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A THIOCHOLINE SURFACTANT: PREPARATION AND KINETIC PROPERTIES

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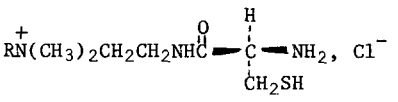
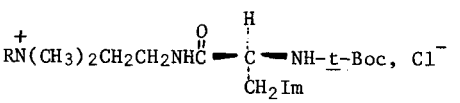
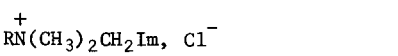
The great nucleophilicity of thiolate anions and the substantial acidity of thiols have made thiol-functionalized surfactant micelles objectives of intense interest,[1-3] both as esterolysis reagents and as analogs of the cysteine proteinases papain and ficin.[4] Initially, "comicellar" reagents were investigated: N-dodecanoyl-D,L-cysteine,[1] alkane thiols,[2] coenzyme A,[3] and glutathione,[3] each solubilized in micellar alkyltrimethylammonium bromides, accelerated the basic cleavage of p-nitrophenyl acetate (PNPA). More recently, we examined the first self-contained, thiol-functionalized surfactant, AS-Cys (I).[5] Although micellar AS-Cys is an excellent reagent for the cleavage of PNPA, previous experience with the imidazole-functionalized surfactants, AS-His-Boc (II)[6] and 16-Im (III),[7] suggested that the unknown thiocholine surfactant IV (16-SH) would be even more reactive than AS-Cys. We now report the preparation of 16-SH and its kinetic behavior with PNPA, where 16-SH proves to be the most reactive self-contained functional micellar reagent yet reported.[8]



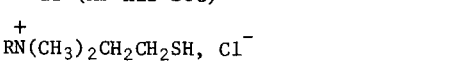
I (AS-Cys)



II (AS-His-Boc)



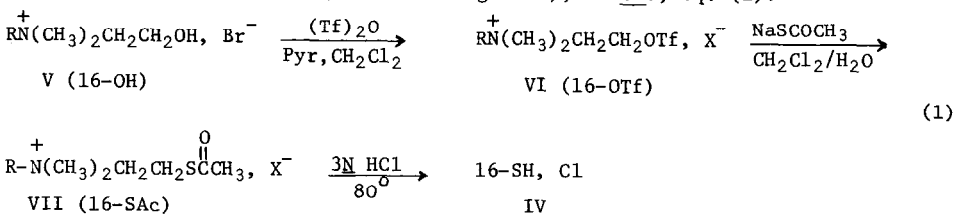
III (16-Im)



IV (16-SH)

[Note: R=n-$C_{16}H_{33}$; Im=4-imidazolyl]

Choline surfactant V (16-OH) was converted to its triflate, VI (2 equiv. Tf_2O, 1 equiv. C_5H_5N, CH_2Cl_2 solution, 25°, 30 min),[9] and the CH_2Cl_2 solution of VI was stirred with excess 1.35 N aqueous sodium thioacetate (25°, 1 hr), dried and stripped, affording VII (16-SAc, X=OTf, mp 60-64°, 81% yield after 3 recryst. from CH_2Cl_2/ether).[10,11] Ion exchange with Dowex 1-X8 (Cl^- form, 25-50 mesh, H_2O, 85-90°, 10 min), followed by filtration and lyophilization, converted 16-SAc, OTf to its water-soluble Cl salt in 90% yield.[10] Treatment of the latter with deoxygenated 3N aq. HCl (N_2 atm, 80°, 1 hr), lyophilization and recrystallization (CH_2Cl_2/ether) gave 80% of 16-SH, Cl, mp 82-84°, > 95% free SH (Ellman's reagent[12]),[13] cf., eq. (1).



(1)

V (16-OH) VI (16-OTf) VII (16-SAc) IV

Cleavage of PNPA by micellar 16-SH[14] was followed by stopped-flow spectrometry at 400 nm and pH 7.0, while nmr experiments demonstrated the sole formation (> 80%) of 16-SAc and p-nitrophenoxide. Variation of [16-SH] over 7 concentrations between 9.24×10^{-4} and 3.02×10^{-2}M gave a (pseudo-first-order) rate constant-[surfactant] profile, Fig. 1, from which $k_{\psi}^{max}=2.16\pm0.02_3$ sec^{-1} at [16-SH]=0.015OM (point C, Fig. 1). At pH 7.96, k_{ψ} was 9.71 sec^{-1} with 0.02OM 16-SH. In Table I, the latter value is compared with analogous rate constants for the cleavage of PNPA by other micellar reagents. For comparison, k_{ψ} for PNPA cleavage by 0.02OM $Me_3\overset{+}{N}CH_2CH_2SH,Br^-$ (prepared by HBr cleavage of commercially available acetylthiocholine bromide) was 0.0197 ± 0.001963 sec^{-1} at pH 8.0 with [thiocholine]=0.0198M. The micellar thiocholine reagent is thus 493 times more reactive than the model compound under comparable conditions.

From the concentration dependence of k_{ψ} at lower concentrations of 16-SH (Gilford model 250 spectrophotometer), the "kinetic" critical micelle concentration (cmc) of 16-SH at pH 7.0[14] is ~4.2×10^{-4}M (point A, Fig. 1).[15,28]

The pH dependence of k_{ψ} was studied in reactions of 4×10^{-3}M 16-SH with 2×10^{-5}M PNPA, μ= 0.05, 23°, employing various buffers at pH 5.00, 6.17, 7.00, 7.97, 9.55, and 9.84. A plot of log (k_{ψ}) vs. pH gave a sharp break-point at pH 7.3, which we take as the pK_a of micellar 16-SH.[18] For comparison, two independent determinations of pK_a for $Me_3\overset{+}{N}CH_2CH_2SH$ afforded 7.81 (30°) to 7.95 (20°)[19a] and 7.80 (temp. not reported).[19b]

From comparisons with other functional micellar reagents (Table I), other surfactant-thiol systems,[1-3,5] and thiocholine, 16-SH emerges as an extraordinarily reactive micellar reagent.[20] It is more reactive than other previously synthesized, self-contained, functional surfactants on the PNPA scale,[21] and relative to the appropriate non-micellar model, displays a greater reactivity enhancement than do other surfactant-thiol systems.[1-3,5] Indeed, toward PNPA, 16-SH (k_{cat}=144, pH 7.0, 23°) is about as reactive as ficin (k_{cat}~173, pH 6.9, 29.6°).[23]

Micellization[14] lowers the pK_a of 16-SH (7.3) relative to that of non-micellar $Me_3\overset{+}{N}CH_2CH_2SH$ (~7.85[19]), such that 29% of the 493-fold greater reactivity of the micellar thiocholine surfactant is attributable to the acid-strengthening effect of micellization and the consequent increase in the concentration of reactive thiolate ions.[24] The remaining catalytic advantage, a factor of 353, must have other origins. The extensive ionization of micellar 16-SH (i.e., zwitterion formation) at pH 8[24] results in substantial "internal" charge neutralization at the micellar surface; the concomitant hydrophobicity and accompanying desolvation may account for much of the residual enhanced reactivity[22] of micellar 16-SH.

Micellar 16-SH is ~3600 times more reactive than its choline analog, 16-OH (Table I, k_{cat}^{rel} scale), for which estimated pK_a's range from 10.5[25] to 12.4.[26] Obviously, much of the catalytic advantage of the thiocholine surfactant micelles derives from greater ionization; just how much of the advantage can be attributed to factors other than acidity differences cannot be determined in the absence of a definitive pK_a for 16-OH.[27] We are continuing our studies of 16-SH.

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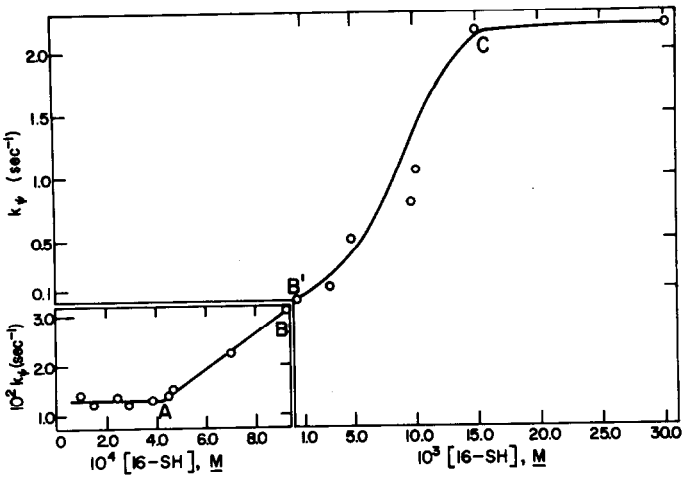

Figure 1. Pseudo-first-order rate constants for the pH 7 cleavage of PNPA by 16-SH vs. [16-SH]. Point A is the kinetic cmc of 16-SH, and point C is taken as k_ψ^{max} for reaction of PNPA with micellar 16-SH. Points B and B' represent identical data plotted on two different scales.

TABLE I. Cleavage of PNPA Catalyzed by Surfactant Micelles[a]

Catalyst	k_ψ^{max} (sec^{-1}) [b]	k_{cat} (1/mol-sec)[c]	k_{cat}^{rel}	Ref.
CTACl[d]	0.00019 [1.35]	0.014	1.0	6
16-OH[e]	0.00190 [1.4]	0.136	9.7	7
AS-His-Boc	0.029 [1.6]	1.8	130.	6
16-Im[e]	0.20 [4.0]	5.0	360.	7
AS-Cys	1.04 [4.0]	26.0	1860.	5
16-SH	9.71[f] [2.0]	485.	34600.	g

[a]See text for catalyst structures; all counterions are chloride. The reaction pH was 8.0; see ref. 14 for other conditions. [b]Values in [ ] are concentrations (Mx100) at which k_ψ^{max} was determined. [c]$k_{cat} = k_\psi^{max}/[surfactant]$. [d]Cetyltrimethylammonium chloride. [e]0.01M PO_4 buffer. [f]pH 7.96. [g]This work.

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(8) Professor U. Tonellato, University of Padua, has independently prepared 16-SH, and will separately report the synthetic and kinetic studies carried out in his laboratory. We thank Professor Tonellato for exchanges of correspondence.
(9) Nmr indicated that the CH_2OH multiplet of 16-OH (centered at δ4.07, $CDCl_3$) was quantitatively replaced by the corresponding multiplet of VI at δ5.07.
(10) Structurally consistent ir and nmr spectra, and a satisfactory elemental analysis were obtained.
(11) The self-phase transfer reaction of 16-OTf and thioacetate is an example of a more general procedure for the synthesis of functional surfactants: R. A. Moss and W. J. Sanders, submitted for publication.
(12) A.F.S.A. Habeeb, Methods Enzymol., 25, 457 (1972); G. M. Whitesides, J. E. Lilburn, and R.P. Szajewski, J. Org. Chem., 42, 332 (1977).
(13) Nmr (δ, $CDCl_3$): 0.87 ("t", 3H, CH_3); 1.27 ["s", 28H, $(CH_2)_{14}$]; 3.24 [brd. m, 12H, $CH_2\overset{+}{N}(CH_3)_2CH_2CH_2SH$]; the 3H S-Ac singlet of VII (δ2.40) was absent in the spectrum of IV.
(14) Conditions: 0.02M N_2-purged PO_4 buffer, μ=0.05, 23°, [PNPA]=2×10^{-5}M.
(15) For the 6 high concentration points of Fig. 1, 16-SH with 72% free SH[13,16] was used, except that >95% free-SH material was employed for point A. For low concentration runs, 16-SH with 55-79% Ellman activity was used in 4 cases; material with >72% activity was used in 4 other experiments. In our experience, all samples of 16-SH with activity >55% gave comparable kinetic results with PNPA, as long as 16-SH was micellar and in substantial excess.
(16) Despite precautions, micellar 16-SH at pH ≥7 rapidly loses activity, presumably by oxidative dimerization.[17]
(17) Cf., S. Shinkai, R. Ando, and F. Yoneda, Chem. Lett., 147 (1977); S. Shinkai and T. Kunitake, Bull. Chem. Soc. Japan, 50, 2400 (1977).
(18) Details will appear in our full paper. Experiments with pH ≥7 employed the stopped-flow spectrometer with double-strength solutions of 16-SH and PNPA at pH 1-2 (several drops of HCl) in syringe A, reacted with NaOH-doped double strength buffer (syringe B); the final pH was determined after reaction.
(19)(a) G. A. Bagiyan, S. A. Grachev, I. K. Koroleva, and N. A. Soroka, Izv. Akad. Nauk SSSR, Ser. Khim., 990 (1976); (b) Ya. L. Kostyukovskii, et. al., Zhur. Obsh. Khim., 42, 2104 (1972).
(20) Analysis of the data in Fig. 1 by methods analogous to those employed in enzymatic catalysis (cf., J. H. Fendler and E. J. Fendler, "Catalysis in Micellar and Macromolecular Systems," Academic Press, New York, 1975, pp. 86ff.) affords k_m=2.53 sec^{-1} and K/N=31.4 for 16-SH + PNPA at pH 7. As anticipated, $k_m \sim k_\psi^{max}$; assuming an aggregation number, N, of ~60, the binding constant, K~0.52, indicating relatively poor binding of PNPA to 16-SH micelles.
(21) This includes AS-Cys,[5] as well as n-dodecyl-(2-hydroxyimidophenethyl)dimethylammonium bromide.[22]
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(24) From the pK_a's, micellar 16-SH is ~83%, and the model thiocholine is ~59% ionized at pH 8.
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(27) Choosing pK_a~10.5 for 16-OH, ionization would be ~0.3% at pH 8. This affords a maximum non-acidity-based catalytic advantage for 16-SH of (0.3/83x3600)~13.
(28) The facile oxidative dimerization of 16-SH precludes simple cmc determinations by conventional static procedures (i.e., surface tension).

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