Vesicle–Micelle Transition of *N*-Acyl Phenylalaninate Salt in Very Dilute Aqueous Solution

Akio Ohta,* Kumiko Tani-i, Aoi Hoshiba, Tsuyoshi Asakawa, and Shigeyoshi Miyagishi
Division of Material Sciences, Graduate School of Natural Science, Kanazawa University, Kakuma, Kanazawa 920-1192

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A unique aggregation behavior of potassium *N*-tetradecanoyl phenylalaninate in dilute aqueous solution was investigated. Surface tension, fluorescence intensity (using probes) and size distribution measurements suggest that a transition from vesicle to micelle takes place. It is presumed that formation of an acid-soap dimer, which is stabilized by an interaction between phenyl groups, brings about construction of vesicle particles at a very dilute concentration.

In the past decade, much attention has been given to the spontaneous formation of thermodynamically stable vesicles, which result from oppositely charged single-tailed surfactant mixtures in aqueous solution.[1,2] It is considered that the vesicle particles are derived from dimers consisting of the two different surfactants, and that these dimers are formed by electrostatic interaction of oppositely charged head groups. Recently, vesicle formation brought about by dimerization of weak electrolytic surfactants through hydrogen bonding has also been reported,[3,4] as well as a example involving acid soaps of fatty acids.[5] All these dimers result from hydrogen bonding between ionic and nonionic species around $pH = pK_a$. Furthermore, some results in which vesicles were formed in aqueous single surfactant systems despite having a $pH \gg pK_a$, have been reported.[3,6,7] Though the detailed mechanism of this vesicle formation is still unknown, it is noticed that the chemical structures of these surfactants had something in common: (1) the polar head was a carboxylate group, and (2) the hydrophobic chain had both functional groups with a double bond (vinyl or phenyl groups) and the ability to hydrogen bond (amide or hydroxy groups).

In this study, the association behavior in dilute aqueous solution of a phenylalanine-type amino acid surfactant which fits the above conditions is investigated using thermodynamic and spectroscopic methods.

N-Tetradecanoyl phenylalanine (C14-Phe) was obtained by reaction of L-phenylalanine with tetradecanoyl chloride as described previously[8] and was recrystallized from acetone–methanol solution. It was then dissolved in 1 mM excess aqueous potassium hydroxide solution. Auramine (Kanto Chemical Co.) and pyrene (Aldrich) were used as fluorescence probes for determination of the critical aggregate concentration (cac). The concentrations of the probe molecules were 1×10^{-5} and 1×10^{-7} M, respectively.

The surface tensions of the aqueous solutions of surfactants were measured at 298.15 K by the drop volume technique using a DVS-2000 system (Yamashita Giken Co. Ltd.), and the results obtained are shown in Figure 1. In the case of a common *N*-acyl amino acid surfactant such as K C14-Ala, the surface tension decreases with increasing concentration and the curve breaks only at the critical micelle concentration (cmc). On the other hand, the curves of K C14-Phe has three break points, which were indicated by arrows, at 0.056, 0.13, and 0.40 mmol kg^{-1}, respectively.

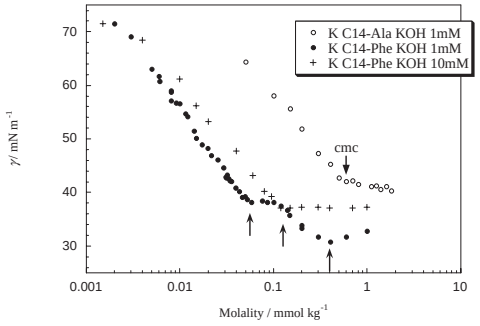

Figure 1. Surface tension vs concentration curves.

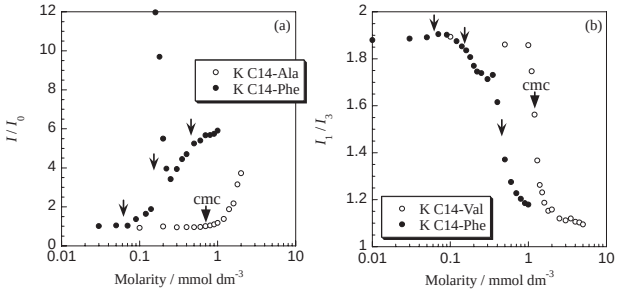

Figure 2. Fluorescence intensity ratio vs concentration curves using (a) auramine, and (b) pyrene as a probe. The arrows indicate the breaks in the surface tension vs concentration curves.

These results suggest transformation or growth of the K C14-Phe aggregates in this concentration range.

Fluorescence measurements were carried out on a Hitachi fluorescence spectrophotometer F-2000 to determine the cmc or cac values of surfactants at 298.2 K. It is known that the ratio of the fluorescence intensity in an aqueous solution containing no surfactant (I_0) to that for a surfactant solution (I) can be used as an indication of the microviscosity of aggregates in aqueous solution, because the fluorescence yield of auramine increased as a result of restriction of its rotational motion when auramine molecules were solubilized.[9] The excitation and emission wavelengths of auramine were 422 and 490 nm, respectively. Figure 2a shows the dependence of the fluorescence intensity ratio (I/I_0) on concentration for two amino acid surfactants. For common surfactants such as K C14-Ala, the cmc can usually be determined as the concentration at which the fluorescence intensity increases sharply. Similarly, the I/I_0 value of K C14-Phe increased at a certain concentration. This concentration corresponded to the concentration at the first break in the surface tension vs concentration curve shown in Figure 1. In contrast to the case for K C14-Ala, a large peak appeared on the increasing section of the curve. The peak concentration was equivalent to that at the second break in Figure 1. Therefore, it is suggested that an

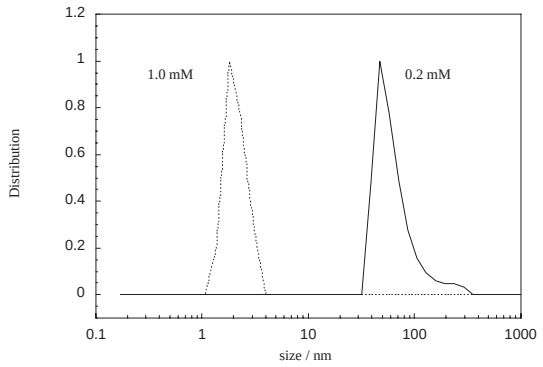

Figure 3. Size distribution of aggregates in alkali aqueous solutions of K C14-Phe at 0.2 mM (solid line) and 1.0 mM (dotted line).

aggregate is formed at the first concentration at which a break occurs in the aqueous K C14-Phe solution, and this grew into a larger one with high viscosity at the point of the second break. However the viscosity of the grown aggregates rapidly decreased with increasing concentration.

A typical pyrene monomer fluorescence spectrum was observed around 380 nm with five vibronic peaks with an excitation wavelength of 335 nm. The ratio (I_1/I_3) of the fluorescence spectra of the first I_1 and the third I_3 peaks from the lower wavelength side was employed as an indicator of the micropolarity of the aggregates in aqueous solution.[10] The values of I_1/I_3 are plotted against the surfactant concentration in Figure 2b. The value of K C14-Phe decreased in two steps with increasing concentration, and that of K C14-Val decreased in one step with the point of inflection corresponding to the cmc. The point of inflection at the lower concentration in the I_1/I_3 vs concentration curve for the K C14-Phe system corresponded to the second break in the curve in Figure 1, and the one at the higher concentration corresponded to the third break. Furthermore, it seems that the I_1/I_3 behavior of K C14-Phe at the second inflection point resembles that of common surfactants accompanied by micelle formation.

Next, the size distribution of the aggregates in aqueous K C14-Phe solution was measured by dynamic light scattering using a Malvern instruments HPP-5001 at 298.2 K. The results are shown in Figure 3. It was found that normal micelle particles about 2 nm in size were present in the solution at 1.0 mM after the third break. On the other hand, relatively large particles appeared at 0.2 mM, which is the concentration halfway between the second and third breaks. Judging from both the size and the high viscosity (see Figure 2), the aggregates appearing after the second break seem to be uni- or multi-lamellar vesicles. The lower I_1/I_3 value of pyrene in the vesicles than in micelles can be interpreted as showing prevention of the incursion of pyrene molecules into the hydrophobic core by the strong packing stress of the vesicle membrane. At the concentration just after the first break, we had no significant results in the distribution diagram. It was concluded, therefore, that the aggregates formed at the first break were relatively small, for example dimers and tetramers.

This type of morphological transition from vesicle to micelle with increasing concentration is often observed in vesicular systems from binary surfactant mixtures. Since the composition of vesicles formed in a binary surfactants system is very different from the prepared composition, one type of surfactant molecules is present in excess in the bulk solution with increasing concentration, and then the excess surfactants finally form micelles.[11] This approach can be applied to this system, if we regard the aqueous solution of K C14-Phe as the mixed solution of an ionic (carboxylate) and a nonionic (carboxylic acid) species. That is, a pre-aggregate composed of an acid–soap dimer unit is formed at the first break, at the second break the small aggregates grow into vesicles, and then micelles are formed by the excess ionic-type surfactant molecules at the third break. The decrease of surface tension after second break implies both changes of size and composition of the vesicle. The pH value of the 1 mM aq solution of potassium hydroxide was about 11, while the pK_a of K C14-Phe was 7.1. This suggests that the amount of ionic-type species is overwhelmingly in surplus compared to the nonionic type. It should be emphasized that the acid–soap dimer can be formed in spite of the considerable imbalance between the amounts in solution. Taking into account that the remarkable growth of aggregate was not observed in such alkali solutions of the other *N*-acyl amino acid surfactants K C14-Ala and K C14-Val or fatty acid type surfactants, it is presumed that formation of the acid–soap dimer of K C14-Phe is unusually stabilized by interaction between phenyl groups.

In order to verify the above hypothesis, some measurements were carried out with a potassium hydroxide concentration higher than 1 mM. It is expected that the acid–soap dimer formation becomes harder with an increase in the alkali concentration. Therefore, the surface tension of an aqueous solution of K C14-Phe with 10 mM KOH was measured and the results have been inserted into Figure 1. It can be seen that the shape of the curve is almost identical to that of a common surfactant such as K C14-Ala. Similarly, there was no abnormal behavior in the fluorescence measurements with 10 mM KOH. This fact strongly supports the vesicle formation being by an acid–soap dimer.

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