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Preparation and Kinetic Properties of Cysteine Surfactants

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Abstract: A cysteine surfactant (IV, AS-Cys) was synthesized by coupling cysteine to *N*-cetyl-*N*,*N*-dimethyl-*N*-β-aminoethylammonium chloride. Under micellar conditions at pH 8.0, excess AS-Cys cleaved *p*-nitrophenyl acetate (PNPA) with $k_\psi^{max} = 1.04\ s^{-1}$ (corresponding to $k_{cat} = 26.0$ L/mol·s), and the formation of *S*-acetyl-AS-Cys. The latter surfactant underwent intramolecular S → N transfer ($k^{max} = 0.44\ s^{-1}$ for nonmicellar conditions at pH 8.0, $k = 0.01\ s^{-1}$ in 5.5×10^{-3} M micellar cetyltrimethylammonium chloride) affording *N*-acetyl-AS-Cys. A second mole of PNPA could be cleaved by the free SH group of micellar *N*-acetyl-AS-Cys ($k_\psi^{max} = 1.45\ s^{-1}$ at pH 8.0, corresponding to $k_{cat} = 36.3$ L/mol·s) yielding *N*,*S*-diacetyl-AS-Cys. S-Deacetylation of the latter was slow ($k_\psi \sim 5 \times 10^{-5}\ s^{-1}$) under micellar conditions at pH 8, but could be accelerated by comicellization with imidazole-functionalized surfactants (e.g., $k_\psi = 9.2 \times 10^{-4}\ s^{-1}$). Various mechanistic aspects of these reactions are discussed in detail.

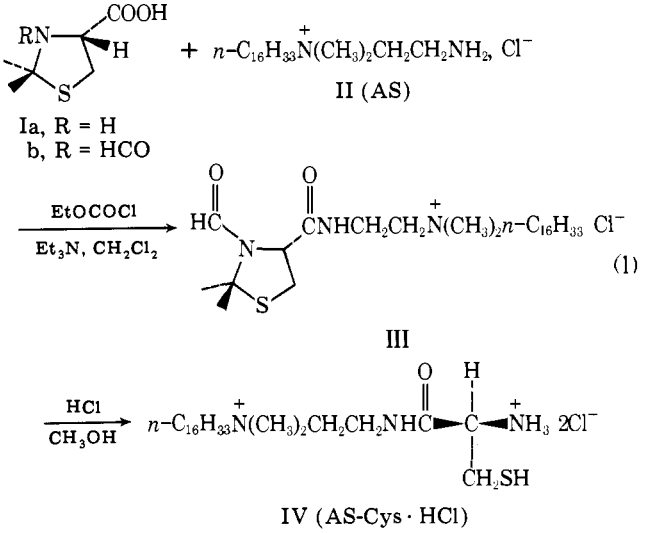
Continued interest in refining the analogy between micellar and enzymic catalysis has greatly stimulated the development of functional micellar reagents.[2-5] Although sulfhydryl surfactants are inherently attractive targets because of the key nucleophilic role played by the SH moiety in the cysteine proteinases papain, ficin, and stem-bromelain,[6] the facile oxidative dimerization of thiols, particularly under micellar conditions,[7] presents synthetic and mechanistic difficulties, and few relevant studies have appeared.

N-Dodecanoyl-DL-cysteine,[8] alkane thiols,[9] coenzyme A,[10] and glutathione,[10] each solubilized in micellar cetyltrimethylammonium[8,10] (CTA) or stearyltrimethylammonium bromides, were shown to accelerate the cleavage of *p*-nitrophenyl acetate (PNPA). Recently, Chaimovitch et al. investigated the effect of micelles on the rate of the *S*- to *N*-acetyl transfer of *S*-octanoyl-β-mercaptoethylamine.[11] In none of these cases, however, have *self-contained* thiol-functionalized surfactants been examined.

Recently, we prepared the first SH-functionalized surfactant catalyst, and offered a preliminary report of its nucleophilic properties toward PNPA.[12] Here, we present full details of the previous work, additional studies of mechanistically relevant comicellar systems, and an examination of the reaction sequence which ensues subsequent to the initial attack of the surfactant on PNPA.

Results

Synthesis. Using the procedure of Sheehan and Yang,[13] L-cysteine hydrochloride was condensed with acetone, affording thiazolidine Ia. The latter, upon formylation, gave Ib, which was converted to the mixed anhydride with ethyl chloroformate, and then coupled to amino surfactant II[14] (AS), yielding protected surfactant III. Deprotection with 1 N HCl in 50% aqueous methanol, followed by trituration with dry ether, gave crystalline surfactant IV (AS-Cys) as the hydrochloride. These reactions are summarized in eq 1.



(1)

AS-Cys is sensitive to air; consequently, it was stored under high vacuum and manipulated under nitrogen. A satisfactory elemental analysis was obtained for immediate precursor III, but was not attempted for AS-Cys itself. The NMR spectrum of the surfactant, however, was definitive ($\delta_{D_2O}^{DSS}$): 0.83, crude "t", $CH_3(CH_2)_{15}$; 1.27, "s", $(CH_2)_{14}$; 3.17, s, $N^+(CH_3)_2$; 3.1-3.9, m, ($N^+CH_2CH_2NHCO + (CH_2)_{14}CH_2N^+ + CH_2SH$); 4.27, t, $J = 6$ Hz, methine. The integral areas appeared in the appropriate ratio. Moreover, an assay with Ellman's reagent[15] at pH 8.0 indicated that AS-Cys possessed 0.99 free SH group per molecule.

Owing to its propensity for oxidation, we did not determine the critical micelle concentration (cmc) of AS-Cys, but we estimate this value to be 5×10^{-4} M (0.02 M phosphate buffer, $\mu = 0.05$ (KCl), 25 °C), based on the cmc's of the related alanine and histidine surfactants under these conditions.[14]

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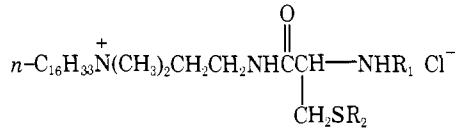
AS-Cys was converted to several related surfactants which were required for the mechanistic studies described below. Treatment of aqueous AS-Cys with *1* equiv of acetic anhydride in dioxane at pH 7.5–8.0 gave, after acidification and lyophilization, the *N*-acetyl derivative V (AS-Cys-NAc) in 80–90% yield. The NMR spectrum of AS-Cys-NAc revealed a signal for CH_3CO at δ_{D_2O} 2.15, but Ellman's assay showed that substantial oxidative dimerization had occurred (only 0.25 free SH group was observed per molecule of V). For kinetic studies, AS-Cys-NAc could be reduced in situ to the SH form by the addition of dithiothreitol,[16] a procedure which did not distort (see below) subsequent rate studies of the reaction of AS-Cys-NAc with PNPA.



IV (AS-Cys), $R_1 = R_2 = H$

V (AS-Cys-NAc), $R_1 = CH_3CO$; $R_2 = H$

VI (AS-Cys-SAc), $R_1 = H$; $R_2 = CH_3CO$

VII (AS-Cys-N,S-Ac_2), $R_1 = R_2 = CH_3CO$

Treatment of AS-Cys·HCl with excess acetyl chloride in CH_2Cl_2 solution under nitrogen, followed by precipitation with dry ether, gave 75% of VI (AS-Cys-SAc) as the hydrochloride. It was not always possible to obtain this compound without also producing some *S,N*-diacetate (VII) as an impurity. Thus, in a typical preparation, NMR revealed the $SCOCH_3$ signal(s) of VI (and of VII) at δ_{CDCl_3} 2.33, but $NCOCH_3$ absorption due to VII was apparent at δ 2.13. The intensity of the latter signal was ca. a third of that due to the δ 2.33 absorption, indicating that the ratio of VI/VII in the mixture was ~2:1.

The diacetyl surfactant, VII (AS-Cys-N,S-Ac_2), was prepared from aqueous AS-Cys at pH 7.5 by portionwise treatment with 2.5 equiv of acetic anhydride in dioxane. Acidification with HCl, lyophilization, and trituration with dry ether gave AS-Cys-N,S-Ac_2, in 55–75% yield, as a white, low-melting solid. NMR revealed $SCOCH_3$ and $NCOCH_3$ singlets at δ_{D_2O} 2.49 and 2.14, respectively, in a ratio of unity.

Further preparative details concerning IV–VII appear in the Experimental Section, where the preparation of the model compound, *N,S*-diacetylcysteine methyl ester, is also described.

Kinetic Studies. The cycle of reactions to be discussed is outlined in Scheme I.

Acylation of AS-Cys (k_1). The cleavage of PNPA by micellar AS-Cys was followed by stopped-flow spectrophotometry at 400 nm and pH 7.0. Pseudo-first-order rate constants observed at various [AS-Cys] are collected in Table I. The highest value of k_ψ (0.238 s^{-1}) was observed at [AS-Cys] = 0.04 M; at pH 8.0, the corresponding k_ψ was 1.04 ± 0.003 s^{-1}. For comparison, we studied the acylation of cysteine methyl ester by PNPA. At pH 8.0, under the conditions of Table I, k_ψ = 0.0289 ± 0.0005 s^{-1} at [Cys-OMe] = 0.04 M.

The pH dependence of the kinetics of PNPA cleavage was studied over the pH range 4.0–9.0; the data appear in Table II. For the six observations, we found log k_ψ to vary linearly with pH, with a slope = 0.61 (correlation coefficient = 0.998, significant at the 99.9% confidence level).

Scheme I

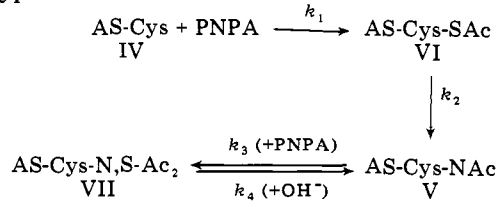


Table I. Cleavage of PNPA by AS-Cys[a]

[AS-Cys], M	k_ψ, s^{-1}	[AS-Cys], M	k_ψ, s^{-1}
0.003	0.0613 ± 0.0045	0.030	0.231 ± 0.002
0.005	0.0887 ± 0.0006	0.040	0.238 ± 0.002
0.010	0.153 ± 0.007	0.040[b]	1.04 ± 0.03
0.020	0.212 ± 0.011	0.050	0.219 ± 0.009

[a] In 0.02 M phosphate buffer, pH 7.0, μ = 0.05 (KCl), 25 °C; [PNPA] = 2.0 × 10^{-5} M. [b] At pH 8.0, other conditions unchanged.

Table II. pH Dependence of the Cleavage of PNPA by AS-Cys[a]

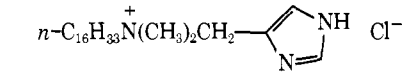
pH	k_ψ, s^{-1}	pH	k_ψ, s^{-1}
4.0[b]	0.0012 ± 0.0001	7.0[c]	0.0887 ± 0.0006
5.0[b]	0.0066 ± 0.0002	8.0[c]	0.437 ± 0.030
6.0[c]	0.0265 ± 0.0020	9.0[d]	1.27_5 ± 0.005

[a] [AS-Cys] = 5.0 × 10^{-3} M; [PNPA] = 2.0 × 10^{-5} M, 25 °C, μ = 0.05. [b] 0.02 M acetate buffer. [c] 0.02 M phosphate buffer. [d] 0.09 M borate buffer.

We attempted to determine the pK_a of 2 × 10^{-4} M AS-Cys spectrophotometrically at 235 nm (thiolate ion),[17] but interpretation of the experimental observations was complicated owing to the presence of the free NH_2 group of AS-Cys,[18] which led to four forms of AS-Cys, prototropically interrelated at pH 8.[19] Using the method of Benesch,[20] we obtained pK_as of ~8.9 for the AS-Cys(NH_2,SH)/AS-Cys(NH_2,S^-) couple and ~7.1 for the AS-Cys(NH_3^+,SH)/AS-Cys(NH_3^+,S^-) couple. Comparable pK_as for cysteine methyl ester are 9.09 and 7.45, respectively.[20] The acidity of AS-Cys(NH_2,SH) therefore appears to be similar to that of cysteine methyl ester. There is no large depression of p*K*a due to cationic micellization. However, in view of our uncertainty about the exact cmc of AS-Cys (see above), we are not sure that 2 × 10^{-4} M AS-Cys is micellar. We can, nevertheless, estimate that AS-Cys is at least 45% converted to S^- forms at pH 8.

In a product study, the reaction of 1 equiv of AS-Cys with 0.75 equiv of PNPA at pH 8 afforded 85% of the theoretical amount of AS-Cys-NAc (V), detectable by NMR (δ_{D_2O} 2.19, singlet due to $NHCOCH_3$). The quantitative analysis was done by NMR integration. On kinetic grounds, however, it is very unlikely that V is the *primary* product of this reaction (see Discussion). Amino surfactants are not nucleophilically competitive with analogous thiol surfactants toward PNPA cleavage at pH 8 where, we estimate, ~45% of the SH moieties of AS-Cys are present in the highly nucleophilic thiolate form. The AS-Cys/PNPA reaction must therefore initially lead to AS-Cys-SAc (VI), which subsequently affords isolable AS-Cys-NAc via a rapid S → N transfer reaction; cf. Scheme I.

In papain, the imidazole moiety of His-159 is believed to activate the thiol residue of Cys-25 by general base catalysis.[6] We attempted to simulate this interaction using a comicellar system of AS-Cys and surfactant VIII (16-Im).[4,5] The resultant kinetic data are summarized in Table III, and will be discussed below.



VIII (16-Im)

S → N Transfer (k_2). It proved most convenient to study S → N transfer using independently prepared AS-Cys-SAc, rather than by monitoring its transient intermediacy during the AS-Cys/PNPA to AS-Cys-NAc sequence (Scheme I). Thioesters absorb in the 229–235-nm range; consequently, S

Table III. Cleavage of PNPA by AS-Cys and 16-Im[a]

catalyst	k_ψ, s^{-1}
AS-Cys	0.0887
16-Im	0.00531
As-Cys + 16-Im[b]	0.0696

[a] Conditions: [surfactant] = 5×10^{-3} M, [PNPA] = 2×10^{-5} M, pH 7.0, 0.02 M phosphate buffer, μ = 0.05 (KCl), 25 °C. [b] 5.0×10^{-3} M in each surfactant.

Table IV. S → N Transfer of AS-Cys-SAc[a]

pH	added surfactant	concn, M	k_{obsd}, s^{-1}
5.00[b]	none		0.0165 ± 0.001
	CTACl	0.0050	0.041 ± 0.001
7.00[c]	none		0.36 ± 0.04
	CTACl	0.0055	0.45 ± 0.04
8.00[c]	none		0.44 ± 0.04
	CTACl	0.0055	0.010 ± 0.001

[a] [AS-Cys-SAc·HCl] = 2.1×10^{-4} M, 25 °C; 1×10^{-4} M V was also present. Kinetics by stopped-flow spectrophotometry at 230 nm. [b] 0.05 M acetate buffer, μ = 0.05 (KCl). [c] 0.02 M phosphate buffer, μ = 0.05 (KCl).

Table V. S → N Transfer of AS-Cys-SAc in CTACl Solution at pH 7.8[a]

added CTACl concn, M	k_{obsd}, s^{-1}	added CTACl concn, M	k_{obsd}, s^{-1}
none	0.39 ± 0.04	0.000 60	0.185 ± 0.005
0.0031	0.065 ± 0.002	0.000 16	0.175 ± 0.004

[a] [As-Cys-SAc·HCl] = 2.4×10^{-4} M (surfactant V was not present in this preparation); 0.02 M phosphate buffer, μ = 0.05, 25 °C. Kinetics were followed on the Gilford spectrometer at 230 nm.

→ N transfer was studied by the rapid decay of the absorbance of AS-Cys-SAc·HCl, at 230 nm, which followed its injection into various buffers.

Typical absorbance changes were 0.15–0.20 units at pH 8.0, with 2.1–2.4 × 10^{-4} M substrate. After the initial rapid decay, a slow decrease in absorbance was observed; this can be attributed to oxidative dimerization of liberated thiol groups. The secondary process was less troublesome at pH 7, and absent at pH 5.

Owing to the strong absorbance of AS-Cys-SAc at 230 nm, we could not work at concentrations much higher than 2 × 10^{-4} M. This is in the cmc region of the surfactant, but may actually be submicellar. Accordingly, the acyl transfers were also studied in 5.5 × 10^{-3} M CTACl solutions. Relevant data appear in Table IV. The initially surprising inhibitory effect of CTACl at pH 8 prompted a more detailed study of the concentration dependence of this phenomenon; cf. Table V.

In a product study, monitored by NMR, subjection of AS-Cys-SAc (δ_{D_2O} 2.43, $SCOCH_3$) to 0.04 M phosphate buffer at pH 7.0 afforded AS-Cys-NAc (δ_{D_2O} 2.22, $NCOCH_3$). The *N*-acetyl surfactant was not initially present in this preparation of AS-Cys-SAc·HCl, so that this experiment is a particularly clean demonstration of S → N transfer.

Acylation of AS-Cys-NAc (k_3). AS-Cys-NAc bears a free thiol moiety, so that the surfactant should be an excellent reagent for the cleavage of PNPA. Moreover, unlike AS-Cys, it does not carry a free amino group. Comparison of the kinetic properties of the two surfactants should then reveal what role, if any, the amino group of AS-Cys plays in its reactivity.

Cleavage of PNPA by AS-Cys-NAc, in phosphate buffer at pH 8, was followed by stopped-flow spectroscopy, monitoring the release of *p*-nitrophenoxide ion at 400 nm. As indicated above, synthetic AS-Cys-NAc could be obtained only in partially oxidized (disulfide) form. Therefore, a stock solution of the monomer was produced in situ by addition of the stoichiometrically required (determined by Ellman's assay[15,16]) quantity of dithiothreitol.[16] Pseudo-first-order rate constants could then be obtained for the cleavage of PNPA, and these appear in Table VI as a function of surfactant concentration.

Table VI. Cleavage of PNPA by AS-Cys-NAc[a]

[AS-Cys-NAc], M	k_ψ, s^{-1}	[AS-Cys-NAc], M	k_ψ, s^{-1}
0.0034	0.10 ± 0.005	0.011	0.87 ± 0.01
0.0055	0.38 ± 0.01	0.040	1.45 ± 0.03
0.0068	0.606 ± 0.002		

[a] In 0.02 M phosphate buffer, pH 8.0, μ = 0.05, 25 °C; [PNPA] = 2.0×10^{-5} M. Kinetics by stopped-flow spectroscopy.

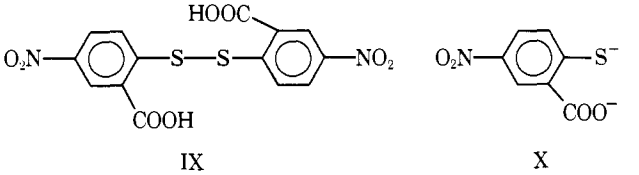
At 0.04 M, k_ψ for AS-Cys-NAc/PNPA was 1.45 s^{-1}, corresponding to a catalytic rate constant of 36.3 L/mol·s. These values may be compared to 1.04 s^{-1} and 26.0 L/mol·s, the analogous rate constants for the AS-Cys/PNPA reaction. In a control experiment, 0.01 M dithiothreitol (a hypothetical unreacted excess) was allowed to react with 2×10^{-5} M PNPA in 0.04 M CTACl at pH 8.0 (0.02 M phosphate buffer). k_ψ^{obsd} was 0.236 s^{-1}, indicating that 0.01 M unreacted dithiothreitol could enhance the value of k_ψ measured for 0.04 M AS-Cys-NAc by a maximum of 20%.

The pK_a of AS-Cys-NAc was determined spectroscopically using thiolate absorbance at 230 nm. In these experiments, [AS-Cys-NAc] was fixed at 2.42×10^{-4} M, CTACl (2.87×10^{-3} M) was added to ensure a micellar system, and KCl was used to set the ionic strength at ~0.053. Nine absorbance values,[21] determined over the pH range 7.30–10.80, were used to construct a graph of $A_{pH}^{230}/A_{max}^{230}$ vs. pH, from which the pK_a was found by the best fit of the data to a theoretical line generated from $A_{pH}^{230}/A_{max}^{230} = (K_a/[H^+])/(1 + K_a/[H^+])$. The p$K_a$ was found to be 8.15, so that, at pH 8, the extent of ionization of AS-Cys-NAc to its $-S^-$ form must be very similar to that of AS-Cys itself (see above).

In a product study, an oxidatively dimerized sample of AS-Cys-NAc was first reduced with a stoichiometric quantity of dithiothreitol at pH 8 (0.02 M phosphate buffer). After 3 min at 25 °C, the AS-Cys-NAc was treated with 1 equiv of PNPA (1 M in dioxane). Acidification with HCl, followed by lyophilization, afforded a product which displayed δ_{D_2O} 2.45 and 2.16, the $SCOCH_3$ and $NCOCH_3$ signals of AS-Cys-N,S-Ac_2.

S-Deacylation of AS-Cys-N,S-Ac_2 (k_4). It could be assumed that turnover of AS-Cys in the presence of excess PNPA would be controlled by S-deacetylation of the *N,S*-diacetate, and we therefore studied the mono- (S)-deacylation of the separately prepared diacetyl substrate. Study of the absorbances at either 230 or 240 nm (thiolate ion[17]) of pH 8 solutions of AS-Cys-N,S-Ac_2 led to data which could not be simply interpreted. A slow increase in absorption was followed by a slow decrease in absorption, perhaps reflecting S-deacylation followed by oxidative dimerization of the resulting AS-Cys-NAc. It was therefore decided to use rapid "trapping" methods to detect hydrolytically liberated AS-Cys-NAc.

In one set of experiments, Ellman's reagent, IX, was employed. Rapid reaction of IX with liberated thiolate afforded



IX X

Table VII. Hydrolysis of AS-Cys-N,S-Ac_2 in the Presence of IX[a]

added surfactant	concn, M	k_{obsd}, s^{-1} [b]
none[c]		$4.8 \pm 0.1 \times 10^{-5}$
CTACl[d]	0.0050	$4.4 \pm 0.2 \times 10^{-5}$
16-Im[d]	0.0050	$9.2 \pm 0.2 \times 10^{-4}$

[a] [AS-Cys-N,S-Ac_2] = $5.6\text{–}6.1 \times 10^{-4}$ M; [IX] varied over several concentrations, in each case, between 1.1×10^{-4} and 1.3×10^{-3} M, pH 8.0, 25 °C. [b] Calculated from extrapolated ([IX] = 0) values of the initial reaction velocity (6–7% of reaction) in units of observed reaction velocity divided by [AS-Cys-N,S-Ac_2]. Blank reactions, containing only buffer and IX, showed no conversion of IX to X; for further details, see text and Experimental Section. [c] 0.1 M phosphate buffer. [d] 0.02 M phosphate buffer, μ = 0.05.

anion X, the strong absorbance of which could be monitored at 412 nm.[15,16,22] This approach, however, was also complicated because, under conditions where [IX] > [AS-Cys-N,S-Ac_2], liberated X was also subject to oxidation, leading to apparent end points which were not valid infinity values.

It proved better to use IX as a trap in a "burst" situation where the first 5–10% of the reaction was followed and the initial rate was determined. In these experiments, [AS-Cys-N,S-Ac_2] was held at $\sim 5 \times 10^{-4}$ M, and [IX] was varied between 1.1×10^{-4} and 1.3×10^{-3} M. Not surprisingly, the large organic anions IX and X were found to inhibit the basic hydrolysis of AS-Cys-N,S-Ac_2, presumably by binding to the cationic surfactant micelles and excluding OH^-.[23] Therefore, initial rates were determined as a function of [IX] and then extrapolated to [IX] = 0. In related experiments, the hydrolysis of AS-Cys-N,S-Ac_2 was studied in the presence of either CTACl or 16-Im (VIII), each at 5×10^{-3} M. Data appear in Table VII.[24]

In a second trapping approach, PNPA was used to intercept liberated AS-Cys-NAc. It was known from the AS-Cys-NAc/PNPA cleavage studies (Table VI) that this reaction was orders of magnitude faster than the hydrolysis of AS-Cys-N,S-Ac_2. The PNPA trapping method presents its own complications, however, because (in contrast to IX) PNPA is subject to direct, micelle-catalyzed hydroxide-mediated cleavage, in competition with cleavage due to liberated AS-Cys-NAc. Moreover, the rate of hydrolysis of micellar AS-Cys-N,S-Ac_2 will also be affected by the binding of PNPA and its *p*-nitrophenoxide cleavage product.

Experiments were done in which 2.6×10^{-3} M AS-Cys-N,S-Ac_2 in 0.02 M phosphate buffer at pH 8.0 was reacted with PNPA (in concentrations varying from 2.5×10^{-4} to 1.0×10^{-3} M), and the appearance of *p*-nitrophenoxide was followed over the first 3–4 min of reaction. Initial rates were determined from $\Delta A/\epsilon_{PNPA}$ (17 800), and the rate constant was extrapolated from the intercept of a graph of initial rate vs. [PNPA]. In this manner, k_4 was estimated to be 9.8×10^{-6} s^{-1}, ~five times smaller than the value obtained using the Ellman's trapping reaction; cf. Table VII.

Although *direct* observation of the hydrolysis of AS-Cys-N,S-Ac_2 to AS-Cys-NAc did not lead to first-order kinetics, such behavior was approximated in the presence of 5.0×10^{-3} M added 16-Im. Under these conditions, monitoring thiolate production at 229 (or 240) nm, with [AS-Cys-N,S-Ac_2] set at 5.8×10^{-5} M (or 1.16×10^{-4} M), gave $k_{obsd} = 3.48 \pm 0.08 \times 10^{-4}$ s^{-1} (or $4.19 \pm 0.08 \times 10^{-4}$ s^{-1}) in 0.02 M phosphate buffer at pH 8.0, μ = 0.05, 25 °C.[25] The average value of 3.8×10^{-4} s^{-1} can be compared with the corresponding 16-Im catalyzed cleavage of AS-Cys-N,S-Ac_2 as followed by the Ellman trapping method (Table VII). The latter method leads to a rate constant which is about twice as large (9.2×10^{-4} s^{-1}).

It is not certain why the k_4 values measured by the Ellman method are larger than the corresponding values obtained by either PNPA trapping or direct observation. Obviously, the first two methods are complicated and, as indicated above, may be perturbed either by the added trapping agent or its reaction product. The "direct method", though free of these problems, requires refinement of the observed infinity values. In view of these difficulties, k_4 is best regarded as only approximately determined, and in the range $\sim 1\text{–}5 \times 10^{-5}$ s^{-1} at pH 8. In the presence of 5.0×10^{-3} M 16-Im, pH 8, the pseudo-first-order k_4 lies in the range $\sim 4\text{–}9 \times 10^{-4}$ s^{-1}. The latter variability will be discussed below.

Table VIII. Rate Constants for the Reactions of Scheme I[a]

rate constant	pseudo-first-order, s^{-1}	second order, L/mol·s[b]
k_1[c]	1.04	26.0
k_2[d]	0.44	
k_3[e]	1.45	36.3
k_4[f]	$1\text{–}5 \times 10^{-5}$	

[a] At pH 8.0, 0.02 M phosphate buffer, μ = 0.05, 25 °C. [b] From k_{obsd} [catalyst]. [c] Maximum value of k_{obsd} at [AS-Cys] = 0.04 M. [d] [AS-Cys-SAc] = 2.1×10^{-4} M. [e] [AS-Cys-NAc] = 0.04 M. [f] [AS-Cys-N,S-Ac_2] = 5×10^{-4} to 2.6×10^{-3} M.

Table IX. Cleavage of PNPA in Surfactant Micelles[a]

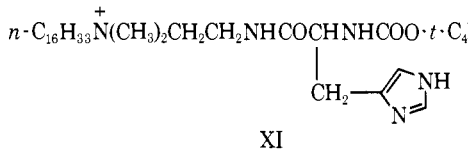
catalyst	k_ψ^{max}, s^{-1} [b]	k_{cat}, L/mol·s[c]	k_{cat}^{rel}	ref
CTACl	0.000 19 [1.3_5]	0.014	1.0	14
AS-His-Boc	0.029 [1.6]	1.8	130.	14
16-Im[d]	0.20 [4.0]	5.0	360.	4
AS-Cys	1.04 [4.0]	26.0	1860.	this work

[a] In 0.02 M phosphate buffers, μ = 0.05, 25 °C. See text for catalyst structures. [b] Values in brackets are concentrations (M × 100) at which k_ψ^{max} was determined. [c] $k_{cat} = k_\psi^{max}/$[surfactant]. [d] 0.01 M phosphate buffer.

Discussion

Table VIII gathers values of the rate constants defined in Scheme I and presented in the Results section. Hydrolysis of AS-Cys-N,S-Ac_2 is clearly the slowest process and would control turnover under conditions in which [PNPA] ≫ [AS-Cys]. The other reactions are comparable in rate at pH 8. It is again convenient to discuss each process separately.

Acylation of AS-Cys (k_1). In Table IX, we collect rate constants for the cleavage of PNPA by various micellar reagents at pH 8; AS-Cys data are taken from Table I. AS-Cys is 1860 times more effective than CTACl at cleaving PNPA at pH 8 (Table IX); relative to buffer alone ($k_0 = 3.5 \times 10^{-5}$ s^{-1}),[12] the enhancement is 29 700. As-Cys is also more reactive than the comparable imidazole surfactant, AS-His-Boc (XI). On



XI

the PNPA scale, AS-Cys is seen to be an extremely potent functional micellar reagent, apparently surpassed in reactivity only by N–OH functionalized surfactants.[26]

It is clearly the SH rather than the NH_2 moiety of AS-Cys which cleaves PNPA, for the observed rate constant is nearly identical with k_3 (Table VIII) for the reaction of PNPA with AS-Cys-NAc (in which the amino function of AS-Cys has been blocked).[27] Thus, although the product isolated from the AS-Cys/PNPA reaction is AS-Cys-NAc, we must infer the initial formation of AS-Cys-SAc on kinetic grounds. This is

perfectly reasonable, because subsequent S → N transfer is very rapid (Tables IV and VIII), so that only the *N*-acetyl surfactant can be isolated under basic reaction conditions.

In comparison to 0.04 M cysteine methyl ester (k_ψ = 0.0289 s^{-1} at pH 8.0), AS-Cys exhibits a catalytic advantage of 36 in the cleavage of PNPA. We attribute this partly to the binding of PNPA by AS-Cys, and partly to enhanced ionization of its SH functionality to the (active) $-S^-$ form due to the cationic micelle. PNPA is not particularly well bound to surfactants analogous to AS-Cys,[14] so that the catalytic advantage is artificially small. Somewhat larger enhancements have been reported (factors of 100–300) for CTABr-solubilized *N*-dodecanoylcysteine,[8,28] coenzyme A,[10] and glutathione,[10] relative to the nonsolubilized compounds. In each case, however, the ratio of rate constants for functional vs. nonfunctional micellar cleavage of PNPA is larger than the analogous ratio for a functional micelle vs. a nonmicellar model bearing the same functionality.

As discussed in our preliminary communication,[12] AS-Cys may also be compared on the PNPA scale with its close relatives, dodecanoylcysteine/CTABr[8] and dodecanethiol/stearyltrimethylammonium bromide (STABr),[9] as well as with the SH enzyme, ficin.[29] At pH 6.0, AS-Cys is kinetically very similar to dodecanoylcysteine/CTABr; k_{cat} values are 5.3 and 4.8 L/mol·s, respectively. At pH 7 (0.07 M Tris buffer), AS-Cys is ~three times more reactive (k_{cat} = 3.38 L/mol·s) than dodecanethiol/STABr (for which k_{cat} = 1.51 L/mol·s may be extrapolated[9]). Relative to ficin (k_{cat} ~ 173, pH 6.9, 29.6 °C),[29] AS-Cys (k_{cat} = 5.95, pH 7.0, 25 °C) is ~29 times less reactive.

From the pH dependence of the AS-Cys/PNPA reaction (Table II), we found log k_ψ to vary linearly with pH, with a slope = 0.61. This is reminiscent of the dodecanethiol/STABr–PNPA reactions, in which the slope of log k_{cat} vs. pH was 0.72 over the pH range 7.0–11.0[9] In both systems, the thio anion is the active form of the SH moiety. The pK_a for the ionization of AS-Cys(NH_3^+,SH) to AS-Cys(NH_3^+,S^-) [but not for AS-Cys(NH_2,SH) to AS-Cys(NH_2,S^-)] appears to be lowered ~0.3 pK unit from the corresponding value of 7.45 reported for cysteine methyl ester.[20] Probably this is a result of cationic micellization,[30] but uncertainty about the cmc of AS-Cys (see above) renders this conclusion tentative.

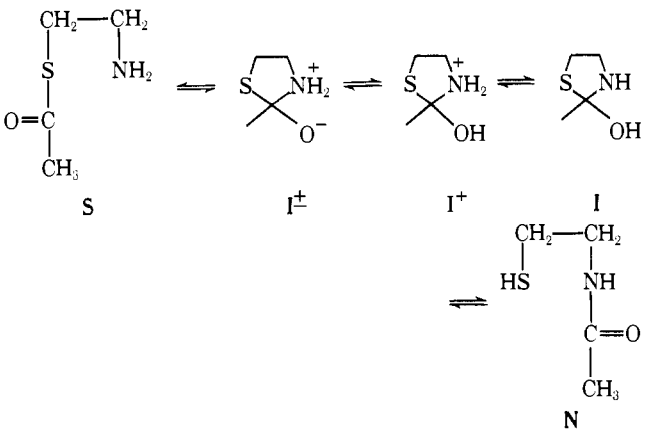
It is clear, however, that the amino group of AS-Cys does not potentiate the micellar surfactant's reactivity by general base activation of the residual thiol moieties (~55% at pH 8): k_3 for the reaction with PNPA of AS-Cys-NAc (in which the amino group has been converted to an amido group) is slightly *greater* than k_1 for the PNPA/AS-Cys reaction.

Similarly (cf. Table III), 16-Im does not act cooperatively with AS-Cys in the cleavage of PNPA at pH 7. AS-Cys is more reactive toward PNPA than is 16-Im at surfactant concentrations of 0.005 M (Table III) or at 0.04 M,[31] where k_ψ^{max} is observed for AS-Cys. Therefore, the kinetically dominant nucleophile in the AS-Cys + 16-Im comicellar system is certain to be supplied by AS-Cys, and the imidazole moiety of 16-Im would have been largely relegated to the role of a base, were it to have played any part in the cleavage reaction.

S → N Transfer (k_2). S → N acyl transfer in β-mercaptoethylamine derivatives is a complex process which has been much studied.[11,32] For the parent molecule, *S*-acetyl-β-mercaptoethylamine, Barnett and Jencks have derived the mechanism shown in Scheme II,[32] the central features of which include intramolecular amino attack on the thioester carbonyl group of **S** leading to cyclic zwitterion, $I^\pm$, followed by a rate-determining proton transfer affording I^+. Acetamide **N** is ultimately formed from I^+ via more rapid reactions which pass through the intermediate hydroxythiazolidine, **I**.

Our results for the S → N conversion of AS-Cys-SAc to AS-Cys-NAc can be related both to Scheme II[32] and also to

Scheme II



the reported effects of cationic micelles on the S → N acyl transfer reaction of *S*-octanoyl-β-mercaptoethylamine (OMA).[11] At pH 6.8, k_0 for the latter reaction was 0.026 s^{-1}. Upon addition of CTABr, k_{obsd} increased to a (calculated) maximum of 0.12 s^{-1}, although [CTABr] > 5.5 × 10^{-3} M (k_{obsd} ~ 0.082 s^{-1}) actually *inhibited* the reaction. The inferred 4.6-fold enhancement of S → N transfer by CTABr micelles at pH 6.8 was attributed to a decrease in pK_a of the amino group of OMA, thus increasing the concentration of the reactive, unprotonated species (**S** in Scheme II).[11,33]

In parallel fashion, 2.1 × 10^{-4} M AS-Cys-SAc[34] undergoes S → N acetyl transfer with k_{obsd} = 0.0165 s^{-1} at pH 5.0, increasing to 0.041 s^{-1} upon the addition of 5.0 × 10^{-3} M CTACl, affording an observed micellar enhancement of ~2.5 (cf. Table IV). We attribute this to a micelle-induced enhancement of the concentration of unprotonated AS-Cys-SAc(NH_2) at pH 5, and a corresponding enhancement of S → N acyl transfer. At pH 7.0, where substantially more AS-Cys is in the free NH_2 form, "k_0" is substantially greater (0.36 s^{-1}), so that micellar enhancement is nearly eliminated (k_{obsd} = 0.45 s^{-1} at [CTACl] = 5.5 × 10^{-3} M).

In contrast, "k_0" at pH 8.0 is 0.44 s^{-1} and the addition of CTACl is inhibiting at all concentrations investigated (Tables IV and V). The simplest explanation of these results is that the NH_2 moiety of AS-Cys-SAc is essentially unprotonated at pH 8.0, so that there is nothing to be gained from the acidity-enhancing effect of cationic micellization; note that "k_0" at pH 8 ≈ k_{obsd} in the presence of CTACl at pH 7. Moreover, if we recall that the rate-determining step of the overall S → N process is conversion of a zwitterion to a cation ($I^\pm$ to I^+ in Scheme II), then it is clear that the addition of cationic micellar CTACl should be anticatalytic; organic cations are well known to inhibit micellar reactions in which net positive charge must be created either before or during the rate-determining step.[35]

The effect of CTACl on the S → N transfer reaction is indeed one of "mixed activation–inhibition", as concluded by Chaimovitch,[11] with activation dominant at the lower pHs, and inhibition pronounced at pH 8. This is quite consistent with both the general principles of micellar catalysis[2] and with the detailed S → N acyl transfer mechanism depicted in Scheme II.[32] Thus, cationic micellization favorably affects the concentration of available reactant, but unfavorably alters the activation energy of the rate-determining step. The relative magnitudes of these competing effects may be presumed to be pH dependent.

Finally, we note that although the S → N transfer reaction of micellar AS-Cys-SAc will be considerably slower than PNPA acetylation of either AS-Cys or AS-Cys-NAc at pH 8 (processes k_1 and k_3 of Scheme I; cf. Tables I, IV, VI, and VIII), the S → N reaction will still be considerably faster than hydrolysis of AS-Cys-N,S-Ac_2 (k_4 in Scheme I; cf. Tables VII

Table X. Hydrolyses of *S*-Acetylcysteine Derivatives[a]

compd	concn, M	added surfactant	concn, M	k_{obsd}, s^{-1} [b]
AS-Cys-N,S-Ac_2	$\sim 6.0 \times 10^{-4}$	none		4.8×10^{-5} [c,d]
AS-Cys-N,S-Ac_2	$\sim 6.0 \times 10^{-4}$	CTACl	5.0×10^{-3}	4.4×10^{-5} [d]
AS-Cys-N,S-Ac_2	$\sim 6.0 \times 10^{-4}$	16-Im	5.0×10^{-3}	9.2×10^{-4} [d]
Cys-OMe-N,S-Ac_2	2.5×10^{-4}	CTACl	5.0×10^{-3}	2.2×10^{-6} [e,f]
Cys-OMe-N,S-Ac_2	2.5×10^{-4}	16-Im	5.0×10^{-3}	2.9×10^{-5} [g]
peptide[h]	$5\text{-}7 \times 10^{-5}$	none		5.2×10^{-4} [i]
peptide[h,j]	$5\text{-}7 \times 10^{-5}$	none		6.0×10^{-6} [i]

[a] At pH 8.0, 0.02 M phosphate buffer, 25 °C, unless otherwise noted. [b] Using IX as trapping reagent; see Results section. [c] 0.1 M phosphate buffer. [d] See Table VII. [e] In the absence of added surfactant, the hydrolysis was too slow to measure. [f] Reproducibility, ±14%. [g] Reproducibility, ±3%. [h] *N*-Ac-Gly-Arg-Phe-Cys(Ac)-Phe-His-Gly-COOH, pH 8.05, 0.02 M Tris–0.1 M NaCl, $2\text{-}3 \times 10^{-4}$ M IX. [i] From ref 22. [j] No IX was present.

and VIII), so that the latter reaction remains the turnover-controlling reaction in Scheme I.[36]

Acylation of AS-Cys-NAc (k_3). Under our standard micellar conditions (Table III), the reaction of PNPA with AS-Cys-NAc is the most rapid of the four processes in Scheme I, slightly faster than the reaction of PNPA with AS-Cys itself (compare Tables I and VI). The comparable reactivity of AS-Cys and AS-Cys-NAc, taken together with the relatively low reactivity of AS-Ala,[14] demonstrates the kinetic unimportance of the free amino group of AS-Cys in its micelle-enhanced basic cleavage of PNPA.[36] AS-Cys-NAc is seen to be a "normal" thiol micellar reagent, quite similar in every respect (including pK_a) to AS-Cys.

The ready synthetic access to AS-Cys-NAc afforded by S-acetylation of AS-Cys, followed by S → N transfer, suggests conceptually simple syntheses of various peptide derivatives of AS-Cys (linkage at its N terminus). The relevant experiments are in progress.

S-Deacylation of AS-Cys-N,S-Ac_2 (k_4). Basic hydrolysis of AS-Cys-N,S-Ac_2 is much the slowest of the four reactions summarized in Scheme I (cf. Tables VII and VIII), even allowing for the uncertainty attached to the actual rate constant. In hypothetical situations where 2[PNPA] > [AS-Cys], hydrolysis of AS-Cys-N,S-Ac_2 would control turnover and the postburst rate.

In Table X we collect rate data for the hydrolyses of AS-Cys-N,S-Ac_2, of *N,S*-diacetylcysteine methyl ester (Cys-OMe-N,S-Ac_2), and of a cysteine-histidine peptide studied by Klotz.[22] All of the rate constants are derived from trapping studies with Ellman's reagent, IX,[22] for convenience of comparison. It should be remembered, however, that these values may be somewhat high for the micellar reactions (cf. Tables VII and VIII, and the relevant considerations in the Results). The following observations can be made.

(1) Deacetylation of 2.5×10^{-4} M Cys-OMe-N,S-Ac_2 was too slow to be measured in the absence of surfactant micelles at pH 8.0, but did occur ($k = 2.2 \times 10^{-6}\ s^{-1}$) in the presence of 5×10^{-3} M CTACl. Presumably this represents cationic micellar catalysis of OH^- attack[23] on the thioester group.

(2) Cys-OMe-N,S-Ac_2 is probably not very well bound to CTACl under the above conditions. Therefore the observation that AS-Cys-N,S-Ac_2 deacetylates more rapidly in the presence ($4.4 \times 10^{-5}\ s^{-1}$) or absence ($4.8 \times 10^{-5}\ s^{-1}$) of 5×10^{-3} M CTACl suggests that micellar hydroxide ion catalysis of this hydrolysis is at least 20-fold, relative to Cys-OMe-N,S-Ac_2, and probably substantially greater.[37]

(3) With 16-Im as the micellar reagent, an additional 13-fold augmentation of the Cys-OMe-N,S-Ac_2 deacetylation rate constant is observed, relative to the CTACl-catalyzed micellar hydrolysis. The parallel reactions of the AS-Cys-N,S-Ac_2/16-Im comicellar system display a 21-fold enhancement, relative to CTACl. These accelerations are reasonably interpreted as nucleophilic attacks of 16-Im's imidazolyl moiety (probably in its anionic form[4]) on the substrate thioester groups. The acetylimidazole–16-Im thus formed will rapidly hydrolyze.[38] It should be noted that these 16-Im catalyzed reactions were carried out in the presence of IX, which rapidly traps the liberated thiol as the thioester is deacetylated.[22] Back attack of thiolate anion on the newly formed acetylimidazole is thus prevented.[39]

(4) Prevention of such back reaction is crucial in intramolecular systems. Under conditions similar to our own, Klotz[22] found that S-deacetylation of Cys-His peptides was considerably faster (87-fold in the example cited in Table X) when trapping reagent IX was present, and concluded that, although there was a "rapid, reversible intramolecular transfer of the acetyl group between the cysteine and histidine residues which greatly favors cysteine, . . . inhibiting back-attack by cysteine (on) the acyl histidine would allow efficient deacylation to occur through intramolecular nucleophilic catalysis by the imidazole group".[22]

With comicellar AS-Cys-N,S-Ac_2 and 16-Im, acetyl transfer is intermolecular, so that back reaction is less of a problem than it is in the analogous but intramolecular peptide reactions.[22,39] Finally, it is interesting to note the similarity, under comparable conditions, of the rate constants for the S-deacetylations of Klotz's peptide[22] and the AS-Cys-N,S-Ac_2/16-Im comicelle (Table X).

Experimental Section[40]

L-4-Carboxy-2,2-dimethylthiazolidine Hydrochloride (Ia). L-Cysteine hydrochloride (10.0 g, 63.7 mmol) was condensed with excess dry acetone, according to the procedure of Sheehan and Yang,[13] yielding 10.2 g (51.8 mmol, 81%) of Ia, mp 156–160 °C (lit.[13] mp 163–165 °C).

L-4-Carboxy-3-formyl-2,2-dimethylthiazolidine (Ib). Hydrochloride Ia (10.0 g, 50.8 mmol) was reacted with 85 mL of 98% formic acid, 3.9 g (57.4 mmol) of sodium formate, and 28 mL of acetic anhydride, according to the procedure of Sheehan and Yang.[13] We obtained 7.2 g (38.1 mmol, 75%) of Ib, mp 220–222 °C (lit.[13] mp 221–222.5 °C).

L-*N*-*n*-Cetyl-*N,N*-dimethyl-*N*-β-(3′-formyl-2′,2′-dimethylthiazolidine-4′-carboxamido)ethylammonium Chloride (III). A 250-mL round-bottom flask was charged with 2.84 g (15 mmol) of Ib and 30 mL of dry CH_2Cl_2. The solution was stirred magnetically and cooled to −6 °C, whereupon 2.1 mL (15 mmol) of freshly distilled triethylamine was added. After the solution was stirred for 10 min, 1.43 mL (1.62 g, 15 mmol) of freshly distilled ethyl chloroformate was added, and stirring was continued for 20 min. Then there was added a chilled suspension of 5.78 g (15 mmol) of amino surfactant II (AS·HCl)[14] and 2.4 mL of triethylamine in 100 mL of dry CH_2Cl_2.[41] The flask which had contained the suspension was washed with 2 × 20 mL of CH_2Cl_2; the washings were added to the reaction vessel. Stirring was continued at 0 °C for 45 min, and then at 25 °C for 5 h. Dilution with 2 volumes of dry ether precipitated triethylamine hydrochloride, which was removed by suction filtration through a fine fritted funnel. The filtrate was reduced to dryness under vacuum; the residue was taken up in 50 mL of methanol and cooled and stirred for 45 min with 4 g

of sodium carbonate. The resulting suspension was filtered, and the filtrate was stripped of solvent. A gummy residue remained which was taken up in chloroform and filtered through a fine fritted funnel. Concentration of the filtrate yielded a yellow residue which was triturated with freshly distilled (Na/benzophenone) ether. Drying under high vacuum then afforded 4.5 g (8.7 mmol, 58%) of white, crystalline III, mp 180 °C.[42]

NMR ($CDCl_3$, Me_4Si): δ 0.88 (crude t, 3 H, $CH_3(CH_2)_{15}$), 1.28 (s, 28 H, $(CH_2)_{14}$), 1.82 and 1.90 (two s, 6 H, 2′-methyls), 3.40 (s, $^+N(CH_3)_2$) and 4.10–3.23 (m, $^+NCH_2CH_2NH + (CH_2)_{14}CH_2N^+ + 5'\text{-}CH_2$) (total of 3.40 + 4.10–3.23 absorptions = 14 H), 4.98 (t, J = 7 Hz, 1 H, 4′-H), 8.33 (s, 1 H, (C=O)H).

Anal. Calcd for III·H_2O ($C_{27}H_{56}ClN_3O_3S$): C, 60.34; H, 10.43; N, 7.82; S, 5.95. Found: C, 59.97; H, 10.00; N, 7.59; S, 6.46.

***N*-Cetyl-*N,N*-dimethyl-*N*-β-(L-cysteinecarboxamido)ethylammonium Chloride Hydrochloride (IV, AS-Cys·HCl).** Protected surfactant III (3.0 g, 5.8 mmol) was stirred in 10 mL of a 1:1 (volume) 1 N aqueous HCl–methanol solution, under nitrogen, at 25 °C. Every 24 h, an additional 5 mL of the methanolic HCl solution was added, until deprotection was complete.[43] This required 5 days. Most of the methanol was then removed by passing a vigorous stream of nitrogen through the solution. Lyophilization of the remaining solution gave a solid which was washed with freshly distilled, dry ether. Drying under high vacuum gave 2.6 g (5.3 mmol, 91%) of off-white solid IV·HCl, mp 180–182 °C dec.

NMR: this spectrum is described in the Results section.

Ellman's Assay.[15] A 0.1-mL aliquot of a solution of 40 mg of 5,5′-dithiobis(2-nitrobenzoic acid) (IX) in 10 mL of 0.1 M phosphate buffer (pH 8.0) was added to 2.0 mL of 0.1 M phosphate buffer (pH 8.0) containing 1.0 mg of EDTA. The absorbance of the resulting solution at 412 nm was 0.14. A stock solution of 30.5 mg (0.062 mmol) of IV·HCl in 10 mL of water was prepared, and 6 μL of this solution (3.7×10^{-5} mmol of IV) was added to the solution of IX, resulting in a new absorbance at 412 nm of 0.38. From the net absorbance change (0.24) and ϵ_{412} of X (13 600), the final [X] in the solution (equivalent to added IV-SH) was 1.76×10^{-5} M, corresponding (in 2.1 mL of solution) to 3.70×10^{-5} mmol of X or added IV (SH form). The fraction of IV in the free SH form was therefore >0.99 for this sample. Our typical samples showed 0.97 free SH group.

***N*-Acetyl Derivative of IV (V, AS-Cys-NAc).** AS-Cys (41.4 mg, 0.085 mmol) was dissolved in 15.0 mL of deoxygenated water. The pH (3.8) was adjusted to 8.0 with 0.12 M aqueous NaOH. A 0.34 M solution of acetic anhydride in dioxane was then added by syringe in five 50-μL portions (0.085 mmol), while the pH was maintained at 7.5 by the addition of NaOH solution as required. After the addition was completed, the solution was maintained at pH 7.5 for 5 min, then acidified to pH 2.5 with 1 N aqueous HCl, and immediately lyophilized. The fluffy, white residue was extracted with 10 mL of CH_2Cl_2, filtered through a 1.2-nm millipore filter, and evaporated to dryness under high vacuum, affording 38.0 mg (0.077 mmol, 91%) of white, crystalline AS-Cys-NAc, mp 124–126 °C.[44] The NMR spectrum (D_2O) was similar to that of AS-Cys, but revealed a 3 H singlet at δ 2.15 (CH_3CONH). Ellman's assay[15] indicated 0.20–0.25 free SH per molecule.

Reduction of oxidized AS-Cys-NAc for use in kinetics experiments was achieved with dithiothreitol (DTT). In a typical example, a solution of 84 mg of "AS-Cys-NAc" (168 μmol, 60% free SH by Ellman's assay, implies a mixture of $(168 - 100)/2 \sim 34$ μmol of AS-Cys-NAc in the disulfide form and 100 μmol of AS-Cys-NAc) in 2 mL of 0.04 M phosphate buffer (pH 8) was treated with 65 μL of 1 M DTT in deoxygenated water (65 μmol, leading to 65 − 34 = 31 μmol of excess DTT). In the solution used for stopped-flow kinetics, the above solution was diluted with 2 mL of 0.04 M phosphate buffer, so that the final concentrations of AS-Cys-NAc and unreacted DTT became 0.042 and 0.0078 M, respectively.

***S*-Acetyl Derivative of IV (VI, AS-Cys-SAc·HCl).** In a typical preparation, 150 mg (31 mmol) of AS-Cys·HCl was placed in a dry 25-mL round-bottom flask equipped with a stirring bar and a fritted glass tube for N_2 purging. The surfactant was dissolved in 3.0 mL of dry CH_2Cl_2, 2.0 mL of freshly distilled acetyl chloride was added, and the solution was stirred under nitrogen for 4 h at 25 °C. Then 20 mL of dry ether was added to precipitate the surfactant. Supernatant was pipetted away, and the residue was triturated with dry ether. Removal of ether and drying under high vacuum gave 120 mg (23 mmol, 74%) of AS-Cys-SAc·HCl, as an off-white solid, mp 112–115 °C dec.

The NMR spectrum ($CDCl_3$) was similar to that of AS-Cys, but revealed signals at δ 2.33 ($SCOCH_3$) and δ 2.13 ($NHCOCH_3$ of VII) in a ratio of 3:1, respectively; see Results section for further discussion. The IR spectrum (Nujol) showed a strong band at 1690 cm^{-1} (*S*-acetyl).

***N,S*-Diacetyl Derivative of IV (VII, AS-Cys-N,S-Ac$_2$).** AS-Cys·HCl (125 mg, 0.25 mmol) was dissolved in 10 mL of deoxygenated water. The initial pH (3.5) was adjusted to 7.5 by the addition of 0.2 N aqueous NaOH. Immediately, four 50-μL portions of 3 M acetic anhydride in dioxane were added (0.6 mmol) by syringe, while maintaining the pH at 7.5 with the NaOH solution. Ellman's assay[15] indicated the absence of free SH groups. After 5 min of standing, the solution was acidified to pH 2.5 with 0.5 N aqueous HCl and lyophilized. The white residue was extracted with 30 mL of ether/ethanol (7:1 by volume); the extract was filtered through a medium sintered glass funnel. Evaporation of solvents under reduced pressure, followed by trituration with dry ether, and drying under high vacuum gave 75 mg (13 mmol, 52%) of AS-Cys-N,S-Ac_2 as a white, amorphous solid, mp 95–99 °C dec.[45]

The NMR spectrum of VII was similar to that of AS-Cys, but showed 3 H singlets at δ (D_2O) 2.49 and 2.14 ($SCOCH_3$ and $NHCOCH_3$). The IR spectrum (Nujol) revealed strong, broad C=O absorptions at 1660 and 1680 cm^{-1} (*N*- and *S*-acetyl).

Anal. Calcd for $C_{27}H_{54}ClN_3O_3S$: C, 60.44; H, 10.17; Cl, 6.63. Found: C, 59.93; H, 10.49; Cl, 7.53. This was the best of three analyses.

***N,S*-Diacetylcysteine Methyl Ester (Cys-OMe-N,S-Ac$_2$).** This compound was prepared from cysteine methyl ester hydrochloride in a manner completely analogous to the preparation of VII from IV (see above). From 120 mg (0.70 mmol) of starting ester, we obtained 75 mg (0.34 mmol, 49%) of white, amorphous solid Cys-OMe-N,S-Ac_2, mp 89.5–90.5 °C.

NMR ($CDCl_3$): δ 2.03 (s, 3 H, $NHCOCH_3$), 2.33 (s, 3 H, $SCOCH_3$), 3.37 (d, J = 6 Hz, 2 H, CH_2SH), 3.77 (s, 3 H, OCH_3), 4.80 (m, 1 H, CH), 6.50 (m, 1 H, NH). The IR spectrum (Nujol) revealed strong carbonyl absorptions at 1640 (*N*-acetyl), 1700 (*S*-acetyl), and 1730 cm^{-1} (methyl ester).

Anal. Calcd for $C_8H_{13}NO_4S$: C, 43.83; H, 5.97; N, 6.39. Found: C, 43.94; H, 6.08; N, 6.14.

Other Materials. Ellman's reagent and dithiothreitol were supplied in 99% purity by Aldrich Chemical Co., and were used without further purification. CTACl was purchased from Chemical Service, Inc. The commercial surfactant was dissolved in a minimum quantity of hot methanol and precipitated with dry ether. This process was repeated five times to give white crystals, mp 201–204 °C dec. 16-Im was available from previous studies.[4,5] PNPA, purchased from Aldrich Chemical Co., was recrystallized from absolute ethanol, mp 78–78.5 °C (lit.[46] mp 79–80 °C).

Kinetic Studies. We used a Gilford Model 250 spectrophotometer coupled to a Gilford Model 6051 recorder to monitor slower reactions (e.g., k_2 and k_4, Scheme I). Faster reactions (k_1, k_2, and k_3) were followed with a Durrum Model D-130 stopped-flow spectrophotometer, equipped with a Beckman DU-2 monochromater and a Tektronix Model 5103N/D15 storage oscilloscope. Constant-temperature circulating baths maintained sample and reference solutions at 25.0 ± 0.2 °C. All buffers were prepared from steam-distilled water which had been deoxygenated with a nitrogen purge. Buffers were purged again before use. Samples for the Gilford instrument were contained in 3.0-mL Teflon-stoppered quartz cuvettes, and were preequilibrated at 25 °C. Unless otherwise indicated in the Results, rate constants were obtained from computer-generated correlations of log ($A^\infty - A^t$) with time, in the standard manner; correlation coefficients were >0.999, and first-order kinetics were followed over >90% of reaction, unless otherwise specified in the Results section.

Details of the measurement of k_1 and k_3, as well as descriptions of pertinent product studies, appear in the Results section and in Tables I–III and VI.

For stopped-flow studies of k_2, a stock solution of AS-Cys-SAc·HCl was prepared by dissolving 2.2 mg of surfactant in 10 mL of water and immediately lowering the pH to 3 with 0.1 N HCl. This afforded a concentration of 4.2×10^{-4} M, which became 2.1×10^{-4} M under stopped-flow conditions. Buffers used in the stopped-flow experiments were at "double" initial concentrations, so that they afforded standard concentrations (see Table IV) after stopped-flow dilution with an equal volume of substrate stock solution. In Gilford studies of k_2, a 0.012 M solution of AS-Cys-SAc·HCl[47] was prepared by dissolving 6.5 mg of surfactant in 1.0 mL of water which was 0.0013 N in HCl.

S → N transfer reactions were initiated by injecting 40-μL aliquots of this solution into 2.0 mL of 0.02 M phosphate buffers (initial pH 8.0). The final surfactant concentration was 2.4×10^{-4} M and the final pH was 7.8. For other details, see the Results section and Table V. A product study is described in the Results.

S-Deacylation of AS-Cys-N,S-Ac_2 (k_4) was studied in three ways, all of which utilized the Gilford spectrophotometer. Direct studies (at 229 or 240 nm)[48] utilized fresh stock solutions of 11.3 mg of surfactant in 1.0 mL of deoxygenated water (0.021 M). Reactions were initiated by injecting appropriate microliter quantities of stock solution into 0.02 M phosphate buffer, which was 5.0×10^{-3} M in 16-Im. For details, see the Results section.

Studies of k_4 in the presence of Ellman's reagent (IX) employed a stock solution of 41.6 mg of IX in 10.0 mL of 0.1 M phosphate buffer, pH 8.0, containing 0.01% of EDTA. Depending on the reaction, 25–100 μL of this solution was added to the reaction cuvette, which also contained 2.0 mL of phosphate buffer, and any second surfactant (Table VII) at 5.0×10^{-3} M. Reactions were initiated by injecting the desired quantity of AS-Cys-N,S-Ac_2 solution (see above), or, in some cases, a stock solution of Cys-OMe-N,S-Ac_2 (see Results and Table X). The formation of anion X was monitored at 412 nm.[49] Experiments were conducted at several [IX] between 1×10^{-4} and 1.3×10^{-3} M and initial rates were extrapolated to [IX] = 0. For a discussion of this procedure, and further details, see the Results section and Table VII.

For studies of k_4 with PNPA trapping, a 2.6×10^{-3} M AS-Cys-N,S-Ac_2 stock solution was prepared by dissolving 14.1 mg of surfactant in 10.0 mL of 0.02 M phosphate buffer at pH 8.0. Aliquots (2.0 mL) of this solution were equilibrated at 25 °C, 5–20 μL of a 0.10 M solution of PNPA in dioxane was added (final [PNPA] = 2.5×10^{-4} to 1.0×10^{-3} M), and the ensuing reaction was followed at 400 nm. Rate constants were obtained from absorbance changes over the first 3–4 min of reaction as described in the Results.

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(25) The observed "infinity absorbance" was refined until the dependence of ln ($A - A_\infty$) vs. time (up to 60–70% of reaction) gave correlation coefficients >0.999. *Observed* infinity absorbances were typically 0.56–0.66 of the theoretical value, for which competitive oxidation of AS-Cys-NAc was probably responsible.
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(36) Interestingly, reaction of 1 equiv of 0.02 M AS-Cys with 1 equiv of PNPA at pH 8 in phosphate buffer gave only AS-Cys-NAc and *no* AS-Cys-N,S-Ac_2 (NMR), which would have been moderately stable under these reaction conditions (see below). Thus, even under these micellar conditions, *initially formed* AS-Cys-SAc undergoes a sufficiently rapid S → N transfer to avoid competitive intermolecular PNPA acetylation of its free NH_2 group; i.e., AS-Cys-SAc is not trapped as the *N,S*-diacetate.
(37) That the hydrolytic rate constant for 6×10^{-4} M AS-Cys-N,S-Ac_2 at pH 8 is not augmented by the addition of 5×10^{-3} M CTACl suggests that the cmc of AS-Cys-N,S-Ac_2 is less than 6×10^{-4} M. This appears to be reasonable, because the cmc of the more hydrophilic AS-Cys can be estimated to be $\sim 5 \times 10^{-4}$ M (see above).
(38) The rate constant for this N-deacetylation reaction is 1.5×10^{-2} s^{-1} when 16-Im$COCH_3$ is formed from 5×10^{-3} M 16-Im and 2×10^{-4} M PNPA in 0.4 M phosphate buffer at pH 8, 25 °C.[5]
(39) Deacetylation of AS-Cys-N,S-Ac_2 with 5×10^{-3} M 16-Im under the conditions of Table X, but monitored *directly* at 229 or 240 nm, gave $k_{deacet} \sim 3.8 \times 10^{-4}$ s^{-1} (see Results). The 2.4-fold decrease in k_{obsd}, relative to the value determined in the presence of IX (Table X), *may* represent back reaction[22] of 16-Im$COCH_3$ with the thiolate group of product AS-Cys-NAc.
(40) Melting points and boiling points are uncorrected. IR spectra were recorded on a Perkin-Elmer Model 727B instrument. UV spectra were recorded on a Cary Model 14 spectrometer. NMR spectra were determined on a Varian T-60 instrument. Microanalyses were done by Robertson Laboratory, Florham Park, N.J.
(41) The suspension had been stirred for 20 min prior to addition, with cooling, and was cooled to 0 °C just prior to addition.
(42) Liquid crystal formation was observed at 130–150 °C.
(43) Deprotection could be followed by NMR (disappearance of the thiazolidone *gem*-dimethyl signals of III) or by Ellman's analysis (see below).
(44) The melting point of AS-Cys-NAc rises to 145–148 °C after oxidative dimerization.
(45) A liquid crystal forms at 62–64 °C.
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(47) This material was free of AS-Cys-NAc (NMR analysis).
(48) Extinction coefficients for the *S*-acetyl band of AS-Cys-N,S-Ac_2 were determined at 229 (5200) and 240 nm (3075); the latter was independent of pH in the range 8.0–10.0.
(49) The extinction coefficient of X is 13 600 at 412 nm, and is pH independent in the pH range 8.0–10.0.