published observations.

(34) Xylene (a mixture of isomers, bp 138–143 °C) was distilled from calcium hydride and stored in sealed bottles. Tetrahydrofuran (THF) was distilled from sodium and benzophenone immediately prior to use. Pyrrolidine and piperidine were freshly distilled from sodium hydride. Anhydrous ether was purchased from Mallinckrodt and used as received. Propargylic alcohols were commercial samples or were prepared[32] in yields of 75–96% from the reaction of aldehydes and 1-lithio-1-alkynes (from *n*-BuLi) in THF at −78 to 25 °C. All reactions were conducted under a nitrogen atmosphere.[35] Concentrations were done using a rotary evaporator under reduced pressure. Commercial plates coated with E. Merck silica gel were used for thin-layer chromatography (TLC). W. R. Grace silica gel (grade 62) was used for column chromatography. ^{1}H and ^{13}C NMR spectra were determined with a Bruker WH-90 spectrophotometer at 90 and 22.6 MHz, respectively. Chemical shifts are reported as δ values in parts per million relative to internal tetramethylsilane = 0. ^{1}H NMR coupling constants (J) are reported in hertz and refer to apparent multiplicities, and not true coupling constants. Abbreviations used are: s, singlet; d, doublet; t, triplet; m, complex multiplet. Infrared spectra were determined with a Beckman Acculab 2 spectrometer. Mass spectra were determined at 75 eV with a Du Pont 21-492B double-focusing spectrometer at the Caltech analytical facility. High-performance liquid chromatography (HPLC) was performed with Waters components consisting of a 6000-A pump, U6K injector, and R401 differential refractometer. Microanalyses were performed by Galbraith Laboratories, and agreed with calculated values within ±0.4%. Melting points were determined in capillary tubes with a Thomas-Hoover apparatus that was calibrated with known standards.

(35) Johnson, W. S.; Schneider, W. P., "Organic Syntheses"; Wiley: New York, 1963; Collect. Vol. IV, p 132.

(36) We have successfully purified a few pseudoureas by chromatography using a Waters Prep LC-500 (silica gel, hexane–triethylamine–ethyl acetate).

(37) German Patent 852 999, 1952; *Chem. Abstr.* **1958**, *52*, P11127a.

(38) This difference in the apparent coupling constant at 60 MHz results from the long-range coupling of the C_4H.

(39) This is a known compound; however, no experimental data have been reported: Hall, N. F.; Springle, M. R. *J. Am. Chem. Soc.* **1932**, *54*, 3469.

(40) Chichibabin, A. E. *Chem. Ber.* **1924**, *57*, 1802.

Nucleophilic Esterolytic and Displacement Reactions of a Micellar Thiocholine Surfactant[1]

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Abstract: The thiol-functionalized surfactant *N*-*n*-cetyl-*N*,*N*-dimethyl-*N*-(β-thioethyl)ammonium chloride, **4** (16-SH), was synthesized. Under micellar conditions at pH 7, excess micellar 16-SH cleaved *p*-nitrophenyl acetate (PNPA) with k_ψ^{max} = 2.16 s^{-1} (corresponding to k_{cat} = 144 L/mol·s) and the formation of 16-SAc. Corresponding data for *p*-nitrophenyl hexanoate (PNPH) were 6.68 s^{-1} and 1037 L/mol·s, respectively. S_N2 reactions between 16-SH and iodoacetamide were also examined; at pH 7, excess micellar 16-SH gave k_ψ^{max} = 1.68 s^{-1} and k_{cat} = 44.8 L/mol·s. Relative to suitable model reactions with thiocholine, micellar kinetic advantages for 16-SH reactions at pH 7 were 251 (PNPA), 2820 (PNPH), and 32 (iodoacetamide); at pH 8, the PNPA cleavage exhibited a micellar advantage of 485. The origins of these catalytic factors are discussed, and the esterolytic reactivity of 16-SH is compared with that of other functional surfactants. Deacetylation of 16-SAc is also examined.

Strong interest in the use of functional surfactant micelles as esterolytic reagents,[2] coupled with the high nucleophilicity of thiolate anions and the appreciable acidity of thiols, has focused attention on the synthesis and properties of thiol surfactants. The micellar reactivity of these reagents is both intrinsically interesting and related to the properties of the cysteine proteinases papain, ficin, and stem bromelain.[3]

Surfactant derivatives of cysteine have been prepared by Heitmann (**1**),[4] Moss et al. (**2**),[5] and Murakami (**3**).[6] A simpler and somewhat more reactive thiol surfactant is the long-chain thiocholine derivative, **4**, prepared in our laboratory[7] and, independently, by Tonellato (Br^- form).[8] In this report, we provide full details of the preparation of **4**, its esterolytic reactivity toward *p*-nitrophenyl acetate (PNPA) and *p*-nitrophenyl hexanoate (PNPH), and its S_N2 reactions with iodoacetamide. Additionally, we discuss the comparative reactivities of surfactants **1**–**4** and of such "comicellar" reagents as alkane thiols,[9] coenzyme A,[10] and glutathione,[10] each solubilized in micellar alkyltrimethylammonium bromide solutions.

n-$C_{11}H_{23}CONHCHCOO^-(H^+)$
|
CH_2SH

1

n-$C_{16}H_{33}\overset{+}{N}(CH_3)_2CH_2CH_2NHCOCHNH_2\ Cl^-$
|
CH_2SH

2

n-$C_{16}H_{33}NHCOCHNHCOCH_2CH_2\overset{+}{N}(CH_3)_3\ Br^-$
|
CH_2SH

3

n-$C_{16}H_{33}\overset{+}{N}(CH_3)_2CH_2CH_2SH\ Cl^-$

4 (16-SH)

Results

Synthesis. Choline surfactant **5** (16-OH) was converted to its triflate derivative with triflic anhydride in CH_2Cl_2/pyridine. Self-phase-transfer catalytic conversion[11] of the triflate to **6** (16-SAc, X = OTf) was achieved by stirring the CH_2Cl_2 solution with excess aqueous sodium thioacetate. Ion exchange of **6** to its water-soluble chloride form, followed by deprotection with deoxygenated 3 N aqueous HCl, lyophilization, and recrystallization from CH_2Cl_2/ether, gave **4** (16-SH). Spectroscopic properties of **4** and its precursors appear in the Experimental Section.

n-$C_{16}H_{33}\overset{+}{N}(CH_3)_2CH_2CH_2OH\ Br^-$

5 (16-OH)

n-$C_{16}H_{33}\overset{+}{N}(CH_3)_2CH_2CH_2SCOCH_3\ X^-$

6 (16-SAc)

0002-7863/80/1502-0754$01.00/0

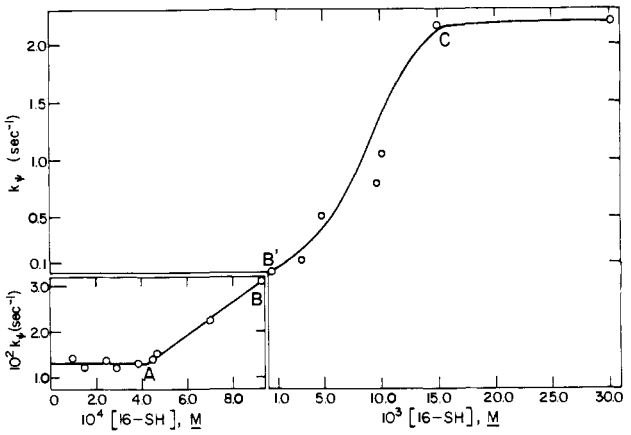


Figure 1. Pseudo-first-order rate constants for the pH 7 cleavage of PNPA by 16-SH vs. [16-SH]. Point A is the kinetic cmc of 16-SH, and point C is taken as k_ψ^{max} for reaction of PNPA with micellar 16-SH. Points B and B′ represent identical data plotted on two different scales.

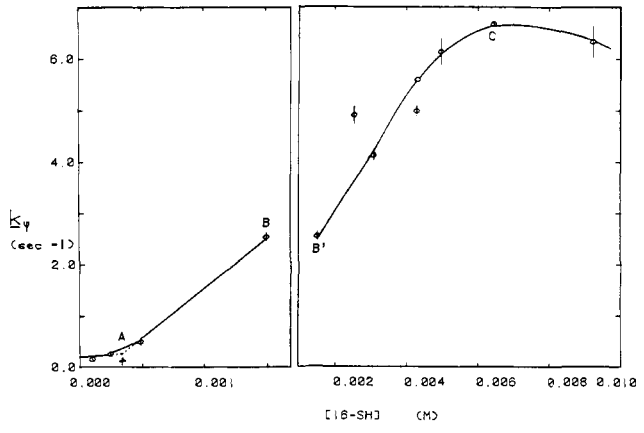


Figure 2. Pseudo-first-order rate constants for the pH 7 cleavage of PNPH by 16-SH vs. [16-SH]. Point A is the kinetic cmc of 16-SH, and point C is taken as k_ψ^{max} for reaction of PNPH with micellar 16-SH. Points B and B′ represent identical data plotted on two different scales.

Table I. Cleavage of PNPA by 16-SH[a]

10^2[16-SH], M	min free SH, %[b]	k_ψ, s^{-1} [c]
3.02	76	2.20
1.50	95	2.16 ± 0.02[d]
1.01	76	1.05
0.969	76	0.798
0.484	76	0.510
0.277	76	0.127
0.0924	72	0.03 ± 0.01
0.0701	72	0.0225 ± 0.0006
0.0467	72	0.0155
0.0459	55-79[e]	0.0139
0.0381	55-79	0.0130 ± 0.0003
0.0287	55-79	0.0122 ± 0.0002
0.0234	55-79	0.0138 ± 0.0014
0.0144	55-79	0.0123 ± 0.0002
0.009 84	55-79	0.0144 ± 0.0015

[a] In 0.02 M phosphate buffer, pH 7.0, μ = 0.05 (KCl), [PNPA] = 2.0 × 10^{-5} M, 25 °C. [b] Analysis by Ellman's assay.[12] [c] Errors are average deviations from the mean of two runs. [d] Three runs. [e] These runs employed successive dilutions of a stock solution which was initially found to contain 79% free SH, but which analyzed for 55% free SH after all dilutions and kinetic runs were completed.

Table II. Cleavage of PNPH by 16-SH[a]

10^3[16-SH], M	k_ψ, s^{-1} [b]	10^3[16-SH], M	k_ψ, s^{-1} [b]
9.23[c]	6.3 ± 0.3$_2$	2.54	4.9 ± 0.2$_3$
6.44	6.68 ± 0.03$_3$	1.50	2.55 ± 0.08$_5$
4.97	6.10 ± 0.25$_4$	0.484[d]	0.50 ± 0.07$_4$
4.31	5.60	0.248	0.26 ± 0.05$_3$
4.29	5.0 ± 0.1$_2$	0.102	0.159 ± 0.009$_2$
3.08	4.13 ± 0.09$_2$		

[a] In 0.02 M phosphate buffer, μ = 0.05 (KCl), pH 7, [PNPH] = 2.0 × 10^{-5} M, 25 °C. [b] Errors are average deviations from the mean of n runs. [c] 79% free SH by Ellman's analysis[12] for this and decreasing [16-SH]. [d] 83% free SH by Ellman's analysis for this and decreasing [16-SH].

Thiol surfactant **4** is prone to aerobic oxidative dimerization. Accordingly, it was routinely stored under high vacuum and manipulated under nitrogen. Our best sample, mp 82-84 °C, displayed >95% free SH activity toward Ellman's reagent,[12] but the micellar kinetic properties toward PNPA of various lots of **4** were essentially constant as long as the Ellman's titer was >55% free SH.

For comparison purposes, the nonsurfactant analogue of **4**, thiocholine bromide (**7**), was prepared by HBr cleavage of commercially available *S*-acetylthiocholine bromide.

$$(CH_3)_3\overset{+}{N}(CH_3)_2CH_2CH_2SH\ Br^-$$
7

Kinetic Studies. Cleavage of Esters. The cleavage of PNPA by excess micellar 16-SH was followed by stopped-flow spectrophotometry at 400 nm and pH 7. NMR experiments demonstrated the sole formation (>80%) of **6** (16-SAc) and *p*-nitrophenoxide under these conditions. Pseudo-first-order rate constants as a function of [16-SH] (3.02 × 10^{-2} to 2.77 × 10^{-3} M) appear in Table I. Slower reactions, [16-SH] = 9.24 × 10^{-4} to 9.84 × 10^{-5} M, were monitored on a Gilford spectrophotometer; these rate constants also appear in Table I. A rate constant/[surfactant] profile, constructed from Table I, appears in Figure 1; the lower value rate constants are shown in the inset at the lower left. The highest observed rate constant, k_ψ^{max}, was 2.16 ± 0.02$_3$ s^{-1} at [16-SH] = 1.50 × 10^{-2} M, point C in Figure 1.

A value in this concentration region was also determined at pH 7.96, k_ψ = 9.71 s^{-1} with [16-SH] = 0.020 M. For comparison, model thiol **7** gave k_ψ = 0.0090 ± 0.0007$_2$ s^{-1} at pH 7.1, [**7**] = 0.0157 M, and k_ψ = 0.020 ± 0.002$_3$ s^{-1} at pH 8.0, [**7**] = 0.0198 M.

Cleavage of PNPH was also studied at pH 7. Table II collects rate constant vs. [16-SH] data and the appropriate profile appears in Figure 2. In this case, k_ψ^{max} = 6.68 ± 0.03$_3$ s^{-1} at [16-SH] = 6.44 × 10^{-3} M, point C in Figure 2. Under similar conditions, model compound **7** cleaved PNPH with k_ψ = 0.001 84 ± 0.000 03$_2$ s^{-1} at [**7**] = 5.01 × 10^{-3} M.

From Figure 1, the kinetic critical micelle concentration (cmc) of 16-SH appears to be ~4.2 × 10^{-4} M (cf. point A) in 0.02 M N_2-purged phosphate buffer, μ = 0.05. The kinetic cmc of the 16-SH/PNPH system is not as apparent (Figure 2), because there is no abrupt "break" in the k_ψ vs. [16-SH] profile at low [16-SH]. Nevertheless, a gentle change of slope is observed, and is extrapolated to [16-SH] = 3.3 × 10^{-4} M (cf. point A, Figure 2).

The pH dependence of the micellar 16-SH/PNPA reaction was studied. Pseudo-first-order rate constants determined over the pH range 5.00-9.84 appear in Table III; log k_ψ vs. pH is rendered in Figure 3. For pH ≤7.00, log k varies linearly with pH with a slope of 0.979; for pH ≥7.97, the corresponding slope is 0.088. The intersection of these two correlation lines, at pH 7.32 (point A, Figure 3), is taken as the pK_a of micellar 16-SH.

In papain, the imidazole moiety of His-159 is believed to activate the thiol residue of Cys-25 by general base catalysis.[3] We attempted to model this interaction with comicellar sys-

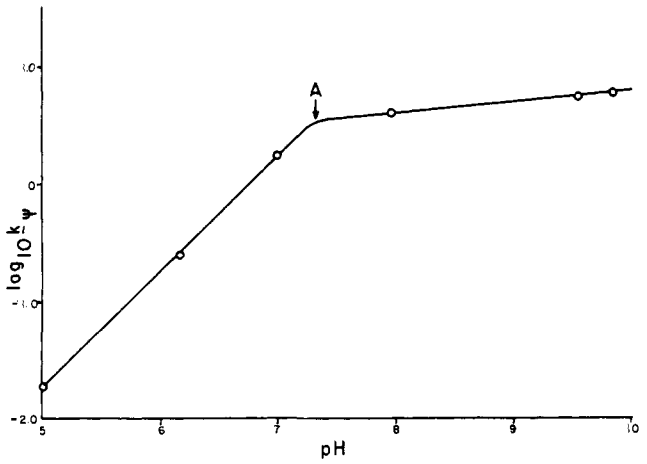


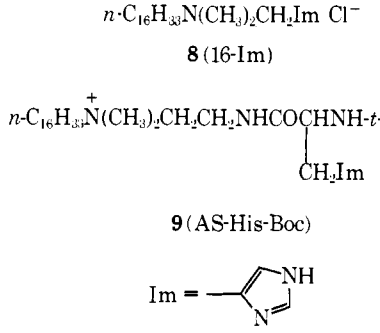
Figure 3. Logarithms of pseudo-first-order rate constants for the 0.004 M 16-SH cleavage of PNPA vs. pH. Point A is taken as the pK_a of 16-SH.

Table III. pH Dependence of the 16-SH/PNPA Reaction[a]

pH	buffer, conc, M	k_ψ, s^{-1} [b]
5.00	acetate, 0.05	$0.0191 \pm 0.0006_2$
6.17	phosphate, 0.02	$0.25 \pm 0.02_2$
7.00	phosphate, 0.02	$1.75 \pm 0.01_2$
7.97	phosphate, 0.02	$4.012 \pm 0.004_2$
9.55	carbonate, 0.05	$5.48 \pm 0.05_3$
9.84	carbonate, 0.05	$5.90 \pm 0.25_3$

[a] Conditions: [16-SH] = 4.0×10^{-3} M; [PNPA] = 2.0×10^{-5} M; μ = 0.05 (KCl); 25 °C. Ellman's assay revealed a range of 77–86% free SH for the several lots of 16-SH used in these experiments. [b] Errors are average deviations from the mean of n runs.

tems of 16-SH and the imidazole surfactants **8** (16-Im)[13] and **9** (AS-His-Boc).[14] Results of these experiments appear in

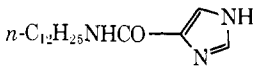


8 (16-Im)

9 (AS-His-Boc)

Table IV. The pK_a values of micellar 16-Im and AS-His-Boc are ~5.8[14] and <4.1,[15] respectively, whereas that of 16-SH is 7.3 (see above). Therefore, the comicellar situations represented in Table IV involve 16-SH >90% in its protonated form, while the imidazole surfactants are ~70 (16-Im) and >90% (AS-His-Boc) in the neutral imidazole form. (The balance in each case is the imidazolium form.) The results in Table IV will be examined below.

Trioctylmethylammonium chloride (TMAC) aggregates at low concentration (10^{-4}–10^{-5} M) in aqueous solution and strongly potentiates the esterolytic reactivity of hydrophobic hydroxamate and imidazole nucleophiles toward PNPA. For example, the second-order rate constant for PNPA cleavage by 7.3×10^{-5} M **10** (pH 9.0, 30 °C) is enhanced ~1170 times



10

Table IV. Cleavage of PNPA by 16-SH Comicelles[a]

surfactant composition	pH	k_ψ, s^{-1}
16-SH + CTACl[b]	6.13	0.362
16-SH + AS-His-Boc	6.07	0.180
AS-His-Boc + CTACl[b]	6.23	0.004 58
16-SH + 16-Im	6.15	0.206
16-Im + CTACl[b]	6.15	0.000 847

[a] Conditions: 5.0×10^{-3} M in *each* surfactant; 0.02 M N_2-purged phosphate buffer, μ = 0.05 (KCl), [PNPA] = 2.0×10^{-5} M; 25 °C. The 16-SH had 85% free SH by Ellman's assay. [b] Cetyltrimethylammonium chloride.

Table V. Cleavage of PNPA by 16-SH/TMAC Aggregates[a]

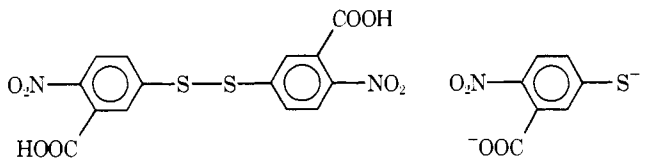
[16-SH], M	[TMAC],[b] M	mol % TMAC	k_ψ, s^{-1} [c]	k_2, L/mol·s[d]
0.000[e]	0.005	100	<0.000 02	
0.001	0.005	83	0.141	141
0.005	0.005	50	0.0386	7.72
0.005	0.001	17	0.107	21.4
0.005	0.000[e]	0	0.362	72.4

[a] Conditions: 0.02 M N_2-purged phosphate buffer, pH 6.15; μ = 0.05 (KCl), [PNPA] = 2.0×10^{-5} M; 25 °C. The 16-SH had 82–84% free SH by Ellman's assay. [b] Trioctylmethylammonium chloride. [c] These rate constants were reproducible to <4%. [d] $k_2 = k_\psi$/[16-SH]. [e] 0.005 M CTACl was added.

by micellar (10^{-3} M) CTABr (cetyltrimethylammonium bromide), but even more so (13 300) by 7.3×10^{-5} M TMAC.[16] The TMAC enhancement was attributed to the formation of tight hydrophobic ion pairs between the imidazole anion form of **10** and TMAC, within the TMAC aggregates. Desolvation of these ion pairs enhances the reactivity of the anionic form of **10**.[16]

We briefly examined the effect of TMAC on the micellar 16-SH/PNPA system. Results appear in Table V, wherein it appears that TMAC-rich micellar coaggregates of 16-SH/TMAC are about twice as reactive as micellar 16-SH alone.

The reaction product of 16-SH and PNPA is **6**, 16-SAc, so that 16-SH turnover in the presence of excess PNPA would be governed by the S-deacylation of 16-SAc. The latter reaction was studied using Ellman's reagent, **11**, to follow the hydrolytic liberation of 16-SH from 16-SAc.[12,17] Reagent **11** rapidly reacts with liberated 16-S^-, affording anion **12**, which can be monitored by its strong absorbance at 412 nm.[12,17] When [**11**] > [16-SAc], liberated **12** is subject to oxidation. Therefore, only the first 5–10% of such reactions was followed, and the initial rate was measured.[5b] [16-SAc] was kept constant at 5.0×10^{-4} M, while [**11**] was varied between 1.1×10^{-4} and 1.3×10^{-3} M. Because the large organic species **11** and **12** inhibit micellar hydrolysis,[5b] four or five rate constants were determined as a function of [**11**], and the dependence of k_{obsd} on [**11**] was extrapolated to [**11**] = 0.



11 **12**

With this method, rate constants for the micellar hydrolysis of 16-SAc were determined in the presence of 0.005 M added CTACl or 16-Im (**8**). Results appear in Table VI. We observed a 217-fold enhancement in the micellar deacylation of 16-SAc by 16-Im relative to CTACl. Analogous experiments with *S*-acetyl-**7** indicated that k_{deacyl} at pH 8 was ~$1-2 \times 10^{-6}$ s^{-1},

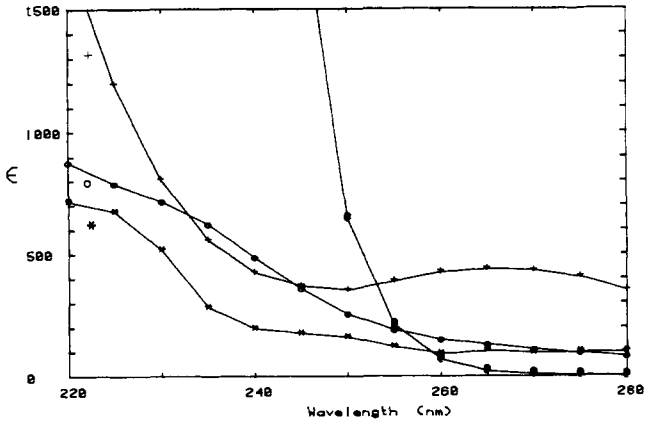


Figure 4. Extinction coefficients (ε) of 16-SH, product **13**, iodoacetamide, and KI vs. wavelength (nm); conditions were pH 7, 0.02 M phosphate buffer, μ = 0.05 (KCl), 0.02 M added CTACl in iodoacetamide and KI solutions. Key: o, 16-SH; *, **13**; +, iodoacetamide; @, KI.

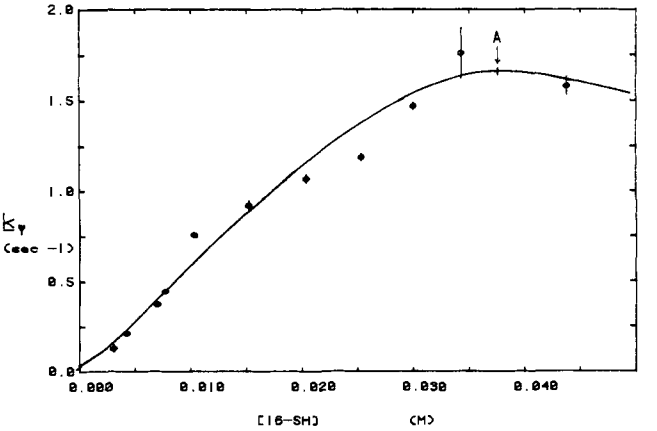


Figure 5. Pseudo-first-order rate constants for the pH 7 reaction of 16-SH and iodoacetamide vs. [16-SH]. Point A is taken as k_ψ^{max}.

Table VI. Hydrolysis of 16-SAc in the Presence of Ellman's Reagent[a]

added surfactant, 5.0×10^{-3} M	k_{obsd}, s^{-1} [b]
none[c]	$5\text{-}8 \times 10^{-6}$
CTACl	1.08×10^{-5} [d]
16-Im	2.34×10^{-3} [d]

[a] [16-SAc] = 5.0×10^{-4} M; 0.02 M phosphate buffer, pH 8.0, μ = 0.05 (KCl), 25 °C; [**11**] = $0.11\text{-}1.3 \times 10^{-3}$ M. [b] Pseudo-first-order rate constant for liberation of **12** from **11**, extrapolated to [**11**] = 0; see text. [c] Determined by NMR spectroscopic observation of 0.5 M 16-SAc in D_2O (pD 8.0); the integral ratio of the $SCOCH_3$ absorption to the $-(CH_2)_{14}CH_3$ absorption was followed over time. Precision was poor. [d] The reproducibility of the rate constants from which k_{obsd} at [**11**] = 0 was extrapolated (see text) permits us to estimate the error in k_{obsd} as 4–6%.

Table VII. Reaction of 16-SH with Iodoacetamide[a]

10^2[16-SH], M	10^4[ICH_2CONH_2], M	k_ψ, s^{-1} [b]
4.38	8.0	$1.58 \pm 0.05_4$
3.43	4.0	$1.76 \pm 0.14_3$
3.00	8.0	$1.47 \pm 0.02_3$
2.53	4.0	$1.19 \pm 0.02_4$
2.04	4.0	$1.07 \pm 0.02_4$
1.52	4.0	$0.921 \pm 0.002_4$
1.03	4.0	$0.76 \pm 0.01_4$
0.770	4.0	$0.44 \pm 0.01_5$
0.700	3.0	$0.37 \pm 0.01_3$
0.429	4.0	$0.21 \pm 0.01_4$
0.306	2.0	$0.13 \pm 0.02_4$

[a] Conditions: 0.02 M phosphate buffer pH 7.0, μ = 0.05 (KCl), 25 °C. Analysis by Ellman's assay indicated 77–80% free SH in the 16-SH. [b] Errors are average deviations from the mean of n runs.

Table VIII. Reaction of Thiocholine Bromide (**7**) with Iodoacetamide[a]

10^2[thiocholine]	k_ψ, s^{-1} [b]
2.89	$0.0399 \pm 0.0005_2$
1.97	$0.0280 \pm 0.0002_3$
1.01	$0.0136 \pm 0.0004_3$
0.762	$0.0128 \pm 0.0004_3$
0.394	$0.0064 \pm 0.0001_3$

[a] Conditions: 0.02 M phosphate buffer, pH 7.0, μ = 0.05 (KCl), 25 °C, [iodoacetamide] = 8×10^{-4} M. Ellman's assay indicated 92.5% free SH for the thiocholine. [b] Errors are average deviations from the mean of n runs.

and that significant enhancement could *not* be elicited upon addition of either CTACl or 16-Im. Apparently, hydrophilic, cationic *S*-acetyl-**7** is excluded from cationic micelles.

S_N2 Reactions. Studies of S_N2 reactions involving functional micellar nucleophiles are very rare; the only well-documented example appears to be Heitmann's report on the reaction of cysteine derivative **1** and iodoacetamide. Relative to the bulk aqueous phase, anionic self-micelles of **1** showed inhibited reactivity toward iodoacetamide, but comicellar **1**/CTABr afforded enhanced second-order rate constants for the S_N2 reaction.[4] We have now examined analogous reactions between self-micellar 16-SH and iodoacetamide.

The reaction product, **13**, was directly prepared from 16-SH and iodoacetamide in water at pH 7 (titrimeter, stat mode). The crude material was ion exchanged to the Cl^- form, recrystallized, and precipitated from water as the triflate salt. Characterization by NMR and elemental analysis appears below. The Me_3N^+ analogue of **13** was similarly prepared from **7** and iodoacetamide.

$$n\text{-}C_{16}H_{33}\overset{+}{N}(CH_3)_2CH_2CH_2SCH_2CONH_2\,X^-$$
13

UV spectra of 16-SH, **13**, iodoacetamide, and KI were determined in aqueous or in micellar CTACl solutions (pH 7) between 220 and 280 nm; see Figure 4. Although all four compounds are present during the reaction of 16-SH with iodoacetamide, Figure 4 shows that the extinction coefficients of 16-SH and **13** are equal at 272 nm, so that their interconversion as the reaction proceeds will cause no change in A_{272}. Iodide ion does not absorb at 272 nm, but iodoacetamide has $\epsilon_{272} \sim 400$. Accordingly, the kinetics of the 16-SH/iodoacetamide reaction were followed by monitoring the disappearance of iodoacetamide at 272 nm.

Pseudo-first-order rate constants for the reactions of excess 16-SH with iodoacetamide were determined by stopped-flow spectroscopy at various [16-SH] and pH 7; data appear in Table VII.[18] A corresponding k_ψ vs. [16-SH] profile appears in Figure 5, from which k_ψ^{max} = 1.68 s^{-1} at [16-SH] = 0.0375 M (point A). Analogous rate constants were determined on the Gilford spectrophotometer for reactions of **7** with iodoacetamide; data appear in Table VIII. k_ψ varied linearly with [thiocholine] according to the relation 1.34[thiocholine] + 0.001 33 = k_ψ. Thus, at [thiocholine] = 0.0375 M (i.e., the concentration for k_ψ^{max} of 16-SH/iodoacetamide), k_ψ^7 = 0.052 s^{-1}. This value, compared with k_ψ^{max} for 16-SH (1.68 s^{-1}), indicates a kinetic advantage of 1.68/0.052 = 32 for the micellar S_N2 reaction.

Table IX. Kinetic Parameters for Reactions of Micellar 16-SH[a]

reaction	pH	k_ψ^{max}, s^{-1} [b]	k_{cat}, L/mol·s[c]	k_ψ^7, s^{-1} [d]	R[e]	k_m, s^{-1} [f]	K/N, M^{-1} [f]
16-SH + PNPA	7.0	2.16 [0.015]	144	0.0090[g]	251	2.52	34.4
16-SH + PNPA	8.0	9.71 [0.020]	485	0.020	485	[h]	[h]
16-SH + PNPH	7.0	6.68 [0.006 44]	1037	0.001 84	2820	11.6	232
16-SH + ICH_2CONH_2	7.0	1.68 [0.0375]	44.8	0.052	32	2.6–2.7[i]	35–40[i]

[a] Conditions: 0.02 M phosphate buffer, μ = 0.05 (KCl), 25 °C. [b] Value in brackets is surfactant concentration (M) at which k_ψ^{max} was observed. [c] $k_{cat} = k_\psi^{max}/[surfactant]$. [d] Pseudo-first-order rate constant for the comparable reaction of thiocholine. [e] $R = k_\psi^{max}/k_\psi^7$ or $k_{cat}^{16\text{-SH}}/k_2^7$; see text. [f] Parameters derived from Lineweaver–Burk analysis of Tables I, II, and VII; see text. [g] pH 7.1. [h] Not available. [i] See text.

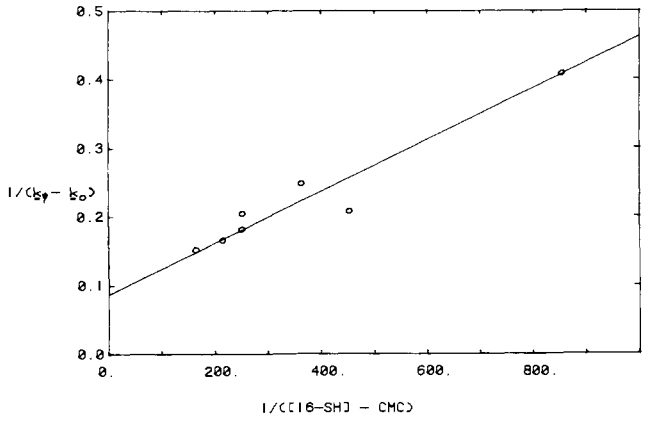


Figure 6. Lineweaver–Burk analysis of the 16-SH/PNPH reaction. See Table II, text, and eq 2.

Discussion

Kinetic Parameters. The data of Tables I, II, and VII afford useful kinetic parameters for characterization of the reactions of 16-SH with PNPA, PNPH, and iodoacetamide. Values of k_ψ^{max} are obtained from the appropriate table or corresponding figure. From these maximal pseudo-first-order rate constants, second-order "catalytic" rate constants, k_{cat}, are derived. Next, by comparison of each k_ψ^{max} with k_ψ for the analogous reaction of thiocholine (7) at identical [RSH], we obtain the ratio R, or $k_\psi^{max,16\text{-SH}}/k_\psi^7$, which is taken as the micellar kinetic advantage of the particular reaction. In cases where the observed k_ψ^7 values were obtained at >1% different [RSH] than those of k_ψ^{max} of 16-SH, R was calculated from $k_{cat}^{16\text{-SH}}/k_2^7$, the denominator being obtained from $k_\psi^7/[7]$. This modification pertains to the pH 7 PNPA and PNPH data in Table IX. The various kinetic parameters are collected in Table IX.

Additionally, the three [16-SH] vs. k_ψ profiles can be analyzed in terms of the micellar pseudophase model. We assume that substrate is partitioned between micellar and bulk phases, and that reaction occurs according to eq 1. Here, M is the

$$\begin{array}{ccc} M + S & \overset{K}{\rightleftharpoons} & MS \\ \downarrow k_0 & & \downarrow k_m \\ P & & P \end{array} \qquad (1)$$

micelle, S is the substrate, MS is the micelle-substrate complex, K is the binding constant of substrate to micelle, and k_0 and k_m are the rate constants for product formation in bulk solvent and micellar phases, respectively. Kinetic analysis of (1) leads ultimately to the Lineweaver–Burk expression:

$$\frac{1}{k_0 - k_\psi} = \frac{1}{k_0 - k_m} + \left(\frac{1}{k_0 - k_m}\right)\left(\frac{N}{K(C_D - \text{cmc})}\right) \qquad (2)$$

where k_ψ is the observed rate constant at a given concentration of surfactant, C_D, and N is the micellar aggregation number. A plot of $1/(k_0 - k_\psi)$ vs. $1/(C_D - \text{cmc})$ is generally linear, and permits the evaluation of k_m and K/N from the observed slope and intercept.[19]

For pH 7 reactions of 16-SH with PNPA, values of k_0 and cmc are taken from point A of Figure 1, and, for the reaction with PNPH, k_0 and cmc are taken as the k_ψ and [16-SH] corresponding to point A of Figure 2. These data are 0.013 s^{-1} at 4.2×10^{-4} M 16-SH for PNPA and 0.10 s^{-1} at 3.3×10^{-4} M 16-SH for PNPH. Insertion of these values and the k_ψ data of Tables I and II into eq 2 generates linear relations (99.9% confidence level) from which are obtained the k_ψ and K/N values gathered in Table IX (see above). The actual correlation for the PNPH data appears in Figure 6.

Reactions of 16-SH with iodoacetamide are more difficult to analyze; because of experimental difficulties the data of Table VII (Figure 5) cannot be extended to sufficiently low [16-SH] to directly afford k_0 and the kinetic cmc. However, these values can be reasonably estimated. We take the cmc as $\sim 4 \times 10^{-4}$ M (see above). At this concentration, thiocholine will react with iodoacetamide with k_ψ = 0.0019 s^{-1} (see Results). Taking this as a *lower limit* for k_0 of 16-SH, application of eq 2 to data of Table VII affords k_m = 2.57 s^{-1} and K/N = 40 M^{-1}. (Note: only k_ψ values for [16-SH] > 0.01 M were used in this calculation, in order to avoid the product oxidation problems encountered at low [16-SH]; see Results.) Owing to enhanced hydrophobic bonding or complexation (see below), $k_\psi^{apparent}$ for the reaction of submicellar 16-SH and iodoacetamide is likely to be greater than k_ψ for the corresponding reaction of thiocholine and iodoacetamide. However, the enhancement is very unlikely to be more than a factor of 32, the value of $k_\psi^{max,16\text{-SH}}/k_\psi^7$ (Table IX). Therefore $0.0019 \times 32 \sim 0.061$ s^{-1} represents an *upper limit* for k_0 of 16-SH. This value, with cmc = 4×10^{-4} M, affords k_m = 2.69 s^{-1} and K/N = 35 M^{-1}, when eq 2 is applied to the data of Table VII. The bracketing values of k_m and K/N appear in Table IX, and their range is seen to be small.

Esterolysis Reactions of Micellar 16-SH. In esterolysis reactions with PNPA and PNPH, micellar 16-SH exhibits maximal second-order cleavage rate constants of 144 and 1037 L/mol·s, respectively, at pH 7. The associated micellar kinetic advantages (R) are 251 and 2820, relative to thiocholine. At pH 8, with PNPA, k_ψ^{max} = 485 L/mol·s and the R value is 485. Compared to other SH-functional surfactants, or to other functional surfactants in general, 16-SH is an extraordinarily powerful micellar esterolysis reagent. Only the N-OH functional surfactants kinetically surpass 16-SH. A more quantitative comparison between functional surfactants appears below.

From the dependence of k_ψ on pH in the micellar 16-SH/PNPA reaction (Table III, Figure 3), it is clear that we require prior ionization of a functional group with $pK_a \sim 7.3$. The pK_a of thiocholine is 7.85,[20a] so that the kinetically determined pK_a of 16-SH is consistent with preequilibrium thiol/thiolate ionization occurring in a cationic micelle with stabilization of the thiolate ion and concomitant 0.5 unit pK_a lowering. Recalling that NMR experiments demonstrate the formation of 16-SAc from 16-SH + PNPA, we may render

the overall micellar esterolysis reaction:

$$RSH \underset{H_2O}{\overset{OH^-}{\rightleftharpoons}} RS^- + ArOCOCH_3 \xrightarrow[-ArO^-]{k_{acyl}} RSCOCH_3 \xrightarrow{OH^-,\ k_{deacyl}} RSH + CH_3COO^- \quad (3)$$

Consider the acylation step. The thiol functions of **7** and 16-SH are β to their quaternary nitrogen atoms, substantially enhancing thiol acidity. Thiolate forms are thus readily available at rather low pH, so that under mild conditions these systems are better esterolysis reagents than many other thiols. At pH 8, micellar 16-SH is ~83% ionized to 16-S^-, whereas thiocholine is ~59% ionized.[20b] A factor of 1.41 of the 485-fold micellar kinetic advantage (R) of 16-SH with PNPA is therefore due to the differential acidity of the micellar vs. nonmicellar reagent. The residual kinetic advantage (344) represents the actual micellar advantage. A parallel treatment of the pH 7 PNPA data (Table IX) reveals that a factor of 2.14 of the 251-fold micellar kinetic advantage is due to differential acidity, leaving ~117 as the intrinsic micellar advantage relative to thiocholine.

Further partition of these residual factors is difficult. The apparently strong binding of PNPA to micellar 16-SH must contribute substantially. Application of eq 2 to the 16-SH/PNPA kinetic profile at pH 7 generates K/N = 34.4 (Table IX). Taking $N \sim 70$[21] affords $K \sim 2400\ M^{-1}$; binding of this strength should account for a sizable portion of the 117-fold actual micellar advantage. In the thiolysis of PNPA by ArSH/CTABr, the maximum micellar kinetic advantages were ~50, and were attributed solely to binding.[22] This result, however, cannot necessarily be extended to aliphatic thiols.[22]

A further complication arises because the cleavage of PNPA by thiol anions suffers a change of rate-determining step around $pK_a(RSH) \sim 7.5$.[23] At higher pK_as formation of a tetrahedral addition intermediate is rate determining, whereas at lower pK_as breakdown of the intermediate is limiting. With micellar 16-SH ($pK_a \sim 7.3$), formation *and* breakdown of the tetrahedral intermediate may be kinetically comparable, making it difficult to predict net micellar kinetic effects. For example, the formation of anionic Meisenheimer addition complexes in nucleophilic aromatic substitution reactions is enhanced by cationic micelles, but breakdown of these intermediates is retarded.[24]

Presently, our assessment is that much of the pK_a-corrected esterolysis rate advantage with 16-SH is due to substrate binding, but that rate-constant enhancement does play some role. The latter may have various origins. We consider micellar zwitterion formation to be important. It results in internal charge neutralization, increased hydrophobicity, thiolate ion desolvation, and enhanced reactivity toward PNPA. Thus at pH 7, micellar 16-SH is 32% zwitterionic and the acidity-corrected micellar advantage, relative to thiocholine (pH 7.1), is 117, whereas at pH 8 micellar 16-SH is 83% zwitterionic and the comparable advantage is 344. An apparent increase in 16-SH reactivity due to increasing hydrophobicity is also suggested by the TMAC comicellization data of Table V.

The importance of substrate binding in micellar esterolytic reactions is reinforced by the PNPH cleavage results. At pH 7, the micellar advantage for 16-SH vs. thiocholine cleavage of PNPH is 2820 (Table IX). Micellar 16-SH is ~32% ionized and thiocholine is ~12% ionized, so that the corrected micellar advantage is ~1060. This is nine times greater than the corresponding value for PNPA cleavage (117), and must in large part reflect the sevenfold stronger binding (cf. K/N, Table IX) of PNPH to micellar 16-SH. Additionally, the 16-SH/PNPH esterolyses may occur in a somewhat more hydrophobic microenvironment than the analogous PNPA reactions, further adding to the advantage of the former.

Although preequilibrium ionization of 16-SH is crucial to the esterolysis, eq 3, the overall rate of reaction was not enhanced in comicelles of 16-SH with either 16-Im or AS-His-Boc. Micellar 16-SH is clearly more reactive with PNPA than either imidazole surfactant comicellized in CTACl (cf. Table IV), so that the kinetically dominant nucleophile in thiol/imidazole comicellar systems is certain to be 16-S^-. In contrast to the His-159/Cys-25 interaction of papain, however, there is no apparent kinetic enhancement of PNPA cleavage with thiol/imidazole comicelles; conditions in their Stern layers are still far from those prevailing in enzymes such as papain and (presumably) residual water prevents significant intramicellar "deprotonation" of thiol by imidazole moieties. Indeed, comicellization of 16-**SH** with either 16-Im or AS-His-Boc *inhibits* PNPA cleavage by factors of 1.7–2.0, relative to 16-SH comicellization with CTACl. The origin of this inhibition is presently unclear, but the effect is surprising because the imidazole surfactants are individually 130–360 times more reactive than CTACl toward PNPA.[14]

PNPA cleavage by 16-SH affords 16-SAc, eq 3. The latter's deacylation would be necessary to regenerate 16-SH and permit it to function as a true catalyst (i.e., "turn over"), but 16-SAc deacylates very slowly at pH 8. Under the conditions of Table VI, *S*-acetylthiocholine shows $k_{deacyl} \sim 1\text{–}2 \times 10^{-6}\ s^{-1}$, so that for micellar 16-SAc ($k_{deacyl} \sim 5\text{–}8 \times 10^{-6}\ s^{-1}$) or comicellar 16-SAc/CTACl ($k_{deacyl} \sim 1.1 \times 10^{-5}\ s^{-1}$) we have, at most, about a tenfold micellar enhancement of deacylation.

Deacylation can be catalyzed by 16-Im; a 217-fold enhancement of k_{deacyl} occurs in 16-SAc/16-Im comicelles at pH 8 (Table VI). Presumably, this originates in facile S → N acyl transfer from 16-SAc to the imidazole group of 16-Im (probably in its anionic form[13]). The acetyl-16-Im thus formed will rapidly hydrolyze at pH 8.[25] Note that the 16-Im catalyzed deacetylations were carried out in the presence of **11**, which rapidly traps liberated 16-S^- and prevents back attack on the 16-Im$COCH_3$. In the absence of **11**, back attack (under the intramolecular conditions prevailing in Cys-His peptides) inhibits efficient external deacylation of an acylhistidine.[17]

Although deacylation of 16-SAc is strongly catalyzed by comicellar 16-Im, the 16-SH/16-Im comicelle is not a likely candidate for catalytic PNPA cleavage with turnover. The reason is that 16-Im cleaves PNPA (k_{cat} = 5.0 L/mol·s, pH 8, 25 °C[13]) faster than it cleaves 16-SAc ($k \sim 0.47$ L/mol·s, pH 8, 25 °C, estimated from Table VI). Under conditions where [PNPA] > [16-SH/16-Im], 16-SH would rapidly give 16-SAc (k_{cat} = 485 L/mol·s), but the 16-Im would then cleave excess PNPA rather than 16-SAc.

Premicellar Complexation. Close examination of Table I and Figure 1 indicates that, below the kinetic cmc (4.2×10^{-4} M 16-SH, point A), the k_ψ vs. [16-SH] profile levels off, and k_ψ remains essentially constant at 0.012–0.014 s^{-1} over the decreasing [16-SH] range, $3.8\text{–}0.98 \times 10^{-4}$ M. One might have expected k_ψ to linearly *decrease* with decreasing [16-SH] below the cmc. It is conceivable that premicellar complexation of PNPA with 16-SH (or with premicellar aggregates of 16-SH) is responsible for this effect in a concentration range where [16-SH] > [PNPA].[26]

Displacement Reactions of Micellar 16-SH. The S_N2 reaction between micellar 16-SH and iodoacetamide yields **13**, with the kinetic parameters gathered in Table IX. These parameters resemble those of the pH 7 16-SH/PNPA reaction; k_m and K/N are almost identical. The former similarity is coincidental because different reaction types are involved, but the near identity of K/N values suggest that PNPA and iodoacetamide are comparably bound to micellar 16-SH. The micellar advantage for esterolysis is ~eight times that for displacement, suggesting that the former reaction profits more, in terms of free energy of activation, by transfer from aqueous

to micellar phase. In the esterolysis transition state considerable negative charge will be localized on the substrate's carbonyl oxygen atom, whereas in the S_N2 transition state this charge is dispersed over the S···C···I triad of atoms. Conceivably, the first example may derive additional electrostatic stabilization from specific Coulombic interactions between "O^-" and the cationic micellar head groups.

Indeed, after correction for ionization differences between 16-SH and thiocholine, the micellar advantage of the present S_N2 reaction is only ~12, less than that observed in the S_N2 reaction between thiophenoxide ion and micellar 16-OMs (mesylate derivative of **5**).[11] There, the ionization-independent micellar advantage was 43, relative to choline mesylate, attributable mostly to binding of thiophenoxide ions by 16-OMs micelles,[11] and the effective conversion of a bimolecular reaction to a quasi-unimolecular reaction.[27] Reactions of comicellar **1**/CTABr with iodoacetamide afford corrected micellar advantages of ~60–80,[4] about six times larger than those observed with 16-SH. The origin of this difference is presently unclear, although it is not large enough to suggest major new mechanistic factors.

Comparisons of Esterolytic Micellar Reagents. The data in Table IX characterize esterolyses of PNPA and PNPH by micellar 16-SH. We can compare the properties of 16-SH with other thiol-functionalized surfactants and with functional surfactants in general.

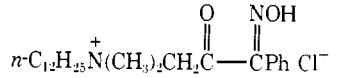
Tonellato independently prepared 16-SH (Br^- form) and studied its reactivity in comicellar CTABr (CTABr/16-SH = 7:1, Tris buffer, μ = 0.1, KCl, pH 7.15). Apparent k_{cat} values were 210 (PNPA) and 1700 (PNPH) L/mol·s, and at pH 7.95 the PNPA cleavage afforded k_{cat} = 580 L/mol·s.[8] These rate constants are generally comparable to our own (Table IX), although Tonellato's are somewhat higher, particularly with PNPH. The origin of these reactivity differences presumably relates to the different microenvironments of 16-SH/CTABr comicelles and 16-SH holomicelles. However, details are presently unavailable.

The overall esterolytic reactivity of 16-SH appears to surpass that of cysteine surfactants **1–3**. Micellar **3** ($pK_a \sim 9.1$) cleaves PNPH (pH 8.65, ~26% ionized, 10% ethanol/water, 30 °C) with k_ψ^{max} = 0.48 s^{-1},[6] whereas 16-SH (pK_a = 7.3) cleaves PNPH (pH 7.0, ~34% ionized, 0.02 M phosphate buffer, 25 °C) with k_ψ^{max} = 6.68 s^{-1}. Micellar **2** (~45% ionized at pH 8[5b]) cleaves PNPA with k_{cat} = 26.0 L/mol·s and a kinetic advantage (compared to cysteine methyl ester) of 36 at pH 8, 25 °C.[5] Under comparable conditions, micellar 16-SH is ~83% ionized and has k_{cat} = 485 L/mol·s, and a micellar advantage (relative to **7**) of 485.

Corrected for the varying extents of ionization to RS^-, micellar **2** and **3** are less effective esterolytic reagents than 16-SH. At low pH, micellar 16-SH is also more reactive than **1**/CTABr, but this appears to be due to their very different pK_a values (~9.1 for **1**/CTABr[4] vs. 7.3 for 16-SH). Thus, at pH 6.0 the comicelle cleaves PNPA with k_{cat} = 4.8 L/mol·s,[4] whereas 16-SH exhibits k_{cat} = 62.5 at pH 6.17 (cf. Table III).

Micellar 16-SH is kinetically comparable to comicellar CTABr/coenzyme A and CTABr/glutathione, for which micellar kinetic advantages of 210 and 100, respectively, have been reported in PNPA cleavage.[10] These factors are based on CTABr-solubilized vs. nonsolubilized thiolate *anions*, and may be compared to our factor of 344 for 16-S^- vs. thiocholine anion (see above). Micellar 16-SH is considerably more reactive at pH 7 (k_{cat} = 144 L/mol·s, 25 °C) than dodecanethiol/stearyltrimethylammonium bromide, for which k_{cat} ~ 1.5 L/mol·s (pH 7, Tris buffer).[9] The 16-SH system is comparable to the SH proteolytic enzyme ficin (k_{cat} ~ 173, pH 6.9, 29.6 °C).[28]

We conclude with a brief comparison of 16-SH with other surfactant esterolytic reagents. For cleavage of PNPA by 0.02 M 16-SH, k_ψ = 9.71 s^{-1} at pH 7.96, corresponding to a second-order esterolytic rate constant, k_2 = 485 L/mol·s. On a scale of "relative micellar catalytic effectiveness" (pH ~8), defined by k_2^{rel} for CTACl ($k_2 = 1.4 \times 10^{-2}$ L/mol·s) = 1.00, 16-SH has k_2^{rel} = 34 600.[29] It is more reactive toward PNPA than AS-Cys (**2**),[5] 16-Im (**8**),[13] AS-His-Boc (**9**),[14] or 16-OH (**5**),[13] for which the k_2^{rel} values are 1860, 360, 130, and 9.7, respectively. Indeed, the reactivity of 16-SH is surpassed only by comicellar blends of N-OH reagents and CTA halides.[30] Comicellar **14**/CTABr (1:7, pK_a = 8.0), for example, cleaves



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PNPA with k_{cat} = 2570 L/mol·s at pH 7.95,[30b] which is to be compared with k_{cat} = 485 L/mol for 16-SH (pK_a = 7.3) under comparable conditions. The comicellar system is ~ eight times more reactive toward PNPA than is micellar 16-SH after correction for differential ionization. The latter, however, remains the most reactive self-contained or "holomicellar" esterolytic reagent yet examined.

Experimental Section[31]

***N*-*n*-Cetyl-*N*,*N*-dimethyl-*N*-(β-thioethyl)ammonium Chloride (4, 16-SH).**[11] Surfactant **5** (16-OH)[32] was converted to its triflate derivative, 16-OTf. Triflic anhydride, 2.86 g (0.0101 mol), was dissolved in 20 mL of CH_2Cl_2 in a 250-mL round-bottom flask. Then 2.00 g (0.0051 mol) of 16-OH and 0.400 g (0.0051 mol) of pyridine dissolved in 20 mL of CH_2Cl_2 were added, and the mixture was allowed to stir at 25 °C for 30 min. NMR then indicated conversion to the triflate surfactant, 16-OTf.[33]

Thiolacetic acid, 2.50 g (0.0328 mol, Aldrich), was dissolved in 10 mL of water and the pH was adjusted to 7.5 with 0.2 M NaOH. This solution was added to the 16-OTf solution, and the two-phase mixture was allowed to stir for 60 min. The CH_2Cl_2 layer and two 10-mL CH_2Cl_2 washes of the aqueous layer were combined and dried over $MgSO_4$. After filtration of the $MgSO_4$, the CH_2Cl_2 was removed under reduced pressure to yield a white residue. This was washed twice with dry ether and then recrystallized three times from CH_2Cl_2/ether to afford 2.15 g (0.0041 mol, 81% yield) of 16-SAc, OTf (**6**): mp 60–64 °C; NMR (δ_{CDCl_3} (Me_4Si)) 0.87 (t, 3 H, CH_2CH_3), 1.28 (s, 28 H, $(CH_2)_{14}$), 2.38 (s, 3 H, $COCH_3$), 3.27 (m, 12 H, $CH_2N^+(CH_3)_2CH_2CH_2S$).

Anal. Calcd for $C_{23}H_{46}F_3NO_4S_2$: C, 52.95; H, 8.89; N, 2.68; S, 12.29. Found: C, 52.66; H, 8.61; N, 2.71; S, 12.34%.

Dry Dowex 1-X8 ion exchange beads, 3.48 g (11.8 mequiv), in the chloride form (J. T. Baker) and 619 mg (1.19 mmol) of 16-SAc in the triflate form were mixed in 20 mL of water and heated to 85 °C for several minutes. The beads were filtered off and washed with water until they were free of surfactant. The aqueous portions were then combined and lyophilized to a dry powder, 16-SAc Cl.[34]

The 16-SAc Cl was then dissolved in 20 mL of nitrogen-purged H_2O, and 7 mL of concentrated HCl was added to give a solution which was 3 N in HCl. This solution was heated to 80 °C under nitrogen for 1.5 h. The HCl was aspirated from the hot solution, which was then lyophilized to a dry powder, 16-SH Cl^-. The product was dissolved three times in CH_2Cl_2 and precipitated each time with ethyl ether. The yield of white solid was 350 mg of 16-SH Cl^- (**4**), 80% based on 16-SAc OTf. The mp was 82–84 °C. Free SH activity was >95% by Ellman's assay.[12,35] NMR (δ, $CDCl_3$): 0.87 (t, 3 H, CH_3); 1.27 (s, 28 H, $(CH_2)_{14}$), 3.24 (br m, 12 H, $CH_2N^+(CH_3)_2CH_2CH_2S$); the 3 H SAc singlet of **6** (δ 2.38) was absent in the spectrum of **4**.

Thiocholine Bromide. *S*-Acetylthiocholine bromide, 483 mg (1.99 mmol, Aldrich), was dissolved in 10 mL of nitrogen-purged H_2O, and 5 mL of 48% aqueous HBr was added to make the solution 3 N in HBr. This solution was then heated to 80 °C for 30 min. The HBr was aspirated from the hot solution, which was then lyophilized to an oil. The oil was triturated with anhydrous ether and then absolute ethanol. More anhydrous ether was then added to precipitate the product. The solid was removed, dissolved in hot absolute ethanol, and precipitated

with anhydrous ether to give 301 mg (1.5 mmol, 75%) of thiocholine bromide, mp 208.5–210.5 °C. NMR (δ_{D_2O} (DSS)): 2.8–3.2 (m, 2 H, CH_2S), 3.2 (s, 9 H, $N(CH_3)_3$), 3.4–3.8 (m, 2 H, CH_2N). Ellman's assay[12,35] indicated 99% free SH.

***N*-*n*-Cetyl-*N*,*N*-dimethyl-*N*-(β-thioacetamidoethyl)ammonium Chloride (13).** This synthesis employed a Radiometer pH meter, coupled to a Radiometer TTT 11 titrimeter and SBR2C titrigraph. The pH was set in the stat mode at 7, and 0.2 N aqueous NaOH served as the titrant. In the titrimeter vessel, 388.5 mg (2.10 mmol) of iodoacetamide (Aldrich) was dissolved in a minimum amount of O_2-free distilled water. Then 731 mg (2.0 mmol) of 16-SH Cl was added. After 10 min, there was no further tendency of the pH to decrease and the reaction was terminated.

The reaction solution was stirred twice with 5-g portions of Dowex 1-X8 beads (Cl^- form) at 80–85 °C for 30 min. Removal of the beads, followed by lyophilization, gave a solid which was recrystallized three times from warm CH_2Cl_2 (+ 3 drops of ethanol)/ether. There was obtained 470 mg (1.11 mmol, 55%) of **13** (Cl^- form). This material analyzed poorly for Cl (9.1% found vs. 8.4% calcd), and was converted to the triflate salt. It was dissolved in 10 mL of water and then 50 mL of triflic acid was added. The resulting precipitate was washed with water and recrystallized from CH_2Cl_2/ether.

NMR ($\delta_{CDCl_3}(Me_4Si)$): 0.92 (t, 3 H, CH_3), 1.23 (s, 28 H, $(CH_2)_{14}$), 3.0–4.2 (m, $CH_2NCH_2CH_2SCH_2$), and 3.73 (s, $N(CH_3)_2$), total 14 H.

Anal. Calcd for $C_{23}H_{47}F_3N_2O_4S_2$: C, 51.5; H, 8.83. Found: C, 51.0; H, 8.60. A slight hygroscopicity of the sample, noted by the analyst, accounts for the marginally low C analysis.

In the same manner, 806 mg (4.36 mmol) of iodoacetamide was reacted with 830 mg (4.15 mmol) of thiocholine bromide to afford 740 mg of the trimethylammonium analogue of **13**. This was ion exchanged to the chloride form, but analyzed high for chlorine (18.2% found vs. 16.7% calcd); NaCl was presumably present.

NMR (δ_{D_2O} (DSS)): 2.90–3.20 (m, 2 H, NCH_2CH_2S), 3.23 (s, 9 H, $(CH_3)_3N$), 3.43 (s, 2 H, SCH_2CO), 3.43–3.87 (m, 4 H, NCH_2CH_2S).

Other Materials. Trioctylmethylammonium chloride (Eastman Organic), *p*-nitrophenyl hexanoate (Sigma), and 5,5′-dithiobis(2-nitrobenzoic acid) (Ellman's reagent, Aldrich) were all used without further purification. *p*-Nitrophenyl acetate was purchased from Aldrich and recrystallized from absolute ethanol, mp 78–78.5 °C (lit. mp 79–80 °C).[36] Cetyltrimethylammonium chloride was purchased from Chemical Services, Inc., and was dissolved five times in hot methanol and precipitated with anhydrous ethyl ether to give white crystals, mp 201–204 °C dec.

UV Studies. Solutions of 9.3×10^{-4} M 16-SH, 1.6×10^{-4} M **13**, 5.4×10^{-4} M iodoacetamide, and 6.2×10^{-5} M KI were made up in 0.02 M ($\mu = 0.05$) pH 7 phosphate buffer. (The buffer for making the 16-SH solution was deoxygenated.) CTACl was added to the iodoacetamide and KI solutions (final [CTACl] = 0.005 M) to afford micellar conditions. The UV spectra of these four solutions were taken over the wavelength range 200–300 nm on the Cary 14 spectrophotometer, coupled with a Cary 1413 specular reflectance unit. The results of these measurements are discussed above and displayed (220–280 nm) in Figure 4.

Kinetic Studies. Reactions with $k_\psi \geq 0.1$ s^{-1} were followed with a Durrum Model D-130 stopped-flow spectrophotometer equipped with a Beckman DU-2 monochromator and a Tektronix Model 5103N/D15 storage oscilloscope. The oscilloscope readout was photographed using a Polaroid camera. Slower reactions ($k_\psi \leq 0.1$ s^{-1}) were monitored on a Gilford Model 250 spectrophotometer coupled to a Gilford Model 6051 recorder. Constant-temperature circulating baths maintained the reaction temperatures at 25.0 ± 0.2 °C. All buffers were prepared from nitrogen-purged steam-distilled water, and were purged again immediately prior to use.

Kinetic esterolysis samples for the Durrum spectrometer, in which the pH was intended to be higher than 7.0, were generated by preparing double-strength solutions of 16-SH and substrate in H_2O to which several drops of 0.2 N HCl had been added, as well as a double-strength buffer solution ($\mu = 0.1$) to which several drops of 0.2 N NaOH had been added. Each solution was loaded into a separate reactant syringe. For stopped-flow experiments in which the pH ≤7.0, a double strength solution of 16-SH in buffer and a double strength substrate-H_2O solution were used in the reactant syringes. A pneumatic driving device caused equal volumes from each syringe to be discharged into a mixing chamber and thence into the optical path. The resultant mixed solution had the proper concentrations of reactants and buffer and ionic strength ($\mu = 0.05$); the pH was measured after the reaction had occurred.

Samples for the Gilford instrument were prepared at the desired concentrations and pHs in 3.0-mL Teflon-stoppered quartz cuvettes. In esterolysis experiments for which [16-SH] ≤ 10^{-3} M, a stock solution of 0.05 M 16-SH in H_2O to which several drops of 0.2 N HCl had been added was prepared and stored under a continuous stream of nitrogen. Aliquots were withdrawn and added to buffer solutions to give the desired concentrations. Reactions were initiated by adding a 20-μL aliquot of a 2×10^{-3} M PNPA or PNPH in *p*-dioxane solution to 2.00 mL of buffered RSH solution in the cuvette. Kinetic data for esterolysis reactions appear in Tables I, II, IV, and V.

Buffer systems utilized were 0.05 M acetate for pH 5.00, 0.02 M phosphate for 6.0 ≤ pH ≤ 8.0, and 0.05 M carbonate for pH ≥9.0. They were prepared using ACS reagent grade electrolytes, adjusted to the proper pH at the pH meter, and adjusted to the appropriate ionic strength using KCl. Kinetic data for PNPA esterolysis as a function of pH appear in Table III.

Kinetic samples for the S_N2 reactions utilized at least a tenfold excess of 16-SH or thiocholine over iodoacetamide to ensure pseudo-first-order kinetics. Iodoacetamide solutions were freshly prepared before each kinetic run.

In the S_N2 reaction of thiocholine with iodoacetamide, stock solutions of 0.41 and 0.16 M thiocholine bromide in N_2-purged 0.02 M phosphate, $\mu = 0.05$ buffer, were prepared. The reaction was initiated by injecting an appropriate aliquot of one of the stock solutions into a cuvette which contained 2.0 mL of a solution of iodoacetamide in the buffer at pH 7. Kinetic data for these reactions appear in Table VIII.

The S_N2 reaction of 16-SH with iodoacetamide was studied with the stopped-flow spectrophotometer. Buffer, surfactant, and iodoacetamide solutions used in these experiments were at "double" initial concentrations, so that they afforded standard concentrations after dilution in the instrument. The reaction product solutions were collected and final pHs were found to be 6.96–7.07. Kinetic data for these reactions appear in Table VII.

Rate constants were obtained from computer-generated correlations of log $(A^\infty - A^t)$ with time in the standard manner. Reactions were generally followed to >70% of completion and exhibited first-order kinetics with correlation coefficients of better than 0.999 unless otherwise indicated. Lineweaver–Burk parameters were computed using a computer-generated least-squares correlation of the $1/(k_\psi - k_0)$ vs. $1/(C_d - \text{cmc})$ data.

Deacylation studies of 16-SAc in the presence of Ellman's reagent (**11**) employed a stock solution of 40 mg of **11** in 10.0 mL of 0.1 M phosphate buffer at pH 8.0, containing 0.01% of EDTA. Depending on the reaction, 20–100-μL aliquots of this solution were added to the reaction cuvette, which also contained 2.0 mL of phosphate buffer, and any second surfactant (Table VI) at 5.0×10^{-3} M. Reaction was initiated by adding an appropriate aliquot of a stock solution of 16-SAc. The initial rate was determined by drawing a line tangent to the reaction trace at the origin. Division by the absorbance of anion **12** (ϵ_{412} 13 600) and by the initial concentration of 16-SAc yielded the pseudo-first-order rate constant. Experiments were conducted at several concentrations of **11** between 1.1×10^{-4} and 1.3×10^{-3} M and initial rates were extrapolated to [**11**] = 0. See the Results section and Table VI for further details.

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Improved Acylation Rates within Cyclodextrin Complexes from Flexible Capping of the Cyclodextrin and from Adjustment of the Substrate Geometry

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Abstract: The acylation of β-cyclodextrin by bound substrates has been studied as a model for serine acylase enzymes such as chymotrypsin. Molecular model building suggested that previously examined substrates, which had given acylation rates only a few hundred times accelerated over the hydrolysis rates, were not optimal geometrically. In our work the geometry for such processes has been improved by fashioning an "intrusive floor" on the cyclodextrin cavity, leading to improved rates. Greater improvements have come from substrate modification, using substrates based on the cinnamic acid, adamantane, and ferrocene frameworks. The rates correlate well with the geometric predictions from molecular models. The best case leads to an acceleration of acylation, relative to hydrolysis, of 10^6–10^7-fold, exceeding that for chymotrypsin with *p*-nitrophenyl acetate.

Introduction

The cyclodextrins (cycloamyloses) have attracted great attention as enzyme models. α-Cyclodextrin (cyclohexaamylose) and β-cyclodextrin (cycloheptaamylose), and to a lesser extent γ-cyclodextrin (cyclooctamylose), have been studied because of their ability to bind large organic molecules into the cavity of the host cyclodextrin by the use of hydrophobic or generalized lyophobic[1] forces. Within the complex there may be reaction *with* the hydroxyl groups of cyclodextrin[2,3] or catalyzed by these hydroxyls.[4] Derivatives of cyclodextrins have also been studied in which catalytic or reactive functional groups are present to attack the bound substrate.[5-8]

Although the ability of the cyclodextrins to bind substrates into a molecular cavity in solution made them interesting enzyme mimics, the accelerations resulting from such binding had been quite modest. For example, Bender et al.[3] had studied

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