Enhanced Aggregation of Stimuli Responsive Surfactants by Esterolytic Reactions

Tsuyoshi Asakawa*, Akina Fujii, Nodoka Yoneda, Akio Ohta, and Hitoshi Asakawa

School of Chemistry, College of Science and Engineering, Kanazawa University, Kanazawa 920-1192, JAPAN

Abstract: Thioester surfactants, [C12H25N(CH3)2(CH2)mSCOCH3] Br (C12mSAc, m = 4, 11, 12), yielded thiol surfactants via thiol-thioester exchange upon addition of dithiothreitol in aqueous solution. The thiol-thioester exchange reaction was enhanced in a micellar system owing to the concentration effect. The enhanced aggregation upon deprotection of the thioacetate group was observed by dynamic light scattering measurements. The thiol surfactants yielded disulfide-linked gemini surfactants upon air oxidation and incubation with hydrogen peroxide. In contrast, the thiol surfactants yielded thioester double-tailed products by esterolytic reactions with p-nitrophenyl hexanoate. The introduction of an alkyl chain to the second side chain significantly decreased the critical micelle concentration and induced the micellar growth.

Key words: thiol-thioester exchange, esterolytic reaction, enhanced aggregation, stimuli responsive surfactant

1 Introduction

Thioesters are very useful as protecting groups and are central to the synthesis of various compounds, e.g.; peptides\(^1\). Thioesters can be hydrolyzed under both acidic and alkaline conditions\(^2\). The reactivity of thioesters is partly due to their poor resonance stabilization in the ground state, because the \(3p\) orbitals of sulfur exhibit poor overlap with the \(2p\) orbitals of the carbonyl group\(^3\). The mechanism of thioester hydrolysis has been investigated in previous kinetic studies\(^4\). The hydrolysis of thioesters is slow near neutral pH, but thiol-thioester exchange is relatively fast in producing new thioesters. A plausible reaction mechanism was reported where the thiolate anions attack the thioester and the rate-determining step is the formation of a tetrahedral intermediate. Thus, thiol-thioester exchange depends on the acidities of the thiol and the solution pH.

Thioester surfactants of quaternary ammonium salts have been prepared by introducing bromoalkylthioacetate to the terminal part of a secondary chain\(^6\). The thioester surfactants with a tetramethylene side chain form typical micelles, while those with longer second chains tend to aggregate and form interdigitated packing in the vesicle bilayers. Monomeric thioacetate surfactants can be hydrolyzed by alkali to generate thiols, followed by disulfide-linked gemini surfactants in aerated solutions. The cross-linking of surfactants is a powerful approach for stabilizing aggregates, and disulfide-linked aggregates can be used to release encapsulated drugs by reduction. Hydrophobic pyrene in disulfide-linked micelles was released by the addition of dithiothreitol (DTT), which is commonly used to cleave disulfide bonds to form thiols\(^8\). Didodecyl dimethylammonium bromide (DDAB) is a well-known surfactant that aggregates to form vesicles\(^9\). However, DDAB contains no stimuli-responsive groups in its amphiphilic structure. When vesicles are used as drug carriers, stimuli-responsive double-tailed surfactants are promising candidates for the release of the loaded cargo from the vesicles. Cationic vesicles have been investigated as chemical carriers for industrial applications, while vesicles composed of phospholipid derivatives have been developed as drug carriers.

2 Experimental Procedures

2.1 Materials

\[[C_{12}H_{25}N(CH_3)_2(CH_2)_mSCOCH_3]Br\] (C12mSAc, m = 4, 11) was prepared by refluxing \(N,N\)-dimethyldodecylamine (Tokyo Kasei Kogyo) and an equimolar amount of \(S-(4\)-bromobutyl)thioacetate or \(S-(11\)-bromoundecyl)thioacetate (Sigma-Aldrich) in acetonitrile for 6 h, as reported previously\(^7\). The compounds were characterized by positive ion ESI-MS (electrospray ionization mass spectrometry). The mass-to-charge ratios for the observed peaks were \(m/z\) = 344.4968, 442.2730 and 456.3942 for C124SAc,
C_{12}11SAc and C_{12}12SAc (Calcd. 344.2987, 442.4083 and 456.4239), respectively. Dithiothreitol (DTT, Wako Pure Chemical Industries, Ltd.), p-nitrophenyl acetate and p-nitrophenyl hexanoate (Tokyo Kasei Kogyo) were used as received. \[ \text{[C}_{12}\text{H}_{25}\text{N}(\text{CH}_3)_2(\text{CH}_2)_2\text{SH}]\text{Br (C}_{12}\text{SH) was prepared as reported previously}^{10}. \text{[C}_{12}\text{H}_{25}\text{N}(\text{CH}_3)_2(\text{CH}_2)_2\text{SCO(\text{CH}_2)}, \text{CH}_3\text{Br (C}_{12}\text{SC}_6)\text{was prepared by the incubation with C}_{12}\text{SH and p-nitrophenyl hexanoate in ethanol for 24h at 40°C. The observed peaks were m/z = 372.2997 (Calcd. 372.3300) for C}_{12}\text{SC}_6.} \]

### 2.2 Measurements

The surfactants were analyzed by high performance liquid chromatography (HPLC) as reported previously\(^7\). A TSKgel ODS-100V (TOSOH Co.) column was used with a methanol/30 mM sodium 1-octanesulfonate (90:10) mixture as an eluting solution. Elution of the cationic surfactant was detected by electrical conductivity measurements. Surfactants containing the thioacetate group were sensitively detected by monitoring the absorbance at 233 nm. Dynamic light scattering (DLS) measurements were performed to determine aggregate diameters using a SZ-100 (HORIBA) instrument. The absorption spectra in aqueous surfactant solutions were recorded using a Hitachi U-2900 spectrophotometer with a 1-mm-pathlength quartz cell. The conductivities of the aqueous surfactant solutions were measured using a Model DS-52 (HORIBA) conductivity meter at 25°C.

![HPLC analysis for 4 mM C_{12}11SAc aqueous solution by the incubation with 8 mM DTT at 25°C. The solid and broken lines were mixing after 0 and 4 days, respectively. DTT was detected around 1 min.](image)

### 3 Results and Discussion

#### 3.1 Generation of thiol surfactants via thiol-thioester exchange

Thioesters have been used as protecting groups for thiols because they can be easily hydrolyzed under alkaline conditions\(^3\). Leclaire et al. reported that thiol-thioester exchange occurred in aqueous solution at neutral pH\(^11\). We reported that the elimination of thioacetate in surfactants upon addition of DTT could be verified by HPLC, LC-MS analysis and \(^1\)H NMR spectroscopy\(^12\). Thiol surfactants can be generated via thiol-thioester exchange in water using water-soluble DTT without side effects on the surfactant aggregation, as reported previously\(^8\). Figure 1 shows the HPLC elution profiles of 4 mM C_{12}11SAc aqueous solutions before and after incubation for 4 days in 8 mM DTT at 25°C. The elution of C_{12}11SAc was sensitively detected at 3.2 min by monitoring absorbance at 233 nm, which is characteristic of thioacetate. The peak area of C_{12}11SAc decreased with incubation time, while the peak area of the conductivity for C_{12}11SAc remained nearly constant. The thioacetate group of C_{12}11SAc was eliminated by DTT, whereas the peak area of DTT eluted at approximately 1 min increased with the introduction of the thioacetate group.

Figure 2 shows the time-course of the peak area related to thioester surfactants as a function of the incubation time at 25°C in the presence of 2 molar equivalents of DTT. It should be noted that C_{12}4SAc is monomer under these conditions while C_{12}11SAc and C_{12}12SAc are micellar systems in 4 mM surfactant, since the CMC of C_{12}4SAc, C_{12}11SAc, and C_{12}12SAc were 6.3, 0.78, 0.45 mM, respectively\(^7\).

![Thiol surfactants via thiol-thioester exchange](image)
peak areas of the C_{12}4SAc monomer system gradually decreased, while those in the CTAB micellar system rapidly decreased with the formation of 3.2 mM C_{12}4SH after 5 days of incubation, as shown in Fig. 2(a). Thiol-thioester exchange was enhanced in the CTAB micellar system owing to the concentration effect. The concentration effect indicates that the cationic CTAB micelles solubilizing C_{12}4SAc attract thiolate anions via electrostatic interactions at the micellar surface. The total concentration of C_{12}4SAc and C_{12}4SH remained almost unchanged without the formation of the disulfide-linked gemini surfactant (2C_{12}4SS) owing to the excess DTT. We reported that C_{12}4SAc in equimolar NaOH aqueous solution was converted to 2C_{12}4SS by hydrolysis and air oxidation after 4 days, and no C_{12}4SH peaks were detected at that time\(^7\). Thus, this method of thiol-thioester exchange is useful for obtaining thiol surfactants without formation of disulfide-linked gemini surfactants.

Figure 2(b) shows that the peak areas of the C_{12}11SAc system significantly decreased, while those of the C_{12}12SAc system slowly decreased. Thiol-thioester exchange was enhanced in a similar manner to that of the CTAB micellar system due to the concentration effect. The DTT thiolate anion will attack to thioester on the cationic micellar surface similar to the base-catalyzed hydrolysis of the CTAB micellar systems reported in the literature\(^10\). In addition, thiol-thioester exchange depends largely on the pK\(^{\prime}\) of DTT, which can vary around pK\(^{\prime}\) = 9.2. The depressed thiol-thioester exchange for the C_{12}12SAc system is likely due to the formation of vesicles in similar to DDAB\(^7\). This behavior suggests that the incorporated C_{12}12SAc in the inner layers of the vesicles will not encounter the water soluble DTT.

3.2 Micellar growth upon deprotection of the thioacetate group

The aggregates diameters were evaluated by DLS measurements at 25°C. The hydrodynamic diameters of the DDAB vesicles were 20.7 ± 6.7 nm, as reported previously\(^7\).

Figure 3(a) shows the size distribution of the 15 mM C_{12}4SAc and 5 mM C_{12}11SAc systems before and after incubation for 3 days with two molar equivalents of DTT at 25°C in comparison with those of the DDAB vesicular systems. The diameters of the 5mM C_{12}11SAc system were 9.6 ± 1.0 nm, which are meaningfully smaller than those of the corresponding DDAB system. Considering the differences in the chemical structure of DDAB and C_{12}11SAc where one terminal methyl in DDAB is replaced with a SCOCH\(_3\) group, the smaller hydrodynamic diameter of C_{12}11SAc suggests that the thioester surfactants adopted an interdigitated packing structure in the vesicle bilayers.

In other words, the thioester group may orient toward the polar water region. The diameters of 15mM C_{12}4SAc were significantly smaller than those of C_{12}11SAc, suggesting the formation micelles with an interdigitated packing structure.

After incubation with DTT, the diameters of the 15 mM C_{12}4SAc and 5 mM C_{12}11SAc systems were enlarged to 2.6 ± 1.0 and 33.5 ± 1.0 nm, respectively. The aqueous solu-
tions of C_{12}11SAc with DTT gradually turned to an opaque blue color, suggesting remarkable growth of the vesicles. The mixed systems of 5 mM C_{12}11SAc-10 mM DTT resulted in 0.4 mM C_{12}11SAc – 4.6 mM C_{12}11SH mixed systems. Deprotection of the hydrophilic thioacetate group induced the observed vesicular sizes for C_{12}11SH. The diameters of the C_{12}11SH vesicles were larger than those of the C_{12}11SAc and DDAB vesicular systems because the weak hydrogen bonding between neighboring thiols in the aggregates promoted further aggregation.

We also examined for 4 mM C_{12}12SAc incubated with 4, 8, and 20 mM DTT for 3 days at 25°C. The diameters of 4 mM C_{12}12SAc were 8.1 ± 2.3 nm, whereas those of the C_{12}12SAc-DTT mixed systems were 10.4 ± 1.9, 9.8 ± 2.3, 11.2 ± 1.8 nm with 4, 8, and 20 mM DTT, respectively, regardless of DTT concentration. Figure 3(b) shows the size distribution of the 4 mM C_{12}12SAc systems before and after incubation for 24 h with 4 mM DTT at 25°C. The diameters of 4 mM C_{12}12SAc were 8.1 ± 2.3 nm, whereas those of 5 mM C_{12}11SAc were 9.6 ± 1.0 nm, similar to the previously reported values. The diameters of the C_{12}12SAc system were somewhat smaller than those of the C_{12}11SAc system. The mismatched length between dodecyl and dodecylthioacetate in C_{12}12SAc likely resulted in the destabilization of vesicles compared to the C_{12}11SAc systems. The thioacetate group of C_{12}12SAc in the inner layer of the vesicles likely protruded toward the outer layer in the interdigitated structure.

Figure 3(b) shows the size distribution of 4 mM C_{12}12SAc, 4 mM C_{12}12SAc + 4 mM DTT and 4 mM C_{12}12SAc + 4 mM DTT + 4 mM H_{2}O_{2} systems at 25°C, respectively.

3.3 Esterolytic reactions using thiol surfactants

Moss et al. prepared thiocholine surfactants for the preparation of functional surfactant micelles. The high nucleophilicity of thiolate anions for thiocholine surfactants facilitates esterolytic reactions with p-nitrophenyl acetate. If p-nitrophenyl hexanoyl (NPH) is used for esterolytic re-
actions, a hydrophobic chain can be introduced into the thiol surfactants during the acylation step. First, we attempted to perform the esterolytic reactions using methyl hexanoate with C12SH. The HPLC analysis showed that no reaction occurred in the aqueous solutions of C12SH micelles even at 40°C. In contrast, the incubation of 10 mM C12SH aqueous solutions with 0.1 mM NPH at 25°C yielded a thioester surfactant C12SC6, as shown in Fig. 4. Elution of C12SH and NPH was observed at 1.6 and 1.8 min, respectively, and the new peaks of C12SC6 and p-nitrophenol appeared at 1.2 and 2.5 min, respectively. The elimination of p-nitrophenol was observed by the absorption spectra shown in Fig. 5(a). The aqueous solution of 2 mM C12SH was incubated with 1 mM NPAc instead of NPH due to the poor water solubility at 25°C. The absorption intensity at 318 nm increased and a new peak corresponding to p-nitrophenol appeared after incubation for 210 min. The absorption at 230 nm also increased and the new peak corresponding to the thioester of C12SC6 appeared.

3.4 Enhanced aggregation upon introduction of a thiohexanoate group

A thiohexanoate group was introduced by incubation with C12SH and p-nitrophenyl hexanoate in ethanol for 24 h. Figure 6 shows the conductivity curves for the C12SC6 aqueous solution at 25°C and a comparison with C12SH. The ratio of the slopes (S2/S1) for the conductivity vs. concentration plot above and below the CMC was used as a measure of the degree of micellar ionization. The S2/S1 values of C12SH and C12SC6 were 0.34 and 0.49, respectively, and the CMC of C12SC6 significantly decreased due to the introduction of an alkyl chain in the second side chain. The CMCs of C12SH and C12SC6 were 9.8 and 1.5 mM, respectively. The CMC decreased with increasing alkyl chain length and could be described using the relation log(CMC) = −An + B, where n is the number of carbons in the alkyl chain and A and B are constants for a series of single chain surfactants. The effect of side chains on the CMC was investigated for a series of alkyldimethylammonium surfactants containing a second alkyl chain. Shorter second alkyl chains did not significantly contribute to micelle formation, whereas the longer alkyl side chain noticeably contributed to the formation of the hydrophobic micellar core, as evidenced by the considerable decrease in CMC. Figure 7 shows plots of log CMC as a function of the total number (n + m) of carbon atoms in the hydrophobic chain for Cn4SAc (C124SAc, C1011SAc, C1211SAc, and C1212SAc) and Cn44Ac (C1244Ac and C1644Ac) compared to that of alkyltrimethylammonium bromide (CnTAB). A = 0.302 of Cn44Ac coincided with that of CnTAB, whereas A = 0.139 of Cn4SAc was less than half of Cn44Ac owing to the presence of the terminal thioacetate group in its alkyl side chain. However, the CMC of C12SC6 corresponded to the series of Cn4SAc, suggesting that the thiohexanoate group acted similar to that of the main alkyl chain. Figure 8 shows the size distribution of the 10 mM C12SAc and 10 mM C12SC6 systems by DLS measurements at 25°C. It should be noted that the C124SAc was prepared by incubation with C12SH and p-nitrophenyl acetate in a similar manner as C12SC6.

![Image](image_url)
C12SAc system formed the usual micellar aggregates, while the diameters of the C12SC6 system increased to 98.2 ± 14.8 nm, indicating enhanced aggregation by the introduction of the thiohexanoate group.

Fig. 5  (a) Absorption spectra for 2 mM C12SH aqueous solution by the incubation with 1 mM NPAc at 25°C. The solid, dotted, broken and dot-dashed lines were mixing after 0, 5, 10 and 210 min, respectively. The peaks for 230 nm and 318 nm correspond to thioester and p-nitrophenol, respectively.
(b) The time-course of absorbance at 318 nm for C12SH aqueous solution by the incubation with 1 mM NPAc at 25°C. Note that data of 4 mM and 6 mM C12SH were coincided.

Fig. 6  The conductivity curves for C12SH and C12SC6 aqueous solution against the surfactant concentration at 25°C.
• C12SH, ○ C12SC6

Fig. 7  Plots of log cmc at 25°C as a function of the number of carbon atoms in hydrophobic chain.
○ alkyltrimethylammonium bromide, (△) CnmASe, (●) Cn4SAc, (■) C12SC6

C12SAc system formed the usual micellar aggregates, while the diameters of the C12SC6 system increased to 98.2 ± 14.8 nm, indicating enhanced aggregation by the introduction of the thiohexanoate group.
Enhanced Aggregation of Stimuli Responsive Surfactants

J. Oleo Sci. 68, (6) 573-580 (2019)

Fig. 8 The size distribution of 10 mM C12SAc and 10 mM C12SC6 systems at 25°C.

4 Conclusion

Functional surfactants containing thioacetate side chains were responsive to thiol compounds. The thioester surfactants, C12mSAc, yielded the corresponding thiol surfactants via thiol-thioester exchange in aqueous solution, resulting in enhanced aggregation due to the elimination of the hydrophilic thioacetate group. Moreover, the thiol surfactants afforded thioester double-tailed surfactants by esterolytic reactions with p-nitrophenyl hexanoate. The introduction of an alkyl chain to the second side chain also enhanced aggregation. The disulfide-linked gemini surfactants, 2C1212SS, showed further enhanced aggregation owing to their hydrophobicity and disulfide bond linkages.

References
1) Rowan, S.J.; Cantrill, S.J.; Cousins, G.R.L.; Sanders, J.K.M.; Stoddart, J.F. Dynamic covalent chemistry. Angew. Chem. Int. Ed. 41, 898-952 (2002).
2) Johnson, E.C.B.; Kent, Stephen B.H. Insights into the mechanism and catalysis of the native chemical ligation reaction. J. Am. Chem. Soc. 128, 6640-6646 (2006).
3) Enrique A. Castro; Kinetics and mechanisms of reactions of thiol, thiono, and dithio analogues of carboxylic esters with nucleophiles. Chem. Rev. 99, 3505-3524 (1999).
4) Dong, J.; Xun, Z.; Zeng, Y.; Yu, T.; Han, Y.; Chen, J.; Li, Y.Y.; Yang, G.; Li, Y. Theoretical study on the alkaline hydrolysis of methyl thioacetate in aqueous solution. J. Phys. Chem. A 115, 13523-13533 (2011).
5) Bracher, P.J.; Snyder, P.W.; Bohall, B.R.; Whitesides, G.M. The relative rates of thiol-thioester exchange and hydrolysis for alkyl and aryl thioalkanoates in water. Orig. Life Evol. Biosph. 4, 399-412 (2011).
6) Takano, Y.; Asakawa, T.; Inai, M.; Ohta, A.; Asakawa, H. Aggregation behavior of disulfide linked gemini surfactants compared to that of double-tailed surfactants. J. Oleo Sci. 66, 1321-1328 (2017).
7) Asakawa, T.; Takano, Y.; Ohta, A.; Asakawa, H. Aggregation and pH responsive behavior of thioester surfactants and formation of disulfide linkages in aqueous solutions. J. Oleo Sci. 67, 199-206 (2018).
8) Asakawa, T.; Shinizu, Y.; Ozawa, T.; Ohta, A.; Miyagishi, S. Aqueous solution properties of disulfide linked gemini and cleaved monomeric thiol surfactants. J. Oleo Sci. 57, 243-249 (2008).
9) Kunitake, T.; Okahata, Y.; Tamaki, K.; Kumamaru, F.; Takayanagi, M. Formation of the bilayer membrane from a series of quaternary ammonium salts. Chem. Lett. 387-390 (1977).
10) Aratono, M.; Mori, A.; Koga, I.; Shigehisa, M.; Onimaru, N.; Tsuchiya, K.; Takiue, T.; Matsubara, H. Spontaneous vesicle formation of mixtures of double-chain cationic surfactants with a different counterion. J. Phys. Chem. B 112, 12304-12311 (2008).
11) Leclaire, J.; Vial, L.; Otto, S.; Sanders, J. K.M. Expanding diversity in dynamic combinatorial libraries: Simultaneous exchange of disulfide and thioester linkages. Chem. Commun. 1959-1961 (2005).
12) Asakawa, T.; Arai, N.; Fujii, A.; Takahashi, K.; Takakuka, K.; Honda, M.; Ohta, A.; Asakawa, H. Aggregation behavior and thiol-thioester exchange for cationic surfactants with propylthioacetate side chain. J. Oleo Sci. 67, 969-976 (2018).
13) Al-Awadi, N.; Williams, A. Effective charge development in ester hydrolysis catalyzed by cationic micelles. J. Org. Chem. 55, 2001-2004 (1990).
14) Ohno, K.; Matsumoto, S.; Aida, M.; Matsura, H. Protonation-induced conformational changes of 2-((N,N-dimethylamino)ethanethiol. Importance of strong N–H···S and N–H···S− hydrogen bonding. Chem. Lett. 9, 828-829 (2003).
15) Moss, R.A.; Bizzigotti, G.O.; Huang, C.-W.; Nucleophilic esterolytic and displacement reactions of a micellar thiocholine surfactant. J. Am. Chem. Soc. 102, 754-762 (1980).
16) Moss, R.A.; Hendrickson, T.F.; Bizzigotti, G.O.; Huang, C.-W.; Esterolytic chemistry of a vesicular thiocholine surfactant. J. Am. Chem. Soc. 108, 5520-5527 (1986).
17) Zana, R. Ionization of cationic micelles: Effect of the detergent structure. J. Colloid Interface Sci. 78, 330-337 (1980).
18) Ikeda, S. Surfactants in Solution (Mittal, K.L.; Lindman, B. eds.), Plenum Press, 2, 825-840 (1982).
19) Hiramatsu, K.; Kameyama, K.; Ishiguro, R.; Mori, M.; Hayase, H. Properties of dilute aqueous solutions of

579

J. Oleo Sci. 68, (6) 573-580 (2019)
double-chain surfactants, alkyldecyldimethylammonium bromides with a change in the length of the alkyl chains. Bull. Chem. Soc. Jpn. **76**, 1903-1910 (2003).