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Micellar Structure of Amphiphilic Statistical Copolymers Bearing Dodecyl Hydrophobes in Aqueous Media

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ABSTRACT: The structure of micellar aggregates formed from ionic statistical copolymers of *N*-acryloyl-amino acids and *N*-dodecylmethacrylamide in 0.05 M aqueous NaCl was studied by light scattering and fluorescence. The experimental results indicated that the tendency of the interchain aggregation increased with the hydrophobic monomer content for each series of the copolymers but decreased with hydrophobicity of the amino acid residue in the copolymers. On the other hand, while the micellar structure of the statistical copolymers strongly depended on the kind of the hydrophobe, it is little dependent on the kind of the electrolyte monomer unit at low ionic strength. Using these data, a theoretical analysis taking into account the chain stiffness effect revealed that uni-core micelles formed from the ionic statistical copolymers were of flower type with a minimum loop size.

Introduction

Amphiphilic polyelectrolytes, which bear hydrophobic and electrolyte units attached to the same polymer chain, form unique micelles in aqueous media depending on the balance between hydrophobic attraction among hydrophobes and electrostatic repulsion among charges.[1] Their association behavior is an important subject of investigation not only because they are used in various fields of applications including cosmetics, drug delivery systems, paints, coatings, and personal care goods[2] but also because they are useful as simple models for the formation of higher order structures of biological macromolecules.

There are two categories in amphiphilic polyelectrolytes: block and statistical copolymers. Among the former, most of diblock copolymers are known to form spherical micelles with a core comprising of insoluble chains and ionic coronal chains in dilute aqueous solutions, and their micellar structures (the aggregation number, micellar and core sizes, critical micelle concentration, and so on) have been most extensively investigated so far.[3] More recently, diblock copolymers with short ionic block chains were reported to form nonspherical micelles, but their detailed micellar structures have not studied yet partly due to the frozen structures.[4]

On the other hand, it is known that ionic statistical copolymers also form micelles in aqueous media. So far, a number of research groups have studied various statistical copolymers by changing (1) contents of hydrophobic and ionic monomers,[5−12] (2) the degree of polymerization of copolymer chains,[13] (3) types of hydrophobic and ionic monomers,[5−7,14−17] and (4) the spacer bonding between hydrophobe and the polymer main chain.[7,18−23] However, the structure of micelles formed from ionic statistical copolymers in aqueous media is less clear than that formed from ionic diblock copolymers due to the complexity of the intramicellar electrostatic and hydrophobic interactions along the copolymer chains.

In the present study, we have investigated dependences of hydrophobicity of both hydrophobic and ionic monomer units on the micellar structure. We have chosen the dodecyl group as the hydrophobe, which is much hydrophobic than the hexyl group studied in a previous work,[13] and also changed hydrophobicity of electrolyte units by choosing different *N*-acryloyl-amino acids, as shown in Scheme 1. Hydrophobicity around the charge was reported to be an important factor for self-association behavior of amphiphilic statistical copolymers,[24,25] but its effect on the micellar structure is an underexamined subject. The use of *N*-acryloyl-amino acids makes us possible a systematic study of the hydrophobicity effect.

We have studied the micellar structure and the interchain aggregation behavior by static and dynamic light scattering combined with fluorescence decay measurements. The results have revealed that the tendency of the interchain aggregation negatively correlates to hydrophobicity of *N*-acryloyl-amino acids and also that the micellar structure of the statistical copolymers is strongly dependent on the kind of the hydrophobe but little on the kind of the electrolyte monomer unit in basic solutions at low ionic strength. Ionic statistical copolymers bearing dodecyl hydrophobes mostly take uni-core micelles of flower type with a minimum loop size, as explained below. This micellar model was also favorably compared with previous results reported by Yamamoto and Morishima[12] on dodecyl-modified statistical copolymers of sodium 2-acrylamido-2-methylpropanesulfonate (AMPS).

Experimental Section

Materials. Glycine, DL-valine, DL-isoleucine, dodecylamine, acryloyl chloride, and methacryloyl chloride were used as received. *N*,*N*-Dimethylformamide (DMF) used as solvent for polymerization was distilled under reduced pressure. 2,2′-Azobis(isobutyronitrile) (AIBN) was recrystallized from ethanol. Methanol used for sedimentation equilibrium measurements was purified by atmospheric distillation. Sodium chloride (NaCl) and pyrene were recrystallized from water and ethanol, respectively. Water was purified with a Millipore Milli-Q system. Other reagents were used without further purification.

N-Acryloylglycine (AGly), *N*-acryloylvaline (AVal), and *N*-acryloylisoleucine (AIle) were prepared from acryloyl chloride and the corresponding amino acids, respectively, according to the procedure of Kulkarni and Morawetz.[26] AGly, AVal, and AIle were purified by recrystallization using ethyl acetate or a mixed solvent of ethyl acetate and methanol. *N*-Dodecylmethacrylamide (C_{12}) was

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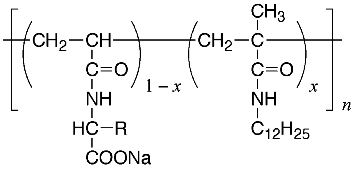
Scheme 1. Chemical Structure of the Copolymers Used in This Study



AGly/$C_{12}(x)$ (R = H)
AVal/$C_{12}(x)$ (R = $CH(CH_3)_2$)
AIle/$C_{12}(x)$ (R = $CH(CH_3)CH_2CH_3$)

prepared from methacryloyl chloride and dodecylamine in benzene in the presence of triethylamine according to the procedure of Morishima et al.[27] C_{12} was purified by recrystallization using benzene.

Preparation of Polymer Samples. A mixture of an *N*-acryloylamino acid (AXaa), C_{12}, and 0.1 mol % (based on the total monomers) AIBN were dissolved in DMF in a flask equipped with a three-way stopcock under an argon atmosphere. The sealed flask was immersed in an oil bath thermostated at 70 °C. After 24 h, the reaction mixture was poured into a large excess of ethyl acetate to precipitate polymer. The polymer obtained was purified by reprecipitation from methanol into a large excess of ethyl acetate three times and dissolved in water. The aqueous solution was dialyzed against water for a week. The polymer was recovered by freeze-drying. According to the same procedure, homopolymer samples of AXaa with different molecular weights were also prepared varying the molar ratio of the monomer and the initiator.

Solutions used for the following measurements were prepared according to the procedure of Morishima et al.[28] That is, each solid polymer sample was dissolved in water at room temperature and heated at 90 °C for 15 min. The solution pH was adjusted to 10 by adding a small amount of aqueous NaOH. The polymer solution was mixed with aqueous NaCl of pH 10 by 1:1 volume ratio to make the salt concentration 0.05 M and stirred overnight for equilibration. This stock solution was diluted with 0.05 M aqueous NaCl of pH 10 to prepare test solutions with different concentrations. Test solutions for light scattering measurements were optically purified by filtration, followed by a 3 h centrifugation at 9000 rpm (10^4 gravities). After these procedures, we did not observe any time dependence of the aggregation behavior.

Copolymer samples were also dissolved in methanol, mixed with methanol containing 0.2 M $LiClO_4$ by 1:1 volume ratio, diluted with methanol containing 0.1 M $LiClO_4$ to prepare test solutions with different concentrations, and used for sedimentation equilibrium measurements to determine the molecular weights.

The copolymers employed in the present study are abbreviated AGly/$C_{12}(x)$, AVal/$C_{12}(x)$, and AIle/$C_{12}(x)$, respectively, where x denotes the C_{12} content in the copolymer (Scheme 1 and Table 1). The C_{12} contents were determined by the C/N ratio in elemental analysis. Values of x for the copolymers were virtually the same as the compositions of C_{12} in monomer feed.

Measurements. (a) Sedimentation Equilibrium. Sedimentation equilibrium experiments were performed for AXaa/$C_{12}(x)$ copolymers dissolved in methanol containing 0.1 M $LiClO_4$, where the copolymers were molecularly dispersed and no large aggregate exists (cf. Supporting Information). Data of sedimentation equilibrium were obtained at 25.0 °C using an Optima XL-I type ultracentrifuge (Beckman-Coulter) equipped with a Rayleigh interference optical system and a diode laser operating at 675 nm and analyzed according to the standard procedure. Specific density increments $\partial\rho/\partial c$ for dialyzed solutions of AXaa/$C_{12}(x)$ copolymers necessary for the data analysis were measured by an oscillation U-tube densitometer (Anton-Paar, DMS5000). As explained in the Supporting Information, the effect of the polydispersity in the copolymer composition was so small that we made no correction to this effect in analyses of sedimentation equilibrium data.

Values of the weight-average molecular weight M_{w1} and the ratio M_{z1}/M_{w1} of the z-average to weight-average molecular weight for AXaa/$C_{12}(x)$ copolymers determined are listed in Table 1. Weight-average degrees of polymerization $N_{0,w1}$ calculated from M_{w1} for all the copolymer samples used range from 151 to 185. From M_{z1}/M_{w1} ranging from 1.5 to 2.1, all the copolymer samples may be expected to have normal distributions of molecular weight for free radical polymerization.

Table 1. Molecular Characteristics of AXaa/$C_{12}(x)$ Copolymer Samples

polymer code	x^a	$M_{w1}/10^{4\,b}$	$N_{0,w1}{}^c$	$M_{z1}/M_{w1}{}^b$
AGly/C_{12}(0.22)	0.22	2.91	172	1.5
AGly/C_{12}(0.31)	0.31	3.37	185	1.7
AGly/C_{12}(0.43)	0.43	3.31	167	1.5
AVal/C_{12}(0.18)	0.18	3.67	180	1.5
AVal/C_{12}(0.31)	0.31	3.76	177	2.1
AVal/C_{12}(0.38)	0.38	3.83	176	2.1
AIle/C_{12}(0.20)	0.20	3.26	151	1.7
AIle/C_{12}(0.30)	0.30	3.90	176	1.5
AIle/C_{12}(0.40)	0.40	3.63	169	2.1

[a] Determined by elemental analysis. [b] Determined by sedimentation equilibrium measurements in methanol containing 0.10 M $LiClO_4$. [c] The weight-average degree of polymerization.

(b) Light Scattering. Simultaneous static and dynamic light scattering experiments were performed for 0.05 M aqueous NaCl solutions of AXaa/$C_{12}(x)$ copolymers as well as AXaa homopolymers at 25.0 °C using an ALV/SLS/DLS-5000 light scattering instrument equipped with an ALV-5000 multiple τ digital correlator using a Nd:YAG (532 nm) or He−Ne (632.8 nm) laser. The procedures of data acquisition were the same as in the previous study.[13,29]

Values of the specific refractive index increment $\partial n/\partial c$ for dialyzed solutions, necessary to analyze static light scattering (and also sedimentation equilibrium) data, were determined with a modified Schulz−Cantow type differential refractometer (Shimadzu) at 25.0 °C. We did not make any corrections to the effect of the polydispersity in the copolymer composition in light scattering data analyses (cf. Supporting Information).

Dynamic light scattering data for 0.05 M aqueous NaCl solutions of AXaa/$C_{12}(x)$ copolymers as well as of AXaa homopolymers showed bimodal relaxations, which indicate that the solutions contain two scattering components with largely different sizes (cf. Figure S2 in Supporting Information). The scattering intensity of the major fast relaxation component in each solution was extracted from the total scattering intensity using the relaxation spectrum obtained by dynamic light scattering and analyzed to determine the weight-average molar mass M_w and the hydrodynamic radius R_H of the major component, as in the previous study[13] (see also Supporting Information).

(c) Fluorescence. A small amount of a concentrated methanol solution of pyrene was added to an aqueous solution of AXaa/$C_{12}(x)$ copolymers (pH 10) prepared as described above, and the solution was stirred overnight. The solution was then mixed with aqueous NaCl of pH 10 (1/1, v/v) and stirred overnight, followed by filtration with a 0.2 μm PTFE membrane filter. After the solution was purged with argon for ca. 30 min, steady-state fluorescence spectrum and fluorescence decay profile of the solution were recorded in the same procedures as the previous.[13] The latter results were analyzed by the Infelta−Tachiya equation[30−33] to estimate the number n_c of hydrophobic cores per micelle in the copolymer solutions as in the previous work.[13]

Results

Conformation of Homopolymers of AXaa. Prior to the discussion of AXaa/$C_{12}(x)$ copolymers, we have to argue the conformation of AXaa homopolymers in aqueous NaCl solutions. In Figure 1, degree of polymerization $N_{0,w}$ dependences of the hydrodynamic radius R_H in 0.05 M aqueous NaCl for the homopolymers are compared with that for AMPS homopolymers recently obtained by Yashiro et al.[34] The dependences of all the homopolymers shown in this figure are almost identical each other, indicating that the homopolyelectrolytes

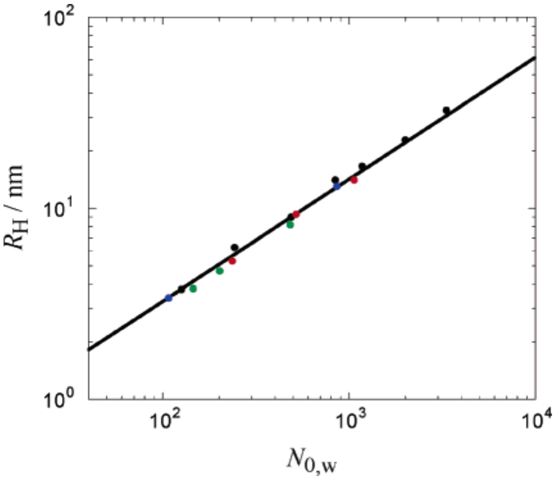


Figure 1. $N_{0,w}$ dependences of R_H for homopolymers of AGly (red circle), AVal (green circle), AIle (blue circle), and AMPS (black circles)[34] in 0.05 M NaCl.

Table 2. Characteristics of Major Aggregating Components of AXaa/C_{12}(*x*) Copolymers in 0.05 M Aqueous NaCl

polymer code	$M_w/10^4$	$N_{0,w}$	$A_2{}^a$	R_H/nm	*m*	ϕ_{in}
AGly/C_{12}(0.22)	5.39	311	5.53	3.6	1.8	0.33
AGly/C_{12}(0.31)	8.32	456	3.24	4.0	2.5	0.40
AGly/C_{12}(0.43)	13.2	677	2.32	4.4	4.0	0.63
AVal/C_{12}(0.18)	5.15	253	5.44	3.6	1.4	0.32
AVal/C_{12}(0.31)	7.50	354	4.66	3.8	2.0	0.43
AVal/C_{12}(0.38)	10.6	491	1.51	4.1	2.8	0.56
AIle/C_{12}(0.20)	4.31	199	6.00	3.4	1.3	0.34
AIle/C_{12}(0.30)	5.93	267	4.74	3.5	1.5	0.45
AIle/C_{12}(0.40)	9.52	422	2.66	4.2	2.5	0.44

[a] In units of 10^{-4} cm^3 mol/g^2.

take the same main-chain conformation in a first approximation in aqueous solution at the low ionic strength. Under such a solvent condition, the electrostatic repulsion plays a main role in the excluded volume and chain stiffness effects. Since all AXaa homopolymers have the same linear charge density at pH = 10 as that of AMPS homopolymers, we expect the strength of the electrostatic repulsion to be almost identical among the polyelectrolytes and thus similar conformation to them at low ionic strength.

Size of AXaa/C_{12}(*x*) Copolymer Aggregates. Table 2 lists weight-average molar masses M_w, second virial coefficients A_2, and hydrodynamic radii R_H of major components of AXaa/C_{12}(*x*) copolymers in 0.05 M aqueous NaCl at 25.0 °C determined by light scattering. Using the results of M_w along with the molecular weight of the same samples determined in methanol with 0.1 M $LiClO_4$, we can estimate the aggregation number *m* of the major component of the copolymer samples in 0.05 M aqueous NaCl (see the sixth column of Table 2). It can be seen that *m* ranges from 1.3 to 4.0 and definitely increases with *x* for each series of AXaa/C_{12}(*x*) copolymers but decreases with hydrophobicity of the amino acid residue in AXaa/C_{12}(*x*) copolymers at the same *x*. The second virial coefficient is a decreasing function of *x* for each series of AXaa/C_{12}(*x*) copolymers.

In Figure 2, the R_H data for AXaa/C_{12}(*x*) copolymer aggregates in 0.05 M aqueous NaCl are plotted against $N_{0,w} = M_w/\bar{M}_0$, where $\bar{M}_0$ denotes the average molar mass per monomer unit for the copolymer. The data points are definitely below the dotted line for the homopolymers in 0.05 M aqueous NaCl (the same as the line in Figure 1), which demonstrates that the aggregates of the copolymers adopt a more compact conformation than do the homopolymers presumably because of the hydrophobic associations of C_{12} units within the aggregate. Intriguingly, all data points of the AXaa/C_{12}(*x*) copolymer aggregates obey the common line indicated (i.e., the thin line), irrespective of the kind of amino acid residues[35] and also of *x*. The common line has a slope of ca. 0.25. This is slightly smaller than that expected for the uniform sphere (=0.33), indicating that the aggregates are spherical but become more compact with increasing $N_{0,w}$.

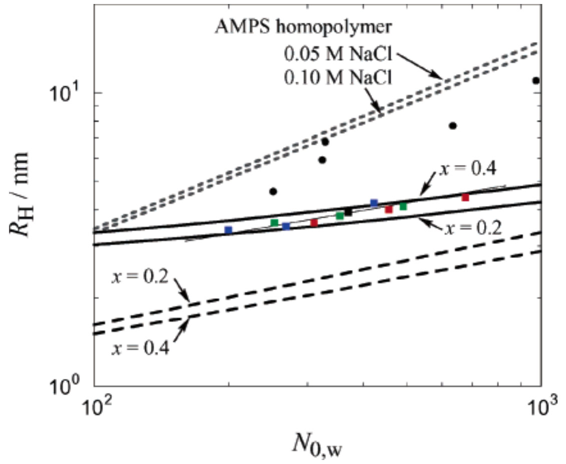


Figure 2. $N_{0,w}$ dependences of R_H for AGly/C_{12}(*x*) (red square), AVal/C_{12}(*x*) (green square), AIle/C_{12}(*x*) (blue square), and AMPS/C_{12}(*x*) (black square) copolymers in 0.05 M NaCl and for AMPS/C_6(*x*) copolymers (black circle) in 0.10 M NaCl[13] along with data for AMPS homopolymers in 0.05[34] and 0.10 M NaCl (gray dotted lines).[13] The thin line is the common line for AXaa/C_{12}(*x*) copolymer aggregates. The black solid lines represent R_H estimated based on the uni-core flower micelle model with the minimum loop size (*x* = 0.2 and 0.4), and the black dashed lines represent maximum values of R_H calculated for the uni-core flower micelle model where all the C_{12} groups in AXaa/C_{12}(*x*) copolymers of *x* = 0.2 and 0.4 are included in the hydrophobic core.

Figure 2 also contains data of AMPS/C_6(*x*)[13] and AMPS/C_{12}(0.34) copolymer aggregates, where the latter copolymer sample was prepared according to the procedure of Morishima et al.[36] The data point for AMPS/C_{12}(0.34) follows the common line for AXaa/C_{12}(*x*) copolymers, with a much weaker $N_{0,w}$ dependence than for AMPS/C_6(*x*) copolymers. Therefore, we can say that the structure of those amphiphilic statistical copolymer aggregates strongly depends on the kind of hydrophobic monomer units but little on the kind of hydrophilic monomer units in 0.05 M aqueous NaCl.

Hydrophobic Core in the AXaa/C_{12}(*x*) Copolymer Aggregates. As in the case of AMPS/C_{12}(*x*) and AMPS/C_6(*x*) solutions, the ratio I_3/I_1 of the intensities of the third (383 nm) to the first (372 nm) vibronic peaks for pyrene solubilized in 0.05 M aqueous NaCl solutions of AXaa/C_{12}(*x*) is ca. 0.9 (Figure 3a), which is much larger than the I_3/I_1 value in the bulk water phase (ca. 0.6)[37] and comparable to that (=0.96) in aqueous micellar solution of sodium dodecyl sulfate.[38] Furthermore, the concentration [Py] of pyrene solubilized in the copolymer solution considerably exceeds the limiting solubility of pyrene in water (<1 μM). These observations demonstrate the formation of hydrophobic microdomains in the aqueous copolymer solutions. It should be noted here that the fluorescence spectrum exhibits a broad band around 480 nm due to pyrene excimer at higher [Py], indicating that plural pyrene molecules are incorporated into a hydrophobic microdomain.

Figure 3b shows decay profiles of the fluorescence intensity $I(t)$ around 400 nm from pyrene solubilized in the solution of AGly/C_{12}(0.31) in 0.05 M NaCl after excitation of pyrene by a 337 nm light pulse of negligible duration at $t = 0$. Whereas $I(t)$

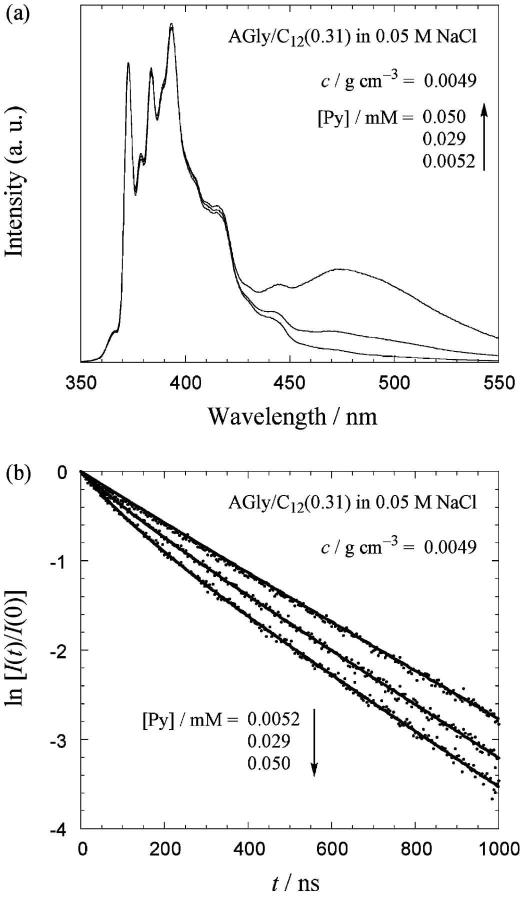


Figure 3. (a) Fluorescence spectra for pyrene solubilized in 0.05 M NaCl solutions of AGly/C_{12}(0.31) ($c = 5 \times 10^{-3}$ g/cm^3). (b) Fluorescence decay profiles around 400 nm for the solutions shown in panel a; curves in panel b, fitting results calculated by the Infelta−Tachiya kinetics.[30−33]

Table 3. Parameters Obtained from the Fluorescence Decay Experiments for AXaa/C_{12}(x) Copolymers in 0.05 M NaCl

polymer code	C_{fast}/ mM	[Py]/ mM	k_0/ μs^{-1}	k_q/ μs^{-1}	k_-/ μs^{-1}	$\bar{n}$	n_c
AGly/C_{12}(0.22)	0.064	0.024	2.6	4.6	2.1	0.33	0.9
AGly/C_{12}(0.31)	0.055	0.029	2.7	4.1	1.1	0.36	1.4
AGly/C_{12}(0.31)	0.055	0.050	2.7	4.4	0.8	0.61	1.4
AGly/C_{12}(0.43)	0.038	0.17	2.6	3.1	1.9	1.8	2.5
AGly/C_{12}(0.43)	0.038	0.21	2.6	3.2	1.9	2.2	2.5
AVal/C_{12}(0.18)	0.081	0.059	1.9	5.0	2.4	0.65	1.1
AVal/C_{12}(0.31)	0.072	0.059	1.9	4.9	1.7	1.1	0.9
AVal/C_{12}(0.38)	0.038	0.14	2.2	3.4	1.1	1.8	2.1
AIle/C_{12}(0.20)	0.17	0.060	1.7	3.0	2.3	0.54	0.8
AIle/C_{12}(0.30)	0.057	0.086	1.7	4.0	1.5	1.3	0.9
AIle/C_{12}(0.40)	0.015	0.13	2.3	5.0	1.8	0.49	1.1

decays single-exponentially with the lifetime of excited pyrene monomer at a lower [Py] (=17 μM), it exhibits a faster decay component at higher [Py]. This faster decay corresponds to the quenching of fluorescence due to pyrene monomer by excimer formation within a hydrophobic microdomain (cf. Figure 3a), which returns to the ground-state emitting light around 480 nm being out of the experimental window.

These fluorescence decay profiles were analyzed on the basis of the Infelta−Tachiya kinetics.[30−33] As shown in Figure 3b, solid curves demonstrate satisfactory fits for the experimental $I(t)$. Fluorescence decay profiles for all AXaa/C_{12}(x) copolymers were also analyzed in the same manner to determine the kinetic parameters, as listed in Table 3. The decay rate constant k_0 of excited pyrene as well as the rate constants k_q for the excimer formation and k_- for exit of pyrene from the microdomain are essentially independent of x and the kind of amino acid residues, although k_0 for AGly/C_{12}(x) aggregates seem to be slightly larger than those for AVal/C_{12}(x) and AIle/C_{12}(x) aggregates. If the copolymer aggregate forms a micelle containing n_c hydrophobic cores,[39] the number $\bar{n}$ of pyrene molecules per hydrophobic core is equal to [Py]/($n_c C_{mic}$), where C_{mic} is the molar concentration of the aggregate calculated by $1000cN_A/M_w$. As shown in the last column of Table 3, n_c is about unity for most of the copolymer aggregates examined. Only AGly/C_{12}(0.43) and AVal/C_{12}(0.38) aggregates with $N_{0,w} \gtrsim 500$ have n_c exceeding 2. As reported previously,[13] AMPS/C_6(0.3) copolymer aggregates of $N_{0,w} \gtrsim 300$ formed multicore micelles. The aggregation number of spherical micelles formed from alkylsulfates, alkylsulfonates, and alkylammonium bromides is known to increase with the length of the alkyl chain.[40] Therefore, it is reasonable that the number of cores is smaller for the C_{12} copolymers than for the C_6 copolymers with the same $N_{0,w}$ and x.

Compactness of Copolymer Micelles. Since the above C_{12} copolymer micelles mostly have a single hydrophobic core and probably spherical shapes (from the weak $N_{0,w}$ dependence of R_H shown in Figure 2), we may estimate the average polymer volume fraction ϕ_{in} inside the micelle by

$$\phi_{in} = \frac{\bar{v} M_w / N_A}{(4\pi/3){R_H}^3} \tag{1}$$

where $\bar{v}$ denotes the polymer specific volume and N_A Avogadro's constant. The result calculated from the experimental M_w and R_H for each copolymer micelle is listed in the last column of Table 2. Values of ϕ_{in} for AXaa/C_{12}(x) copolymers of $x \geq 0.3$ and AMPS/C_{12}(0.34) are larger than or equal to 0.4. Since ϕ_{in} for globular proteins ranges 0.4−0.6,[41] the aggregates of these copolymers may be expected to be almost as compact as the globular proteins in aqueous salt solution due to the significant hydrophobicity of the C_{12} unit. On the other hand, ϕ_{in} for AMPS/C_6(x) copolymers is considerably smaller than 0.4, indicating that aggregates of AMPS/C_6(x) copolymers do not take a compact shape like globular proteins. As shown in our previous work,[13] AMPS/C_6(x) copolymers with low molar masses forms a starlike micelle in aqueous NaCl.

Discussion

Micellar Structure of Amphiphilic Statistical Copolymers. As described in the former section, the light scattering and fluorescence measurements have demonstrated that most of AXaa/C_{12}(x) and AMPS/C_{12}(0.34) copolymers exist as spherical uni-core micelles of small numbers (i.e., 1−4) of chains in 0.05 M aqueous NaCl. Here, we investigate the structure of the micelles in more details using the relation between R_H and $N_{0,w}$ shown in Figure 2.

If all the C_{12} hydrophobes of AXaa/C_{12}(x) copolymers are included in a spherical hydrophobic core, the polymer aggregate must be the flower micelle with many small loops, as depicted in Figure 4a. The average contour length $l_{contour}$ of the AXaa sequence in the random copolymer can be calculated by $\sum_{\mu=0}^{\infty} l\mu x(1-x)^m = l(1-x)/x$ from the mole fraction $1-x$ and the contour length l (= 0.25 nm) of the AXaa monomer unit, and the loop size is at most the half of $l_{contour}$. On the other hand, the volume V_{core} of the core formed from C_{12} hydrophobes may be calculated by $\upsilon_{C12} x N_{0,w}$ with the molecular volume υ_{C12} of the dodecyl group. According to Tanford,[41] υ_{C12} is calculated to be 0.35 nm^3. Therefore, the radius of the spherical uni-core

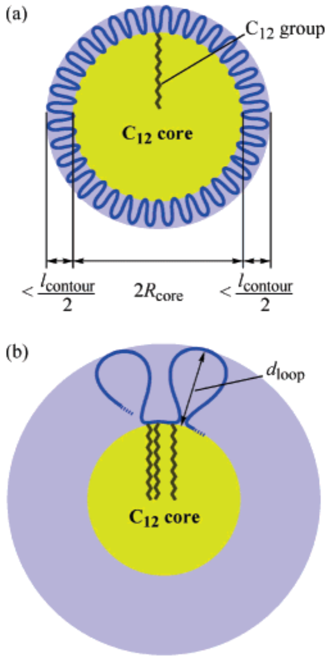


Figure 4. Schematic representations of the flower-like micelle models, in which all the C_{12} hydrophobes are included in the hydrophobic core (a) and with the minimum loop size (b) (see text). For simplicity, only a part of hydrophobic chains are drawn, and those attached to the loops outside the hydrophobic core are omitted.

micelle should be at most $l_{contour}/2 + (3V_{core}/4\pi)^{1/3}$. Dashed lines in Figure 2 indicate the values of $l_{contour}/2 + (3V_{core}/4\pi)^{1/3}$ calculated for $x = 0.2$ and 0.4, both of which are much smaller than the experimental R_H for the uni-core micelles of AXaa/$C_{12}(x)$ and AMPS/C_{12}(0.34) copolymers. This indicates that not all C_{12} hydrophobes in the copolymer chains are included in the hydrophobic core of the micelle.

Because the copolymer chains are not perfectly flexible due to the intrinsic local stiffness and the intrachain electrostatic repulsion, it is impossible for the copolymer chains to take very small loops. The above calculation of the micellar size ignored this stiffness of the copolymer chains. We next calculate the micellar size taking into account this chain stiffness effect.

A wormlike chain needs a minimum contour length l_{min} to form a loop or ring. Yamakawa and Stockmayer[42] calculated the ring closure probability of the wormlike chain as a function of the Kuhn segment number N. They demonstrated that the probability sharply increases from zero at $N \gtrsim 0.8$. Therefore, we may have $l_{min} = 0.8 \times 2q$, where q is the persistence length of the wormlike chain. The number of loops per copolymer chain in the uni-core micelle is then given by $n_{loop} = lN_{0,w1}/l_{min}$, where l is the contour length per monomer unit and $N_{0,w1}$ is the degree of polymerization of the copolymer chain, and each copolymer chain in the micelle contacts with the hydrophobic core $n_{loop} + 1$ times. If λx hydrophobes are inserted into the hydrophobic core at each contact (cf. Figure 4b, where $\lambda x = 3$), the core consists of $\lambda x(n_{loop} + 1)m$ hydrophobes. In what follows, we regard λ as a constant. Thus, the radius R_{core} of the spherical hydrophobic core is calculated by

$$\frac{4\pi}{3}R_{core}^{\ 3} = \nu_{C12}\lambda x\left(\frac{lN_{0,wl}}{1.6q} + 1\right)m \tag{2}$$

Furthermore, Yamakawa and Stockmayer[43] calculated the most probable configuration of the minimum loop of the wormlike chain. According to their result, the loop size d_{loop} may be written as

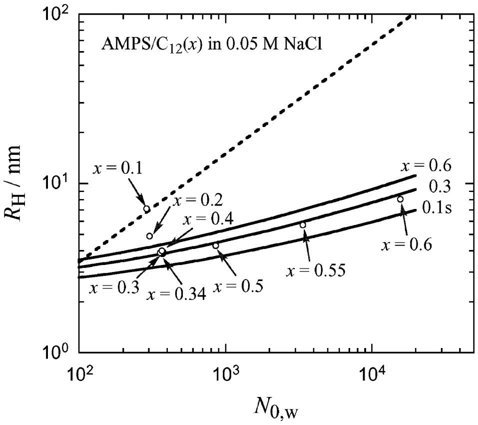


Figure 5. $N_{0,w}$ dependences of R_H for AMPS/$C_{12}(x)$ copolymers in 0.05 M NaCl obtained in this study (filled circle) and by Yamamoto and Morishima (unfilled circles).[12] The black solid and dotted lines represent R_H values estimated on the basis of the uni-core flower micelle model with the minimum loop size and for AMPS homopolymers in 0.05 M NaCl,[34] respectively.

$$d_{loop} = 1.22l_{min}/\pi = 0.62q \tag{3}$$

If interactions among hydrophobic and ionic side chains attaching to the loop chain do not seriously affect the loop chain conformation, the radius of the spherical uni-core flower micelle with the minimum loops may be calculated by $R_{core} + d_{loop}$ using eqs 2 and 3.[44] Equations 2 and 3 contain four molecular parameters, q, l, λ, and υ_{C12}, and we may choose q (= 3.0 nm) determined by Yashiro et al.[34] for AMPS homopolymers in 0.05 M aqueous NaCl, l (= 0.25 nm) estimated from the bond length and angle for the C−C single bond, and υ_{C12} (= 0.35 nm^3) estimated by Tanford. Thus, the only adjustable parameter is λ, and the choice of 15 for λ leads a successful fitting to the experimental R_H for uni-core micelles formed from AXaa/$C_{12}(x)$ and AMPS/C_{12}(0.34) copolymers, as shown by the solid curves in Figure 2. The curves did not essentially depend on the choice of m from 1 to 4. It is noted that the loop size of the micelle is not determined by x but by q, and the core size only slightly increases with x. Therefore, the little x dependence of R_H is naturally explained by this flower micelle model with the minimum loop size. Furthermore, if q is insensitive to the hydrophilic side chains, the micellar size is also insensitive to the kind of the hydrophilic monomer unit.

As mentioned above, among the copolymer samples examined, only AGly/C_{12}(0.43) and AVal/C_{12}(0.38) with $N_{0,w} \gtrsim 500$ form multicore micelles.[45] This indicates that the maximum aggregation number of the dodecyl group forming the hydrophobic core (= $500\lambda xl/1.6q$) is ca. 80 at $x = 0.2$ and 160 at $x = 0.4$.

Yamamoto and Morishima[12] studied the micellization behavior of AMPS/$C_{12}(x)$ copolymers over a wide range of x from 0.1 to 0.6 in 0.05 M aqueous NaCl at 25 °C. Their $N_{0,w}$ dependence of R_H is shown in Figure 5 by unfilled circles along with our result for AMPS/C_{12}(0.34) (filled circle) along with theoretical curves for our flower micelle model at $x = 0.1$, 0.3, and 0.6. The data points for $0.3 \leq x \leq 0.4$ are close to the theoretical values at the corresponding x, and those for $x \leq 0.2$ deviate from the theoretical prediction to approach the dotted line for AMPS homopolymers (the same as the dotted line for 0.05 M NaCl in Figure 2). When x decreases, $l_{contour}$ (= $l(1 - x)/x$) becomes larger than l_{min} (= $1.6q$) at a some critical x. For copolymers with x less than this critical value, the loop size must be calculated by $1.22 l_{\mathrm{contour}}/\pi$ instead of eq 3, and finally R_H of the copolymer approaches that of the homopolymers. If $q = 3$ nm, this critical x is 0.05. The experimental critical x is considerably larger than this theoretical one for AMPS/C$_{12}$(x) copolymers. At present, we have no suitable reason for this disagreement. It may be interesting to study this critical composition for various amphiphilic statistical copolymers. The data point for $x = 0.6$ is slightly below the theoretical curve for the same x. Although Yamamoto and Morishima[12] did not determine the number n_c of hydrophobic cores in the micelles, we may expect multi-core micelles at $x \geq 0.5$ with $N_{0,\mathrm{w}} > 500$. Thus, our uni-core flower micelle model may not be suitable to compare with the data points at $x \geq 0.5$.

In the present flower micelle model, the hydrophobic core is definitely separated from the loop domain, and distributions of hydrophobic and ionic groups in the latter domain are not considered. We may expect, however, that the hydrophobic and ionic groups segregate each other in the loop domain, and the hydrophobic groups distribute near the hydrophobic core. Therefore, the actual hydrophobic domain may have a diffuse structure. Our flower micelle model approximates this diffuse domain boundary simply by a sharp interface. It is difficult to argue the detailed hydrophobic domain structure in the statistical copolymer micelle only by the light scattering results obtained in the present study. For such an argument, we need some complementary information, e.g., molecular dynamics simulation.

Conclusion

We have investigated the micellar structure of ionic statistical copolymers bearing hydrophobic dodecyl groups as the hydrophobe and different amino acid residues as the hydrophilic side chain in 0.05 M aqueous NaCl by light scattering and fluorescence. The statistical copolymer micelles consist of 1.3−4.0 copolymer chains, mostly have a single hydrophobic core, and take compact conformations comparable to typical globular proteins. The dependences of the hydrodynamic radius on the number of monomer units for the micelles of the above copolymers and most of previously studied statistical copolymers composed of AMPS and *N*-dodecylmethacrylamide[12] were favorably compared with the uni-core flower micelle model with the minimum loop size.

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Supporting Information Available: Effects of the polydispersity in the copolymer composition on light scattering and sedimentation equilibrium and light scattering data analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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(45) Figure 2 indicates that the R_H data for AGly/C_{12}(0.43) and AVal/C_{12}(0.38) agree with the theoretical values (the black solid lines). However, the agreements seem to be by accident because the theory is based on the uni-core flower micelle.

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