratios of the intensities of the fluorescence emitted by the Py and 2-Np labels as a function of the polymer concentration for the LA-, CD-, and AD-containing terpolymer systems. The 2-Np labels can be predominantly excited at 290 nm, but a slight contribution of direct excitation of the Py labels cannot be eliminated. The polymer concentrations were varied by adding the nonlabeled copolymers having the same

Chart 3



hydrophobic groups (Chart 3), keeping the concentration of the labeled polymers constant. In the case of the LA-containing polymer system, the Py fluorescence intensity increased significantly with increasing the polymer concentration following onset of the Py fluorescence at ca. 0.2 wt %. In contrast, the CD- and AD-containing terpolymers showed no such increase in the Py fluorescence until the concentration was increased to ca. 7 wt %. These observations indicate that the CD and AD residues in the present polymers have much stronger tendency for intramolecular association than does the LA residue. In the LA-containing polymer, hydrophobic association seems to be an intramolecular event only at concentrations below 0.2 wt %. By contrast, in the CD- and AD-containing polymers, intramolecular association seems to be dominant and the polymers can exist as unimers at much higher concentrations up to ca. 7 wt %. These findings indicate that, although the numbers of the carbon atom are approximately the same (C_{12} for LA and CD and C_{11} for AD), the stabilities of the hydrophobic clusters are very different depending on whether the structure of the hydrophobic group is cyclic (CD or AD) or linear (LA).

In methanol solutions of the CD- and AD-containing polymers, the intermolecular energy transfer between the 2-Np label and Py label occurs at much lower concentrations than in aqueous solution (Figure 2). In methanol, the polymers should adopt a random-coil or slightly extended conformation due to the fact that hydrophobic interaction is absent and electrostatic interaction is much less than that in water because of a much less degree of dissociation of the sodium sulfonate units in the polymer. Therefore, it is much more likely that the polymer chains interpenetrate in methanol than in water, which facilitates the intermolecular energy transfer. A comparison of the intermolecular energy transfer in water (Figure 1) and in methanol (Figure 2) confirms the conclusion that the CD- and AD-containing polymers form unimers in water. It should be noted that, in the case of LA-containing polymer, there is not much difference in the ease of the intermolecular energy transfer between methanol and water. This is an indication that the LA groups tend to associate intermolecularly in aqueous solution prevailing over intermolecular charge repulsion.

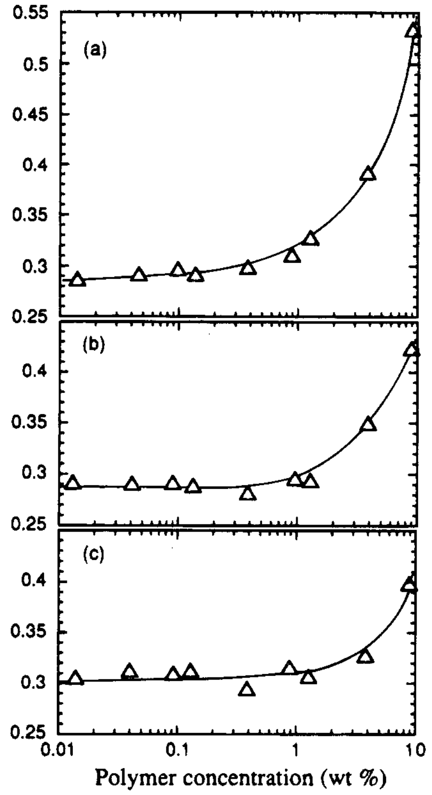


Figure 2. Intensity ratio for the fluorescence of the Py and 2-Np labels (I_{Py}/I_{Np}) as a function of the concentration of the total polymers in methanol solutions of the mixtures of the Py-labeled and 2-Np-labeled polymers: (a) poly(A/LA/Py) and poly(A/LA/2-Np); (b) poly(A/CD/Py) and poly(A/CD/2-Np); (c) poly(A/AD/Py) and poly(A/AD/2-Np). The total polymer concentrations were increased by adding the nonlabeled polymers having the same hydrophobic groups (see the Experimental Section).

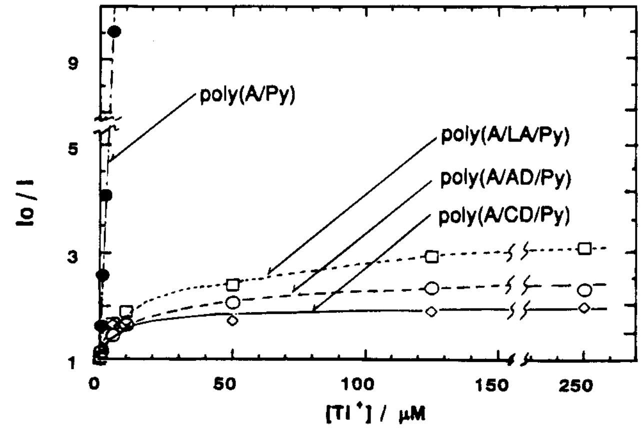


Figure 3. Stern–Volmer plots for fluorescence quenching by Tl^+ for the Py labels in poly(A/LA/Py), poly(A/CD/Py), poly(A/AD/Py), and poly(A/Py) in aqueous solution.

Fluorescence Quenching by Thallium Nitrate. The encapsulation of the Py labels in the hydrophobic microdomains and their protection from the aqueous phase are indicated by a sharp suppression of fluorescence quenching by Tl^+ as shown in Figure 3. The fluorescence of pyrene is known to be quenched by Tl^+ due to an external heavy-atom effect that requires a short-range interaction.[43] In the reference copolymer poly(A/Py) which assumes an open chain conformation, Tl^+ ions are electrostatically concentrated in the vicinity of the anionic polymer chain and can come into contact with the Py residues. Thus, the Py fluorescence in the reference copolymer is efficiently quenched by Tl^+ (Figure 3). In sharp contrast, because the Py labels in the terpolymers are buried inside the hydrophobic microdomains, Tl^+ ions cannot reach the Py sites,

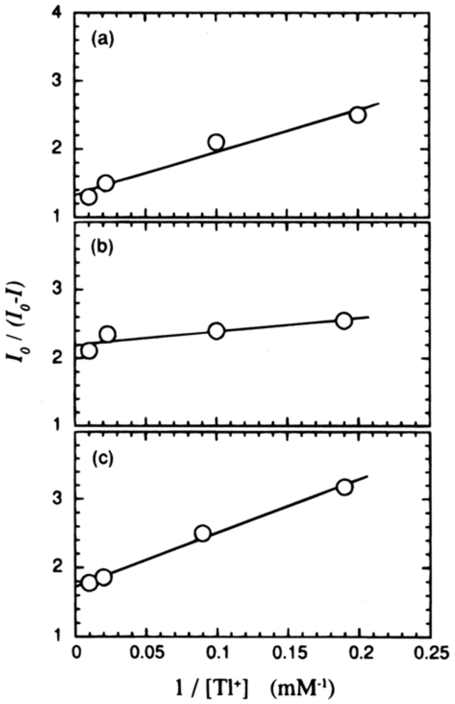


Figure 4. Modified Stern−Volmer plots for fluorescence quenching by Tl$^+$ for the Py labels in poly(A/LA/Py) (a), poly(A/CD/Py) (b), and poly(A/AD/Py) (c) in aqueous solution.

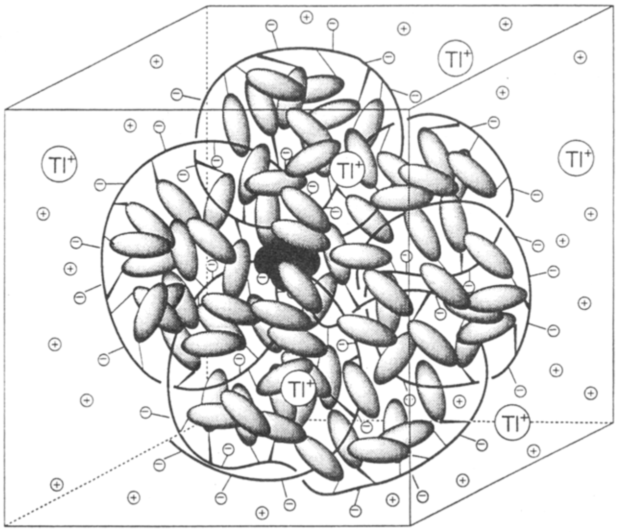


Figure 5. Conceptual illustration of a model for the unimer. The Py label buried in the hydrophobic cluster and Tl$^+$ ions electrostatically concentrated on the unimer are also illustrated.

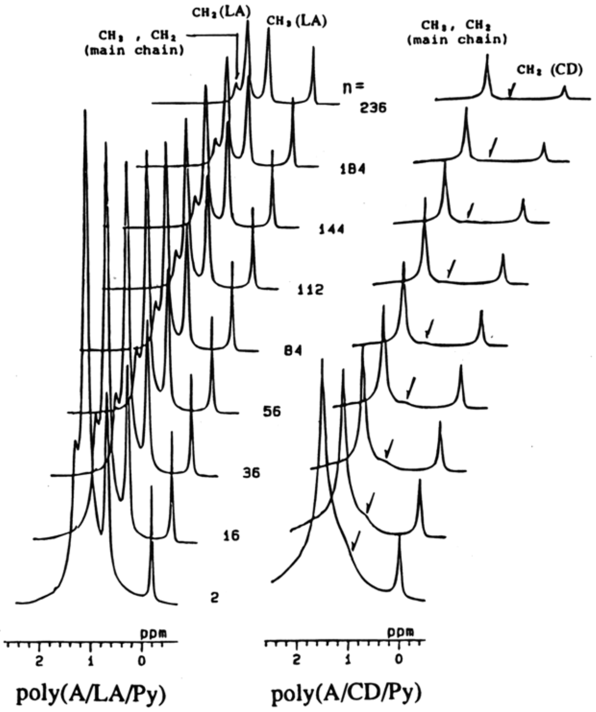


Figure 6. Comparison of ^{1}H-NMR stack plots for poly(A/LA/Py) and poly(A/CD/Py) in D_2O at 25 °C. *n* represents the repeat number of the 180° pulse.

leading to a sharp suppression of the fluorescence quenching (Figure 3).

In the case where there are two chromophore sites, one is accessible to quenchers and the other is not, the Stern−Volmer equation can be modified as[44]

$$I_0/(I_0 - I) = 1/fK[\mathrm{Tl}^+] + (1/f) \qquad (1)$$

where I and I_0 are the fluorescence intensity in the presence and absence of the quencher, respectively, K is the Stern−Volmer constant for the accessible chromophores, and f is the fraction of the accessible chromophores. Figure 4 shows the plots of the quenching data according to eq 1 for the terpolymers. Apparently, the data for all the terpolymers follow eq 1. From the intercepts, f values were estimated to be 0.76, 0.45, and 0.58 for poly(A/LA/Py), poly(A/CD/Py), and poly(A/AD/Py), respectively. These values imply that 55 and 42% of the Py labels are protected from the access of Tl$^+$ in the CD- and AD-containing terpolymers, respectively, while only 24% of the Py labels are protected in the LA-containing terpolymer. The protection is much less effective in the LA-containing polymer than in the CD- and AD-containing polymers, the CD-containg polymer giving the most effective protection. The polymer concentration for these quenching experiments is low enough (0.01 wt %) for the terpolymers, including poly(A/LA/Py), to exist as unimers. A proposed model for the unimer with the Py label confined within the hydrophobic clusters is illustrated in Figure 5. It should be pointed out that although a dominant part of the fluorescence quenching by Tl$^+$ is due to the heavy-atom interaction, quenching by electron transfer may be involved to some extent. Since electron transfer can occur at a longer distance than does the heavy-atom interaction, fluorescence from the Py labels buried near the surface in the hydrophobic microdomain may be quenched by Tl$^+$ via the electron transfer, though the Py sites are too far from Tl$^+$ for the heavy-atom interaction to occur. If such electron transfer quenching is involved, f values must be overestimated.

NMR Relaxation. For an understanding of the difference in the extent of the protection of the Py labels in the hydrophobic microdomains of the LA and CD groups, the NMR relaxation times for the protons in these hydrophobic groups were compared. The NMR relaxation techniques provide useful information about local segment motions in polymers.[45−47] Figure 6 shows stack plots of the ^{1}H-NMR spectra from which spin−spin relaxation times (T_2) were determined for poly(A/LA/Py) and poly(A/CD/Py) in D_2O at 25 °C. The peaks at 0.9 and 1.4 ppm for the LA-containing terpolymer are attributable to the methyl and methylene protons in the LA groups, respectively. In the CD-containing terpolymer, on the other hand, the methylene protons

Table 1. Values of T_1 and T_2 for the Methyl and Methylene Protons in the LA Groups in Poly(A/LA/Py) and for the Methylene Protons in the CD Groups in Poly(A/CD/Py)

		T_1 (ms)[a]		T_2 (ms)[a]	
terpolymer	solvent	CH_3-	$-CH_2-$	CH_3-	$-CH_2-$
poly(A/LA/Py)	D_2O	472	438	34	15
	DMF-d_7	2916	627	805	211
poly(A/CD/Py)	D_2O		904		4
	DMF-d_7		442		13

[a] Relaxation times were determined at chemical shifts of 0.9 and 1.2 ppm for the CH_3- and $-CH_2-$ protons, respectively.

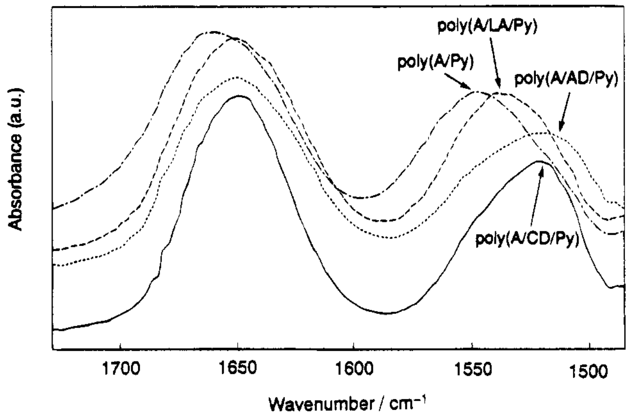


Figure 7. Expanded-scale FTIR spectra for poly(A/LA/Py), poly(A/CD/Py), poly(A/AD/Py), and poly(A/Py) measured as KBr pellets.

in the CD groups gives no peaks but a broad shoulder at ca. 1.2 ppm, despite the fact that the number of the CD methylene protons is larger than the total of the AMPS methyl and main-chain methylene protons which give an unresolved broad peak at ca. 1.5 ppm. This is because the resonance line of the CD methylene in the terpolymer is extremely broad due to highly restricted motions. The values of T_2 are listed in Table 1 in comparison with the values of spin−lattice relaxation times (T_1). The T_2 value for the CD methylene protons in the CD-containing terpolymer estimated at 1.2 ppm (shoulder) is significantly smaller than those for the La methyl and La methylene protons in the LA-containing terpolymer. This indicates that the mobility of the CD groups in the clusters is much more restricted than that of the LA groups. The T_1 value for the CD methylene in the CD-containing terpolymer is much larger than that for the LA methylene in the LA-containing terpolymer. The spin−lattice relaxation occurs most efficiently through molecular motion whose frequency is comparable to the NMR frequency.[48] Therefore, T_1 decreases concurrently with T_2 as a molecular motion decreases. Reaching a minimum value, T_1 then increases with a further decrease in the molecular motion, while T_2 remains as a minimum value. The large T_1 values, along with the small T_2 value, for the CD methylene in poly(A/CD/Py) is indicative of highly restricted motions of the CD groups, as compared with the LA groups in poly(A/LA/Py), as a result of the cluster formation.

FTIR. In these terpolymers, the hydrophobic pendant groups are linked to the main chain via amide spacer bonds. Figure 7 shows FTIR spectra in the region 1500−1700 cm^{-1} recorded on an expanded scale. All the spectra were measured as KBr pellets prepared with the polymers recovered from their aqueous solutions by freeze-drying. An IR spectrum for poly(A/Py) is also presented in Figure 7 for comparison. All the polymers show two characteristics IR absorption bands in this wavenumber region due to the amide bond, the peaks at higher and lower wavenumbers attributable to ν(C=O) and δ(NH), respectively. It should be noted that these bands for the terpolymers are shifted toward lower wavenumber as compared to those for the reference copolymer, more significant shifts observed in the δ(NH) band than in the ν(C=O) band. These lower-wavenumber shifts are ascribable to the hydrogen bonding of the amide spacer bonds. Furthermore, there are significant differences in the extent of the lower-wavenumber shift in the δ(NH) band among the terpolymers. The LA-containing terpolymer exhibited a lower-wavenumber shift by 8 cm^{-1} as compared with the δ(NH) band of the reference copolymer, while the CD- and AD-containing terpolymers showed much larger shifts of ca. 30 cm^{-1}. These observations suggest that hydrogen bonds are formed between the spacer amide bonds more strongly in the CD- and AD-containing terpolymers in the solid samples than are in the LA-containing terpolymer.

Table 2. Light Scattering and SAXS Data for LA-, CD-, and AD-Containing Terpolymers in Aqueous Solution

polymer	M_w[a]	R_S (nm)[b]	d (nm)[c]
poly(A/LA/Py)	1.2×10^5	7.0	
poly(A/CD/Py)	5.1×10^5	5.5	11
poly(A/AD/Py)	3.5×10^4	6.2	7.3

[a] Weight-average molecular weight determined by SLS. [b] Average Stokes radius determined by DLS. [c] Spacing calculated from the scattering angle in SAXS.

The solid polymer samples for the FTIR measurements were recovered from ca. 5 wt % aqueous solutions of the polymers by a freeze-drying technique. In general, conformations of polymers in solution may not always be retained in freeze-dried solid polymers. In the case of the present amphiphilic polysulfonates, however, the self-organized structures, either unimolecular or multimolecular aggregates, may be retained to some extent because the self-organized structures have essentially no dynamic nature and may be viewed as solid particles sustained in water by negatively charged surface, as is revealed by the characterization in this work.

Light Scattering. In Table 2 are listed static and dynamic light scattering data for poly(A/LA/Py), poly(A/CD/Py), and poly(A/AD/Py) in aqueous solution containing 0.1 M NaCl. Weight-average molecular weights (determined by SLS) of poly(A/LA/Py) and poly(A/CD/Py) are on the order of 10^5 and that of poly(A/AD/Py) is on the order of 10^4. A characteristic feature for these terpolymers is that the Stokes radii (determined by DLS) are considerably small for their molecular weights. This is an indication that the terpolymers adopt a very compact conformation due to self-association of the hydrophobic groups in aqueous solution. It should be pointed out that poly(A/CD/Py) has the most compact conformation of all three terpolymers as indicated by the largest ratio of mass to dimension ($M_w = 5.1 \times 10^5$ against $R_s = 5.5$ nm).

SAXS. Figure 8 shows SAXS data for poly(A/CD/Py) and poly(A/AD/Py) in a concentrated (ca. 10 wt %) aqueous solution. Scattering peaks were observed at $2\theta = 0.8$ and 1.2° for the CD- and AD-containing terpolymers, respectively, which correspond to the spacings of 11 and 7.3 nm, respectively. A spacing of 11 nm for the CD-containing terpolymer coincides with the Stokes diameter determined by DLS (Table 2). These

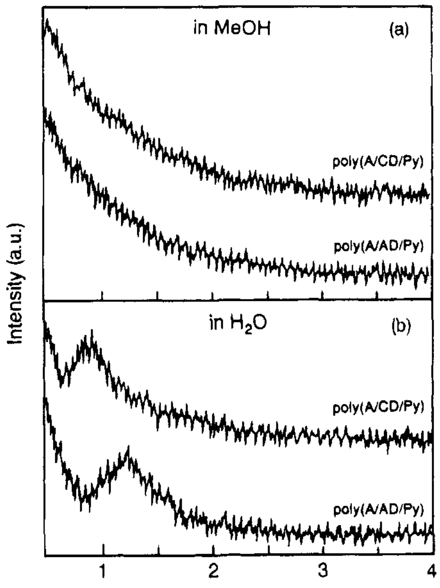


Figure 8. SAXS patterns for poly(A/AD/Py) and poly(A/CD/Py) in methanol (a) and in aqueous solution (b). Concentration of the polymers, ca. 10 wt %.

scattering peaks can be interpreted to be due to closely packed unimer particles in the concentrated aqueous solutions. The CD- and AD-containing terpolymers remain as unimers even at high concentrations, and the unimers may exist independently (with no significant interpenetration) even at the high concentration (ca. 10 wt %) for the SAXS measurements. As is evident in Figure 8b, no such scattering peaks were observed in the methanol solution. These terpolymers adopt random coils in methanol, and the random coils shuold interpenetrate each other at the high concentration (ca. 10 wt %) employed for the SAXS measurements.

It should be noted, however, that in the case of poly(A/LA/Py), no scattering peaks were recognized in aqueous solution in the small-angle region. This is because the LA-containing terpolymer can only exist as unimer at concentrations below ca. 0.2 wt %, and at higher concentrations the intermolecular association occurs.

We previously reported that an AMPS terpolymer with 59 mol % 1-Np and 1 mol % Py groups emitted entirely Py fluorescence when the 1-Np chromophores were selectively excited at 290 nm in aqueous solution.[15] The Py groups are confined within the hydrophobic clusters of the 1-Np groups in the unimer of this 1-Np-containing terpolymer in aqueous solution. Upon excitation of the 1-Np chromophores, singlet-excited energy of 1-Np migrates throughout the unimer and is eventually trapped by the Py groups. This observation suggests that, even if there are a number of cluster units in a unimer, all the cluster units are in contact with each other such that the 1-Np singlets can migrate from one cluster unit to another. This situation may apply to unimer-forming aliphatic hydrophobic groups including CD and AD groups.

Conclusions

Random copolymers of AMPS and methacrylamides *N*-substituted with LA, CD, or AD groups form self-organized microphase structures in aqueous solution. The CD and AD groups in the polymers show a much stronger tendency for the intramolecular self-association than do the LA groups. The CD- and AD-containing polymers exist as unimers in a wide range of concentrations up to ca. 7 wt %. In contrast, the LA-containing polymers can exist as unimers only at much lower concentrations (<ca. 0.2 wt %), intermolecular aggregates being formed at higher concentrations. The unimers of the CD-containing polymers are extremely compact; e.g., the polymer with a weight-average molecular weight of 5.1×10^5 forms unimers with a mean hydrodynamic radius of 5.5 nm. ^{1}H-NMR relaxation times indicated that the local motions of the CD groups in the unimers were much more restricted than those of the LA groups. Such compact unimer structures are primarily the consequence of extensive hydrophobic interactions between the bulky hydrophobes, but hydrogen bonding between the spacer amide bonds may contribute to the retention of the unimer structures to some extent.

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