Formation of Giant Liposomes from Crystalline Complexes of Monoalkylammonium Surfactants and 4-Hydroxybiphenyl

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Single-chain alkylammonium halides are well-known cationic surfactants that form micelle structures in water. A micelle is a small globular aggregate containing low numbers of surfactants which does not transform into other larger aggregates, such as a liposome, under normal conditions. On the other hand, a low-viscosity micelle solution sometimes transforms into a highly viscous solution when a certain aromatic compound, for example the salicylate ion, is added.[1] Transmission electron microscope (TEM) pictures reveal that the viscous solutions contain rodlike structures.[2] Recently, several investigators[3–5] and some of us[6] reported that ammonium surfactants form crystalline complexes with various aromatic compounds. These results are suggestive of the formation of an aggregate larger than a micelle from a binary mixture of an ammonium surfactant and an aromatic compound. We thus studied the aggregation behavior of several crystalline complexes in water, and found that the complexes formed giant liposomes on the supermicron scale. The giant liposomes exhibited a dynamic morphological transformation in a manner similar to that observed for aqueous biolipids[7] and for aqueous dialkylammonium salts which form bilayer structures.[8]

Dodecyltrimethylammonium chloride (DTAC), dodecyltrimethylammonium bromide (DTAB), cetyltrimethylammonium chloride (CTAC), and cetyltrimethylammonium bromide (CTAB) were used in the study as typical micelle-forming surfactants. These surfactants dissolved well in water despite the differences in chain length and/or kind of counter anion, giving a foamy and transparent solution with low viscosity. We observed no aggregate in the aqueous solutions (1–400 mM) using an optical microscope equipped with a dark-field condenser; therefore, small globular micelles should have been formed. We then examined binary mixtures of the surfactants and aromatic compounds, namely, biphenyl, 2-naphthol, 4-hydroxybiphenyl, and 4,4′-dihydroxybiphenyl. These arenes can form crystalline complexes with the previously mentioned surfactants when they are ground with an agate mortar and pestle or when the mixture of the aromatic compound and the surfactant is recrystallized from a suitable organic solvent.[3–6] The crystalline complexes are divided into guest-dependent complexes, whose thermal behavior is very similar to that of the guest crystal,[6c] and host-dependent complexes, whose thermal behavior is very similar to that of the host crystal.[6b] All of the crystalline complexes except for the DTAB/4-hydroxybiphenyl combination form either a guest- or a host-dependent complex (Table 1). The DTAB/4-hydroxybiphenyl combination can form both types of complexes depending on the ratio that is mixed.

Table 1. Crystal types and morphology in aqueous crystalline complexes.[a]

	DTAX		CTAX	
	$X = Cl^-$	$X = Br^-$	$X = Cl^-$	$X = Br^-$
biphenyl	N, no data	N, no data	N, no data	N, h-d (2:1)
4-hydroxybiphenyl	L, g-d (1:1)	L, g-d (1:1)	L, g-d (1:1)	LC, no data
4,4′-dihydroxybiphenyl	C, no data	C, h-d (2:1)	C, h-d[b]	C, no data
2-naphthol	L, g-d (1:1)	L, no data	L, no data	LC, no data

[a] The surfactant and the aromatic compound were mixed in a mortar in a 1:1 and a 2:1 molar ratio. The aggregate morphology was unchanged with the mixing ratio, except for the case of DTAB/4-hydroxybiphenyl. This mixture formed a host-dependent complex when the DTAB/4-hydroxybiphenyl ratio was 2:1. At a mixing ratio of 1:1, crystallites were observed together with liposomes (see text). N: no crystallites and no liposomes were observed, L: liposomes were observed, LC: liposomes and crystallites were observed; h-d: host dependent, g-d: guest dependent. [b] CTAC:4,4′-dihydroxybiphenyl:H_2O = 2:1:2.

Three different methods were employed for the observation of morphology (see the Experimental Section). Method I was used in general, whereas the other two methods were used to observe the dissolving process of the complexes or the aromatic compounds in water and the surfactant solution, respectively. The results of the microscopic observations are summarized in Table 1. Figures 1 and 2 show typical microscope pictures of the aqueous DTAC/4-hydroxybiphenyl complex. Liposomes with various shapes were observed (Figure 1a). Large liposomes were seen when the sample was observed according to method II (Figure 1b). The diameter of these liposomes was in the range of 10–50 μm, and the largest liposome has a diameter of greater than 100 μm. The concentration strongly affected the liposome formation. In the case of DTAC/4-hydroxybiphenyl, we observed crystallites instead of liposomes in dilute solutions at concentrations below 10 mM, crystallites and liposomes at 10–20 mM, and only liposomes above 20 mM. The liposomes were maintained without precipitates in the 20-mM solution for more than several months, and, hence, the liposomes were stable at a relatively high concentration. However, it is unusual that high water content favors the crystallite instead of the liposome. Although the phenomenon may have a thermodynamic explanation, it is still under experimental study.

A liposome structure was also observed when 4-hydroxybiphenyl was added to the surfactant solutions (method III). Figures 1c and 2 show the dissolving process of 4-hydroxybiphenyl in the DTAC solution. Myelin figures immediately appeared when the 4-hydroxybiphenyl crystals were saturated with about 30 mM DTAC micelle solution (Figure 1c). The myelin figures grew into long tubular structures (Figure 2a), which transformed into liposomes (Figure 2d) via a rosarylike structure (Figures 2b and 2c). The morphological dynamics is exactly the same as that observed in bilayer membrane systems such as aqueous lecithin[7] and aqueous dialkylammonium bromide.[8] The other surfactant/4-hydroxybiphenyl systems and surfactant/2-naphthol also exhibited the same

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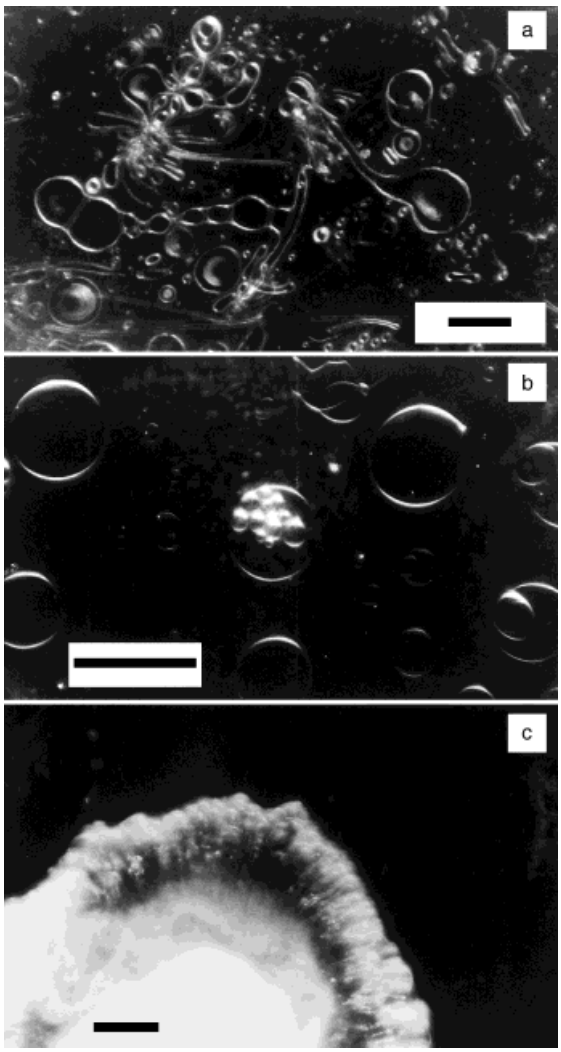

Figure 1. Dark-field light microscope pictures of liposomes formed from the DTAC/4-hydroxybiphenyl complex; scale: 50 μm. a) A 20 mM aqueous solution was used (method I); b) liposomes with a diameter of 10–50 μm were observed at room temperature (method II); c) the crystal of 4-hydroxybiphenyl was swollen, and a myelin figure appeared immediately after mixing (method III).

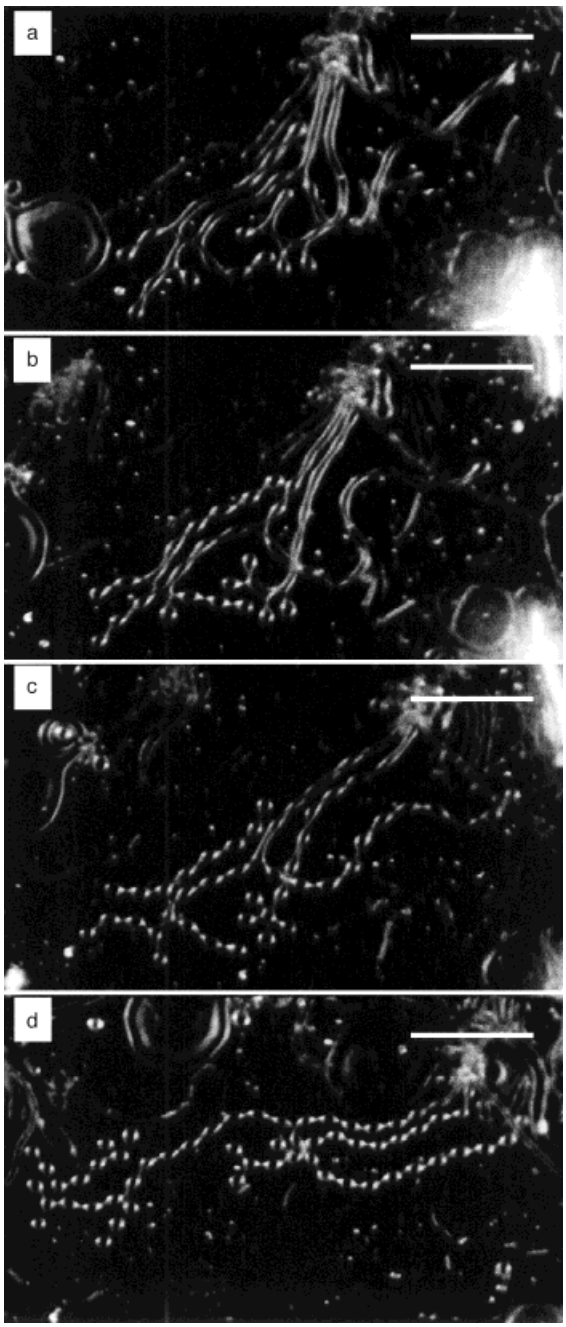

Figure 2. Dynamic morphological transformation of 4-hydroxybiphenyl promoted by the addition of aqueous CTAC solution (ca. 30 mM; method III); scale: 25 μm. a) A flexible tube isolated from the swollen crystal of 4-hydroxybiphenyl (b); c) a rosarylike aggregate transformed from the flexible tube shown in (a); d) small liposomes which appeared from the rosarylike aggregate shown in (c).

morphological dynamics. However, the stability of the liposomes depended upon the kind of surfactant because the surfactants with a shorter chain (DTAC and DTAB) maintained a liposome structure without precipitation at 20 mM for more than several months, whereas those with a longer chain (CTAC and CTAB) produced precipitates after incubation for one day at room temperature.

No liposome was observed for the surfactant/biphenyl and the surfactant/4,4′-dihydroxybiphenyl crystalline complexes. Although the former dissolved in water, we observed no aggregate using an optical microscope (method I). The crystals of the biphenyl disappeared within one day upon mixing with a surfactant solution (method III). The surfactant/4,4′-dihydroxybiphenyl crystalline complex did not dissolve in water, and the 4,4′-dihydroxybiphenyl crystals remained intact in the surfactant solution when method III was employed. These results suggest that the chemical structure of the guest molecule is of essential importance. The spontaneous liposome formation requires the presence of a phenol group.

Bilayer structures are predominantly formed from double-chain ammonium surfactants, whereas single-chain ammonium amphiphiles form micelle structures.[9] However, the single-chain ammonium amphiphile can form bilayer vesicles upon mixing with an anionic micelle surfactant,[10] because the resultant ion pair possesses two hydrophobic chains. In the present case, if a dissociated phenolate group was produced, it could interact with the cationic ammonium head group and

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should form a pseudo-double-chain amphiphile similar to the cation–anion pair surfactant, because the hydroxy proton of the 4-hydroxybiphenyl dissociates within the hydrophilic region where the hydroxide anions are condensed. However, liposome formation was observed in 0.1M HCl, in which the dissociation of the phenol group should be prevented. Furthermore, the bromide anion was detected, and the dissociated phenolate was not observed in the single crystal from the aqueous solution of the CTAB/4-hydroxybiphenyl mixture. The crystal structure was entirely the same as that of the crystal recrystallized from organic solvents. Therefore, electrostatic interactions are not the essential factor in the spontaneous liposome formation.

The liposome formation is strongly related to the structure of the crystalline complexes, because a liposome structure was formed only from the guest-dependent complexes. The structural difference between the host- and guest-dependent complexes is in the packing arrangement of aromatic molecules (Figure 3). In the guest-dependent complexes, the long

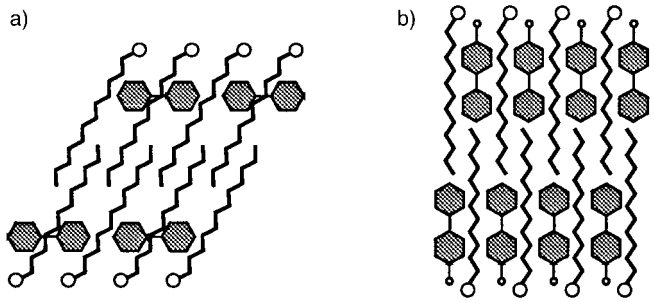

Figure 3. Schematic illustration of the packing arrangement of aromatic compound in crystals of host-dependent (a) and guest-dependent complexes (b).

axis of an aromatic molecule is parallel to that of the surfactant molecules, and, hence, the hydrophobic biphenyl part is buried in the hydrophobic region in the surfactants. In contrast, the aromatic compounds are largely tilted in the host-dependent complexes, and, hence, the long axis of the aromatic compounds is almost perpendicular to that of the surfactant molecules. In this case, the hydrophobic biphenyl part binds to the hydrophilic region of the surfactant and diminishes the hydrophilicity of the surfactants. According to the previous studies on an aqueous bilayer structure, the molecular orientation is almost the same as that in the crystal of the component.[11] In the present case, the local structure within the giant liposome should also be similar to that in the single crystal of a complex. Therefore, a parallel rather than a perpendicular alignment would be preferred to form a liposome structure, because the perpendicular alignment should reduce the hydrophilicity of the aggregate. In fact, DTAB/4-hydroxybiphenyl formed a liposome when the mixing ratio was 1:1, whereas it produced precipitates when the ratio was 2:1 (Table 1).

Experimental Section

Materials: All of the materials used in the study are commercially available from Wako Pure Chemical Ind., Ltd (Osaka, Japan). The crystalline complexes were prepared by recrystallization of an equimolar mixture of a surfactant and an aromatic compound from acetone/ethyl acetate (1/1). The single crystal of the complex was obtained in the same manner as described in ref. [6b, c].

Microscopic observations: Three different methods were employed for the observation. Method I: The crystalline complex was dispersed in water by sonication (1–20mM) and then observed. Method II: A very small amount of the crystalline complexes was sandwiched between a slide glass and a cover glass. The space between the glasses was filled with water and the complex then observed. Method III: A very small amount of the aromatic compounds was sandwiched between a slide glass and a cover glass. The space between the glasses was filled with aqueous surfactant solutions (10–30mM) and the complex then observed. Excess water or solution was blotted by filter paper from the edge of the cover glass. The sample was sealed with colorless manicure to prevent water evaporation. Observations were performed using a dark-field light microscope (OLYMPUS BHF) with a 200-W high-pressure mercury lamp (OSRAM HBO 200W/2) as the light source. We obtained a negative of 132 magnifications on KODAK Tri-X pan film (ISO 400) using a camera module (OLYMPUS PM-10AD; Figure 1) or obtained an image using a digital still camera system (Fuji Photo Film Co., Ltd.; FUJIX HC-300; Figure 2).

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