Video-Enhanced Differential Interference Contrast Microscope Observation of pH-Dependent Vesicle **Formation by Single-Chain Surfactants**

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Aqueous solutions of dipotassium dodecenesuccinate (C12SAK), N-tetradecanoyl-L-aspartic acid (C14-Asp), and hexadecenesuccinic acid ($C_{16}SA$) have been observed at room temperature with a video-enhanced microscope. While many needle-shaped crystals precipitated in aqueous surfactant solutions at low pH, vesicles and small particles were observed in solutions at medium pH. Vesicles disappeared at high pH, and the number of small particles decreased with increasing pH. Globular vesicles with diameters 2-7 μ m generally contained a few small particles.

Introduction

It has been known that surfactants or amphiphiles can associate into vesicles or liposomes in the same manner as natural lipids. Although these surfactants were generally double-tailed, vesicle formation by single-chain surfactants was also seen in some cases.¹⁻⁵

Gebicki and Hicks¹ have confirmed the existence of liposomes with an unsaturated single-chain fatty acid (oleic acid) membrane. Hargreaves and Deamer² have found that saturated fatty acids, monoalkanoic acids, form spontaneous liposomes in dilute aqueous solutions. They also observed vesicles of anionic surfactants and surfactant mixtures. The vesicle formation depended on surfactant concentration, pH, temperature, and the addition of salt.

Haines³ has discussed the stability of oleic acid and dodecanoic acid liposomes as functions of pH and protonation state of the carboxylic acid groups. Subsequently, Li and Haines⁴ developed the procedure for preparation of large unilamellar vesicles of oleic acid and surfactant mixtures with narrow size distributions.

Kunitake et al.⁵ have investigated the aggregate morphology of a large number of single-chain amphiphiles with a rigid segment in addition to a hydrophilic head group and a flexible tail. They discussed the relationship between structural elements of the amphiphile and aggregate morphology.

In this paper, the pH dependence of vesicle formation in aqueous solutions of single-chain amphiphiles with dicarboxylic acid hydrophilic head groups was examined by use of the video-enhanced differential interference contrast microscope (VEM).

Experimental Section

Samples of C₁₂SAK and C₁₂SA were the same as previously used.⁶ They take the double bond geometry of the trans isomer. C14Asp was kindly supplied by Dr. T. Kanekiyo of Mitsubishi Petrochemical Co., Ltd., Mie, Japan. It was synthesized from tetradecanoyl chloride and L-aspartic acid on the basis of the Schotten-Bouman method.

$$\begin{array}{c} C_{9}H_{19}CH = CHCH_{2}CH - COOK \\ I \\ CH_{2} - COOK \\ C_{12}BH_{27}C - NH - CH - COOH \\ I \\ O \\ CH_{2} - COOH \\ C_{14}Asp \\ C_{13}H_{27}CH = CHCH_{2} - CH - COOH \\ CH_{2} - COOH \\ C$$

The surfactant concentration was $0.22 \times 10^{-2} \text{ g cm}^{-3}$ for all three surfactants. Aqueous solutions at several pH values were made by adjusting the pH with HCl for C12SAK or NaOH for $C_{14}Asp$ and $C_{16}SA$. The surfactants were dissolved at high temperature and the solutions were then stored at room temperature.

Microscopic observation was performed at room temperature on the VEM equipped with a video camera and a digital image processing system. The equipment and procedures were described in detail in previous papers.⁷⁻⁹ Micrographs were taken from the video monitor with a Polaroid camera. The mixing experiment was carried out by using a flow cell with a 100-200 µm depth.9

Results and Discussion

Aqueous solutions of C₁₂SAK precipitated as needleshaped crystals below pH 4.9 were turbid at pH 5.2-6.2 and were clear above pH 6.4. $C_{14}Asp$ in water precipitated at pH 5.8, was a turbid solution at pH 6.2, and cleared at pH 6.5–9.1. Translucent solutions of $C_{16}SA$ appeared at

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 ⁽¹⁾ Gebicki, J. M.; Hicks, M. Nature (London) 1973, 243, 232; Chem.
Phys. Lipids 1976, 16, 142.
(2) Hargreaves, W. R.; Deamer, D. W. Biochemistry 1978, 17, 3759.
(3) Haines, T. H. Proc. Natl. Acad. Sci. U.S.A. 1983, 80, 160.

⁽⁴⁾ Li, W; Haines, T. H. Biochemistry 1986, 25, 7477.

⁽⁵⁾ Kunitake, T.; Okahata, Y.; Shimomura, M.; Yasunami, S.; Takarabe, K. J. Am. Chem. Soc. 1981, 103, 5401.

⁽⁶⁾ Imae, T.; Suzuki, S.; Abe, A.; Ikeda, S.; Fukui, Y.; Senoh, M.; Tsujii,

K. Colloid Surf. 1988, 33, 75.
(7) Kachar, B.; Evans, D. F.; Ninham, B. W. J. Colloid Interface Sci. 1984, 100, 287.

⁽⁸⁾ Brady, J. E.; Evans, D. F.; Kachar, B.; Ninham, B. W. J. Am. Chem. Soc. 1984, 106, 4279.

⁽⁹⁾ Miller, D. D.; Bellare, J. R.; Evans, D. F.; Talmon, Y.; Ninham, B. W. J. Phys. Chem. 1987, 91, 674.



Figure 1. VEM photographs of aqueous solutions of $C_{12}SAK$ of 0.22×10^{-2} g cm⁻³ at room temperature: (a) pH 5.2; (b) pH 6.2; (c) pH 7.3. The bar represents 10 μ m. An arrow points to a vesicle.



Figure 2. VEM photographs of aqueous solutions of $C_{14}Asp$ of 0.22×10^{-2} g cm⁻³ at room temperature: (a) pH 6.2; (b) pH 8.9. The bar represents 10 μ m. An arrow points to a vesicle.

pH 7.1-8.3 and became transparent at pH 10.4.

With the VEM, many vesicles and small particles were seen coexisting with crystals in an aqueous solution of $C_{12}SAK$ at pH 4.9. As seen in Figure 1a, vesicles and small particles filled the solution at pH 5.2, although crystals had disappeared. The number of vesicles decreased at pH 6.1–6.2 (Figure 1b), and the vesicles vanished above pH 6.4. The number of small particles decreased gradually with increasing pH, but a small quantity of particles remained even at pH 7.3 (Figure 1c).

In the aqueous solution of $C_{14}Asp$, small particles

coexisted with crystals at pH 5.8 and were seen together with vesicles at pH 6.2 (Figure 2a). Small particles disappeared gradually at pH 6.5 - 9.1, and vesicles were not observed above pH 6.5 (Figure 2b).

Many $C_{16}SA$ vesicles and small particles were seen at pH 7.1–7.8 (Figure 3a,b). The vesicles disappeared above pH 8.1 (Figure 3c), and the number of small particles gradually decreased to a negligible amount as the pH was increased to 10.4.

There were similarities in the behavior of the three surfactants. Vesicles and/or small particles were generated



Figure 3. VEM photographs of aqueous solutions of $C_{16}SA$ of 0.22×10^{-2} g cm⁻³ at room temperature: (a) pH 7.1; (b) pH 7.8; (c) pH 8.1. The bar represents 10 μ m. An arrow points to a vesicle.

with crystals in the aqueous solutions at low pH. While the crystals disappeared at medium pH, the vesicles and small particles remained. The vesicles vanished at high pH as the number of small particles decreased gradually with an increase in pH. Vesicles were formed at about the same pH as solution translucency occurred. The pH region of vesicle formation or translucent solution seemed to be higher for surfactants with a longer alkyl chain.

All three surfactants formed globular vesicles with diameters of 2–7 μ m, and some of them enclosed a few small particles. Small particles of $C_{12}SAK$ and $C_{16}SA$ were spherical, whereas those of C₁₄Asp appeared ellipsoidal. Submicrometer particles may have been small vesicles or fine droplets; this could not be ascertained from the VEM observation.

By use of a flow cell with two inlet ports, a stream of an aqueous solution of $C_{12}SAK$, pH 5.2, was placed in contact with a parallel stream of an aqueous NaOH solution, pH 8.9. The flow of both streams was stopped simultaneously, and vesicles at the interface were observed as diffusion occurred. Figure 4a shows the interface when the flow was stopped. Parts b to f of Figure 4 show the time-dependent disappearance of vesicles and small particles in the solution of $C_{12}SAK$ near the interface, as the C12SAK solution was mixed with the NaOH solution by diffusion. Although numerous vesicles and small particles were still in existence after 6 min, the vesicles diminished after 12 min and vanished at 20 min. On the other hand, some small particles continued to exist after 20 min, and a tiny number were seen after 40 min.

Additional flow cell experiments were carried out for aqueous solutions of C₁₄Asp at pH 6.2 and of C₁₆SA at pH 7.5, which were allowed to contact an aqueous NaOH solution at pH 11.4. Vesicles and small particles started to disappear after several minutes. After scores of minutes, vesicles vanished, and small particles became infrequent.

In summary, the pH-dependent vesicle formation by single-chain surfactants with a dicarboxylic acid group, which was first seen in the solutions prepared at different pH values, may also be accomplished by the flow cell experiments.

The relationship between structural elements of amphiphile and aggregate morphology has been discussed by some workers.^{5,10,11} The balance of hydrophilic head and hydrophobic tail was emphasized¹¹ and, on the other hand, the existence of a rigid segment was necessary for vesicle formation by single-chain amphiphiles.⁵

When only the balance of head and tail groups is considered, single-chain amphiphiles with a bulky dicarboxylic acid hydrophilic head group like C12SAK, C14Asp, and $C_{16}SA$ should form spherical micelles. Actually, as shown in this paper, they are preferentially associated into vesicles. Moreover, these amphiphiles have no rigid segment possessing the liquid crystalline property; therefore, they do not satisfy the conditions for vesicle formation described above.

There are reports of vesicle formation by simple singlechain amphiphiles like oleic acid, 1-4 monoalkanoic acids, 2,3 octadecyl phosphate, and octadecyl sulfate² and by amphiphile mixtures like alkanoate-alkyl alcohol, oleic acidcholesterol, sodium dodecyl sulfate-dodecanol, sodium dodecyl sulfate-hexadecyltrimethylammonium bromide,² dodecanesulfonate-dodecanol, and oleic acid-oleyl alcohol.⁴ Recently, we¹² have found that vesicle-like assemblies are also formed in iridescent solutions of single-chain surfactants, alkyldimethylamine oxides ($C_n DAO$), $C_{16}SA$, and diglycerol isostearyl ether ($C_{18}DGE$). C_nDAO are a simple amphiphile and C₁₈DGE has a bulky head group.

We must find an alternate mechanism for vesicle formation by single-chain amphiphiles. For weak acidic and basic amphiphiles and amphiphile mixtures, the complex formation by hydrogen bonding or by ionic pairing might be important for the stabilization of vesicles. Amphiphiles with peptide residues may be linked by hydrogen bonding in vesicles. In addition, the vesicle formation by fatty acids must be considered in connection with their droplet formation.2,3,13

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⁽¹⁰⁾ Tanford, C. The Hydrophobic Effect. Formation of Micelles and Biological Membranes; Wiley-Interscience: New York, 1973. (11) Israelachvili, J. N. Intermolecular and Surface Forces; Academic

Press: London, 1985. (12) Imae, T.; Trend, B. J. Colloid Interface Sci., in press.

⁽¹³⁾ Drzymala, J. J. Colloid Interface Sci. 1985, 107, 442.



Figure 4. Time-dependent disappearance of vesicles and small particles in an aqueous solution of $C_{12}SAK$ of 0.22×10^{-2} g cm⁻³ at room temperature: (a) the interface, (b-f) aqueous solution of $C_{12}SAK$ near the interface; time (min) (b) 1, (c) 6, (d) 13, (e) 22, (f) 42. The solution at pH 5.2 was in contact with an aqueous NaOH solution at pH 8.9. Arrows in part a represent the direction of interface. The bar represents 10 μ m.

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