Efficient Deacylation of *N*-Acylimidazoles by Functionalized Surfactant Micelles

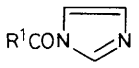
Yasuji Ihara *
Yamaguchi Women's University, 3-2-1 Sakurabatake, Yamaguchi 753, Japan
Mamoru Nango and Joichi Koga
Department of Applied Chemistry, University of Osaka Prefecture, Sakai, Osaka 591, Japan

Hydroxylated surfactant micelles are powerful catalysts for the deacylation of *N*-acylimidazoles under neutral conditions; the deacylation rates of hydrophobic acylimidazoles are accelerated remarkably by functionalized micelles containing three hydroxy groups at the polar head.

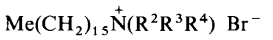
Studies on the catalytic hydrolysis by α-chymotrypsin and related enzymes of *p*-nitrophenyl esters suggest that reaction occurs by initial acylation of the active site of the imidazole followed by acyl transfer to the serine hydroxy group.[1] The catalytic hydrolysis of *p*-nitrophenyl esters by micelles composed of functionalized surfactants normally occurs by a nucleophilic mechanism with subsequent decomposition of an acylated micellar intermediate.[2–6] We have previously demonstrated that large rate enhancements of the deacylation process during the hydrolysis of *p*-nitrophenyl esters by *N*-acylhistidine or dipeptide catalysts occur in the presence of hydroxylated surfactant micelles.[7,8]

We now examine simple enzyme models for acyl transfer reactions. The present communication describes the remarkable effect of hydroxylated surfactant micelles on the deacylation of *N*-acylimidazoles under neutral conditions.

Kinetic studies were carried out at a fixed concentration of the surfactant. The deacylation of the substrates was examined under the following conditions; [surfactant] ≫ [substrate], pH 7.30, 0.02M MOPS buffer, and 25 °C. Table 1 summarizes the results for the deacylation of four acylimidazoles (**1a—d**) in the presence of micelles of surfactants (**2a—d**). The deacylation rates were dependent on the concentration of the surfactants used. The deacylation of the *N*-acylimidazoles in the presence of (**2a**) (CTAB) is only 1.0—2.7 times faster than in bulk solution, whereas the hydroxylated surfactants (**2b—d**) greatly enhance the deacylation rate constants. Thus, the reactions of hydrophobic acylimidazoles with (**2d**) gave the largest deacylation rate enhancements; the relative deacylation rate constant ratio compared to the bulk solution was 1 370 for (**1c**) and 2 830 for (**1d**) in 1.00×10^{-3}M surfactant. These large deacylation enhancements can be ascribed to intermolecular acyl transfer from the imidazole to the hydroxy groups. This behaviour is associated with the incorporation of the acylimidazoles on to the surface of the micelles, leading to an effective orientation for the attack of the hydroxy groups.



(1)



(2)

a; R^1 = Me
b; R^1 = $Me(CH_2)_4$
c; R^1 = $Me(CH_2)_8$
d; R^1 = $Me(CH_2)_{12}$

a; $R^2 = R^3 = R^4$ = Me(CTAB)
b; $R^2 = R^3$ = Me, $R^4 = (CH_2)_2OH$
c; R^2 = Me, $R^3 = R^4 = (CH_2)_2OH$
d; $R^2 = R^3 = R^4 = (CH_2)_2OH$

The most interesting result in this experiment is the large difference in deacylation rates among the hydroxylated surfactants (**2b—d**). The deacylation rate constants increase sharply with the number of hydroxy groups in the surfactant

Table 1. Rate constants for deacylation of *N*-acylimidazoles in the presence of surfactant micelles[a]

	$k_d \times 10^3/s^{-1}$ (relative rate compared to bulk solution)			
Surfactant[b]	(**1a**)	(**1b**)	(**1c**)	(**1d**)
None	0.120 (1)	0.109 (1)	0.130 (1)	0.107 (1)
(**2a**)	0.120 (1)	0.112 (1.03)	0.271 (2.08)	0.289 (2.70)
(**2a**)[c]	0.127 (1.06)	0.157 (1.40)	0.203 (1.58)	
(**2b**)	0.266 (2.22)	1.73 (15.9)	21.2 (163)	34.6 (323)
(**2b**)[c]	1.38 (11.5)	9.50 (87.2)	22.3 (172)	
(**2c**)	0.731 (6.09)	6.54 (60.0)	74.0 (569)	105 (981)
(**2c**)[c]	4.48 (37.3)	31.4 (291)	70.0 (538)	
(**2d**)	1.26 (10.5)	8.66 (79.4)	178 (1 370)	303 (2 830)
(**2d**)[c]	11.8 (98.3)	80.2 (735)		

[a] At pH 7.30, 0.02M MOPS buffer, and 25 °C; [surfactant] = 1.00×10^{-3}M, [substrate] = 1.00×10^{-4}M. From three or more independent experiments, we estimate that the rate constants are reproducible to ±4%. [b] The c.m.c. values of the surfactants are $2—5 \times 10^{-4}$M under the experimental conditions used (determined from the kinetic measurements). [c] [Surfactant] = 1.00×10^{-2}M.

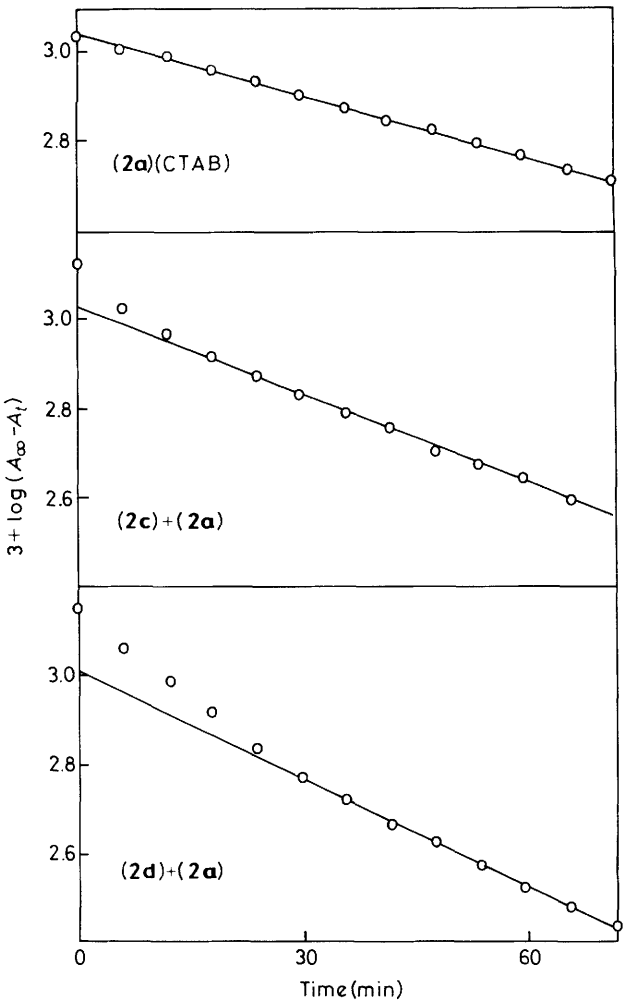


Figure. The product-time curves for the deacylation of *N*-acylimidazoles in the presence of surfactant micelles. *Conditions:* pH 7.30, 0.02M MOPS buffer, 25 °C; [(**2a**)] = 1.00×10^{-2}M, [(**2c**) or (**2d**)] = 1.00×10^{-3}M, [substrate] = 1.0×10^{-2}M.

molecule, suggesting that the acyl transfer reaction is sensitive to catalytic activity of the hydroxy functions of the surfactant. This probably reflects the microenvironment and acidity of the hydroxy groups, and suggests that a hydroxy group in micelles of (**2d**) is strongly activated by intramolecular hydrogen bonding which causes the lowering of the pK_a values.[9,10] The ^{1}H n.m.r. chemical shifts (in $CDCl_3$) of the hydroxy proton signals for (**2c**) and (**2d**), δ 4.80 and 4.74 respectively, were displaced to low magnetic field relative to that for (**2b**) at δ 5.06, consistent with such an activation of the hydroxy groups in (**2c**) and (**2d**). The micellar deacylation of (**1a**) with (**2b**—**d**) was carried out in the pH range 8—10. The slopes of the log k_d *versus* pH plots show a linear increase in the order (**2d**) > (**2c**) > (**2b**) in these pH regions. This result also suggests an apparent pK_a shift of the hydroxy group by the action of the surface charge of the micelle. Above pH10 the reaction rates were too great to be measured.

In order to obtain more information about the mechanism of the deacylation process, the reactions in (**2a**) were examined using an excess of substrate over the hydroxylated surfactants (see Figure). In the presence of the functionalized surfactants, the reaction kinetics have two phases; an initial 'burst' process, then a slower stationary state process. The reaction products

Table 2. Kinetic analysis under the burst conditions[a]

Surfactant system	Reaction ratio[b] (**1a**)	Reaction ratio[b] (**1b**)	$k_d \times 10^4$ (s^{-1}) After burst (**1a**)	$k_d \times 10^4$ (s^{-1}) After burst (**1b**)
(**2a**)			1.22	1.81
(**2b**) + (**2a**)	0.7	0.8	1.45	1.86
(**2c**) + (**2a**)	1.1	1.2	1.53	1.82
(**2d**) + (**2a**)	1.8	1.7	1.72	2.34

[a] At pH 7.30, 0.02M MOPS buffer, 25 °C; [(**2a**)] = 1.00×10^{-2}M, [(**2b**—**d**)] = 1.0×10^{-3}M, [substrate] = 1.0×10^{-2}M. [b] The mole ratios of the reaction products based on (**2b**—**d**) were determined from the intercepts of the *y* axis of the product time plots.

corresponding to the burst process are formed in 0.7—0.8, 1.1—1.2, and 1.7—1.8 molar ratios with respect to the (**2b**), (**2c**), and (**2d**) concentrations in the total surfactants, respectively (Table 2). Kinetic analysis of the stationary state process (after the burst process) shows that the deacylation rates obtained in the presence of hydroxylated surfactants are similar to those obtained with a non-functionalized surfactant. These results show that acyl transfer from the imidazolyl to the hydroxy group is the probable deacylation mechanism in the present catalytic system.

In conclusion, the present study clearly shows that hydroxylated surfactants are powerful catalysts for the deacylation of *N*-acylimidazoles. The deacylation involves interactions between hydroxy groups and the acylimidazoles, and the *N*-decanoyl (**1c**) or *N*-myristoyl-imidazole (**1d**) incorporated in the micelles is then positioned for attack by the hydroxy function in the micellar phase, resulting in great acceleration of deacylation rates.

Experimental

The synthesis of surfactants (**2b**—**d**) has been reported.[7] *N*-Acylimidazoles (**1b**—**d**) were prepared and purified by literature procedures.[11]

The deacylation rate constant (k_d) was measured spectrophotometrically by following the disappearance of the substrate absorption at 240—250 nm. In most experiments the surfactant concentration was much larger than that of the substrate. The kinetics were first order and good least-squares rate constants were obtained ($r > 0.999$). We also examined the reaction using an excess of substrate over the functionalized surfactant. Excess of non-functionalized surfactant (CTAB) was added because of the limited solubility of the substrate.

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