Functional Micellar Catalysis. Part 5.[1] Catalysis of Activated Amide Hydrolysis by Hydroxy and Imidazole Functionalized Surfactant Systems

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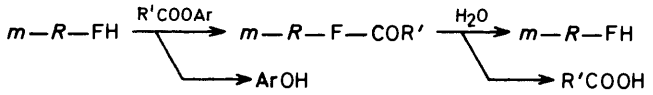
The hydrolysis of *N*-(n-butyl)-2,4-dinitrotrifluoroacetanilide (IX), *N*-acetylimidazole (Xa), and *N*-hexanoylimidazole (Xb), in the pH range 7—8.5, is catalysed by micelles and co-micelles (with cetyltrimethylammonium bromide) of functional surfactants containing the hydroxy-group and/or the imidazole ring [(I)—(VIII)]. The kinetic effects here observed are significantly different from those previously reported for the same micellar reagents in the hydrolysis of *p*-nitrophenyl alkanoates. Such differences are ascribed to a change in the rate-limiting step of the acylation of the micellar functions, assuming a common nucleophilic mode of action of the functional micelles in the cleavage of activated esters and amides.

In Part 4,[1] we reported on the micellar catalytic properties [2] of a number of functional surfactants containing the hydroxy-group (also in the activated form of oxime and hydroxamic acid derivatives) and/or the imidazole ring, as models of trypsin-like enzymes, in the hydrolysis of *p*-nitrophenyl esters (acetate, PNPA, and hexanoate, PNPH). The mode of action of the micellar surfactants (*m*-*R*-FH in Scheme 1) was shown to involve (nucleophilic) acylation and deacylation of the FH function. The acylation rates in the pH range 7—8 were found to depend on the apparent pK_a of the micellar functions, *i.e.* on the fraction of dissociated FH groups: once corrected for such fraction, the apparent catalytic rate constants, k_c, were found to be similar for all the functional surfactants investigated, their apparent pK_a values ranging from 8 to 12.8.



Scheme 1

A quite different trend was observed in an analogous study of the catalytic activity of the same functional surfactants in the hydrolysis of activated amides. We here report the kinetic results obtained for micellar solutions of surfactants (I)—(VIII) for the hydrolysis of *N*-(n-butyl)-2,4-dinitrotrifluoroacetanilide (IX). Some of these surfactants have also been tested in the hydrolysis of the *N*-acylimidazoles (Xa and b). A preliminary account of this investigation has been reported.[3]

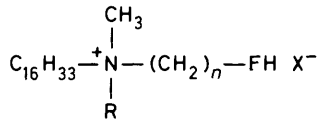
RESULTS

The synthesis and general properties of surfactants (I)—(VII) have been reported.[1,4-7]

A systematic kinetic analysis of the micellar effects of the surfactants for the hydrolysis of anilide (IX) was made for aqueous solutions of co-micelles of cetyltrimethylammonium bromide (CTABr) and the functional surfactant, D^f, under the conditions used in previous studies: [1,8] (CTABr) : $[D^f]$ = *ca.* 6 : 1, Tris buffers of constant ionic strength (μ 0.05, KCl). With the conditions $[D^f] \gg$ [substrate], the pseudo-first-order rate constants, k_ψ, obtained by following the appearance of *N*-(n-butyl)-2,4-dinitroaniline at 365 nm, may be expressed [8] by equation (1) where k_c

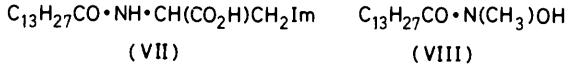
$$k_\psi = (k_o + k_c[D^f]_m)/(1 + K_s[D^t]_m) \qquad (1)$$

is the apparent catalytic rate constant, K_s the binding constants of the substrate to the micellized surfactants, $[D^f]_m$ the concentration of micellar functional detergent, and $[D^t]_m$ the total detergent concentration in micelles. The k_o term is the pseudo-first-order rate constant which may be approximated [8] to that observed for micellar solutions of CTABr alone at $[CTABr]_m = [D^t]_m$. Under the chosen conditions, the hydrolysis of (IX), at variance with that of *p*-nitrophenyl alkanoates,[1] is catalysed by CTABr ($k_{\psi max.}$/

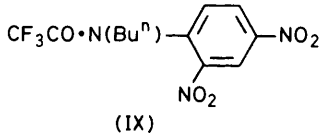


(I) - (VI)

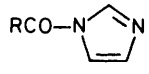
(I) R = Et, *n* = 2, FH = OH, X = Br
(II) R = Me, *n* = 1, FH = C(Ph)=NOH, X = Br
(III) R = Me, *n* = 1, FH = CO·C(Ph)=NOH, X = Cl
(IV) R = Me, *n* = 1, FH = Im, X = Cl
(V) R = Me, *n* = 2, FH = NH·CO·Im, X = Cl
(VI) R = $(CH_2)_2OH$, *n* = 1, FH = Im, X = Cl



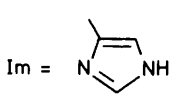
(VII) (VIII)



(IX)



(X)
a; R = CH_3
b; R = C_5H_{11}



k_{buffer} 8.5 at pH 7.15 and 16 at pH 7.95) and the raw kinetic data were corrected as described [8] to evaluate the k_c and K_s parameters. In the case of the imidazole functionalized surfactants (IV) and (V), the k_ψ values are very close to those of k_o: the k_c constants are therefore very approximate estimates. In the case of myristoylhistidine (VII), the rate–concentration profile was lower than that of CTABr alone, *i.e.* $k_\psi = (0.6—0.7)k_o$, corresponding to an inhibitory effect of (VII). The k_c constants are in Table 1; the K_s values are in the range $(1.9—2.5) \times 10^3$ l mol^{-1}.

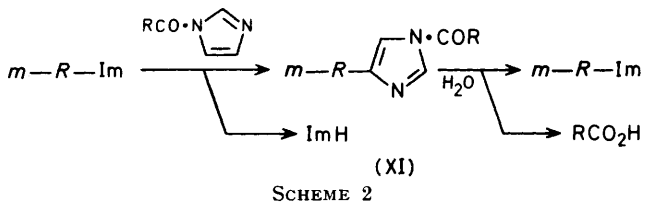
TABLE 1

Apparent catalytic rate constants (k_c/l mol^{-1} s^{-1}) for the hydrolysis of (IX) in Tris buffers [a] at 25 °C

pH	(I)	(II)	(III)	(IV)	(V)	(VI)	(VII)	(VIII)
7.15	9.9	6.2	4	*b*	1.0	10.5	*c*	3.2
7.95	53	37	25	2.8	6.6	49	*c*	13

[a] Other conditions: see text. [b] The k_ψ values are too close to k_o to allow an estimate of k_c. [c] Inhibition is observed (see text).

In a complementary set of kinetic measurements, we tested the catalytic effect of homogeneous micelles of (I) and (III)—(VI) in the hydrolysis of amide (IX), and of (I) and (IV)—(VI) in the hydrolysis of (Xa and b), in 0.02M-borate buffer at pH 8.4. The rate–concentration profiles of the acylimidazoles (X) were obtained from k_ψ values determined by following the disappearance of the substrates at 243 (Xa) and 247 nm (Xb). In this case, when imidazole functionalized surfactants (IV)—(VI) were tested, we were concerned with the possibility that the spectrophotometric measurements at those wavelengths might be complicated by the formation and build-up [4b,9] of the intermediates (XI) ($\lambda_{max.}$ *ca.* 245 nm) following acyl transfer from the substrate



SCHEME 2

to the functionalized surfactant, *m*-*R*-Im (Scheme 2). We have verified that under the conditions used, [surfactant] ≫ [substrate], the hydrolysis of PNPA and PNPH promoted by micellar reagents (IV)—(VI) proceeds without any detectable accumulation of intermediates (XI): an isosbestic point is observed in the region 245—247 nm. Since acylation of the imidazole ring of surfactants (IV)—(VI) is faster in the case of PNPA and PNPH than in that of (Xa and b) respectively, we inferred that in the hydrolysis of acylimidazoles, intermediates (XI), if formed, do not accumulate and are therefore hydrolysed faster than they are generated. In each case, the disappearance of (X) followed clean first-order kinetics. The apparent catalytic rate constants are in Table 2. They were obtained by the application [8] of equation (2), equating k_o to k_{buffer}. The K_s values were in the range $(2.0—2.5) \times 10^3$ for (IX),

$$k_\psi = (k_o + k_c[D^f]_m)/(1 + K_s[D^f]_m) \qquad (2)$$

$(3—4.2) \times 10^2$ for (Xa), and $(1.4—1.9) \times 10^3$ l mol^{-1} for (Xb). Under these conditions, the hydrolysis of the acylimidazoles (X) is little affected by CTABr {$k_{\psi max.}/k_{buffer}$ 3.3, [(Xb)], [CTABr] = 4×10^{-3}M} while the hydrolysis of (IX) is much accelerated by the nonfunctionalized surfactant ($k_{\psi max.}/k_{buffer}$ 165, [CTABr] 4.5×10^{-3}M; see also Table 2).

Micellar effects on the hydrolysis of the amides (IX) and (X), in particular of (X), are more pronounced [3] in 0.02M-borate than in Tris (μ 0.05) of the same pH. Additional experiments also showed that micellar effects are very sensitive to changes in the ionic strength.

TABLE 2

Apparent catalytic rate constants [a] (k_c/l mol^{-1} s^{-1}) for the hydrolysis of amides [b] (IX) and (Xa and b) in 0.02M-borate buffer at pH 8.4 and 25 °C

	(I)	(III)	(IV)	(V)	(VI)
(IX)	870	450	27	40	530
(Xa)	10.5		0.3		6.6
(Xb)	97		2.3	3.5	38

[a] The 'apparent' k_c values for CTABr are 32 (IX), 0.6 (Xa), and 1.9 (Xb). [b] $10^4 k_{buffer}$ 3.3 (IX), 9.2 (Xa), and 3.5 s^{-1} (Xb).

DISCUSSION

The main features emerging from the present study are the following. (a) The catalytic effects on the hydrolysis of activated amides, in the pH range 7—8.5, of the hydroxy and imidazole functionalized surfactants (I)—(VIII) are not greatly differentiated, the k_c values of the various micellar reagents for each amide (see Tables 1 and 2) being within a factor of <40. (b) The choline-type surfactant (I) is in each case the most effective reagent, the hydroxy and imidazole bifunctionalized surfactant (VI) is only slightly less reactive than (I) while the imidazole functionalized reagents (IV), (V), and (VII) are the least effective surfactants of the series. This picture is dramatically different from that obtained [1] in the case of *p*-nitrophenyl esters: (a) in the pH range 7—8, huge differences (>10^4) in the k_c values are observed. (b) Surfactant (I) is by far the least effective catalyst, much less reactive than any of the imidazole-containing surfactants (IV)—(VII).

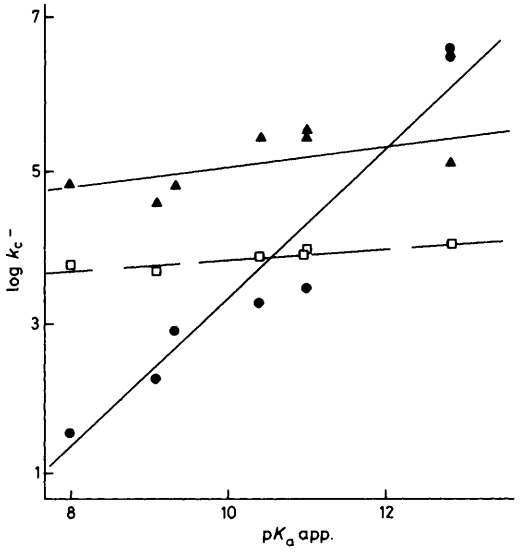
It is here assumed that the mechanism of amide cleavage [10] by the functional surfactants is similar to that proven in the case of *p*-nitrophenyl alkanoates, involving acylation and deacylation (a fast process in the case of the trifluoroacetanilide) of the nucleophilic function. It is also assumed that the effective nucleophilic species in the acylation step are the conjugate bases of the functional groups, F^-, of the surfactants with the exception of myristoylhistidine (VII) which is not dissociated [6,11] to any significant extent in the pH range explored. A limited support to this assumption comes from the increase of the k_c values on going from pH 7.15 to 7.95 (see Table 1) and from the fact that myristoylhistidine (VII) is not a reagent in the hydrolysis of the anilide (IX) (see below).

Particularly in the hydrolysis of (IX), the effect of functional cationic micelles is complicated by concurrent counterion, probably OH^-, catalysis [12] as revealed by the strong effect of micelles of the non-functionalized surfactant CTABr. The k_c values of Table 1 are a measure of the differential catalytic effect of the micellar functional surfactants relative to that of micellar CTABr alone.

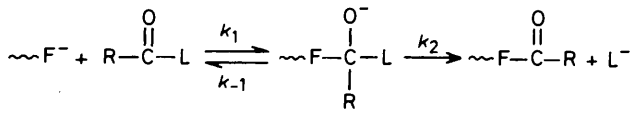
The inhibitory effect of myristoylhistidine (VII) in co-micelles may well be due to partial neutralization of the positive charge of the cationic micelles due to the carboxylate residue of the histidine derivative which may determine a lesser concentration of reactive counterions at the micellar surface. Among the other functional surfactants, only (III) (pK_a app. 8.0 [1]) whose function is significantly dissociated to its anionic form and (VIII) which is a neutral reagent, may give partial neutralization of the positive charge of the co-micelles relative to that of micelles of CTABr alone and the k_c values of (III) and (VIII) of Table 1 may therefore be taken as lower limits of the apparent catalytic rate constants.

The k_c values of Tables 1 and 2 for the bifunctional reagent (VI) are very close to those of the choline-type surfactant (I) and larger than those of any of the imidazole functionalized surfactants (IV), (V), and (VII). This is better illustrated by the kinetic data for homogeneous micelles in Table 2: here, the k_c values are a measure of the gross catalytic effect, including counterion catalysis. When the latter effect is taken into account, the k_c values are rather different. Micelles of the imidazole functionalized surfactant (V) are even less effective than those of CTABr [at least in the hydrolysis of (IX) and (Xa)], while those of the bifunctional (VI) are only less than twice less reactive than those of (I). A similar trend was observed by O'Connor and Porter [13] in the hydrolysis of *N*-ethyl-4-nitrotrifluoroacetanilide. Thus, according to the kinetic evidence, the effective group in bifunctional (VI) is the hydroxy-group and not imidazole (as in the hydrolysis of *p*-nitrophenyl esters).

With the above assumptions, the catalytic rate constants of Table 1 may be corrected for the fraction of anionic forms of the micellar reagents here investigated from known apparent pK_a values and the approximation [8] $k_c^- = k_c(1 + [H^+]/K_a \text{ app.})$. A plot of log k_c^- *versus* pK_a app. is in the Figure together with those obtained for the same functional surfactants in the hydrolysis of PNPA and PNPH. Although one cannot attach any precise meaning to the slopes of these Brønsted plots for such widely differing nucleophilic reagents,[14] the evident variation between the esters and (IX) indicates a substantial change in the detailed mechanism of the acylation process. The simplest explanation assumes a full change in the rate-limiting step, assuming the operation of Scheme 3, from formation of the tetrahedral intermediate (XIII) (PNP esters) to its collapse to the transient product [anilide (IX)]. This seems reasonable considering the nature of R and L of (XII) for the two types of substrates. A change of R from alkyl to trifluoromethyl and L from *p*-nitrophenolate (pK_a 7.02 [15]) to *N*-(n-butyl)-2,4-dinitroanilide ion (pK_a *ca.* 15 [16]) must dramatically increase the ratio $k_1 : k_2$ (and $k_{-1} : k_2$) on going from the esters to the anilide.



log k_c^- *versus* pK_a app. for the functional reagents (I)—(VIII), in the hydrolysis of PNPH ▲, PNPA □, and anilide (IX) ●



(XII) (XIII)

SCHEME 3

The kinetic analysis in the case of acylimidazoles (X) has been restricted to a few functional surfactants: the observed trend (Table 2) parallels that observed in the case of anilide (IX). Here too, we assume that the rate-limiting step is the breakdown of the tetrahedral intermediate (XIII). Interestingly, in the case of surfactants (IV) and (V) both F^- and L^- (Scheme 3) are imidazolyl residues; however, F^- is a weaker base than L^- by at least 3 pK_a units [4] and thus the ratio $k_2 : k_1$ must be correspondingly low.

Based on these and previous results, the catalytic activity of functional micelles, such as those here explored, in the hydrolysis of carboxylic acid derivatives and their analogy with enzymes are better defined: large kinetic benefits, near to those of enzyme reactions, can only be expected for activated substrates with good leaving groups.

EXPERIMENTAL

The synthesis of surfactants (I)—(VIII) has been reported.[1,4-7] *N*-Acetylimidazole (Xa), m.p. 104 °C (lit.,[17] 104 °C), *N*-hexanoylimidazole (Xb), m.p. 24—35 C° (lit.,[17] 35 °C), and *N*-(n-butyl)-2,4-dinitroaniline, m.p. 89—90 °C (lit.,[18] 90 °C), were prepared and purified by literature procedures.

N-(*n-Butyl*)-2,4-*dinitrotrifluoroacetanilide* (IX).—*N*-(n-Butyl)-2,4-dinitroaniline (2.0 g) was dissolved in freshly distilled trifluoroacetic anhydride and one drop of concentrated H_2SO_4 was added to the solution. After 2 h at room temperature, the mixture was poured into an iced (1 : 1) carbon tetrachloride–water mixture and rapidly extracted. The organic layer was washed with iced water and dried over Na_2SO_4 and Drierite. Removal of the solvent yielded an oily residue which was purified by chromatography on a

short column packed with silica gel, using chloroform–pentane as eluant. The *product*, a yellow oil (0.15 g) (Found: C, 42.7; H, 3.75; N, 12.3. $C_{12}H_{12}F_3N_3O_5$ requires C, 43.0; H, 3.6; N, 12.55%) upon hydrolysis in Tris buffer at pH 7.95 yielded 99.7% ($\lambda_{max.}$ 363 nm, ε 15 700) *N*-(n-butyl)-2,4-dinitroaniline.

The general procedure for kinetic measurements has been described.[1,4] In the hydrolysis of (IX) the appearance of the aniline was monitored at 360 nm, in that of (Xa and b) the disappearance of the substrate was followed at 243 and 247 nm respectively, using a Gilford 2400 or Varian Cary 219 spectrophotometer.

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