Aggregation Behavior of Disulfide Linked Gemini Surfactants Compared to that of Double Tailed Surfactants

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Abstract: Disulfide linked gemini surfactant having a long spacer chain, \([C_{10}H_{21}N(CH_3)2(CH_2)_{11}SS(CH_2)_{11}N(CH_3)2C_{10}H_{21}]2Br\) (2C1011SS), was prepared by the hydrolysis and oxidation process of thioester group for \([C_{10}H_{21}N(CH_3)2(CH_2)_{11}SCOCH_3]Br\). The critical vesicle concentrations of double tailed surfactants such as dialkyldimethylammonium bromide were observed by the conductivity and light scattering methods. The disulfide bonds of gemini surfactant, \([C_{12}H_{25}N(CH_3)2CH_2CH_2SSCH_2CH_2N(CH_3)2C_{12}H_{25}]2Br\) (2C12SS), were rapidly cleaved by the addition of water-soluble dithiothreitol. However, it took long time to cleave the disulfide bonds of so-called double tailed surfactants 2C1011SS due to vesicle formation. The dynamic light scattering method showed that the diameters of 2C12SS micelles were increased with the cleavage of disulfide bonds, whereas those of 2C1011SS aggregates remained almost constant at 17.6 ±1.3 nm in similar size with dialkyldimethylammonium bromide vesicles. The time course of disulfide cleavage was examined by the conductivity and HPLC analysis. The produced thiol surfactants were returned to their original gemini surfactants by the addition of \(H_2O_2\).

Key words: double tailed surfactant, critical vesicle concentration, diameters of aggregates, cleavage and formation of disulfide linked gemini surfactant

1 INTRODUCTION

Thioester is known as protecting group because it can be easily hydrolyzed by alkali or acids\(^1\)\(^-\)\(^2\). The expected hydrolysis product, thiol compound, can be oxidized to disulfide compound. Therefore, monomeric thioester surfactants will produce to disulfide linked gemini surfactants by the hydrolysis and oxidation process. We aimed at the disulfide linked gemini surfactant having a long spacer chain, as well as analogous structures of double tailed surfactants, dialkyldimethylammonium bromide. That is to say, the chemical structure of \([C_{10}H_{21}N(CH_3)2(CH_2)_{11}SS(CH_2)_{11}N(CH_3)2C_{10}H_{21}]2Br\) (2C1011SS) can be referred to as the disulfide linked double tailed surfactant.

Didodecyldimethylammonium bromide (DDAB) is well known to aggregate into vesicles\(^3\)\(^-\)\(^4\). If vesicles are used as drug carriers, the controlled release of solubilizates will be desired. However, DDAB have no cleavable or responsive groups in the amphiphilic structure. Stimuli responsive surfactants will be designed to the release of the loaded solubilizates from the vesicle. The stimulation such as redox, light and pH has been used as trigger of release\(^5\)\(^-\)\(^8\).

Since the disulfide bond can be cleaved and formed by redox, it will be worthwhile to introduce the disulfide bond into double tailed surfactants. We have reported that the transformation from the gemini to monomeric surfactants was performed by the addition of dithiothreitol, which is commonly used to cleave the disulfide bond into free thiols\(^9\). Subsequently, the produced thiol surfactants can be expected to return to the original geminis by the formation of disulfide when exposed to air. That is to say, the control of surfactant aggregation will lead to the controlled release of the solubilized substances in the micelles.

In this paper, we focused on the aggregation behavior of disulfide linked gemini surfactant, in other words, the disulfide linked double tailed surfactant. The critical micelle concentration (cmc) was examined by pyrene fluorescence probe method. The critical vesicle concentration (cvc) was evaluated by the conductivity and light scattering measurements. The diameters of aggregates were determined by dynamic light scattering method in comparison with double tailed surfactants.
2 EXPERIMENTAL PROCEDURES

2.1 Materials

\[ \text{[C}_{10}\text{H}_{21}\text{N}((\text{CH}_2)_2\text{SCH}_2\text{Br})\text{C}_{10}\text{H}_{11}\text{Ac}] \text{Br} \] was prepared by refluxing \( \text{N,N-dimethyldecylamine} \) and equimolar \( \text{S-(11-bromoundecyl} \) thioacetate (Sigma-Aldrich) in acetonitrile for 6 h, as shown in Scheme 1. After the evaporation of acetonitrile, the products were washed with petroleum ether. The products were hydrolyzed in ethanol by the addition of equimolar \( \text{NaOH} \) at 40°C for 5 h. After the oxidation for several days, the elution peak of \( \text{C}_{10}\text{H}_{11}\text{Ac} \) disappeared and the elution of \( \text{2C}_{10}\text{H}_{11}\text{SS} \) was observed in HPLC chromatogram. \( \text{2C}_{10}\text{H}_{11}\text{SS} \) was extracted with acetone and dried under reduced pressure over \( \text{P}_2\text{O}_5 \). The addition of excess \( \text{DTT} \) in \( \text{2C}_{10}\text{H}_{11}\text{SS} \) aqueous solutions gave \( \text{C}_{10}\text{H}_{11}\text{SH} \) for 7 days at 25°C. \( \text{[C}_{12}\text{H}_{25}\text{N}((\text{CH}_2)_2\text{SSCH}_2\text{SCH}_2\text{N}((\text{CH}_2)_2\text{Br})_2\text{C}_{12}\text{H}_{25}\text{SS}]\text{Br} \) was prepared in similar procedures as reported previously. \( \text{Didodecyldimethylammonium bromide (DDAB)} \) was purified by recrystallization from acetone. \( \text{Didodecyldimethylammonium bromide (DeDAB)} \) and \( \text{dithiothreitol (Wako Pure Chemical Industries, Ltd.)} \) were used as received. The chemical structures of surfactants are summarized in Scheme 2.

2.2 Measurements

The conductivities of aqueous surfactant solutions were measured using a Model DS-52 (HORIBA) conductivity meter. The absorption spectra were recorded using a Hitachi U-2900 spectrophotometer. The fluorescence spectra of \( \text{10}^{-7} \text{M pyrene} \) in surfactant solutions were measured from 300 nm to 450 nm under the excitation at 335 nm using a Hitachi F-2700 spectrophotometer. The intensities of first and third peaks of pyrene fluorescence were recorded as well as the intensity of light scattering at 335 nm. Dynamic light scattering (DLS) measurements were performed to determine the diameter of aggregates using SZ-100 (HORIBA). The cleavage of disulfide bond was attempted by the addition of dithiothreitol (DTT). The produced compounds by the addition of DTT and \( \text{H}_2\text{O}_2 \) were analyzed by HPLC as reported previously.

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Scheme 1 Preparation of disulfide linked gemini surfactant and the cleaved monomeric surfactant.

Scheme 2 Chemical structures and the abbreviations of surfactants.
3 RESULTS AND DISCUSSION

3.1 HPLC analysis of disulfide linked gemini surfactant

The surfactants were analyzed by reversed phase chromatography using 90:10 methanol:30mM sodium 1-octanesulfonate mixtures as eluting solutions. The elutions of surfactants were monitored by electrical conductivity detector. Figure 1 shows the chromatograms of C_{10,11}SAc, 2C_{10,11}SS and C_{10,11}SH, that were detected at 2.1, 5.8, 2.3 min, respectively. The retention time of C_{10,11}SAc was short due to the hydrophilic thioester group, whereas that of 2C_{10,11}SS was long due to the hydrophobic alkyl chains. The complete cleavage of disulfide bonds for 2C_{10,11}SS was time-consuming probably due to the vesicle formation because the water-soluble dithiothreitol (DTT) could hardly react with the disulfide bonds in the hydrophobic region. DTT can cleave the disulfide bonds for monomeric 2C_{10,11}SS in equilibrium state with vesicles, as shown in Scheme 3. That is to say, 2C_{10,11}SS will be exchanged between vesicles and bulk water phase, and then the monomeric 2C_{10,11}SS will be attacked by DTT, resulting in the formation of C_{10,11}SH.

3.2 Aqueous solution properties of surfactants

The conductivity method was used to examine for the differences in aqueous solution properties between short and long spacer chain in disulfide linked gemini surfactants. Figure 2 shows the conductivity curves for aqueous solutions of 2C_{12}SS and 2C_{10,11}SS against the surfactant concentration. The experimental data of 2C_{12}SS gave linear plots at concentrations below the cmc. The ratio of the slopes for the conductivity vs. concentration plot above and below the cmc (S_2/S_1) has been used as a measure of the degree of micellar ionization \(^1\). The cmc and S_2/S_1 values were 0.92 mM and 0.36 for 2C_{12}SS, respectively.

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Fig. 1  HPLC elution profiles of C_{10,11}SAc, 2C_{10,11}SS and C_{10,11}SH.

Fig. 2  The conductivity curves for aqueous solutions of 2C_{12}SS and 2C_{10,11}SS against the surfactant concentration. (○)2C_{12}SS, (●)2C_{10,11}SS.

Scheme 3  The cleavage of disulfide spacer chain could occur in bulk water phase for vesicle system. Note that the disulfide linkage in vesicles can be anticipated as illustrated.
However, the cmc of 2C_{10}11SS was too low for the accurate determination using the conductivity method. The first inflection about 0.1 mM for 2C_{10}11SS might correspond to the cvc of 2C_{10}11SS. The vesicles of 2C_{10}11SS will exist above 0.11 mM, which was detected by the following measurements using pyrene. The slope for the conductivity curve gradually decreased with increasing surfactant concentration. The S_2/S_1 value was 0.27 indicating the formation of aggregates, but those greatly decreased to S_3/S_1 0.06 with increasing surfactant concentration. The second inflection point was observed around at 1.7 mM far above the expected cvc, suggesting the growth of 2C_{10}11SS vesicles along with counterion binding. The cmc of 2C_{10}11SS was equal to about half of that for DDAB, whereas the cvc of 2C_{10}11SS was only one fifth of that for DDAB, suggesting the contribution of disulfide bond for vesicle formation in similar with protein chemistry. The disulfide bond is known to be stable and provide the structural stability for protein. The disulfide bond usually exists in the inside of protein and acts as a hydrophobic group. The disulfide linkage between inner and outer surfactant molecules in vesicles will contribute the stabilization of vesicles, resulting in the significant lower cvc in compared with DDAB.

Micelle formation has been evaluated by using pyrene fluorescence method\textsuperscript{12}. The fluorescence intensity ratio of the first and third vibronic peaks of pyrene is sensitive to the solvent polarity in the solubilization site\textsuperscript{13}. The I_1/I_3 values decreased with the solubilization of pyrene into the micelles. Figure 3(a) shows the variation in I_1/I_3 values against surfactant concentrations of 2C_{10}11SS, 2C_{12}SS, DDAB and DeDAB. The I_1/I_3 values of pyrene in water gave reproducible value of 1.87 ± 0.1, which are in fair agreement with the reported values. The I_1/I_3 value of 2C_{10}11SS significantly decreased in low surfactant concentration, which became similar values for DDAB micelle system. The data obtained by the conductivity and pyrene fluorescence probe methods for the cmc, degree of micellar ionization and index of micellar micropolarity are summarized in Table 1.

The vesicle formation of DDAB was already indicated by the cryogenic transmission electron microscopy\textsuperscript{3, 4}. Supposing DDAB will form vesicles at a certain concentration,
we checked the cvc of DDAB by convenient light scattering method. Figure 3(b) shows the variations in the intensity of light scattering \( I_s \) at 335 nm. The gradual increase in \( I_s \) occurred at around 1 mM 2C_{12}SS, which suggests the micelle formation. In contrast, the significant increase in \( I_s \) was observed at 0.58 mM DDAB, which can be determined as cvc. The opaque blue color of solutions obviously appeared in 6 mM DDAB suggesting vesicle formation.

DLS measurements have been applied for the estimation of hydrodynamic diameter of aggregates\(^{14,15}\). Figure 4 shows the distribution of aggregates diameter for 2C_{10}11SS, 2C_{12}SS, DDAB and DeDAB. The average diameter of 2C_{12}SS micelles gave 2.6 ± 1.5 nm, whereas that of 2C_{10}11SS system gave 17.6 ± 1.3 nm indicating sharp distributions in comparison with DDAB and DeDAB. This suggests that the disulfide linkage could involve in stabilizing the vesicle formation.

### 3.3 Cleavage of disulfide bond by DTT

Next, we examined the cleavage of disulfide bonds of 2C_{12}SS and 2C_{10}11SS systems. DTT is known to a reducing reagent with the formation of stable cyclic disulfide. Figure 5(a) shows the change in absorption spectra for 5 mM 2C_{12}SS aqueous solutions with the addition of 5, 10, 15 mM DTT. The reaction product of a cyclic disulfide exhibited their absorption maxima at 285 nm. The isosbestic point was observed at 268 nm and the appeared absorption maxima became almost constant by adding twice mole of DTT. We confirmed the complete cleavage of 2C_{12}SS by HPLC analysis as shown in Fig. 5(b). The elution peak of 2C_{12}SS was observed at 3.5 min, whereas the new peak of C_{12}SH appeared at 1.7 min by the addition of equimolar DTT. The elution peak of 2C_{12}SS was disappeared by the addition of twice mole of DTT.

Figure 5(c) shows the change in absorption spectra for 5 mM 2C_{10}11SS aqueous solutions with the addition of 10 mM DTT at 25°C for 24 h. The baseline of absorption spectrum was shifted upward due to the opaque blue color of solution. Therefore, the differences in absorption spectra between 2C_{10}11SS and 2C_{12}1011SS + 10 mM DTT were plotted against wavelength as shown in Fig. 5(d). The absorption maxima 290 nm originated from cyclic disulfide was revealed in 2C_{10}11SS system as well as 2C_{12}SS system. This result indicated that 14% of disulfide bond for 2C_{10}11SS were cleaved after the incubation with twice mole of DTT.

### 3.4 Change in conductivity along with cleavage of disulfide bond

The time-course of disulfide cleavage can be followed by the conductivity measurement. The produced C_{12}SH gave cmc 9.7 mM, which was one order higher than 0.92 mM for 2C_{12}SS\(^9\). Thus the conductivity of 5 mM 2C_{12}SS micelle solution will increase with increasing of monomeric C_{12}SH concentration by the cleavage of disulfide bonds. In addition, the cyclic disulfide is not surface active, and is not ionic conducting species\(^9\). Figure 6(a) shows the time-course of conductivity for 5 mM 2C_{12}SS with addition of 5 mM DTT in comparison with 5 mM 2C_{10}11SS. The conductivities for 5 mM 2C_{12}SS system increased quickly and reached almost constant within 10 min. On the other hand, the increases in conductivities were relatively sparse, and then were noteworthy to a certain extent after 24 h incubation for 5 mM 2C_{10}11SS system. As mentioned above, the complete cleavage of disulfide bonds for 2C_{10}11SS was time-consuming because DTT could hardly react with the disulfide bonds buried in the hydrophobic region of vesicles.

Hydrogen peroxide is a mild oxidizing agent that can oxidize thiols producing H_{2}O by-product\(^{10}\). Thus the appropriate addition of H_{2}O_{2} can be expected to hardly influence the aggregation behavior. Figure 6(b) shows the changes in conductivities of 5 mM 2C_{12}SS aqueous solution with the addition of equimolar DTT and H_{2}O_{2} at 25°C for 24 h. The addition of DTT produced monomeric C_{12}SH leading to increase in conductivity, and the addition of H_{2}O_{2} produced 2C_{12}SS micelles leading to decrease in conductivity. The several cycles of the cleavage and linkage of disulfide bond were obviously realized, but the conductivities for 2C_{12}SS systems increased due to the incomplete formation of 2C_{12}SS. The formation of 2C_{12}SS by H_{2}O_{2} was confirmed by HPLC analysis. The elution peaks of C_{12}SH and 2C_{12}SS were detected at 1.7 min and 3.5 min, respectively.
peak area for C₁₂SH decreased and that for 2C₁₂SS increased with the addition of 5 mM H₂O₂. However, the C₁₂SH was detected somewhat even if equimolar H₂O₂ was added and incubated at 25°C for 24 h, resulting in the increase of conductivities along with several cycles as plotted solid circles in Fig. 6(b).

3.5 Size distribution of aggregates by DLS measurements

DLS measurements have been applied for the estimation of hydrodynamic diameter of aggregates. Figure 7(a) shows the aggregates diameter of 5 mM 2C₁₂SS and 5 mM 2C₁₂SS + 5 mM DTT systems. We measured at regular time intervals for 2C₁₂SS aqueous solutions after the addition of equimolar DTT. The length of the fully extended tail was given as 1.7 nm in alkyl chain of 12 carbon numbers. The average diameter 2.6 ± 1.5 nm of 2C₁₂SS, i.e., observed 1.3 nm for micelle radius was small in comparison with the length including the hydrophilic group. This suggests that the disulfide spacer chain could prevent the dense packing of surfactant in micelles, leading to the interdigitated alkyl chains with smaller micelle size. With the addition of DTT, 2C₁₂SS micelles regenerate along with the cleavage of disulfide spacer chain, producing C₁₂SH as indicated by HPLC analysis. The micelle diameter was increased up to 7.6 ± 3.3 nm for 2C₁₂SS-C₁₂SH mixtures. The monomeric C₁₂SH induced the molecular packing of mixed micelles. We have reported that the surface area per surfactant of C₁₂SH was considerably smaller than half the value of 2C₁₂SS. The closer packing of C₁₂SH resulted in the lower surface tensions compared with those of dodecyltrimethyl-
ammonium bromide (DTAB). Thus we deduced that the thiol group could be advantageous for micelle formation because of the ability to form a weak hydrogen bond with thiol group of neighboring surfactant molecules in the micellar palisade layer. The ethanethiol group may be incorporated into the palisade layer in mixed micelles.

On the other hands, the diameter of 2C1011SS system was almost independent on the addition of DTT even after 7 days incubation at 25°C as shown in Fig. 7(b). The produced C1011SH is also a double tailed surfactant like dialkyldimethylammonium bromide, forming vesicles with opaque blue color of solutions. The thiol group in the terminal of alkyl chain can be incorporated into the hydrophobic core of aggregates. The size distributions of C1011SH system were more sharp in comparison with DeDAB and DDAB vesicular systems. This suggests that the vesicles might be stabilized with the weak hydrogen bond of thiol groups in the vesicular hydrophobic region.

4 CONCLUSION

We succeeded in preparing disulfide linked gemini surfactants with a long spacer chain. The sharp size distribu-
tions of 2C₁₀₄₁₁SS vesicles suggested that the disulfide bonds buried in the hydrophobic region could contribute the stability of vesicles. The complete cleavage of the disulfide bonds for 2C₁₀₄₁₁SS was time-consuming and hardly affected the size of vesicles. In contrast, the cleavage of the disulfide bonds for 2C₈₋₄₁₁SS occurred readily and contributed the micellar growth. Moreover, the cleaved thiol surfactants can be reverted to the original gemini surfactants by the addition of H₂O₂. The reversibility between cleavage and linkage of disulfide bond can be expected to control the aggregation behavior.

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