Tetrahedron Letters No. 15, pp 1275 - 1278, 1975. Pergamon Press. Printed in Great Britain.

MECHANISM OF THE CATALYSIS BY FUNCTIONAL MICELLES CONTAINING A HYDROXY GROUP. A MODEL OF ACTION OF SERINE PROTEINASES

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(Received in UK 6 January 1975; accepted for publication 5 March 1975)

Surfactant micelles catalyze the alkaline hydrolysis of esters (see reviews [1,2]). Introduction of a hydroxy group to a surfactant molecule greatly enhances micellar catalysis [3,4]. Unfortunately, no adequate explanation has so far been offered to account for the role of this functional group in the acceleration of these reactions. Hypothetical mechanisms for micellar catalysis offered in the literature are contradictory, cf. ref's 5 and 6. We have studied the kinetics of the hydrolysis of p-nitrophenylheptanoate (NPH) in the presence of cationic micelles of octadecyldiethylhydroxyethylammonium bromide, $HO\text{-}CH_2CH_2\text{-}N^+Et_2C_{18}H_{37}Br^-$. The reaction rate was followed spectrophotometrically, by the liberation of p-nitrophenol (400 nm); the experimental procedure is described in detail in ref. 7.

We have found that the hydroxy group which is a part of the surfactant molecule (SA-OH) behaves as a nucleophilic agent. This is indicated, among other things, by the pattern of the "product-time" curve for the conditions when the initial concentration of the ester exceeds by far the initial concentration of micelle-forming nucleophile, i.e. at $[NPH]_o \gg [SA\text{-}OH]_o$ (see Fig. A). At these ratios of the reagents' concentrations, the reaction kinetics has two phases, i.e. at first a rapid release (a burst) of p-nitrophenol,

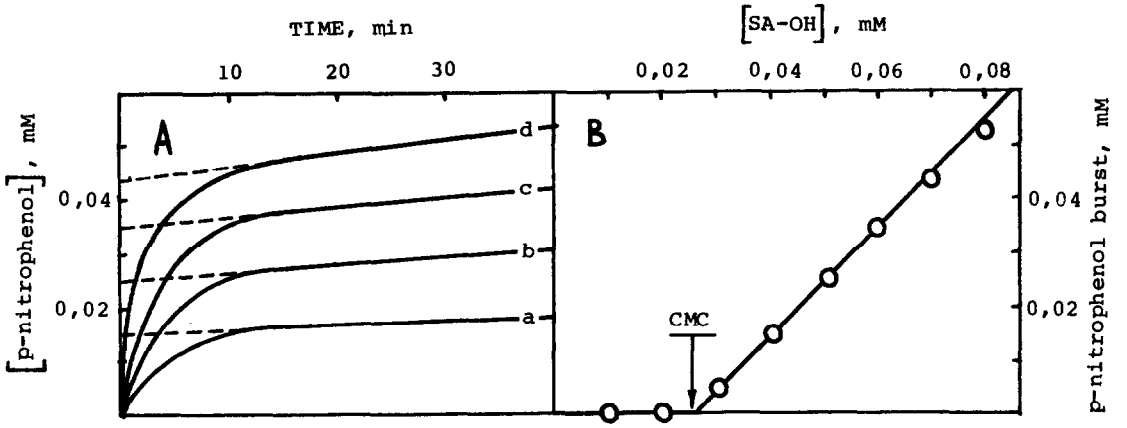

Figure A The product-time curves for p-nitrophenol liberation during NPH hydrolysis in the presence of SA-OH micelles.

B The dependence of p-nitrophenol burst upon the concentration of the surfactant.
Conditions: 30°, pH 8.5 (0.02 M borate-buffer), 0.02 M KCl; 1 v% DMSO, $[NPH]_o$ = 2,5 x 10^{-4}M, $[SA-OH]_o$ x 10^{2}M: a - 4, b - 5, c - 6, d - 7

then a slower stationary process. Such kinetics cannot be explained in terms of the inhibition of the reaction by the products, since adding p-nitrophenol and heptanoic acid does not change this biphasic character of the reaction. The kinetic mechanism of this phenomenon is the following: (i) in the rapid (prestationary) stage there occurs acylation of the hydroxy group in the SA-OH molecule; (ii) then follows the hydrolysis of the intermediate acylsurfactant, which is the rate-determining step of the process. That this mechanism is correct is proved by the following facts: (i) p-nitrophenol corresponding to the primary release (see the region of the ordinate in Fig. A) is formed in an equimolar ratio with respect to the SA-OH concentration in the micelles, as is shown by the data in Fig. B; (ii) the stationary rate of the over-all process (found by the slope of the linear part of any curve in Fig. A) is equal to the ratio of hydrolysis of the intermediate heptanoyl derivative of the micelle-forming surfactant. To prove this, we have specially synthesized $SA-OCOC_6H_{13}$ according to the procedure described in ref. 7 (cf. the data in the Table).

TABLE. Equilibrium and rate constants for the scheme (1)-(3). Conditions: pH 8.5 (0.02 M borate buffer), 30°, 0.02 M KCl, 1 v % DMSO

$k_{2,m}K/V$ $l\ min^{-1}mole^{-1}$	K $l\ mole^{-1}$	$k_{2,m}/V$ min^{-1}	$k_{3,m}$ min^{-1}	
-	-	-	0.007 ± 0.002	a
-	-	-	0.005 ± 0.0005	b
19000	1900	10	-	c
1350	5000	0.27	-	d
-	5250	-	-	e

[a] At $[NPH]_o \gg [SA\text{-}OH]_o$, where $[SA\text{-}OH]_o$ slightly exceeds CMC

[b] Hydrolysis of the specially synthesized $SA\text{-}O\text{-}COC_6H_{13}$ (in the absence of NPH)

[c] At low ionic strength and $[NPH]_o < [SA\text{-}OH]_o$

[d] At high ionic concentration of the solution (0.2 M KNO_3) neutralizing the electrostatic potential of the micelle and $[NPH]_o < [SA\text{-}OH]_o$

[e] Found in an independent experiment from the dependence of the solubility on the surfactant concentration at pH 5.5 (the procedure is described in ref.9)

So the mechanism of hydrolysis of NPH in the presence of SA-OH micelles may be presented in the following way:

$$NPH_{\text{in water}} \underset{\text{instantaneously}}{\overset{K}{\rightleftharpoons}} NPH_{\text{on the micelles}} \qquad (1)$$

$$(NPH + SA\text{-}OH)_{\text{on micelles}} \xrightarrow[\text{rapidly}]{k_{2,m}} SA - O\text{-}COC_6H_{13} + \text{p-nitrophenol} \qquad (2)$$

$$(SA\text{-}O\text{-}COC_6H_{13} + OH)_{\text{on micelles}} \xrightarrow[\text{slowly}]{k_{3,m}} SA\text{-}OH + \text{heptanoate} \qquad (3)$$

where $K = (P-1)V$, P is the partition coefficient equal to $[NPH]_{\text{on micelles}}/[NPH]_{\text{in water}}$, V is the molar volume of the surfactant and k_{2m}, k_{3m} are the rate constants. The experimental data on the dependence of the stationary rate of the over-all process (1)-(3) on the surfactant concentration, obtained at different concentration ratios of the ester and the micelle-forming nucleophile, were analyzed in terms of the kinetic theory [8-10]; the constants pertaining to scheme (1)-(3) are listed in the Table (a more detailed kinetic analysis is given in ref. 7).

The following comparison may characterize the effectiveness of the micellar catalysis in system (1)-(3): the second order rate constant for NPH alcoho-

lysis under the action of triethylhydroxyethyl ammonium bromide $Br^{-}Et_3N^{+}CH_2$-CH_2OH, which forms no micelles, has been found by us to be 0.062 $min^{-1}mole^{-1}$, which is 10^{-5} of the corresponding rate constant of the "micellar" reaction, $k_{2,m}$ K/V (see the Table). Such a high acceleration occurs not only as a result of a heightening of the ester concentration on nucleophile micelles, but also due to the apparent pK_a shift of the hydroxy group by the action of the surface charge of the micelle. The value of the latter factor (more than one order of magnitude) may be estimated from the decrease of the catalytic effect (the decrease in the rate constant $k_{2,m}$ K/V), observed when the ionic strength of the solution increases, see the Table. Besides, that, the pK_a value for $Br^{-}Et_3N^{+}CH_2CH_2OH$ (forming no micelles) has been found by us in an independent experiment as being equal to 12.8 ± 0.3, whereas the pK_a for SA-OH in the micelle is 10.5 ± 0.5 (for details see ref. 7).

It should be noted by way of conclusion that the micellar model studied has a certain similarity to catalysis by serine proteinases, both with respect to the mechanism (the participation of the alkoxy ion as a nucleophile) and by formal kinetic manifestations (three steps of the reaction involving the sorption and acylated intermediates) and by the order of magnitude of the rate acceleration [7,11].

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