Catalytic Efficiency of Cationic Micellar Catalysts Bearing a Mercapto Group as the Reaction Center†

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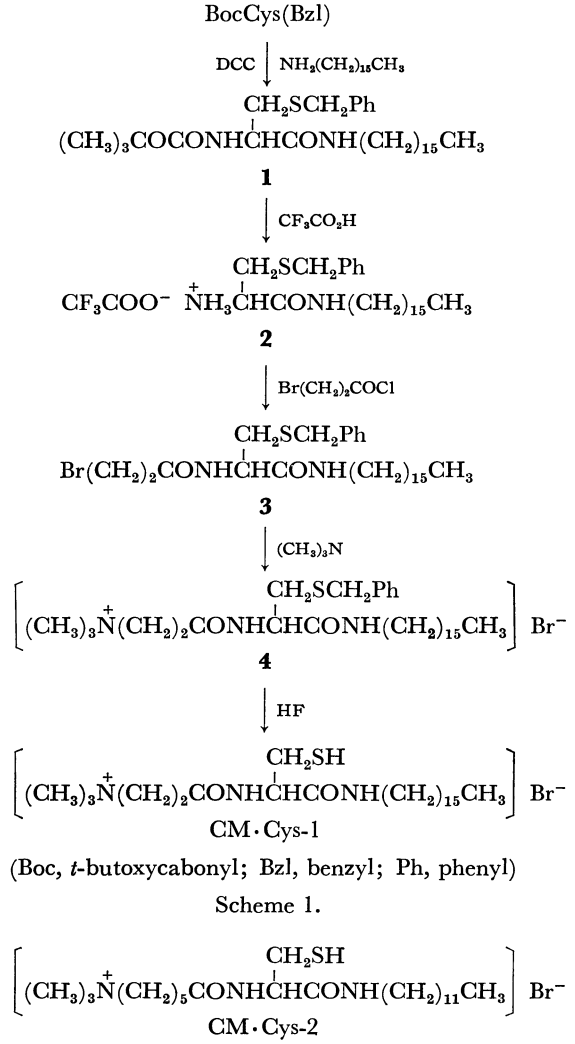
In order to clarify micro-environmental effects on the reactivity of mercapto groups in enzymes, cationic surfactants bearing a mercapto group, *N*-hexadecyl-N^{α}-(3-trimethylammoniopropionyl)-L-cysteinamide bromide (CM·Cys-1) and *N*-dodecyl-N^{α}-(6-trimethylammoniohexanoyl)-L-cysteinamide bromide (CM·Cys-2), were synthesized and their kinetic behavior investigated. The surfactants above their critical micelle concentrations markedly catalyzed the decomposition of *p*-nitrophenyl hexanoate (PNPH) and acetate (PNPA), the concentration-rate profiles being found to be those for typical micelle-catalyzed reactions. The rate constants for the degradation of PNPH as catalyzed by CM·Cys-1 and -2 in the micellar phase are 0.482 and 0.197 s^{-1}, respectively, in 9.8% (v/v)-ethanol–1.0% (v/v)dioxane–1.0% (v/v)methanol–water at 30.0±0.1 °C, pH 8.65, and μ 0.10 (KCl). The difference in catalytic activity can be attributed partly to the micro-environmental effect on the pK_a value of the mercapto group lying at the reaction center. The rate constants for the thiolate anions (true reactive species) of CM·Cys-1 and -2 to react with PNPH were identical with each other irrespective of the nature of the surfactants. The electrostatic effect provided by the cationic charge in the Stern layer, which acts to reduce the pK_a value of the reactive mercapto group, seems to play a more important role than the desolvation effect on the thiolate anion by the hydrophobic field.

Functionalized surfactants involving a reactive group such as the imidazolyl,[1] carboxyl,[2] or hydroxyl group[3] have been developed as enzyme models. However, only a limited number of surfactants bearing a mercapto group have been synthesized; their catalytic activity for degradation of *p*-nitrophenyl carboxylates has been examined.[4] The active mercapto group of these surfactants seems to be located in the electrostatic Stern layer judging from their molecular structures. In spite of the fact that the catalytically active groups of enzymes are located in their hydrophobic pockets, no successful attempt has been made to locate the catalytic groups in the hydrophobic micellar core so as to simulate the function of an enzymatic reaction center. The anionic surfactants involving a L-cysteinyl residue as the reaction center markedly accelerate the degradation of *p*-nitrophenyl carboxylates below their critical micelle concentrations, the reactivity of the mercapto group being lost upon formation of the anionic micelles.[5] The surfactants show pronounced reactivity even in a neutral pH region when mixed with hexadecyltrimethylammonium bromide (CTAB) in the micellar phase. The electrostatic field effect provided by the cationic head of CTAB micelle enhances the nucleophilicity of the mercapto group in the mixed micelles for such reactions.

In order to develop novel surfactants which can incorporate the multi-functions provided by the comicelle constructed by CTAB and *N*-hexadecyl-N^{α}-glutaryl-L-cysteinamide, we have prepared cationic surfactants bearing a mercapto group, *N*-hexadecyl-N^{α}-(3-trimethylammoniopropionyl)-L-cysteinamide bromide (CM·Cys-1) and *N*-dodecyl-N^{α}-(6-trimethylammoniohexanoyl)-L-cysteinamide bromide (CM·Cys-2). The mercapto group is expected to be placed in a hydrophobic core of the micellar phase. The micro-environmental effect on the nucleophilicity of the mercapto group provided by the present cysteine-containing cationic surfactants has been investigated as regards the deacylation of *p*-nitrophenyl carboxylates. The synthetic procedure for CM·Cys-1 is shown in Scheme 1.



(Boc, *t*-butoxycabonyl; Bzl, benzyl; Ph, phenyl)

Scheme 1.

† Contribution No. 512 from this Department.

Experimental

Spectroscopic data were taken on a JASCO DS-403G grating IR spectrophotometer, a Varian A60 NMR spectrometer, and a Hitachi 124 spectrophotometer. pH-Measurements were carried out with a TOA HM-9A pH meter equipped with a TOA GC-125 combined electrode after calibration with a combination of appropriate aqueous standard buffers.

p-Nitrophenyl carboxylates were prepared by the reaction of the corresponding carbonyl chlorides with *p*-nitrophenol. The esters were identified by elemental analyses and spectroscopic measurements.[6] 2,2′-Dinitro-5,5′-dithiodibenzoic acid (DTNB, Nakarai Chemicals, bio-analytical grade) was used without further purification.

The synthetic procedure for *N*-hexadecyl-N^{α}-(3-trimethylammoniopropionyl)-L-cysteinamide bromide (CM·Cys-1) is outlined in Scheme 1. *N*-Hexadecyl-N^{α}-*t*-butoxycarbonyl-*S*-benzyl-L-cysteinamide (**1**) was prepared as described previously.[5]

N-Hexadecyl-N$^{\alpha}$-(3-bromopropionyl)-S-benzyl-L-cysteinamide (3). Trifluoroacetic acid (25.0 g) was added to a dichloromethane solution (17 ml) of **1** (2.4 g) and the mixture was stirred at room temperature for 1 h. Evaporation of excess trifluoroacetic acid *in vacuo* gave pale yellow oil (**2**); yield 2.5 g (quantitative). Elimination of the *t*-butoxycarbonyl group was confirmed by means of NMR spectrum. Amine component **2** (1.74 g) and triethylamine (0.97 g) were dissolved in dichloromethane (20 ml) and cooled down to 0 °C. 3-Bromopropionyl chloride (1.09 g) dissolved in dichloromethane (5 ml) was added to the solution in 10 min at this temperature. The mixture was stirred for 30 min at 0 °C and at room temperature for 2 h. The reaction mixture, after dichloromethane (50 ml) had been added, was washed with water (50 ml×3), 5% aqueous sodium hydrogencarbonate (50 ml ×3), 5% aqueous citric acid (50 ml×3), and saturated aqueous sodium chloride (50 ml×3). After being dried over anhydrous sodium sulfate, the mixture was evaporated *in vacuo* at 40 °C to give a glassy solid of pale yellow; yield 1.65 g (99%), mp 86—88 °C. NMR ($CDCl_3$, TMS): δ 0.88 (3H, broad t, $C\underline{H}_3(CH_2)_{14}CH_2$–), 1.25 (28H, s, $CH_3(C\underline{H}_2)_{14}CH_2$–), 2.65—2.95 (4H, m, $BrCH_2C\underline{H}_2CO$– and $-C\underline{H}_2SCH_2$-Ph), 3.22 (2H, broad t, $CH_3(CH_2)_{14}C\underline{H}_2NH$–), 3.65 (2H, t, $BrC\underline{H}_2CH_2CO$–), 3.80 (2H, s, $-CH_2SC\underline{H}_2Ph$), 4.55 (1H, broad t, $-C\underline{H}(CH_2SCH_2Ph)CO$–), and 7.37 (5H, s, phenyl H's).

N-Hexadecyl-N$^{\alpha}$-(3-trimethylammoniopropionyl)-S-benzyl-L-cysteinamide Bromide (4). Dry trimethylamine gas was introduced into a benzene solution (60 ml) of **3** (1.6 g) for 6 h, and the solution was stirred at room temperature for 14 h. After benzene had been evaporated off *in vacuo*, methanol was added to the residue and removed *in vacuo*. The treatment with methanol was repeated three times. A white solid was recovered and recrystallized from ethyl acetate–petroleum ether; yield 1.60 g (86%), mp 88—95 °C, Dragendorff positive. NMR (methanol-d_4, TMS): δ 0.88 (3H, broad t, $C\underline{H}_3(CH_2)_{14}CH_2$–), 1.25 (28H, s, $CH_3(C\underline{H}_2)_{14}CH_2$–), 2.60—3.05 (4H, m, $(CH_3)_3\overset{+}{N}CH_2C\underline{H}_2CO$– and $-C\underline{H}_2SCH_2Ph$), 3.18 (9H, s, $(C\underline{H}_3)_3\overset{+}{N}$–), 3.04—3.41 (4H, m, $(CH_3)_3\overset{+}{N}C\underline{H}_2CH_2CO$– and $CH_3(CH_2)_{14}C\underline{H}_2NH$–), 3.80 (2H, s, $-CH_2SC\underline{H}_2Ph$), 4.55 (1H, broad t, $-C\underline{H}(CH_2SCH_2Ph)CO$–), and 7.35 (5H, s, phenyl H's).

N-Hexadecyl-N$^{\alpha}$-(3-trimethylammoniopropionyl)-L-cysteinamide Bromide (CM·Cys-1). Anisole (1.0 ml) and **4** (700 mg) were placed in a reaction vessel into which hydrogen fluoride (10 ml) was introduced. The mixture was stirred at 0 °C for 1 h, at 15 °C for 30 min, and then evaporated *in vacuo* to remove hydrogen fluoride completely. Methanol (30 ml) was added to the residue and white precipitates were recovered by filtration. After methanol had been evaporated off, 2-mercaptoethanol (5 ml) was added to the residue and the mixture was stirred at room temperature for 14 h. Methanol (10 ml) was added to the mixture and the product was purified by gel-filtration chromatography (Sephadex LH-20, methanol as eluant) to give a hygroscopic solid; yield 180 mg (30%), nitroprusside positive. IR (neat): 3240 (NH str.); 2900 and 2840 (CH str.); 1655 and 1545 cm^{-1} (C=O str.). NMR (methanol-d_4, TMS): δ 0.90 (3H, broad t, $C\underline{H}_3(CH_2)_{14}CH_2$–), 1.30 (28H, s, $CH_3(C\underline{H}_2)_{14}CH_2$–), 2.65—3.20 (6H, m, $CH_3(CH_2)_{14}C\underline{H}_2NH$–, $-C\underline{H}_2SH$, and $(CH_3)_3\overset{+}{N}CH_2C\underline{H}_2CO$–), 3.18 (11H, s, $(C\underline{H}_3)_3\overset{+}{N}CH_2CH_2CO$–), and 4.35 (1H, broad, $-C\underline{H}(CH_2SH)CO$–). Found: C, 56.25; H, 9.46; N, 6.99%. Calcd for $C_{25}H_{52}BrN_3O_2S$: C, 55.79; H, 9.72; N, 7.80%.

N-Dodecyl-N^{α}-(6-trimethylammoniohexanoyl)-L-cysteinamide bromide (CM·Cys-2) was prepared in a manner similar to that described for CM·Cys-1.

N-Dodecyl-N$^{\alpha}$-t-butoxycarbonyl-S-benzyl-L-cysteinamide. Yield 51%, needles (recrystallized from ethyl acetate), mp 57—59 °C. NMR ($CDCl_3$, TMS): δ 0.88 (3H, broad t, $C\underline{H}_3(CH_2)_{11}$–), 1.25 (20H, s, $CH_3(C\underline{H}_2)_{10}CH_2$–), 1.45 (9H, s, $(C\underline{H}_3)_3CO$–), 2.76 (2H, broad d, $-C\underline{H}_2SCH_2Ph$), 3.25 (2H, m, $CH_3(CH_2)_{10}C\underline{H}_2$–), 3.75 (2H, s, $-CH_2SC\underline{H}_2Ph$), 4.21 (1H, broad t, $-C\underline{H}(CH_2SCH_2Ph)CO$–), and 7.37 (5H, s, phenyl H's).

N-Dodecyl-N$^{\alpha}$-(6-bromohexanoyl)-S-benzyl-L-cysteinamide. Yield 81%, pale yellow oil. IR (neat): 3250 (NH str.); 2900 and 2830 (CH str.); 1635 and 1535 cm^{-1} (C=O str.). NMR ($CDCl_3$, TMS): δ 0.88 (3H, broad t, $C\underline{H}_3(CH_2)_{11}$–), 1.26 (20H, s, $CH_3(C\underline{H}_2)_{10}CH_2$–), 1.90 (6H, m, $BrCH_2(C\underline{H}_2)_3CH_2CO$–), 2.50 (2H, broad t, $BrC\underline{H}_2(CH_2)_3CH_2CO$–), 2.78 (2H, d, $-C\underline{H}_2SCH_2Ph$), 3.19 (2H, broad t, $CH_3(CH_2)_{10}C\underline{H}_2$–), 3.42 (2H, t, $BrC\underline{H}_2$–), 3.76 (2H, s, $-CH_2SC\underline{H}_2Ph$), 4.51 (1H, broad t, $-C\underline{H}(CH_2SCH_2Ph)CO$–), and 7.33 (5H, s, phenyl H's).

N-Dodecyl-N$^{\alpha}$-(6-trimethylammoniohexanoyl)-S-benzyl-L-cysteinamide Bromide. Yield 97%, hygroscopic glassy solid of pale yellow (recrystallized from ethyl acetate–petroleum ether), mp 68—75 °C, Dragendorff positive. IR (Nujol): 3260 (NH str.); 1630 and 1540 cm^{-1} (C=O str.). NMR (methanol-d_4, TMS): δ 0.88 (3H, broad t, $C\underline{H}_3(CH_2)_{11}$–), 1.25 (20H, s, $CH_3(C\underline{H}_2)_{10}CH_2$–), 2.00 (6H, m, $(CH_3)_3\overset{+}{N}CH_2(C\underline{H}_2)_3CH_2CO$–), 2.31 (2H, broad t, $(CH_3)_3\overset{+}{N}(CH_2)_4C\underline{H}_2CO$–), 2.75—3.20 (4H, m, $-C\underline{H}_2SCH_2Ph$ and $CH_3(CH_2)_{10}C\underline{H}_2NH$–), 3.40 (11H, s, $(C\underline{H}_3)_3\overset{+}{N}CH_2$–), 3.75 (2H, s, $-CH_2SC\underline{H}_2Ph$), 4.60 (1H, broad, $-C\underline{H}(CH_2SCH_2Ph)CO$–), and 7.35 (5H, s, phenyl H's).

N-Dodecyl-N$^{\alpha}$-(6-trimethylammoniohexanoyl)-L-cysteinamide Bromide (CM·Cys-2). Yield 32%, glassy solid, mp 133—145 °C, nitroprusside positive. IR (Nujol): 3260 (NH str.); 1630 and 1530 cm^{-1} (C=O str.). NMR (methanol-d_4, TMS): δ 0.90 (3H, broad t, $C\underline{H}_3(CH_2)_{11}$–), 1.30 (20H, s, $CH_3(C\underline{H}_2)_{10}CH_2$–), 1.10—2.00 (6H, m, $(CH_3)_3\overset{+}{N}CH_2(C\underline{H}_2)_3CH_2$–), 2.40 (2H, broad, $(CH_3)_3\overset{+}{N}(CH_2)_4C\underline{H}_2CO$–), 2.65—3.10 (4H, m, $-C\underline{H}_2SH$ and $CH_3(CH_2)_{10}C\underline{H}_2NH$–), 3.18 (11H, s, $(C\underline{H}_3)_3\overset{+}{N}CH_2$–), and 4.35 (1H, broad, $-C\underline{H}(CH_2SH)CO$–). Found: C, 55.23; H, 9.12; N, 7.08%. Calcd for $C_{24}H_{50}BrN_3O_2S$: C, 54.95; H, 9.61; N, 7.08%.

Kinetic Measurements. The concentration of free thiol for CM·Cys-1 and -2 was determined by using Ellman's reagent (DTNB)[7] before kinetic runs. Under the conditions employed, the reaction between surfactant and ester species

was fast enough to neglect oxidation of the mercapto group of the surfactants. Rates of *p*-nitrophenol liberation from *p*-nitrophenyl esters were measured at 400 nm with a Hitachi 124 spectrophotometer. Each run was initiated by adding a dry dioxane solution (30 μl) of a substrate ester to a mixture of a reaction medium (3.0 ml) and a dry methanol solution (30 μl) of a catalyst which was pre-equilibrated at 30.0±0.1 °C in a thermostatted cell set in the spectrophotometer. The reaction medium was prepared as follows: 10.0 ml of 1.0 M aqueous potassium chloride, 10.0 ml of an appropriate aqueous buffer, and 10.0 ml of dry ethanol were placed in a 100-ml volumetric flask; the flask was filled with deionized and distilled water. Aqueous buffer solutions adopted in the present study were prepared by combining 1/10 M potassium dihydrogenphosphate and 1/20 M sodium borate.

The mole fractions of CM·Cys-1 and -2, which bear the free mercapto group and were used for kinetic runs, were 0.63 and 0.67, respectively. Thus, all the rate data were corrected to a 100% content of the free mercapto group. In order to find the effect of disulfide content on kinetic behavior, the thiol surfactants involving the free mercapto group in the mole fraction range 0.33—0.67 were also examined for their catalytic activity. All the kinetic data thus obtained after correction for the disulfide content were identical regardless of disulfide content. The substrate-binding ability of the surfactants was not affected by the disulfide content.

Results and Discussion

Degradation of *p*-nitrophenyl carboxylates as catalyzed by CM·Cys-1 and -2 has been studied in 9.8%(v/v)ethanol–1.0%(v/v)dioxane–1.0%(v/v)methanol–water at 30.0±0.1 °C and μ 0.10 (KCl). Apparent first-order rate constants (k_{obsd}) were obtained by measuring the amount of liberated *p*-nitrophenol. The first-order kinetics was found to hold up to 90% conversion of the substrate for each kinetic run, no noticeable hydrolysis of the acylated thiol ester being observed.

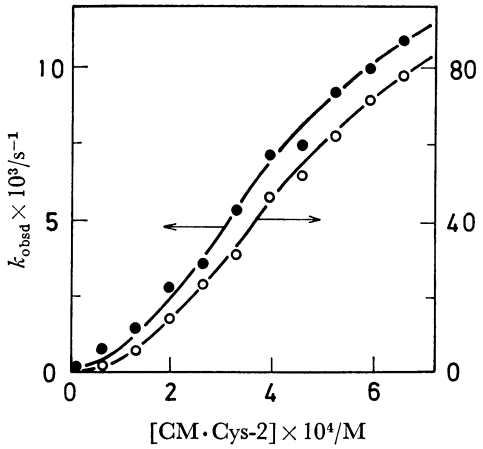


Fig. 1. Plots of apparent first-order rate constant *vs.* surfactant concentration (C_D) for the deacylation of *p*-nitrophenyl hexanoate (PNPH, ○) and acetate (PNPA, ●) as catalyzed by CM·Cys-2 in 9.8%(v/v)-ethanol–1.0% (v/v)dioxane–1.0% (v/v)methanol–water at 30.0±0.1 °C, pH 8.65, and μ 0.10 (KCl). Initial concentrations: PNPH, 0.992×10^{-5} M; PNPA, 0.990×10^{-5} M.

Rate(k_{obsd})-concentration profiles (Fig. 1) for the degradation of *p*-nitrophenyl hexanoate (PNPH) and acetate (PNPA) as catalyzed by CM·Cys-2 at pH 8.65 are typical for the micellar catalysis. The reaction pathway for the degradation of *p*-nitrophenyl carboxylates as catalyzed by the thiol surfactants is given by Scheme 2, where S denotes an ester substrate (PNPH or PNPA), M a micelle constructed by a thiol surfactant

$$\mathrm{M} + \mathrm{S} \overset{K_b}{\rightleftharpoons} \mathrm{MS} \xrightarrow{k_m} \mathrm{P}$$
$$\downarrow k_s$$
$$\mathrm{P'}$$

Scheme 2.

(CM·Cys-1 or -2), MS a complex formed with the micelle and the substrate, and P and P′ denote reaction products. The k_s and k_m values are defined as follows.

$$k_s = k_{hyd} + k_{SH}(\text{monomer}) \quad (1)$$

$$k_m = k_M^{OH} + k_{SH}(\text{micelle}) \quad (2)$$

where k_{hyd} denotes the rate constant for the alkaline hydrolysis, k_{SH}(monomer) that for the acyl transfer from a substrate to the mercapto group of a monomeric surfactant in the bulk phase, k_M^{OH} that for the alkaline hydrolysis by concentrated hydroxide ions in the Stern layer of the micelle, and k_{SH} (micelle) that for the acyl transfer from a bound substrate to the mercapto group of the micellar surfactant. The observed first-order rate constant (k_{obsd}) is given by the following equation on the basis of Scheme 2.[8]

$$k_{obsd} = \frac{k_s + K_b k_m[\mathrm{M}]}{1 + K_b[\mathrm{M}]} \quad (3)$$

This can be rearranged to give

$$\frac{1}{(k_{obsd} - k_s)} = \frac{1}{(k_m - k_s)} + \frac{N}{(k_m - k_s)K_b(C_D - \mathrm{CMC})} \quad (4)$$

where N denotes the aggregation number and C_D total concentration of the thiol surfactant. The critical micelle concentration (CMC) was kinetically estimated

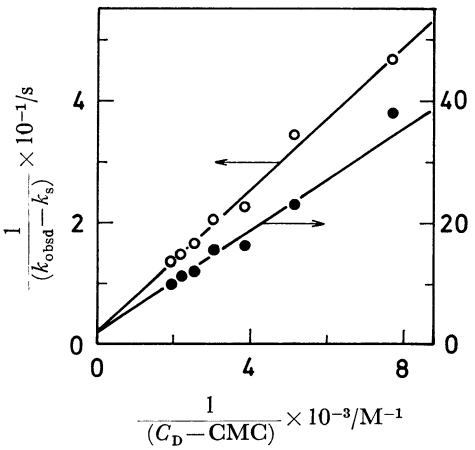


Fig. 2. Plots of $1/(k_{obsd}-k_s)$ *vs.* $1/(C_D-\mathrm{CMC})$ for the deacylation of PNPH (○) and PNPA (●) as catalyzed by CM·Cys-2 in 9.8%(v/v)ethanol–1.0%(v/v)dioxane–1.0%(v/v)methanol–water at 30.0±0.1 °C, pH 8.65, and μ 0.10 (KCl). Initial concentrations: PNPH, 0.992×10^{-5} M; PNPA, 0.990×10^{-5} M.

Table 1. Kinetic parameters for the deacylation of *p*-nitrophenyl hexanoate and acetate as catalyzed by the thiol surfactants[a)]

Surfactant	$k_s \times 10^3$ / s^{-1}	k_m / s^{-1}	K_b/N / M^{-1}	k_m/k_{hyd}	k_{obsd}^{max} / s^{-1}	k_{cat}[b)] / $s^{-1}M^{-1}$
			PNPH			
CM·Cys-1	0.91	0.482	2560	3680	0.224	424
CM·Cys-2	1.03	0.197	884	1500	0.066	99
			PNPA			
CM·Cys-1	1.15	0.0586	962	376	0.0211	31.9
CM·Cys-2	0.99	0.0508	485	326	0.0109	16.7

a) In 9.8 %(v/v)ethanol–1.0%(v/v)dioxane–1.0%(v/v)methanol–water at 30.0±0.1 °C, pH 8.65, and μ 0.10 (KCl). Initial concentrations: PNPH, 0.992×10^{-5} M; PNPA, 0.990×10^{-5} M. Apparent first-order rate constants for the alkaline hydrolysis: PNPH, 1.31×10^{-4} s^{-1}; PNPA, 1.56×10^{-4} s^{-1}. Approximate CMC's: CM·Cys-1, 4×10^{-5} M; CM·Cys-2, 9×10^{-5}M. 1 M=1 mol dm^{-3}. b) $k_{cat}=k_{obsd}^{max}/[\text{surfactant}]_{max}$; k_{obsd}^{max}, maximum observed rate constant under present conditions with a surfactant concentration, $[\text{surfactant}]_{max}$.

from the corresponding rate-concentration profile. The apparent first-order rate constants observed at the critical micelle concentrations were referred to the k_s values. Linearity holds between the left-hand side term of Eq. 4 and the reciprocal concentration of micellized surfactant (C_D—CMC) as shown in Fig. 2 for the CM·Cys-2 catalysis as an example. The kinetic parameters are summarized in Table 1. The k_s values for the reaction between thiol surfactants and ester substrates indicate that the reactivity of CM·Cys-1 is identical with that of CM·Cys-2 in the bulk phase. The pseudo-intramolecular rate constant (k_m) and the binding ability (K_b/N) reflect the catalytic activity of CM·Cys-1 and -2 in the micellar phase. In order to clarify the difference in catalytic activity, k_m and K_b/N values for the degradation of PNPH as catalyzed by CM·Cys-1 and -2 were evaluated at several pH's from the corresponding data of saturation kinetics (Fig. 3, CM·Cys-2 catalysis). The kinetic parameters obtained are given in Table 2. The pH-rate profiles (Fig. 4) suggest that the reactive species is the thiolate anion in the micellar phase as shown by

Table 2. Kinetic parameters for the deacylation of *p*-nitrophenyl hexanoate as catalyzed by CM·Cys-1 and -2[a)]

pH	$k_{hyd} \times 10^4$ / s^{-1}	$k_s \times 10^3$ / s^{-1}	k_m / s^{-1}	K_b/N / M^{-1}
		CM·Cys-1		
8.98	2.15	1.66	0.546	1760
8.69	1.40	0.91	0.483	2570
8.45	0.92	0.55	0.248	5260
8.06	0.47	0.24	0.121	4150
		CM·Cys-2		
8.95	2.29	1.66	0.407	950
8.67	1.31	1.04	0.198	880
8.47	0.94	0.87	0.099	1620
7.94	0.41	0.28	0.034	1710

a) In 9.8%(v/v)ethanol–1.0% (v/v) dioxane–1.0%(v/v)-methanol–water at 30.0±0.1 °C and μ 0.10 (KCl). Initial concentrations: PNPH, 0.992×10^{-5} M; CM·-Cys-1, $(0.660—6.60) \times 10^{-4}$ M; CM·Cys-2, $(0.664—6.64) \times 10^{-4}$ M. Approximate CMC's: CM·Cys-1, 4×10^{-5} M; CM·Cys-2, 9×10^{-5} M.

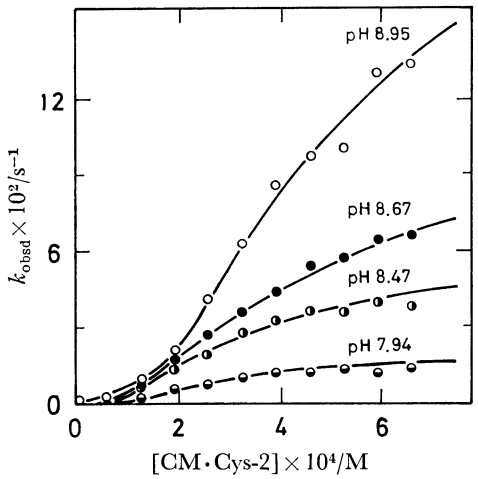


Fig. 3. Plots of apparent first-order rate constant *vs.* surfactant concentration (C_D) for the deacylation of PNPH as catalyzed by CM·Cys-2 in 9.8%(v/v)ethanol–1.0%(v/v) dioxane–1.0%(v/v) methanol–water at 30.0±0.1 °C and μ 0.10 (KCl); initial concentration of PNPH, 0.992×10^{-5} M.

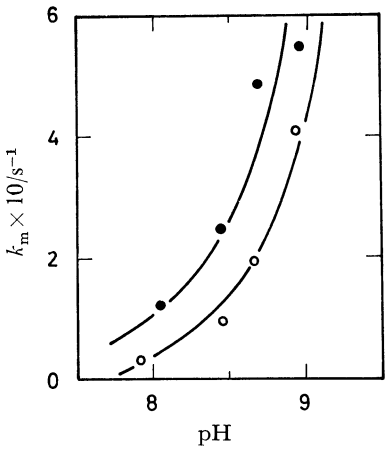


Fig. 4. pH-rate profiles for the deacylation of PNPH as catalyzed by CM·Cys-1 (●) and -2 (○) in 9.8%-(v/v)ethanol–1.0%(v/v)dioxane–1.0%(v/v)methanol–water at 30.0±0.1 °C and μ 0.10 (KCl); initial concentration of PNPH, 0.992×10^{-5} M.

$$S\cdot M(SH) \overset{K_a}{\rightleftharpoons} S\cdot M(S^-) \overset{k_m^*}{\longrightarrow} P$$

Scheme 3.

where M(SH) and M(S^-) denote micellar surfactants bearing neutral mercapto and anionic sulfido groups, respectively. The following relations are established.

$$k_m\{[S\cdot M(SH)] + [S\cdot M(S^-)]\} = k_m^*[S\cdot M(S^-)] \quad (5)$$

$$K_a = \frac{[S\cdot M(S^-)][H^+]}{[S\cdot M(SH)]} \quad (6)$$

By combination and rearrangement of Eqs. 5 and 6, we obtain the relation between the k_m and k_m^* values:

$$\frac{1}{k_m} = \frac{1}{k_m^*} + \frac{[H^+]}{k_m^* K_a} \quad (7)$$

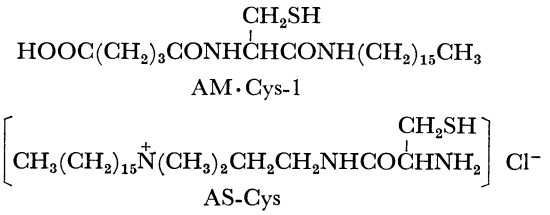
Plots of $1/k_m$ against $[H^+]$ for the deacylation of PNPH as catalyzed by CM·Cys-1 and -2 provide k_m^* and pK_a values (Table 3).

Table 3. True rate constants (k_m^*) and kinetic pK_a values for the deacylation of *p*-nitrophenyl hexanoate as catalyzed by CM·Cys-1 and -2[a]

Surfactant	k_m^*/s^{-1}	pK_a
CM·Cys-1	1.36	9.09
CM·Cys-2	1.28	9.52

a) Calculated from data given in Table 2.

Catalytic Efficiency of the Micellar Thiol Surfactants. The more effective micellar catalyst was provided by CM·Cys-1, the k_m value for the deacylation of PNPH being 3680 times as large as the corresponding k_{hyd} value (Table 1). The remarkable efficiency of CM·Cys-1 is comparable to that of the mixed micelle formed with CTAB and *N*-hexadecyl-N^{α}-glutaryl-L-cysteinamide (AM·Cys-1); k_m/k_{hyd}, 3080.[5] For the degradation of



AM·Cys-1

AS-Cys

PNPA, CM·Cys-1 shows large catalytic efficiency as compared with [2-(cysteinylamino)ethyl]hexadecyldimethylammonium chloride (AS-Cys) reported by Moss and his coworkers (k_{cat}, 26.0).[4b] The catalytic efficiency (k_m) of CM·Cys-1 for the degradation of PNPH is larger than that of CM·Cys-2 by 2.44-fold at pH 8.65. Nevertheless, both micelles show similar reactivity toward the substrate in terms of k_m^* (Table 3). The result suggests that the reactivity of the thiolate anion of these surfactants does not depend on the depth of its location in a hydrophobic micellar core. On the contrary, the pK_a value for the mercapto group of CM·Cys-2 increases by 0.5 pK_a unit relative to that for CM·Cys-1. Moss and his coworkers reported pK_a for the dissociation of the thiol proton of AS-Cys (at 2×10^{-4} M) as 8.9.[4c] The mercapto group of AS-Cys is presumably located in the cationic Stern layer. The results suggest that the pK_a value is affected by the depth of the active group in the micellar phase and its micro-environment is subject to the electrostatic field effect as well as to the hydrophobic effect. The difference of the catalytic efficiency among CM·Cys-1 and -2 at pH 8.65 for the deacylation of PNPH can be explained by the pK_a effect. On the other hand, the catalytic efficiency of CM·Cys-1 is nearly comparable to that of CM·Cys-2 for the deacylation of PNPA. The catalytic efficiency of these surfactants for the deacylation of PNPA seems to be affected by its orientation behavior in the micellar phase which is different from that of PNPH. PNPH would be so arranged in the micellar phase that an attack of the mercapto group on it is facilitated in the hydrophobic core, PNPA being incorporated into the electrostatic Stern layer as shown in Fig. 5. This is in line with the results reported by Brown and Schofield.[9] They indicated that hexane is solubilized in the micellar interior, enforcing tighter packing and reduced mobility, while benzene and 1-pentanol disrupt the micellar structure by binding close to the Stern layer.

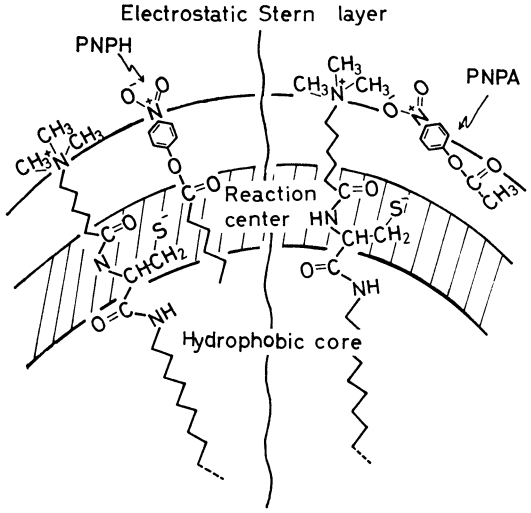


Fig. 5. Schematic representation for the orientation of ester substrates (PNPH and PNPA) in the CM·Cys-2 micelle.

Substrate-binding Ability of the Micellar Thiol Surfactants. The K_b/N values (Table 1) indicate that both micelles have stronger binding ability for PNPH than for PNPA, and that CM·Cys-1 has stronger binding ability for both substrates than CM·Cys-2. The result suggests that the bulky side chain (mercaptomethyl group) located in the hydrophobic micellar core disturbs the micelle structure; *i.e.*, the hydrophobicity of micelles. The binding ability due to the hydrophobic interaction between the substrate and the micelle seems to be affected by the depth of the bulky branched group along the hydrophobic alkyl chain of the surfactant molecule. The K_b/N value decreases with increasing pH of the medium (Table 2). Thus, the binding ability decreases with increasing the fraction of the anionic sulfido group, indicating that the ionic charge on the hydrophobic chain perturbs the structure of micellar

core so as to reduce the hydrophobic micro-environmental effect. The critical micelle concentration of these surfactants is practically not affected by pH under the present conditions.

Conclusion. Both CM·Cys-1 and -2 show profound catalytic efficiency for the degradation of carboxylic esters to an extent comparable to that of the CTAB–AM·Cys-1 comicelle.[5] The large efficiency of CM·Cys-1 and -2 in the micellar phase is due to the electrostatic field effect provided by the cationic charge placed in the Stern layer which acts to stabilize the anionic transition state and to reduce pK_a of the mercapto group. An anionic nucleophile in general would be subjected to the desolvation effect in the hydrophobic micellar core. However, the reactivity of the anionic sulfido group of CM·Cys-1 is comparable to that of CM·Cys-2 in the micellar phase. This indicates that the desolvation effect does not play a primary role for the development of the thiol reactivity. Being a soft nucleophile, the thiolate anion would not sustain the solvation effect by water molecule as effectively as a hard nucleophile, such as the carboxylate anion.

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