Micelle–Vesicle Transition by Cleavage of Disulfide Spacer Chain for Gemini Surfactant in Didodecyldimethylammonium Chloride Aqueous Solutions

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Abstract: We examined the “micelle-vesicle transition” through the mixing effect of single–tailed thiol surfactants, [C_{12}H_{25}N(CH_{3})_{2}CH_{2}SSCH_{2}N(CH_{3})_{2}C_{12}H_{25}]2Cl (C_{12}SSC_{12}), which have a disulfide bond in the spacer chain. Phase diagrams of C_{12}H_{25}N(CH_{3})_{2}CH_{2}SHCl-didodecyldimethylammonium chloride (C_{12}SH-DDAC) and C_{12}SSC_{12}-DDAC were determined by conductivity and pyrene fluorescence probe methods. The aggregate diameters were evaluated by dynamic light scattering (DLS). The critical vesicle concentration (CVC) was confirmed by the abrupt increase in the intensity of light scattering with excitation at 335 nm. Vesicle formation was confined to the DDAC-rich region of the C_{12}SSC_{12}-DDAC system, while the vesicle formation region for the C_{12}SH-DDAC system spread out with the addition of dithiothreitol (DTT) to C_{12}SSC_{12}-DDAC. This implies that single–tailed surfactants can induce a more favorable environment for molecular packing of the vesicular surface. The micelle–vesicle transition occurs with disulfide spacer chain cleavage of gemini surfactants at a particular specific concentration range.

Key words: micelle-vesicle transition, disulfide linked gemini surfactant, cleavage of disulfide spacer chain

1 INTRODUCTION

Controlled release of solubilizates is desirable when vesicles are used as drug carriers. Stimuli such as redox, light, and pH have been used to trigger release\(^1\)\(^-\)\(^9\). Since double–tailed surfactants such as didodecyldimethylammonium chloride (DDAC) are known to form vesicles\(^6\), hydrophilic solubilizates can be encapsulated inside the aqueous core of the vesicle. However, DDAC has no cleavable or responsive groups in the amphiphilic structure. In order to exploit conventional amphiphiles for drug-delivery systems, the addition of functional surfactants should be designed for the release of loaded solubilizates from the vesicle. The addition of single–tailed surfactants has been reported to affect the concentration range of vesicle formation as well as the packing of vesicles\(^6\)\(^-\)\(^8\).

The production of single–tailed surfactant by cleavage of disulfide linked gemini surfactants with the addition of dithiothreitol (DTT) has been reported in our previous work\(^3\)\(^,\)\(^10\), shedding light on single–tailed thiol surfactants which influence the vesicular region in comparison with conventional single–tailed or gemini surfactants\(^11\). The critical micelle concentration (CMC) was determined using conductivity and pyrene fluorescence probe methods. The critical vesicle concentration (CVC) was evaluated using light scattering measurements. Phase diagrams of single–tailed/double–tailed surfactant (C_{12}SH-DDAC) and gemini/double–tailed surfactant (C_{12}SSC_{12}-DDAC) systems are reported in this paper. Vesicle formation was examined with the addition of DTT to C_{12}SSC_{12}-DDAC. The disruption of vesicles, and thus the release of encapsulated solubilizates, will be realized by the disulfide linkage along with oxidation in the concentration region of micelle-vesicle transition\(^10\).

2 EXPERIMENTAL PROCEDURES

2.1 Materials

\[[C_{12}H_{25}N(CH_{3})_{2}CH_{2}SS(CH_{2})_{2}N(CH_{3})_{2}C_{12}H_{25}]2Cl (C_{12}SSC_{12})\] gemini surfactants were prepared in similar procedures as reported previously\(^10\). Bis[2-((N,N-dimethylaminoethyl)disulfide dihydrochloride (Wako Pure Chemical Industries, Ltd.) was mixed with a double molar concentration of sodium ethoxide in ethanol. After the removal of precipitated NaCl, the solution was stirred with...
1-iodododecane in 1:2 molar ratio of at 40°C for 3 days. After the evaporation of ethanol, the products were washed thoroughly with acetone. Then, ion exchange from iodide to chloride ion was performed in methanol solution using Amberlite IRA-410. After the evaporation of methanol, the products were purified by repeated recrystallization from acetone-ethanol mixtures. C<sub>12</sub>H<sub>25</sub>N(CH<sub>3</sub>)<sub>3</sub>Cl (DTAC) and didodecyldimethylammonium chloride (DDAC) were purified by repeated recrystallization from acetone. The C<sub>12</sub>SSC<sub>12</sub> were synthesized using a procedure similar to the one reported previously<sup>9</sup>. DTT (Wako Pure Chemical Industries, Ltd.) was used as received.

2.2 Measurements

The conductivity measurements of aqueous surfactant solutions were carried out using a conductivity meter, Model DS-52 (HORIBA). The fluorescence spectra of 10<sup>-7</sup> M pyrene in surfactant solutions were measured from 300 nm to 450 nm, under excitation at 335 nm, using a Hitachi F-2700 spectrophotometer. The ratios of the first and third peaks of pyrene fluorescence were recorded, as well as the intensity of light scattering at 335 nm. The diameter of the aggregates was determined by DLS using a Model SZ-100 (HORIBA) analyzer. The disulfide bond was cleaved by the addition of DTT with equimolar C<sub>12</sub>SSC<sub>12</sub>, and the cleavage process was completed within 60 min. The concentrations of the produced C<sub>12</sub>SH were determined by high-performance liquid chromatography (HPLC) analysis as reported previously<sup>9</sup>. All measurements were performed at 25°C.

3 RESULTS AND DISCUSSION

3.1 Aqueous solution properties of surfactants

The conductivity method was used to examine the differences in aqueous solution properties between methylene and disulfide spacer chain in gemini surfactants. Figure 1(a) shows the conductivity curves for aqueous solutions of C<sub>12</sub>-6-C<sub>12</sub> and C<sub>12</sub>SSC<sub>12</sub> against the surfactant concentration. The slope of C<sub>12</sub>SSC<sub>12</sub> at concentrations below the CMC was agreement with that of C<sub>12</sub>-6-C<sub>12</sub> suggesting no difference in the monomeric state. The ratio of the slopes for the conductivity vs. concentration plot above and below the CMC (S<sub>2</sub>/S<sub>1</sub>) has been used as a measure of the degree of micellar ionization<sup>13</sup>. The CMC values were identified by a distinct inflection point at 1.79 mM for C<sub>12</sub>-6-C<sub>12</sub> and 1.24 mM for C<sub>12</sub>SSC<sub>12</sub>, which was about 1.7 times that of C<sub>12</sub>SSC<sub>12</sub> with bromide counterion<sup>9</sup>. The S<sub>2</sub>/S<sub>1</sub> values were 0.59 for C<sub>12</sub>-6-C<sub>12</sub> and 0.45 for C<sub>12</sub>SSC<sub>12</sub>, respectively. It was reported that a hexamethylene spacer chain of C<sub>12</sub>-6-C<sub>12</sub> is well stretched at micellar surface<sup>14</sup>, while diethyldisulfide (CCSSCC) has a gauche-gauche-gauche form in water<sup>15</sup>. The conformation of the disulfide spacer chain of C<sub>12</sub>SSC<sub>12</sub> results in the lower degree of micellar ionization. The electrostatic repulsion between the head groups at C<sub>12</sub>SSC<sub>12</sub> micelle surface is weaker than that of C<sub>12</sub>-6-C<sub>12</sub>. Thus, the CMC of C<sub>12</sub>SSC<sub>12</sub> is somewhat lower than that of C<sub>12</sub>-6-C<sub>12</sub>.

Next, the single–tailed surfactant was examined by the cleavage of C<sub>12</sub>SSC<sub>12</sub> using DTT. Figure 1(b) shows the conductivity curves for DTAC and C<sub>12</sub>SH against the surfactant concentration. We confirmed that the CMC of DTAC was unaffected by the presence of DTT. The CMC of C<sub>12</sub>SH was 14.3 mM, which was about 12 times the concentration of 1.24 mM for C<sub>12</sub>SSC<sub>12</sub>. This is in agreement with the CMC of

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**Fig. 1** (a) Conductivity for aqueous solutions of gemini surfactants. (○) C<sub>12</sub>-6-C<sub>12</sub>, (●) C<sub>12</sub>SSC<sub>12</sub>. (b) Conductivity for aqueous solutions of single–tailed surfactants. (○) DTAC, (●) C<sub>12</sub>SH.
the single–tailed surfactant DTAC being more than 14 times higher than that of the gemini surfactant C\textsubscript{12-6-C}_{12}.

The CMC of 14.3 mM for C\textsubscript{12}SH was meaningfully low as compared with 25.6 mM for DTAC. Zana reported the effect of side alkyl chain on CMC of dodecyldimethylalkylammonium bromides\textsuperscript{16}, and indicated that the side alkyl chain started to be incorporated into the micellar hydrophobic core when the carbon number is greater than three. This suggests that the ethanethiol group may not be incorporated into the micellar hydrophobic core. However, the thiol group could be advantageous for micelle formation because of its ability to form a weak hydrogen bond with the thiol group of neighboring surfactant molecules in the micellar palisade layer\textsuperscript{17}. This situation may result in the lower CMC of C\textsubscript{12}SH as compared with that of DTAC.

Micellar micropolarities were evaluated by the pyrene fluorescence method. The fluorescence intensity ratio of the first and third vibronic peaks of pyrene was sensitive to the solvent polarity at the solubilization site\textsuperscript{18}. Figure 2 shows the variation in the I\textsubscript{1}/I\textsubscript{3} values against the surfactant concentrations of C\textsubscript{12-6-C}_{12}, C\textsubscript{12SSC}_{12}, DTAC, and C\textsubscript{12}SH. The I\textsubscript{1}/I\textsubscript{3} values in water were 1.87 \pm 0.1, which are in reasonable agreement with the reported values. The I\textsubscript{1}/I\textsubscript{3} values decreased significantly with the solubilization of pyrene into the micelles. The micellar micropolarity observed for the alkyltrimethylammonium system became rather high in comparison with that of anionic dodecylsulfate micelles. The solubilization site of pyrene will be close to the micellar surface because of the interaction of pyrene molecules and trimethylammonium groups\textsuperscript{19}. The I\textsubscript{1}/I\textsubscript{3} value of C\textsubscript{12-6-C}_{12} above CMC was 1.64, which is higher than that of C\textsubscript{12SSC}_{12}. This suggests water penetration for the C\textsubscript{12-6-C}_{12} micelles because of the well–stretched hexamethylene spacer chain at the micellar surface. The CMC values determined by pyrene fluorescence method were in fair agreement with those determined by the conductivity methods. The data for the CMC, degree of micellar ionization, and index of micellar microplarity are summarized in Table 1.

### 3.2 Double–tailed surfactant–rich systems

The aggregate formation of didodecyldimethylammonium chloride (DDAC) was investigated by analyzing the surface tension, turbidity, and electrical conductivity data. The vesicle formation of DDAC has been investigated previously by the cryogenic transmission electron microscopy (cryo–TEM)\textsuperscript{5}. The CVC of DDAC was verified using the pyrene fluorescence probe method, to determine if DDAC forms vesicles at a certain concentration. Figure 3 shows the variations in I\textsubscript{1}/I\textsubscript{3} and the intensity of light scattering (I\textsubscript{s}) at 335nm. I\textsubscript{s}\textsuperscript{0} stands for the intensity of light scattering for water as a standard. The I\textsubscript{1}/I\textsubscript{3} values significantly decreased and gradually lowered with linear regression. An abrupt increase in I\textsubscript{s} occurred near 7.7 mM DDAC, which is

![Fig. 2](image)

**Fig. 2** (a) Fluorescence intensity ratio of pyrene for aqueous solutions of gemini surfactants. (○) C\textsubscript{12-6-C}_{12}, (●) C\textsubscript{12SSC}_{12}. (b) Fluorescence intensity ratio of pyrene for aqueous solutions of single–tailed surfactants. (○) DTAC, (●) C\textsubscript{12}SH.

| surfactant      | Conductivity | Pyrene |
|-----------------|--------------|--------|
|                 | CMC/mM | S\textsubscript{2}/S\textsubscript{1} | CMC/mM | I\textsubscript{1}/I\textsubscript{3} |
| DTAC            | 25.6   | 0.41   | 27.2   | 1.49   |
| C\textsubscript{12}SH | 14.3   | 0.40   | 13.8   | 1.46   |
| C\textsubscript{12SSC}_{12} | 1.24   | 0.45   | 1.35   | 1.48   |
| C\textsubscript{12-6-C}_{12} | 1.79   | 0.59   | 1.70   | 1.64   |
determined as the CVC. Obvious turbidity of the solutions was observed at 10 mM DDAC, suggesting vesicle formation.

We also examined the mixing effect of surfactants on DDAC vesicles. The single–tailed and gemini surfactants were added to 10 mM DDAC. Figure 4 shows the variations in $I_1/I_3$ and $I_s$ against the added surfactant concentrations. The $I_1/I_3$ values increased with the addition of C12-6-C12, suggesting the relaxation of the tight vesicle surface by the inserted gemini surfactants. On the other hand, the $I_1/I_3$ values were observed to decrease for the DTAC and C12SH systems, suggesting that the single–tailed surfactants show the tighter molecular packing at the vesicle surface. However, the $I_1/I_3$ values were restored upon the further addition of single–tailed surfactants. The micelles of single–tailed surfactants solubilized DDAC, as was evident from not only the CMC but also the decrease in $I_s$, as shown in Fig. 4(b). The decrease in $I_s$ indicates the disintegration of DDAC vesicles. The $I_s$ values abruptly decreased above the CMC for gemini surfactants, whereas it increased with the addition of single–tailed surfactants. This result suggested that single-tailed surfactants provide more favorable conditions for vesicle formation. DTAC would be more advantageous for insertion toward the vesicle surface owing to the greater compactness of the hydrophilic groups as compared with the case of C12SH. This indicates that the wedge-shape of single–tailed surfactant has a predominant effect on vesicle surface.

![Fig. 3](a) Fluorescence intensity ratio of pyrene (b) Intensity of light scattering at 335 nm for aqueous solutions of double–tailed surfactant. (○) DDAC.

![Fig. 4](a) Effect of added surfactants on $I_1/I_3$ (b) Effect of added surfactants on $I_s$ for 10 mM DDAC vesicular aqueous solutions. (○) C12-6-C12, (●) C12SSC12, (△) DTAC, (▲) C12SH.
DLS measurements have been used to estimate the hydrodynamic diameter of aggregates. Figure 5 shows the diameter of the aggregates in a 10 mM DDAC system and the effects of adding the single-tailed and gemini surfactants for DDAC vesicles. The diameter of the aggregates in a 10 mM DDAC system was found to be 64 nm, which decreased significantly to 2.2 nm with the addition of gemini surfactants, suggesting the disruption of vesicles. The arrows in Fig. 5 show the experimental sequence. The 10 mM DDAC + 6 mM C12SH system corresponds to the addition of DTT to 10 mM DDAC + 3 mM C12SSC12 system. In this sequence, DDAC vesicles disappeared with the addition of C12SSC12, and then, the DDAC-rich vesicles were regenerated with the cleavage of the disulfide spacer chain by DTT. The diameter of the aggregates increased to 57 nm for the C12SH-DDAC system.

3.3 Single-tailed surfactant-rich systems

The composition and concentration regions of micelle-vesicle transition should be determined in order to realize the release model of solubilizates. Figure 6 shows the variations in I1/I3 and Is against the total surfactant concentrations for X_{DDAC}=0.2 DTAC-DDAC and C12SH-DDAC systems. Because the CMC of 0.18 mM for DDAC is much lower than those of single-tailed surfactants, the I1/I3 values decrease to 1.4 or less. This indicates the formation of closely packed DDAC-rich aggregates in single-tailed surfactant-rich systems. The increase in Is indicates the formation of DDAC-rich vesicles. The CVC for mixed systems are considerably lower than 7.7 mM for the DDAC single system due to the effect of wedge-shaped of the single-tailed surfactant. However, the I1 values decrease around 10 mM with increasing total surfactant concentration. At the fixed composition X_{DDAC}=0.2, the monomeric C12SH increases with increasing total concentration. When monomeric concentration of C12SH reaches the CMC, the generated C12SH micelles can solubilize DDAC, resulting in disappearance of vesicles. The transition from vesicles to mixed micelles is defined as CMC*. The slight increase in I1/I3 at 12.5 mM also suggests the transition from vesicles to mixed micelles at higher concentrations.

3.4 Phase diagrams of DDAC mixed system

The CMC and CVC values of C12SSC12-DDAC and C12SH-DDAC were determined using conductivity, pyrene fluorescence probe, and the light scattering methods. The CMC
decreased with an increase in $X_{\text{DDAC}}$ as expected. The CMC*, i.e., the transition from vesicle to mixed micelle, was observed only for the C$_{12}$SSC$_{12}$-DDAC system, and it increased with increasing $X_{\text{DDAC}}$. The CVC for the C$_{12}$SSC$_{12}$-DDAC system increase to 9.0 mM at $X_{\text{DDAC}} \neq 0.8$, that is to say, vesicles formation was observed only in DDAC-rich region for C$_{12}$SSC$_{12}$-DDAC system. On the other hand, the vesicle formation region was found to be spread out for the C$_{12}$SH-DDAC system. The CVC for C$_{12}$SH-DDAC system decreased to 5.7 mM or lower with a decrease in $X_{\text{DDAC}}$ due to the effect of the wedge shape of the single–tailed surfactant. Therefore, the transition from C$_{12}$SSC$_{12}$-DDAC micelles to C$_{12}$SH-DDAC vesicles can be induced by the addition of DTT, as shown in Fig. 7.

4 CONCLUSION

We found that the micelle-vesicle transition occurs upon the addition of DTT to a C$_{12}$SSC$_{12}$-DDAC mixed system. Therefore, DDAC vesicles containing C$_{12}$SH are generated along with the cleavage of the disulfide spacer chain of C$_{12}$SSC$_{12}$. DDAC vesicles disappear with the generation of C$_{12}$SSC$_{12}$ along with oxidation of thiol groups for C$_{12}$SH, as shown in Scheme 1. Therefore, the solubilizes in the aqueous core of the vesicle can be released by the generation of C$_{12}$SSC$_{12}$. The release model of solubilizes is activated by controlling the oxidation of thiol groups.

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