Macromolecules **1998,** *31,* 3588−3594

Fluorescence Studies of Hydrophobic Association of Random Copolymers of Sodium 2-(Acrylamido)-2-methylpropanesulfonate and *N*-Dodecylmethacrylamide in Water

Hiroshi Yamamoto, Masanobu Mizusaki, Kenjiro Yoda, and Yotaro Morishima*

Department of Macromolecular Science, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan

Received January 7, 1998; Revised Manuscript Received April 1, 1998

ABSTRACT: Intrapolymer self-association of random copolymers of sodium 2-(acrylamido)-2-methylpropanesulfonate (AMPS) and *N*-dodecylmethacrylamide (DodMAm) with varying compositions in aqueous solution was investigated by various fluorescence techniques. The polymers were labeled with 1 mol % of naphthalene (Np) or pyrene (Py) or doubly labeled with Np (4 mol %) and Py (1 mol %). Vibronic fine structures of Py fluorescence, lifetimes of Np and Py fluorescence, intrapolymer nonradiative energy transfer (NRET) from singlet excited Np to ground-state Py labels, and fluorescence quenching by thallium cations were investigated as a function of the DodMAm content (f_{Dod}) in the copolymers with or without added salt. Results from all these fluorescence studies indicate that with increasing f_{Dod}, hydrophobic association commences at $f_{\mathrm{Dod}} \cong 20$ mol % in pure water and at $f_{\mathrm{Dod}} \cong 10$ mol % in 0.1 M NaCl, showing a saturation tendency near $f_{\mathrm{Dod}} \cong 40$ mol % in the salt solution. In contrast, viscosity data show that the polymer size markedly decreases in the regime $5 < f_{\mathrm{Dod}} < 20$ mol % owing to intrapolymer hydrophobic association of dodecyl groups. This decrease in the macroscopic size of the polymer in the low f_{Dod} regime could not be detected by any fluorescence techniques employed. Although the viscosity data do not show any further contraction of the polymer chains at $f_{\mathrm{Dod}} > 20$ mol %, the NRET results indicate a considerable increase in the compactness of polymer chains at $f_{\mathrm{Dod}} > 20$ mol %. The combination of these fluorescence techniques proved to provide a sensitive tool to detect hydrophobic associations and conformational changes in the hydrophobically modified polymers, while viscosity reflects only global changes in the polymer size.

Introduction

In the past decades, various types of hydrophobically modified water-soluble polymers have been synthesized and studied with a focus of their self-associating behavior in water.[1] The self-association of hydrophobes in such polymers occurs either within a single polymer chain or among different polymer chains, or both at a time, depending on the chemical structure of the polymers. In general, in highly dilute aqueous solutions, hydrophobic associations may preferentially occur within a polymer chain, but with an increase in the polymer concentration, a tendency for interpolymer association increases.

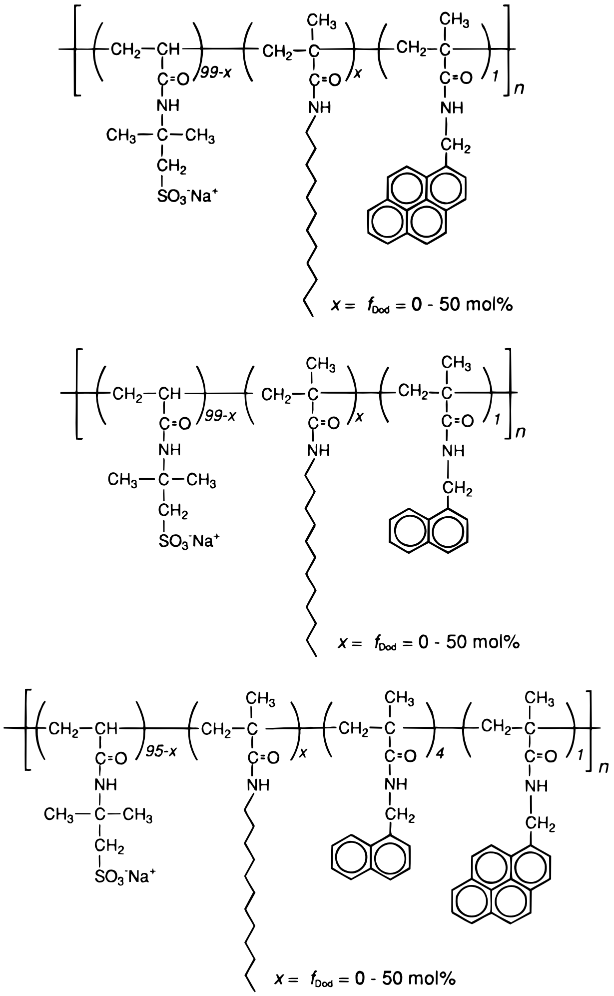
We previously reported that random copolymers of sodium 2-(acrylamido)-2-methylpropanesulfonate (AMPS) and methacrylamides N-substituted with bulky hydrophobes have a strong preference for intrapolymer self-association even in a concentrated aqueous solution, leading to the formation of “unimolecular micelles” (unimer micelles).[2−4] The type of hydrophobes, sequence distribution of the electrolyte and hydrophobic monomer units in the polymer chain, and the type of spacer bonds between the hydrophobes and polymer chain are some of the critical parameters that determine whether the hydrophobic association occurs in an intra- or interpolymer fashion.

Copolymers of AMPS and *N*-cyclododecylmethacrylamide or *N*-adamantylmethacrylamide show a particularly strong tendency for intrapolymer hydrophobic association even at high concentrations on the order of 10 wt % in water, while copolymers of AMPS and *N*-dodecylmethacrylamide (DodMAm) show a modest tendency for intrapolymer association.[3] The latter copolymers form unimer micelles only when the concentration is lower than ca. 0.5 wt %. In these copolymers, where AMPS and hydrophobic units are randomly distributed along a polymer chain, the intrapolymer hydrophobic interaction is competing with electrostatic repulsion between AMPS units within a polymer chain. If the hydrophobe content in the copolymer is sufficiently low, the polymer chain will adopt an extended conformation characteristic of fully ionized polyelectrolytes in water. However, with an increase in the hydrophobe content (or a decrease in the AMPS content), hydrophobic association begins to occur, resulting in a decrease in polymer size in aqueous solution. With a further increase in the hydrophobe content, the polymer size will further decrease, eventually collapsing into a highly compact structure that is referred to as a unimer micelle.[3]

In the present study, we attempted to characterize the hydrophobic self-association behavior of the copolymers of AMPS and DodMAm in water as a function of the DodMAm content by using various fluorescence techniques in combination. Fluorescence is a sensitive tool to monitor associative behavior of hydrophobically modified water-soluble polymers.[5] For fluorescence characterization, a fluorescent chromophore is often labeled on a polymer by copolymerization employing a monomer substituted with an appropriate chromophore. In this work, we labeled the AMPS−DodMAm copolymers with naphthalene (Np) or pyrene (Py) or doubly labeled them with Np and Py in the same polymer chain (Chart 1). With use of these labeled polymers, we investigated self-association behavior by focusing on the vibronic fine structures of Py fluorescence spectra,

S0024-9297(98)00022-9 CCC: $15.00

Chart 1



lifetimes of Np and Py fluorescence, fluorescence quenching by thallium (Tl^+) cations, and intrapolymer nonradiative energy transfer (NRET) from Np to Py. The vibronic fine structures and lifetimes will report on the polarity of microenvironments where fluorophores exist, while Tl^+ quenching will report on the extent of the "protection" of fluorophores from the bulk aqueous phase. Intrapolymer NRET will give us information about the compactness of polymer conformation. Thus, the combination of these different fluorescence methods is of use for comprehensive characterization of the self-association behavior of polymers.

Experimental Section

Monomers. *N*-Dodecylmethacrylamide (DodMAm),[6] *N*-(1-pyrenylmethyl)methacrylamide (1PyMAm),[7] and *N*-(1-naphthylmethyl)methacrylamide (1NpMAm)[8] were prepared as reported previously. 2-(Acrylamido)-2-methylpropanesulfonic acid (AMPS) was used as received from Nitto Chemical Industry Co.

Singly-Labeled Polymers. A general procedure for the preparation of naphthalene- or pyrene-labeled polymers is as follows: A mixture of AMPS, DodMAm (0−50 mol %), 1PyMAm (1 mol %) or 1NpMAm (1 mol %), and 0.1 mol % (based on the total monomers) of 2,2′-azobis(isobutyronitrile) (AIBN) was dissolved in *N*,*N*-dimethylformamide (DMF) in a glass ampule, and the ampule 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 ether to precipitate polymers. The polymer was purified by three reprecipitations from methanol into ether and then dissolved in dilute aqueous NaOH. The alkaline solution was dialyzed against pure water for a week and finally lyophilized. The compositions of the singly-labeled polymers were determined by elemental analysis (N/C ratios) and UV absorption spectroscopy.

Doubly-Labeled Polymers. Polymers labeled doubly with naphthalene and pyrene were prepared and purified in a manner similar to the preparation of singly-labeled polymers with the use of AMPS, DodMAm (0−50 mol %), 1NpMAm (4 mol %), and 1PyMAm (1 mol %). The compositions of the doubly-labeled polymers were determined by elemental analysis (N/C ratios) and UV absorption spectroscopy.

Other Materials. Analytical grade thallium nitrate, sodium chloride, and sodium acetate were used without further purification. Milli-Q water was used for fluorescence measurements.

Measurements. **a. Absorption and Fluorescence Spectra.** Absorption spectra were recorded with a JASCO V-520 spectrophotometer. Steady-state fluorescence spectra were measured on a Hitachi F-4500 fluorescence spectrophotometer by use of a 1-cm path length quartz cuvette with excitation at 290 and 343 nm for naphthalene and pyrene labels, respectively.

b. Fluorescence Lifetime. Fluorescence decays were measured by a time-correlated single-photon counting technique using a Horiba NAES 550 system equipped with a flash lamp filled with H_2. The decay function and response function were both measured simultaneously. The decay curves were analyzed by conventional deconvolution techniques.

c. Nonradiative Energy Transfer (NRET). Aqueous solutions (0.05 mg/mL) of doubly-labeled polymers with varying DodMAm contents were excited at 290 nm, and fluorescence spectra were recorded in the wavelength range 300−550 nm. The intensity of pyrene fluorescence due to NRET from singlet excited naphthalene was estimated at 376 nm. Contribution from direct pyrene excitation was corrected by subtracting from each spectrum the emission spectrum of the corresponding pyrene singly-labeled polymer of the same pyrene concentration.

d. Fluorescence Quenching with Thallium Nitrate. Small amounts of a stock solution of thallium nitrate were added to 0.05 M sodium acetate aqueous solutions of naphthalene-labeled polymers such that the concentrations of Tl^+ ions ranged 0−8 mM while the concentration of the polymers was kept constant at 1 mg/mL. Fluorescence was measured with excitation at 290 nm.

Results and Discussion

Fluorescence Spectra and Lifetimes. We previously reported that the copolymerization of AMPS and DodMAm is an "ideal copolymerization", which leads to copolymer compositions equal to monomer feed compositions and completely random distributions of the monomer sequences.[7] When the content of dodecyl (Dod) groups (f_{Dod}) (mol %) in the copolymers is low enough, Np and Py labels will be exposed to the aqueous phase, but when f_{Dod} is sufficiently high, hydrophobic association will occur to form hydrophobic microdomains in which the hydrophobic labels may be incorporated.

The Np and Py labels show only monomeric fluorescence because their loading amount in the polymers is low enough, i.e., 1 mol %. Fluorescence spectra of Py labels provide information about their local environments because the ratio of the third to first vibrational fine structures, I_3/I_1, in their fluorescence spectra is sensitive to the environmental polarity.[9] It is generally known that the I_3/I_1 ratio is larger in less polar media.[9] The I_3/I_1 ratios for the Py-labeled polymers at varying f_{Dod} in pure water and in 0.1 M NaCl are plotted against f_{Dod} in Figure 1. In pure water, the I_3/I_1 ratios at $f_{Dod} <$ 10 mol % are ca. 0.6, reflecting the polarity of bulk

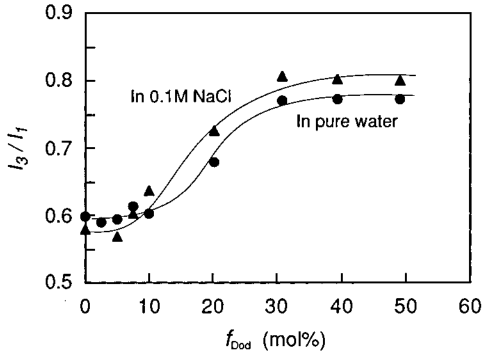


Figure 1. Plot of the I_3/I_1 ratio in pyrene fluorescence spectra of the Py-labeled AMPS–DodMAm copolymers as a function of the DodMAm content.

Table 1. Fitting Parameters for Fluorescence Decays for Np and Py Labels in the AMPS–DodMAm Copolymers with Varying DodMAm Contents (f_{Dod}) Measured in 0.1 M NaCl[a]

f_{Dod} (mol %)	τ (ns)/α	χ^2
	Py-Labeled Polymer	
0	182/1.0	1.65
2.5	183/1.0	1.39
5	186/1.0	1.41
7.5	153/0.639, 365/0.361	1.02
10	159/0.627, 392/0.373	0.996
20	164/0.507, 373/0.493	0.959
30	173/0.498, 375/0.502	1.14
40	162/0.293, 350/0.707	1.08
50	173/0.240, 358/0.760	1.11
	Np-Labeled Polymer	
0	37.6/1.0	1.23
10	42.2/1.0	1.51
20	49.6/1.0	1.57
30	52.6/1.0	1.53
40	54.3/1.0	1.70
50	54.4/1.0	1.50

[a] Fitting function: $I(t) = \sum \alpha_i \exp(-t/\tau_i)$, where τ_i is the fluorescence lifetime of the ith component and α_i is its fraction.

water. The I_3/I_1 ratio increases in the region $10 < f_{Dod} < 30$ mol %, reaching a maximum value of 0.77 at $f_{Dod} > 30$ mol %. In 0.1 M NaCl, this increase in the I_3/I_1 ratio commences at a lower f_{Dod}, reaching a maximum value of ca. 0.8 at $f_{Dod} \cong 30$ mol %. This value is slightly larger than that in pure water. These observations suggest the following. It is likely that the polymers adopt an extended conformation in pure water and an open coil in 0.1 M NaCl when $f_{Dod} < 10$ mol %. In these situations, Py labels are exposed to the aqueous phase. Hydrophobic microdomains begin to form at $f_{Dod} \cong 10$ mol % in pure water and at a somewhat lower f_{Dod} in 0.1 M NaCl, and Py labels are incorporated in the microdomains. With an increase in f_{Dod} the extent of the label incorporation increases, reaching a maximum extent at $f_{Dod} \geq 30$ mol %. Microenvironments about Py labels in 0.1 M NaCl are slightly more hydrophobic than those in pure water when compared at the same f_{Dod}.

Fluorescence lifetimes for aromatic chromophores tend to become longer in less polar media.[7] Figure 2 shows fluorescence decay data for Np and Py labels measured in 0.1 M NaCl at 25 °C. All the decay data for Np labels were best fitted with a single-exponential function regardless of f_{Dod}. The lifetime increases with increasing f_{Dod} as listed in Table 1. In the case of the Py labels, however, the decays are single-exponential only when $f_{Dod} \leq 5$ mol %. At $f_{Dod} \geq 7.5$ mol % the decays are biphasic with a slower decay component with a lifetime on the order of 350–390 ns. The fraction of the slower decay component increases progressively with f_{Dod}, as can be seen from Table 1. These observations suggest that there are two states of Py labels with respect to the extent of the incorporation of each label

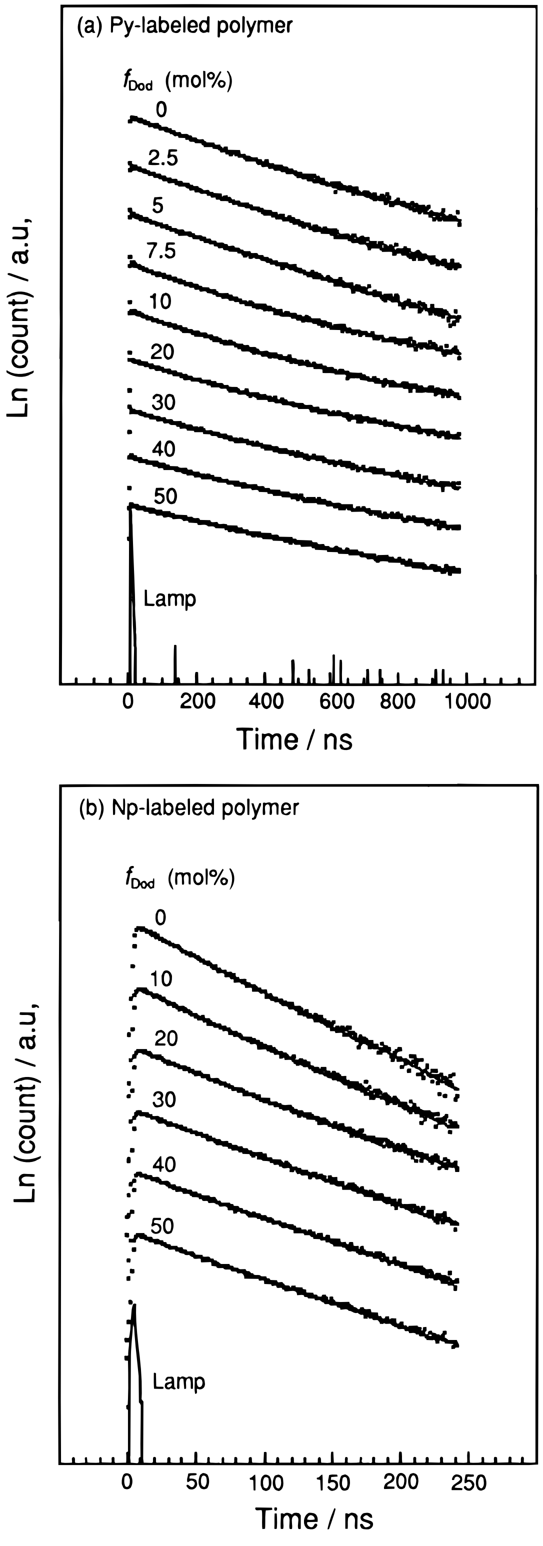


Figure 2. Fluorescence decay profiles for Py-labeled (a) and Np-labeled (b) AMPS–DodMAm copolymers with varying DodMAm contents in 0.1 M NaCl aqueous solutions. The decay plots are presented in the figure with their baselines vertically shifted for ease of comparison. Best fit curves from the use of a single- or double-exponential function are indicated on the data plots.

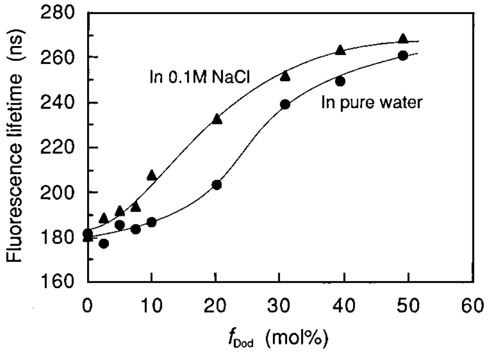


Figure 3. Plot of the fluorescence lifetime for the Py-labeled AMPS−DodMAm copolymers as a function of the DodMAm content.

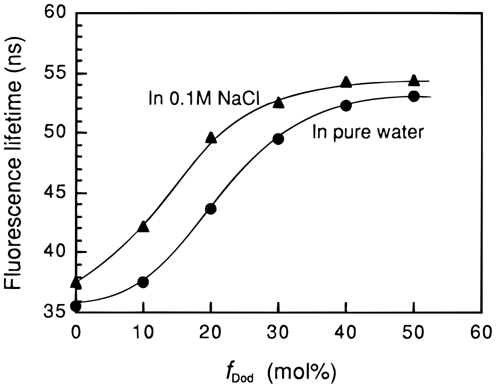


Figure 4. Plot of the fluorescence lifetime for the Np-labeled AMPS−DodMAm copolymers as a function of the DodMAm content.

into a hydrophobic microdomain, i.e., completely incorporated labels and nonincorporated or only partially incorporated ones. The former may be responsible for the slower decay and the latter for the faster one. By contrast, it seems that all Np labels are quite uniformly incorporated into hydrophobic microdomains such that at a low f_{Dod} each Np species is partly incorporated in a Dod microdomain, but there is not much difference in the extent of the incorporation. These speculations are based on the fact that the molecular size of naphthalene is only half that of pyrene.

The fluorescence lifetimes for the Py and Np labels are plotted as a function f_{Dod} in Figures 3 and 4, respectively. For the Py-labeled polymers with $f_{Dod} \geq$ 7.5 mol %, average lifetimes are plotted in Figure 3. The lifetimes measured in pure water are also plotted for comparison. For both Np and Py labels the dependence of the lifetime on f_{Dod} appears to parallel that of the I_3/I_1 ratio on f_{Dod} (Figure 1), except that no plateau was observed for the lifetimes at $f_{Dod} > 30$ mol %; the lifetimes in pure water and in 0.1 M NaCl increase further in the region $f_{Dod} > 30$ mol %, while the I_3/I_1 ratio shows a saturation. We will discuss this point in a later subsection.

Fluorescence Quenching by Thallium Nitrate. Fluorescence quenching of polymer-bound aromatic chromophores by Tl^+ ions provides a useful tool to study conformational and dynamic behavior of the polymer in aqueous solution.[3,10−17] This technique is particularly useful for the characterization of self-association of hydrophobically modified water-soluble polymers labeled with aromatic chromophores.[3,14−17] The quenching of aromatic fluorophores by Tl^+ ions requires a short-range interaction due to an external heavy atom effect,[18] and the quenching of polyanion-bound aromatic fluorophores by Tl^+ ions is very efficient because the electrostatic attraction by polyanions can bring Tl^+ ions close to fluorophore sites.[11−13] However, if aromatic chromophores are encapsulated in hydrophobic microdomains formed from amphiphilic polyanions, the chromophores are protected from the access of Tl^+ ions.[3,10−17] This situation leads to a suppression of fluorescence quenching, and the degree of the suppression reflects the extent of the protection of the chromophores in the microdomain, thus allowing one to verify the formation of hydrophobic microdomains in hydrophobically modified polyanions.

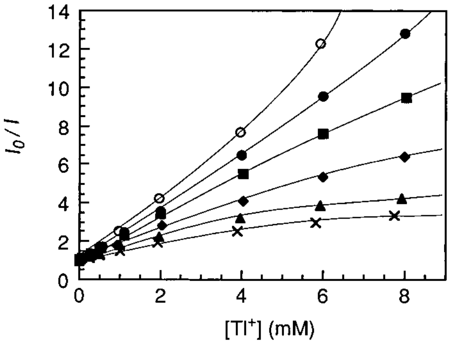


Figure 5. Stern−Volmer plots for fluorescence quenching by Tl^+ for the Np-labeled AMPS−DodMAm copolymers with varying DodMAm contents in 0.05 M sodium acetate aqueous solutions at pH 8. f_{Dod} = 0−50 mol % from top to bottom.

Figure 5 shows Stern−Volmer plots for the Np-labeled polymers with varying f_{Dod}. All the quenching experiments were performed in a 0.05 M sodium acetate buffer solution of pH 8. For the Np-labeled polymer without dodecyl groups ($f_{Dod} = 0$), which assumes an open chain conformation, the quenching is very efficient because Tl^+ ions are electrostatically concentrated on the polymer, the plot showing an upward curvature. However, the quenching is progressively suppressed and the plot becomes a downward curvature as f_{Dod} is increased, which arises from increasing protection of Np labels from Tl^+ ions.

In the case where there are two fluorophore sites, one accessible to quenchers and the other not, the Stern−Volmer equation can be modified as[18]

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

where I and I_0 are the fluorescence intensities in the presence and absence of the quencher, respectively, K is the Stern−Volmer constant for the accessible chromophores, and ϕ is the fraction of the accessible chromophores. By applying eq 1 to the data in Figure 5, we can estimate the fraction of fluorophores that are not accessible to the quencher, $1 - \phi$, for the Np-labeled polymers with varying f_{Dod}. Results are plotted in Figure 6. The value of $1 - \phi$ significantly increases in the range $20 < f_{Dod} < 40$ mol %. This tendency is quite different from that of the fluorescence lifetime, which shows a considerable increase in the range $10 < f_{Dod} <$ 30 mol % and a tendency for level off at $f_{Dod} \cong 30$ mol % (Figure 4). This implies that although the micropolarity that the Np label can experience tends to level off at f_{Dod} near 30 mol %, the extent of the steric protection of the Np label further increases with increasing f_{Dod} in the region $f_{Dod} > 30$ mol %.

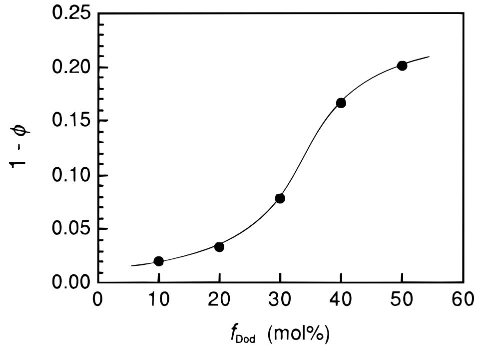


Figure 6. Plot of the fraction of fluorophores not accessible to Tl^+ quenchers ($1 - \phi$) as a function of the DodMAm content in the Np-labeled AMPS−DodMAm copolymers in 0.05 M sodium acetate aqueous solutions at pH 8.

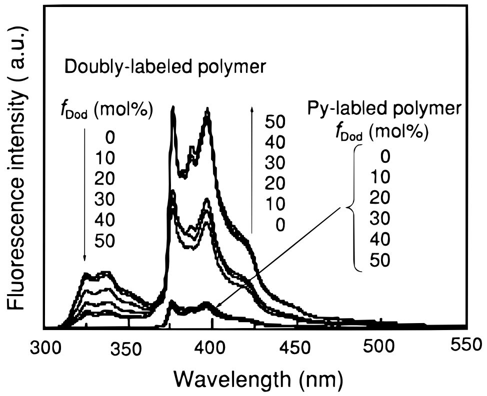


Figure 7. Fluorescence spectra for the Py and Np doubly-labeled AMPS−DodMAm copolymers with varying DodMAm contents in 0.1 M NaCl. The spectra for the Py singly-labeled polymers are also indicated. Excitation at 290 nm.

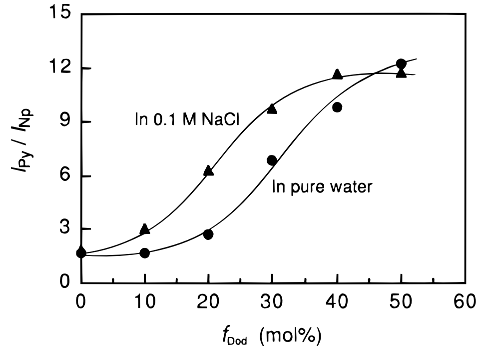


Figure 8. Ratio of the intensities of fluorescence from Py and Np labels (I_{Py}/I_{Np}) as a function of the DodMAm content in the Np and Py doubly-labeled AMPS−DodMAm copolymers in 0.1 M NaCl with excitation at 290 nm.

NRET in Doubly-Labeled Polymers. NRET between an energy donor and energy acceptor covalently attached to the same polymer chain or separate polymer chains provides a sensitive tool to probe into conformational changes.[19−23] Naphthalene and pyrene are often used as an energy donor and acceptor, respectively, because this donor/acceptor pair has a large spectral overlap, and naphthalene can be almost selectively excited at a wavelength near 290 nm. The occurrence of NRET is indicated by an increase in pyrene fluorescence with excitation of naphthalene.

Figure 7 shows fluorescence spectra of the doubly-labeled polymers with different f_{Dod} in 0.1 M NaCl aqueous solutions. For comparison, fluorescence spectra of the pyrene-labeled polymers are presented in Figure 7. The concentrations of Py labels for both the doubly- and singly-labeled polymers are the same, and all the sample solutions were excited at 290 nm. The intensity of characteristic Py fluorescence in the wavelength region 360−500 nm increases significantly in the doubly-labeled polymers, indicating intrapolymer NRET from photoexcited Np to Py labels. With an increase in f_{Dod}, the intensity of Py fluorescence increases and, in turn, the intensity of Np fluorescence in the wavelength region 310−360 nm decreases. This is due to a decrease in the average distance between the Np and Py labels within the polymer chain; i.e., with increasing f_{Dod}, increasing fractions of Np and Py labels come close to each other within the Förster radius ($R_0 = 2.86$ nm for transfer from 1-methylnaphthalene to pyrene[24]). This decrease in the donor−acceptor distance results from an increase in the extent of hydrophobic association between Dod groups with increasing f_{Dod}. McCormick and co-workers[23] discussed that if a constant naphthalene/pyrene ratio was employed, changes in the extent of NRET were proportional to the intensity ratio of pyrene fluorescence to naphthalene fluorescence (I_{Py}/I_{Np}). Thus, this ratio is a practical measure for the NRET quantum efficiency. In Figure 8, I_{Py}/I_{Np} is plotted as a function of f_{Dod}, the I_{Py}/I_{Np} ratio reflecting the average separation between Np and Py labels. Although Np labels can be predominantly excited at 290 nm, a slight contribution of direct excitation of Py labels was corrected for the estimation of I_{Py}/I_{Np} (see Experimental Section). In pure water, the I_{Py}/I_{Np} ratio commences to increase significantly at $f_{Dod} \cong 20$ mol % and continues to increase even at $f_{Dod} = 50$ mol %. This suggests that a decrease in the donor/acceptor separation or a decrease in the polymer size due to the hydrophobic association of Dod groups begins to occur significantly at $f_{Dod} \cong 20$ mol % in pure water, and the compaction of the polymer size continues over the entire range of the Dod content ($f_{Dod} < 50$ mol %) shown in Figure 8. In 0.1 M NaCl, on the other hand, the I_{Py}/I_{Np} ratio slightly increases at $f_{Dod} \leq 10$ mol % and more significantly increases in the range $10 < f_{Dod} < 40$ mol %, reaching a plateau at $f_{Dod} \cong 40$ mol %. This indicates that the polymer compaction begins and completes at lower Dod contents than those in pure water. These results are virtually consistent with those from the I_3/I_1 ratio (Figure 1) and lifetime data (Figures 3 and 4). NRET reflects the conformational compactness, while the I_3/I_1 ratio and fluorescence lifetime reflect the micropolarity about the labels. Accordingly, as f_{Dod} is increased, the polymer compaction occurs in parallel with an increase in the micropolarity about the fluorescence labels, which is an indication of the increasing hydrophobic self-association of Dod groups with increasing Dod content.

All the NRET results described above were obtained at a 0.05 mg/mL polymer concentration (see Experimental Section). In a separate experiment, we examined NRET as a function of f_{Dod} at a higher polymer concentration (0.3 mg/mL) and confirmed that results were virtually identical to those obtained at 0.05 mg/mL. Furthermore, in our earlier work we confirmed that a mixture of two singly-labeled polymers (i.e., a 1 mol % Py-labeled polymer with $f_{Dod} = 49$ mol % and a 4 mol % Np-labeled polymer with $f_{Dod} = 32$ mol %) exhibited no NRET at polymer concentrations <ca. 0.5 wt %.[3] Thus, we can conclude that interpolymer NRET is absent in the results in Figures 7 and 8.

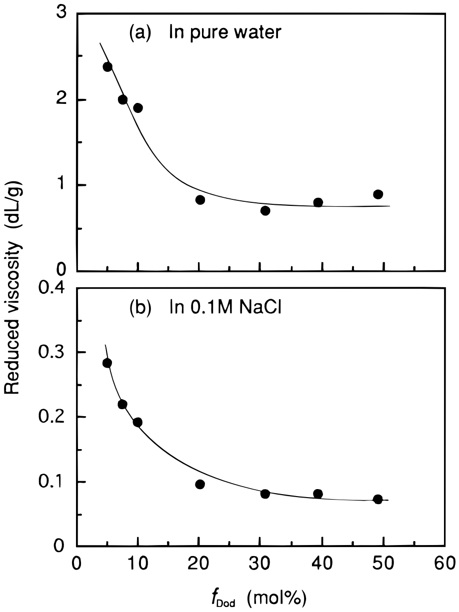


Figure 9. Plot of the reduced viscosity at 0.1 g/dL as a function of the DodMAm content in the Py-labeled AMPS−DodMAm copolymers.

Viscosity. As discussed above, the dependence of the extent of the hydrophobic self-association on f_{Dod} was able to be observed through various photophysical phenomena, which include the I_3/I_1 ratio in pyrene fluorescence, fluorescence lifetimes, Tl^+ quenching, and NRET. The I_3/I_1 ratio, lifetimes, and NRET consistently indicated that the extent of the hydrophobic association significantly increased in the regime $10 < f_{Dod} < 30$ mol % in 0.1 M NaCl, but the Tl^+ quenching indicated that the extent of the chromophore protection significantly increased in the regime $20 < f_{Dod} < 40$ mol %. This means that although the hydrophobicity around the labels and the polymer chain contraction can nearly be maximized at $f_{Dod} \cong 30$ mol %, more Dod groups are necessary to maximize the chromophore protection. The chromophore protection does not seem to be maximized even at $f_{Dod} = 50$ mol %. Fluorescence provides only microscopic information about polymer chains. Thus, it is important to clarify how the microscopic information from fluorescence is related to the macroscopic dimension of polymer chains.

In Figure 9, reduced viscosities (at 0.1 g/dL) are plotted as a function of f_{Dod}. The reduced viscosities in 0.1 M NaCl are nearly 1 order of magnitude lower than those in pure water. Important observations, as compared to the observations from fluorescence, are that the reduced viscosity sharply drops in the range $5 < f_{Dod} < 20$ mol %, and that no further significant decrease in the viscosity is observed in the region $f_{Dod} > 20$ mol %. These observations indicate that the collapse of the polymer coil due to the intrapolymer hydrophobic association of Dod groups occurs at $f_{Dod} < 20$ mol %, and essentially no further chain contraction at $f_{Dod} > 20$ mol % is detectable by viscometry. This is in contrast to the fluorescence results; e.g., the NRET data show that a significant decrease in the microscopic polymer size occurs at $f_{Dod} > 20$ mol %. Furthermore, the effect of added salt on the microscopic chain contraction observed by NRET is much smaller than that on the macroscopic chain contraction observed by viscosity. The comparison of viscosity and fluorescence data leads us to conclude that macroscopic chain collapse occurs in the range $5 < f_{Dod} < 20$ mol %, which can be detected by viscosity but not by fluorescence and that significant microscopic chain contraction occurs in the range $20 < f_{Dod} < 30$ mol %, which can only be detected by fluorescence but not by viscosity. Similar results have been reported by McCormick and co-workers[23] for fluorescence-labeled copolymers of acrylamide and sodium 11-(acrylamido)-undecanoate. On the basis of excimer emission and NRET data for the pyrene- and naphthalene-labeled polymers, they have shown that the conformational changes on the angstrom level are only observable by photophysical analysis, while such changes are not observable by macroscopic methods such as viscosity.

Conclusions

Intrapolymer hydrophobic association of Dod groups in the copolymers of AMPS and DodMAm with varying f_{Dod} was investigated by various fluorescence techniques with the use of the polymers singly labeled with Np or Py and doubly labeled with Np and Py. The I_3/I_1 ratio in Py fluorescence, fluorescence lifetimes of Np and Py labels, and NRET from Np to Py in the doubly-labeled polymers consistently indicated that the extent of the hydrophobic association increased significantly in the f_{Dod} range between 10 and 30 mol % in 0.1 M NaCl, but the Tl^+ quenching indicated that a significant increase in the chromophore protection occurred in the range $20 < f_{Dod} < 40$ mol %. This means that although the hydrophobicity around the labels and the polymer chain contraction can nearly be maximized at $f_{Dod} \cong 30$ mol %, more Dod groups are necessary to maximize the chromophore protection. In contrast, viscosity data show that the hydrodynamic volume of the polymers decreases markedly at $f_{Dod} < 20$ mol % but no significant decrease in the hydrodynamic volume was observed at $f_{Dod} > 20$ mol %. This decrease in the macroscopic size of the polymers in the low f_{Dod} regime was not able to be detected by any of the fluorescence techniques employed. Although the viscosity data showed no further contraction of polymer chains at $f_{Dod} > 20$ mol %, the NRET results indicated a significant increase in the microscopic compactness of the polymer chains in this high f_{Dod} regime.

Acknowledgment. This work was supported in part by a Grant-in-Aid on Priority-Area-Research, “New Polymers and Their Nano-Organized Systems” (No. 277/08246236), from the Ministry of Education, Science, Sports, and Culture, Japan.

References and Notes

(1) For reviews, see: (a) McCormick, C. L.; Bock, J.; Schulz, D. N. *Encyclopedia of Polymer Science and Engineering;* John Wiley: New York, 1989; Vol. 17, p 730. (b) Bock, J.; Varadaraj, R.; Schulz, D. N.; Maurer, J. J. In *Macromolecular Complexes in Chemistry and Biology*; Dubin, P., Bock, J., Davies, R. M., Schulz, D. N., Thies, C., Eds.; Springer-Verlag: Berlin and Heidelberg, 1994; p 33. (c) Valint, P. L., Jr.; Bock, J.; Schulz, D. N. In *Polymers in Aqueous Media: Performance through Association*; Glass, J. E., Ed.; Advances in Chemistry Series 223, American Chemical Society; Washington, DC, 1989; p 399. (d) *Polymers as Rheology Modifiers*; Schulz, D. N., Glass, J. E., Eds.; Advances in Chemistry Series 462; American Chemical Society: Washington, DC, 1991.
(2) Morishima, Y. *Trends Polym. Sci.* **1994**, *2*, 31.
(3) Morishima, Y.; Nomura, S.; Ikeda, T.; Seki, M.; Kamachi, M. *Macromolecules* **1995**, *28*, 2874.
(4) Morishima, Y. In *Solvents and Self-Organization of Polymers*; Webber, S. E., Tuzar, D., Munk, P., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1996, p 331.

(5) See for example: *Photophysical and Photochemical Tools in Polymer Science*; Winnik, M. A., Ed.; Reidel: Dordrecht, The Netherlands, 1986.

(6) Morishima, Y.; Kobayashi, T.; Nozakura, S. *Polym. J.* **1989**, *21*, 267.

(7) Morishima, Y.; Tominaga, Y.; Kamachi, M.; Okada, T.; Hirata, Y.; Mataga, N. *J. Phys. Chem.* **1991**, *95*, 6027.

(8) Morishima, Y.; Tominaga, Y.; Nomura, S.; Kamachi, M. *Macromolecules* **1992**, *25*, 861.

(9) Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1977**, *99*, 2039.

(10) Chu, D.-Y.; Thomas, J. K. *Macromolecules* **1984**, *17*, 2142.

(11) Morishima, Y.; Tominaga, Y.; Nomura, S.; Kamachi, M. *J. Polym. Sci., Polym. Chem. Ed.* **1993**, *31*, 789.

(12) Morishima, Y.; Ohgi, H.; Kamachi, M. *Macromolecules* **1993**, *26*, 4293.

(13) Morrison, M. E.; Dorfman, R. C.; Clendening, W. D.; Kiserow, D. J.; Rossky, P. J.; Webber, S. E. *J. Phys. Chem.* **1994**, *98*, 5534.

(14) Cao, T.; Yin, W.; Webber, S. E. *Macromolecules* **1994**, *27*, 7459.

(15) Cao, T.; Yin, W.; Armstrong, J. L.; Webber, S. E. *Langmuir* **1994**, *10*, 1841.

(16) Procházka, K.; Kiserow, D. J.; Webber, S. E. *Acta Polym.* **1995**, *46*, 277.

(17) Kramer, M. C.; Welch, C. G.; Steger, J. R.; McCormick, C. L. *Macromolecules* **1995**, *28*, 5248.

(18) Hashimoto, S.; Thomas, J. K. *J. Am. Chem. Soc.* **1985**, *107*, 4655.

(19) Webber, S. E. *Chem. Rev.* **1990**, *90*, 1469.

(20) Winnik, F. M. *Polymer* **1990**, *31*, 2125.

(21) Ringsdorf, H.; Simon, J.; Winnik, F. M. *Macromolecules* **1992**, *25*, 5353 and 7306.

(22) Hu, Y.; Kramer, M. C.; Boudreaux, C. J.; McCormick, C. L. *Macromolecules* **1995**, *28*, 7100.

(23) Kramer, M. C.; Steger, J. R.; Hu, Y.; McCormick, C. L. *Macromolecules* **1996**, *29*, 1992.

(24) Berlman, I. B. *Energy Transfer Parameters of Aromatic Compounds*; Academic Press: New York, 1973.

MA980022U