Effect of Detergents on the S- to N-Acyl Transfer of S-Acyl-β-mercaptoethylamines

Iolanda M. Cuccovia, Elsie H. Schröter, Roberto C. de Baptista and Hernan Chaimovich*

Instituto de Química, Departamento de Bioquímica, Universidade de São Paulo, Caixa Postal 20780, São Paulo, Brasil

Received March 23, 1977

The rate of S- to N-acyl transfer of S-octanoyl-β-mercaptoethylamine (OMA) is enhanced by hexadecyltrimethylammonium bromide (CTAB) micelles by 4.6-fold and slightly inhibited by the nonionic detergent Brij-35. The rate of the S to N transfer of S-acetyl-β-mercaptoethylamine (AMA) is unaffected by CTAB or Brij. Micelles of a negative detergent, sodium dodecyl sulfate, inhibit the rate of S to N transfer of AMA by 100-fold; the inhibition in the case of OMA is 5×10^3-fold. An increase in the apparent pK of the ammonium ion and a decrease in the conformational mobility of OMA are proposed to account for the observed results.

Micelles modify the rates and/or product distribution of a number of reactions in both aqueous and nonaqueous solvents (for recent reviews, see ref 1–3). The usefulness and limitations of the "micelle model" for the understanding of the catalytic mechanism of enzymes have been discussed.[1-3] Since distortion of a part of the substrate has been shown to be an important factor in some enzyme-catalyzed reactions,[4] it might be expected that micellar modeling of distortion would be more feasible in intra- rather than intermolecular reactions due to the high fluidity and mobility of micelles.[3]

This work is concerned with the effect of detergent on the S- to N-acyl transfer in mercaptoethylamines. The mechanism of this reaction is well known as a result of a number of studies.[5-10] In the early work of Martin and co-workers,[6] it was demonstrated that acyl transfer occurs via the formation of a cyclic intermediate of the type previously demonstrated for the S- to O-acyl transfer in the $CH_3COS(CH_2)_nOH$ series. Above pH 6, partitioning of the cyclic intermediate back to the starting thioester is negligible, resulting in the production of the amide as the only product.[11] The occurrence of a rate-limiting proton-transfer step was later proposed by Barnett and Jencks.[10] The fact that the rate of the S- to N-acyl transfer in mercaptoethylamine is insensitive to the addition of high salt concentration or of solvents like butanol[10] facilitates the analysis of the effect of detergent on this complex reaction; thus microsolvent effects[3] or the high effective ionic strength at the stern layer of a micelle would not be expected to alter significantly the reaction rate.

The data for the effect of detergents on the rate of S- to N-acyl transfer on mercaptoethylamines presented here demonstrates a strong rate inhibition by negative detergents, a negligible effect by nonionic detergents, and a small catalysis by positive detergents.

Experimental Section

CTAB was obtained from Merck, Darmstadt, Germany (pro Analysis grade Lot 252534), and was recrystallized three times from acetone–ethanol.

SDS was obtained from BDH, Poole, England (specially pure grade Lot 1262984), and was recrystallized from ethanol and shown to be free from sulfate, alcohols, or higher analogues by acid hydrolysis and vapor phase chromatography of the ether extracts.

S-Acetyl-β-mercaptoethylamine·HCl (hereafter referred as AMA) was synthesized by a published procedure;[12] mp 137–138 °C (lit. mp 137 °C[12]).

S-Octanoyl-β-mercaptoethylamine·HCl (OMA) was synthesized as described; mp 105–108 °C (lit. mp 111–113 °C[11]); S analysis; IR, UV, and NMR spectra are all in accord with structure.

All other reagents were analytical grade. Water which had been deionized and twice distilled in glass was used throughout.

Kinetics. The kinetics of the intramolecular conversion of S-acylmercaptoethylamines to the corresponding amides was followed spectrophotometrically at 25 °C (Masterline Forma Scientific bath) in the thermostated compartment of a Gilford recording spectrophotometer. The reaction was followed at 229 nm, and the measured extinction coefficient of the thioesters corresponded to those described.[8] The reaction cuvettes, containing all additions except the thioester, were checked for pH, after temperature equilibration, with a Metrohm pH meter equipped with a microcombination electrode. The pH rechecked after each kinetic run did not change. All reactions were started by adding 10 μL of a freshly prepared aqueous stock solution of the thioester (ca. 10^{-3} M). The stock solution was never used for more than 1 h. Reactions were followed for at least 4 half-lives. The first-order rate constants were calculated from log $(A_0 - A_t)$ vs. time plots.

NMR measurements were carried out in a Varian T-60 spectrometer.

Results

The cationic detergent hexadecyltrimethylammonium bromide (CTAB) catalyzed the S- to N-acyl transfer of S-octanoyl-β-mercaptoethylamine (Figure 1), the rate constant increasing sharply at CTAB concentrations greater than 3×10^{-4} M. The critical micelle concentration (cmc) of CTAB in water is 9×10^{-4} M.[3] It is known that both the addition of salts or amphiphilic electrolytes[1,3] can lower the cmc of this detergent. Taking 3×10^{-4} M as the effective cmc under our kinetic conditions, it is possible to calculate the maximum rate acceleration and the partition coefficient of the substrate between water and the micellar pseudophase.[13] According to this model, a unimolecular reaction can be treated using

$$k_\psi = \frac{k_m K(C_D - \text{cmc}) + k_o}{K(C_D - \text{cmc}) + 1} \quad (1)$$

where k_ψ is the observed first-order constant, C_D is the total detergent concentration, k_o is the observed first-order rate constant in the absence of detergent, K is the distribution coefficient of the reagent, and k_m is the rate constant in the micellar phase. k_m and K were iterated from an initial value of k_m taken as the observed first-order rate constant at the plateau (Figure 1). Only the data up to 5.5×10^{-3} M CTAB were used in this calculation. At pH 6.8 in 0.01 M NaH_2PO_4, k_o is 0.026 s^{-1}, the calculated k_m under the same conditions is 0.12 s^{-1}, so the rate acceleration by CTAB is 4.6-fold.

In order to discard the possibility of self-aggregation of the substrate that would modify the observed rate constant for the uncatalyzed reaction, the concentration of OMA was varied between 2.8×10^{-5} and 4.2×10^{-4} M. It was found that between these limits the first-order rate constant did not change. The values of k_ψ obtained at high detergent concentration deviate from the predicted values, indicating that the model described by eq 1 accounts only partially for all the data.

The inhibitory effect of sodium dodecyl sulfate (SDS) in this system is more pronounced than the (small) catalytic effect observed with CTAB (Figure 2).

Various models were considered in order to fit all the inhibition data. These models were based on assumptions of formation of aggregates containing substrate and SDS in definite

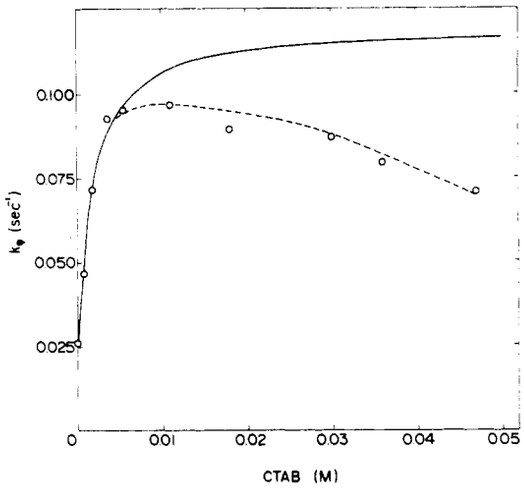


Figure 1. Catalysis of the S- to N-acyl transfer of S-octanoyl-β-mercaptoethylamine by CTAB. Solid line is calculated (see text).

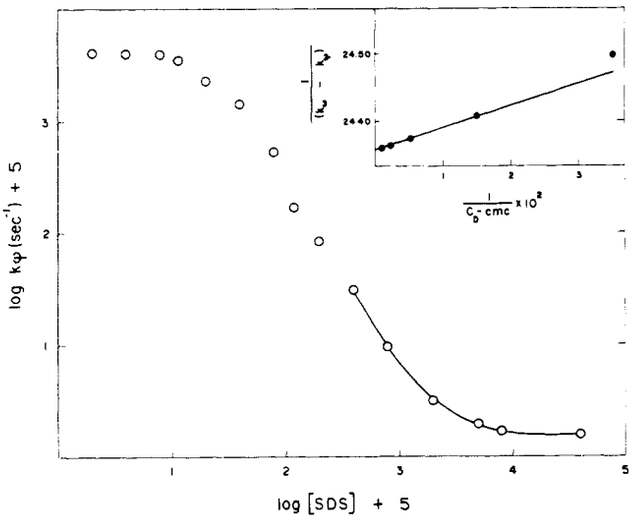
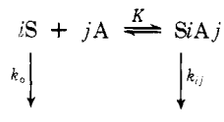


Figure 2. Effect of SDS on the S- to N-acyl transfer of S-octanoyl-β-mercaptoethylamine (OMA). Solid line is calculated, inset shows this same region of SDS concentration plotted according to eq 2 (see text). Initial concentration of OMA, 2×10^{-4} M.

proportion over the entire SDS concentration range used as shown in Scheme I. In this scheme, i represents the moles of OMA in the substrate–detergent aggregate, j the number of moles of detergent in the aggregate, K the equilibrium constant, and k_{ij} the rate constant for the S to N transfer in an aggregate with i moles of OMA and j moles of SDS. This type of model has been successfully employed in other aminolysis systems.[14] However, these models failed to give internally consistent results in this case. This failure is probably a reflection of multiple equilibrium between SDS and the substrate that results in an ensemble of mixed aggregates between OMA and SDS with varying i, j (Scheme I), and consequently, different k_{ij}. The i to j ratio would be expected to change with detergent concentrations. As the inhibition by SDS is readily observable at OMA/SDS ratios higher than 1 (Figure 2), the aggregates may even consist of $i > j$.

Scheme I



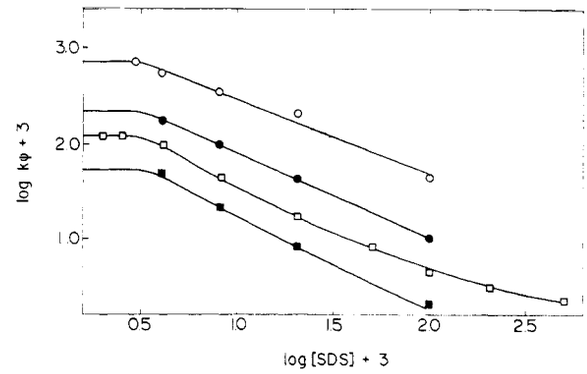


Figure 3. Effect of SDS on the S- to N-acyl transfer of S-acetyl-β-mercaptoethylamine. Solid lines were calculated according to eq 1; (■) pH 6.4, (□) pH 6.8, (●) pH 7.14, (○) pH 7.8. All buffers contained 0.02 M NaH_2PO_4 adjusted with NaOH to the desired pH.

Table I. Effect of Brij 35 on the Rate of S- to N-Acyl Transfer of S-Octanoyl-β-Mercaptoethylamine[a]

Brij 35, M × 10^4	$k_\psi \times 10^2$, s^{-1}
	4.4
2.7	4.3
5.5	4.4
11.0	3.7
22.0	4.0
55.0	2.5

[a] All reactions were carried out at 25 °C in 4.0×10^{-2} M Na phosphate buffer, pH 6.80; initial concentration of OMA was 2.54 × 10^{-4} M.

The experimental points obtained at SDS/OMA > 10, when the aggregate can be viewed as a typical SDS micelle, were fitted to the same distribution model[13] outlined above for the catalysis of the same reaction by CTAB (solid line in Figure 2) and is amplified in the inset using another form of eq 1:

$$\frac{1}{k_o - k_\psi} = \frac{1}{k_o - k_m} + \frac{1}{K(k_o - k_m)} \times \frac{1}{C_D - \text{cmc}} \qquad (2)$$

The iterative procedure gives $K = 8.2 \times 10^4$. Distribution coefficients of this order of magnitude had previously been observed in an intermolecular aminolysis system using a reactive micelle and oppositely charged ester as a substrate.[14] The k_m was calculated to be 1.5×10^{-5} s^{-1} and under those conditions cmc was taken as 1.2×10^{-3} M.

The rate of the S to N transfer is slightly inhibited by a nonionic detergent (Brij 35) (Table I).

In order to make an assessment of the relative importances of hydrophobic and electrostatic contributions to the effects of detergents described above, the S to N transfer reaction was studied using S-acetyl-β-mercaptoethylamine (AMA) as substrate. The rate of the S to N transfer reaction of AMA is unaffected by the addition of a positive detergent up to 100-fold the critical micelle concentration. This (lack of) effect is to be expected on the basis of previous data on aminolysis of charged esters[14] and indicates that AMA does not incorporate significantly in the CTAB micellar phase. SDS inhibits the rate of the S to N reaction of AMA (Figure 3). The inhibition results can be fitted to a simple distribution model using eq 1. The calculated parameters (k_m and K) were further analyzed varying the pH between 6.4 and 7.8 (Table II).

The maximum inhibition (k_o/k_m) by micellar SDS is consistently 100-fold at all the pHs studied. The rate increase with pH is consistent with the previously reported effect of pH in this reaction.[10] Both the effect of pH on k_m and on the distribution coefficient of the substrate (Figure 3 and Table II) indicate that the protonated form of II binds better to the

Table II. Effect of Sodium Dodecyl Sulfate on the Rate of *S*- to *N*-Acetyl Transfer in *S*-Acetyl-β-mercaptoethylamine[a]

pH	k_o, s^{-1}	k_m, s^{-1}	K
6.4	0.054	4.0×10^{-4}	322
6.8	0.122	1.52×10^{-3}	375
7.14	0.234	2.0×10^{-3}	279
7.8	0.722	7.0×10^{-3}	202

[a] All reactions were carried out at 25 °C in 2.0×10^{-2} M Na phosphate buffers. Initial substrate concentration was usually 2×10^{-4} M. See text for the description of the calculation of k_m (rate constant in the micellar phase) and K (distribution constant of substrate). k_o represents the rate constant in the absence of added detergent.

Table III. Line Broadening of the ^1H NMR Signal of the Bridge Methylene Hydrogens of $CH_3(CH_2)_6COS(CH_2)_2NH_3Cl$ (OMA) by Sodium Dodecyl Sulfate (SDS)[a]

OMA, M	SDS, M	Line width, Hz
0.025		1.6
0.025	0.125	2.6
0.025	0.188	2.0
0.025	0.250	2.0
0.025	0.375	2.0
0.025	0.500	2.0
0.050		1.7
0.050	0.220	5.6
0.050	0.325	3.6
0.050	0.375	3.6
0.050	0.500	4.0

[a] See Results and Experimental Section for details. Under the conditions used the uncertainty in the determination of the width at half-height is 0.1 Hz.

micelle, as expected for an association that is predominantly ionic.

As the effect of SDS on OMA can, as a limit, be ascribed to a simple effect on the acid dissociation constant of the amine (vide infra), we have carried out a limited nuclear magnetic resonance investigation of the OMA/SDS complexes in order to try to distinguish effects other than a pK shift of the terminal amine. A series of aqueous solutions of OMA containing various concentrations of SDS were prepared. It was calculated that under these conditions (pH 5.0 ± 0.2; measured at 1:100 dilution) due to both the pH decrease (see Table II) and the addition of SDS, NMR measurements could be carried out before any measurable change in the concentration of OMA could occur. The width at half-height of the signal from methylenic bridge group increases (Table III) and then decreases to a plateau value which is significantly higher than that of the width of the free compound. This variation in the width of the lines is consistent with our previous contention of the existence of a continuum of OMA/SDS aggregates with varying proportions. Detailed investigations of this complex were limited by the very low solubility of the complex(es) at these high OMA concentrations. Moreover, the $C_2(H_2)$ triplet of the octanoyl chain broadens considerably with the first SDS additions, making it impossible to resolve clearly. These results constitute an indication of decrease of conformational freedom of OMA upon incorporation to SDS aggregates.

Discussion

Micellar catalysis or inhibition often arises from concentration of the reagents in the micellar phase; this effect is, of course, absent in mono- and intramolecular reactions. If the uncatalyzed reaction is very sensitive to changes in the solvent or to the addition of electrolytes, rate modifications by micelles will reflect directly the incorporation of the substrate into a medium different from water. Thus, rate modifications by micelles are seldom interpretable in terms of intrinsic effects of the detergent aggregates on the reactivity of the substrate.

The most probable source of the inhibitory effect of SDS on the *S*- to *N*-acyl transfer of mercaptoethylamines would be a pK shift of the terminal ammonium ion caused by electrostatic interaction with the negative surface of the micelle. In the case of long-chain acyl substrates, a decrease in the flexibility of the substrate caused by both surface and hydrophobic contributions could lead to additional stabilization of the initial state, and thus increased inhibition, by the negatively charged micelle.

The simplest explanation for the rate decrease of the *S*- to *N*-acyl transfer of AMA is an increase in the pK of the ammonium ion. Indeed a 100-fold inhibition, that is observed for AMA, can be accounted for by an increase in pK of 2 pH units. pK displacements of this magnitude have been measured or inferred[1-3] in a number of micelle modified reactions. Were this effect to be the only inhibitory factor, the rate of reaction of the unprotonated form in the Stern layer would be unaffected. This would suggest little penetration of the amine into the micellar phase and is in accordance with the small degree of hydrophobic stabilization afforded by a single CH_3 group and the small distribution constant measured for AMA. As the pK of this ammonium ion has been reported as 9.1[8], the apparent pK on the SDS micelles would be of the order of 11.

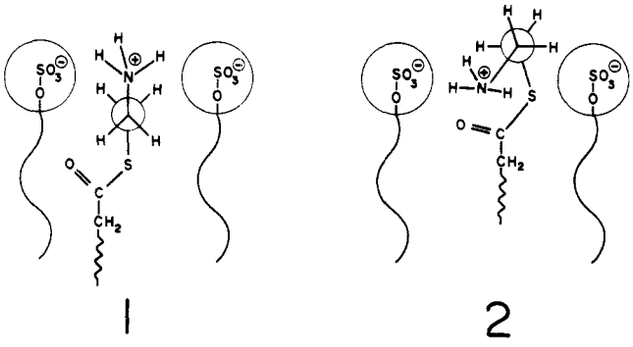
At high SDS/OMA ratios, where the inhibitory effect can be quantitatively analyzed, the micellar rate is slower than the water rate by a factor of 5×10^3. A pK shift is unlikely to be the sole cause of this inhibition. Although the effect of SDS on the pK of aliphatic amines has not been described, its effect on H_o for primary, secondary, and tertiary aromatic amines is, at the maximum, 1.25 units.[15] Moreover, the decrease in pK of dodecylammonium upon micellization (which can be visualized as the reverse of the effect of incorporation of an alkyl ammonium ion into a negative micelle) is not higher than 1.4 pH units.[16] This consideration and the effect of SDS on AMA allows one to set an upper limit of 2 pH units to the pK displacement produced by SDS on OMA. Thus, even taking into account the pK shift contribution, leading to a 100-fold inhibition, a rate decrease of, at least, 50-fold has to be explained.

This latter inhibition factor is most readily accounted for by assuming a decrease in the flexibility of substrate. Indeed the NMR data are unambiguous in the indication that the signal due to the methylene bridge protons is broadened by SDS. No signal splitting is observed under our conditions, strongly suggesting that the broadening is due not to a removal of the degeneracy, but rather to a decrease of the conformational freedom of this segment of the substrate.

Solutes have considerable mobility in the micellar phase,[3,17,18] and the motional freedom along the surfactant chain is only modestly restricted; however, the maximum motional restriction has been observed near the polar end of the detergent chain,[19] and the type of movement of the substrate can be restricted according to the relationship between the substrate and the micellar surface.[20] A good example of this is the observation that the addition of SDS causes an increase in the population of one of the isomers of *N*-octanoylsarcosinate,[21] demonstrating that favorable interactions between substrate and micelle can stabilize, significantly, one particular configuration of the substrate.

From the values of association constants of hydrophobic substrates with oppositely charged micelles,[14] it was expected that protonated OMA would interact favorably with SDS micelles, and this is reflected in the large value for the distri-

Scheme II



1 2

bution coefficient for this substrate. That this interaction has both electrostatic and hydrophobic components is implied by the 250-fold smaller distribution coefficient for AMA. The hydrophobic, or electrostatic interaction(s), will tend to stabilize the amine segment in the Stern layer; the hydrophobic contribution, on the other hand, will favor the insertion of the long acyl chain in the interior of the micelle. These interactions will favor an elongated configuration of the substrate (form 1, Scheme II). The molecular movement of OMA in SDS micelles will thus be nonisotropic, in the sense that the attainment of the bent conformation, necessary for attack leading to products, will be highly unfavorable (form 2, Scheme II) due, in part, to the exposure of the methylenic bridge to the solvent. Rotational anisotropy of a negatively charged spin probe (*N*-oxyl-4′,4′-dimethyloxazolidine derivative of 5-ketostearic acid) incorporated into a positively charged micelle has recently been described.[22] It has also been proposed, in a SDS inhibited system, that the ionic array of SDS with a positively charged substrate is tight in order to explain the observed stereoselectivity[23] of the reaction.

The rate of S to N transfer in OMA is enhanced about fivefold in the micellar phase of CTAB. The simplest explanation of this (small) effect would be a decrease in the pK of the terminal amine, thus increasing the concentration of the reactive (unprotonated) species. In a related system, it has been shown that micellization of dimethyl dodecyl ammonium chloride produces both an increase in the proton-exchange rate and a decrease in the pK of the ammonium ion of 1.4 pH units.[13] Taking this latter system as references, it would be expected that, in the absence of other effects, the rate acceleration caused by CTAB in the S to N transfer of OMA should be at least 30-fold. The rate acceleration obtained is significantly smaller, and the kinetic results can not be accommodated within the framework of a simple distribution model. This constitutes an indication of the occurrence of a mixed activation–inhibition effect by CTAB on this reaction.

Acknowledgments. This work was partially supported by Project Bioq/FAPESP. The authors thank Dr. F. Quina for his helpful comments and revision of the manuscript.

Registry No.—CTAB, 57-09-0; SDS, 151-21-3; AMA, 17612-91-8; OMA, 17612-92-9; Brij 35, 9002-02-0.

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Mechanism of the Meyer–Schuster Rearrangement

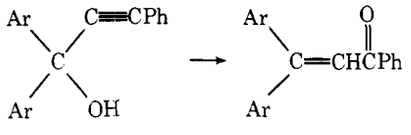
Michael Edens, David Boerner, Carol Roane Chase, David Nass, and Melvyn D. Schiavelli*[1]

Department of Chemistry, College of William and Mary, Williamsburg, Virginia 23185

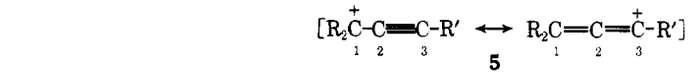
Received January 25, 1977.

The mechanism of the Meyer–Schuster rearrangement of tertiary arylpropargyl alcohols to α,β-unsaturated carbonyl compounds is discussed. The data, inverse solvent isotope effects, k_{H_2O}/k_{D_2O} = 0.36–0.48, ρ vs. σ^+ = −2.3 at the reaction site and −1.6 at the rearrangement terminus, $(k_H/k_d)_\alpha$ at the rearrangement terminus = 0.92, and relatively large negative $\Delta S^\ddagger$, all suggest an ion–dipole intermediate undergoing nucleophilic attack by H_2O as the rate-determining step. The rearrangement of eight triaryl- and diarylpropargyl alcohols is reported.

In 1922 Meyer and Schuster reported that triarylpropargyl alcohols, **1** and **2**, were converted in good yield to α,β-unsaturated ketones **3** and **4** by a variety of acidic catalysts such as CH_3COOH/H_2SO_4, HCl in ether, acetic anhydride, and acetyl chloride.[2] Several reviews concerned with the Meyer–Schuster and related Rupe rearrangements have appeared within the last 10 years.[3,4] Each suggests that alkynyl cations such as **5** are involved in the Meyer–Schuster rear-



1 Ar = Ph **3**
2 Ar = *p*-ClC_6H_4 **4**



5