Self-organization and Microstructures of Sodium **11-Acrylamidoundecanoate in Water**

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The aggregation behavior of sodium 11-acrylamidoundecanoate in aqueous solution has been investigated. The critical micelle concentration was determined by surface tension measurement. Steady-state fluorescence quenching of pyrene was used to determine micellar aggregation number. The micropolarity of the micellar aggregates was determined from the intensity ratio (I_3/I_1) in the fluorescence spectrum of pyrene. The fluorescence probe studies indicated that the microenvironment of the probe molecule was nonpolar in nature. FT-IR spectra were measured to elucidate the role of intermolecular hydrogen bonding between amide groups on the formation of supramolecular self-assemblies. The dynamic light scattering measurements have shown the existence of large cylindrical micellar aggregates in dilute aqueous solutions. The light and transmission electron micrographs were measured to study the microstructures of the supramolecular assemblies in aqueous solutions of the amphiphile. In this work, it has been demonstrated that bilayer structures appearing as vesicles, tubules, and wormlike micelles can be formed spontaneously in dilute aqueous solution even from a simple single-tail surfactant.

Introduction

Amphiphilic molecules tend to self-organize in aqueous solution to produce diverse submicroscopic and supramolecular structures above a critical concentration.¹ The shape and size of these structures depend on the surfactant concentration, the length of the hydrophobic tail, temperature, and the concentration of electrolyte (in the case of ionic surfactants). Depending upon the shape and size of these structures, they are called spherical micelles, rods, tubules, wormlike micelles, and vesicles. Amphiphiles containing two tails usually form layer structures called vesicles, and those containing a single tail usually form micelles. Synthetic amphiphiles with different chemical structures and composition of headgroups and hydrocarbon tail(s) that can self-organize into vesicles in dilute aqueous solutions have been reported in the recent literature.² However, the formation of vesicles from singletail surfactants in dilute aqueous dispersions is determined by the balance between the hydrophobic tails and that among the hydrophilic headgroups and also by the molecular geometry. Recently, it has been shown that simple micelle-forming single-tail amphiphiles can also form vesicles induced by suitable counterions including metal ions, organic cations and anions, and cosurfactants. Luisi and co-workers⁴ have reported that 1-dodecylphosphoric acid spontaneously forms stable vesicles in water at pH \sim 2 at room temperature in the absence of Li⁺, Na⁺, and K⁺ ions. More recently, it has been reported that N-acylamino acids, alkylamine oxides, and aldonamides self-organize in dilute aqueous dispersions to form aggregates with various morphologies: spherical micelles

and vesicles, fibers, chiral bilayer ribbons, tubules, and helical structures.⁵ In these reports, the intermolecular hydrogen bonding has been implicated to be responsible for the formation of bilayer structures or fibrous gels.⁶

In this work, we have investigated the aggregation behavior of sodium 11-acrylamidoundecanoate, SAAU (see Figure 1 for molecular structure), in aqueous solutions. This molecule has a long C_{14} (including the amide group) hydrocarbon chain, and if desired, the tails may be tethered through polymerization of the acrylamido group very easily. Although the surface properties of SAAU and of the corresponding polymer have already been studied,⁷ the information regarding micellar structure is missing.

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Figure 1. Plot of γ vs log *C*. Inset: molecular structure of SAAU.

Table 1. Surface and Self-organization Properties of 2.5mM SAAU in Water

R _H (nm)	cmc (mM)	$\gamma_{\rm cmc} \ ({ m mN} \ { m m}^{-1})$	A _m (nm ²)	$N_{ m agg}$	I_{3}/I_{1}	$10^{-10}D$ (m ² s ⁻¹)
76	0.4	48.0	1.0	171 ± 10	0.69	2.7

We thought that introduction of an acrylamido group at the end of the alkyl chain might inhibit the chain mobility and thereby could promote formation of supramolecular self-assemblies. The aim of the present study is to investigate (i) the influence of the terminal amide group on the micellar properties such as cmc and aggregation number (N_{agg}), (ii) the microenvironment of the self-assemblies, (iii) the microstructures of the self-assemblies in aqueous solutions, and (iv) the role of intermolecular hydrogen bonding between amide groups upon aggregation toward microstructure formation.

Results and Discussion

Among the available methods that can be used to estimate critical micellar concentration (cmc), the surface tension method is versatile not only because cmc can be extracted from the plots of surface tension versus log concentration but also because one can get information on the nature of adsorbed layers at the air/water interface.⁸ A typical plot of surface tension versus log C for an aqueous solution of SAAU is shown in Figure 1. The surface tension values decrease linearly with log C and show a characteristic break and remain constant thereafter. The break point corresponds to the cmc value. No minimum around the cmc can be observed. This confirms the purity of the surfactant. The surface properties are tabulated in Table 1. The cmc value (0.40 mM) of SAAU is close to the value (0.43 mM in 0.01 M NaOH) already reported in the literature.⁷ However, the cmc value of SAAU is less than that of the corresponding fatty acid soap.⁹ The surface area per surfactant molecule, $A_{\rm m}$, at the interface estimated from the slope of the linear part by using the Gibbs adsorption equation¹⁰ is equal to 1.0 nm², which suggests formation of bilayer structures in solution.¹¹



Figure 2. Plot of $\ln(I_0/I)$ vs [CPC]. Inset: size distribution of 2.5 mM SAAU aggregates in aqueous solution.

Assuming monodispersed micelles, the average aggregation number (N_{agg}) was obtained from the slope of the plot (Figure 2) of $\ln(I_0/I)$ as a function of quencher concentration according to the equation¹²

$$\ln(I_0/I) = N_{agg}[CPC]/(C - cmc)$$
(1)

where I_0 and I are the intensities of pyrene fluorescence in the absence and presence of quencher, respectively, Cis the total surfactant concentration, and [CPC] is the concentration of the quencher, *N*-cetylpyridinium chloride (CPC). It can be seen from Table 1 that the N_{agg} value in a 2.5 mM solution of SAAU is 171. This value is much larger than the predicted value (64)¹³ for the corresponding fatty acid soap and therefore suggests the presence of larger aggregates.

The presence of larger aggregates in aqueous solutions of SAAU is also suggested by the dynamic light scattering (DLS) studies. A second-order cumulant analysis of the intensity autocorrelation function results in a broad distribution of micellar size (inset, Figure 2) in a 2.5 mM surfactant solution. The calculated values of the apparent diffusion constant, D, and the hydrodynamic radius, $R_{\rm H}$, are listed in Table 1. The values of D and $R_{\rm H}$ are respectively far too small and too large for a normal spherical micelle.¹⁴ For a normal spherical micelle the translational diffusion constant is of the order of 10⁻⁸ m s^{-1} , and the hydrodynamic radius is about 2.0 nm.¹⁴ Therefore, it can be concluded that the self-assemblies formed by the SAAU amphiphile in dilute aqueous solutions have either rodlike or tubular structures. The size of the aggregates increased upon increase of amphiphile concentration.

Since the ratio of the intensities at the wavelengths corresponding to the first (I_1) and third (I_3) vibronic bands of pyrene fluorescence changes as a function of solvent polarity,¹⁵ it is often used to estimate the polarity of the microenvironment of the fluorophore in micellar solutions. We have measured the I_3/I_1 ratio of pyrene fluorescence in a 2.5 mM solution of SAAU. The ratio I_3/I_1 (0.69) thus obtained was used to get the apparent dielectric constant from the calibration curve constructed from the known values of the I_3/I_1 ratio in organic solvents.¹⁵ The I_3/I_1 value suggests that the fluorophore is solubilized in the nonpolar region of the micellar core, and the micropolarity corre-

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sponds to that of ethyl acetate ($\epsilon = 6.02$).¹⁵ The low polarity of the microenvironments of the aggregates is indicative of ordering of the micellar interface, which reduces the degree of water penetration in the hydrocarbon layer.

The above results clearly suggest the existence of bilayer structures in aqueous solutions of SAAU. The low cmc and high Nagg values of SAAU compared to those of sodium tetradecanoate9,13 indicate enhanced hydrophobic interaction of the long hydrocarbon tails of the amphiphile. To incorporate a large number of hydrocarbon chains in a micelle, a distortion in the micellar shape is required. The molecular structure of SAAU shows that there can be formation of stable intermolecular hydrogen bonding between -NH- and -CO- in the amide groups of two neighboring hydrophobic tails that induces a stable linear state. Therefore, FT-IR spectra were recorded for (i) the pure aqueous sample (20 mM), (ii) the solution in dry tetrahydrofuran (THF) solvent, and (iii) the solid material in KBr. The appearance of the -NH- stretching frequency (3306 cm^{-1}) and the amide-I band (1653 cm^{-1}) of the aqueous solutions of SAAU indicates the existence of an amide group. The N-H stretching frequency is similar to that for the solid sample and is shifted to lower frequency compared to that in THF (3324 cm⁻¹) solvent. The amide-I band (1653 cm⁻¹) in the aqueous sample resembles the corresponding stretching frequency measured for the solid (1655 cm^{-1}) and THF (1652 cm^{-1}) solutions. The existence of intermolecular hydrogen bonding is further evidenced by the amide-II band in the solid state (1561 cm⁻¹) and in aqueous solution (1559 cm⁻¹), which is shifted to higher frequency as compared to that in an unassociated state (1542 cm⁻¹) in THF solvent. The intermolecular hydrogen bonding between -NH- and -CO- in the amide group induces molecular packing leading to the formation of a flat-layered structure. This is further indicated by the micropolarity of the self-assemblies. The bilayer sheets with strong surface binding interactions may also tend to form ribbons and mono- or multilayer tubules. The role of amide hydrogen bonding at the surfactant headgroup in the formation of bilayer vesicles has been reported for many *N*-acylamino acid amphiphiles.⁶

To investigate the microstructure of the aggregates, we have performed optical as well as transmission electron microscopic (TEM) studies for solutions of different concentrations of SAAU. The light micrographs (Figure 3A and B) revealed a variety of morphologies. When optical microscopic measurements were carried out for the transparent aqueous SAAU solutions (2.5 mM, pH 8.4) after 2 h of sample preparation, no spherical micelles or vesicles could be observed. However, occasional tube and rodlike aggregates of variable diameters and length were observed, as shown in micrographs A and B, respectively. The picture in the inset of micrograph A is a tubule. The hollowness of the tubes is clearly visible in both the images. The widths of the tubes were approximately 14 μ m, and the lengths were up to a few millimeters. The wall thickness of the water-filled tubular vesicle in Figure 3A is about 3.5 μ m, which is about 1750 times thicker than the theoretical molecular length (~2.0 nm) of the amphiphile. That is, the tubules are multilayered structures. Similar light micrographs of tubular vesicles have been reported for lecithin.¹⁶ The micellar rods have a diameter in the range 40–50 μ m and a length of a few millimeters. The absence of spherical micelles and vesicles in the light micrographs at low concentrations is perhaps due to their

small sizes. However, at higher concentrations (>10 mM) of the amphiphile, only rodlike structures could be observed.

The electron micrographs of a negatively stained specimen prepared from a transparent aqueous solution of SAAU (2.5 mM) at pH 8.4 are depicted in Figure 3C and D. Like optical micrographs, they also revealed various structures: regular spherical micelles (not shown), spherical vesicles (Figure 3C), long water-filled tubules (Figure 3D), and rods (inset of Figure 3D). Similar structures could also be observed after aging the same sample for a day. The spherical vesicles have different sizes (diameters in the range 100-800 nm). The wall thickness of the vesicles is of the order of 140 nm, which indicates that the vesicles are multilayered structures. The micrograph in the inset of Figure 3C shows how smaller vesicles fuse together to form large vesicles. The diameter of the tubules with the wall thickness of \sim 50 nm is in the range 1–2 μ m, and they have a length of about 1 mm. On the other hand, the long rods have a diameter of about 450 nm. The dimensions of the structures observed in electron micrographs are smaller as compared to those in light micrographs. This might be due to an artifact of the sample preparation method for electron micrographs. The formation of rods/ tubules has been observed previously for other systems,¹⁷ including fatty acids.¹⁸ The micrographs (not shown) obtained from concentrated solutions (10 mM) of SAAU also revealed vesicles, tubules, and rodlike structures of bigger sizes. Therefore, it can be concluded that the bilayer structures are present in a wide range of concentration. The existence of tubular and rodlike structures is consistent with the results obtained from light scattering studies.

The bilayer arrangement is obtained by the hydrophobic interaction between hydrocarbon chains. In the case of SAAU, the two-layer arrays of the intermolecular hydrogen-bonding interactions through the amide bonds of the neighboring tail of the surfactant molecules result in the formation of a parallel arrangement of the corresponding hydrocarbon chains such that the SAAU molecules can self-organize into bilayer structures in water. The existence of the tubules indicates that ordered bilayer membranes are formed. The bilayer sheets roll up to form tubules or cigarlike scrolls. It is not clear whether the rodlike structures were formed from vesicles or the tubules converted to rods, as they are present at all concentrations in the range 2-10 mM. However, since at higher concentrations (>10 mM) of the amphiphile only rodlike structures could be observed, the fluid rods may have formed either by slow swelling of the multilayer systems or by fusion of vesicles or spherical micelles. As they consist of multiple bilayers, their diameter is in the range of micrometers.

In conclusion, we have shown for the first time that a simple fatty acid with a secondary amide linkage at the end of the hydrocarbon chain can spontaneously form stable ordered bilayer self-assemblies in dilute aqueous solutions. Indeed, sodium 11-acrylamidoundecanoate spontaneously forms multilayered spherical vesicles and tubules along with normal spherical and rodlike micelles in dilute aqueous solutions at room temperature. These structures are completely converted to bigger rodlike

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Figure 3. Optical (upper panel) and electron miocrographs (lower panel) of a 2.5 mM solution of SAAU: (A) long tubules (inset: wormlike tubules); (B) long rod; (C) spherical vesicle; (D) tubules and rods (inset).

micelles at higher concentrations of the amphiphile. Dynamic light scattering measurements show that the micellar aggregates have a very low translational diffusion coefficient and have a large apparent hydrodynamic radius, suggesting the formation of elongated self-assemblies in aqueous dispersions. The existence of spherical vesicles and tubular structures suggests that ordered bilayer membranes are spontaneously formed from the amphiphile in aqueous solution. The driving force for the bilayer formation is the intermolecular hydrogen bonding between the secondary amide groups of the neighboring molecules, which promote the formation of a linear array of the amphiphile molecules.

Experimental Section

The 11-acrylamidoundecanoic acid was synthesized and purified according to the reported procedure.⁷ The sodium salt was prepared by stirring equimolar mixtures of NaOH and 11-acrylamidoundecanoic acid in dry methanol for 24 h. The salt was obtained after evaporation of the solvent. It was recrystallized from ethanol until it was free from unreacted acid. The structure was confirmed by IR and ¹H NMR spectra.

 $^1\rm H\,$ NMR spectra were recorded on a Bruker SEM 200 instrument in CDCl₃ solvent using TMS as standard. The FT-IR spectra in aqueous solution were measured with a Thermo Nicolet Nexus 870 spectrometer. A thin layer of a 10 mM solution of the compound was placed between zinc selenide plates. The background spectrum of the pure solvent was subtracted from the raw data using the instrumental software.

All aqueous solutions were made in Milli-Q water (18.2 M Ω). The cmc was measured by the surface tension method using a Torsion Balance (S.D. Hurdson & Co., Kolkata) at room temperature (~28 °C). The fluorescence measurements were performed with a SPEX Fluorolog-3 spectrofluorometer. Pyrene was employed as a micropolarity sensitive probe. The average micellar aggregation number (N_{agg}) was determined by steady-state fluorescence quenching of pyrene following the method reported by Turro et al. using cetylpyridinium chloride (CPC) as quencher.¹²

The DLS measurements were performed using a Photal DLS-7000 (Otsuka Electronics Co. Ltd., Osaka, Japan) optical system equipped with an Ar⁺ ion laser (75 mW) operated at an output power of 16 mW at $\lambda_0 = 488$ nm. The surfactant solution was filtered into the quartz cell by use of a membrane filter (0.22 μ m). The diffusion coefficient was deduced from the standard second-order cumulant analysis of the autocorrelation functions measured at a 90° angle. From the apparent diffusion coefficient, the hydrodynamic radius, $R_{\rm H}$, of the micelles was calculated using the Stokes–Einstein equation:

$$R_{\rm H} = k_{\rm B} T/6\pi\eta D \tag{2}$$

where $k_{\rm B}$ is the Boltzmann constant, *T* is the absolute temperature, *D* is the diffusion constant, and η is the viscosity of the solvent. For all light scattering measurements, the temperature was 22 \pm 0.5 °C.

The light micrographs were obtained from a Leica-DMRXP microscope. The images taken with a video camera were analyzed by Leica Qwin software. A drop of the filtered (through a 0.22 μ m, Millex Millipore filter) surfactant solution was placed on a

thoroughly cleaned glass plate. For electron microscopic studies, a drop of the aqueous solution was placed on the carbon-coated copper grids, blotted with filter paper, and negatively stained with a freshly prepared 2% aqueous solution of phosphotungstic acid. The specimens were examined on a Phillips 120 CM electron microscope operating at 120 kV at room temperature (\sim 28 °C).

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