Deacylation Effect in the Hydrolysis of *p*-Nitrophenyl Esters by Bifunctional Comicelles Containing Imidazolyl and Hydroxyl Groups

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Synopsis. The rate constants for hydrolysis of *p*-nitrophenyl acetate, hexanoate, and dodecanoate catalyzed by bifunctional catalysts containing the imidazolyl and hydroxyl groups have been determined in the presence of hexadecyltrimethylammonium bromide micelles at 25 °C. The kinetic analysis suggests a nucleophilic mechanism which involves acylation followed by deacylation at the imidazolyl group. Although no appreciable differences in cooperative catalytic efficiencies are observed between the bifunctional groups in the acylation step, it is found that the deacylation rates are accelerated by the hydroxyl group of the catalyst in the presence of micelles.

In the past several years, many micellar enzyme models have been investigated in order to gain further insight into the nature of enzyme reactions[1-5] and, particularly, bifunctional micellar catalysis has been reported as models of esterolytic enzymes for the hydrolysis of *p*-nitrophenyl esters. The catalytic hydrolysis of *p*-nitrophenyl esters by micelles derived from functional surfactants normally occurs by a nucleophilic mechanism with subsequent decomposition of an acylated micellar intermediate.[3a,4,5] We have previously demonstrated the large rate enhancements in deacylation process during the hydrolysis of *p*-nitrophenyl esters by *N*-acylhistidine or dipeptide catalysts in the presence of hydroxyl functionalized surfactant micelles.[6]

The present study describes the results for deacylation effect of bifunctional comicellar catalysts containing the imidazolyl and hydroxyl groups for the hydrolysis of *p*-nitrophenyl acetate (PNPA), hexanoate (PNPH), and dodecanoate (PNPL) in the presence of hexadecyltrimethylammonium bromide (CTAB) micelles. The catalysts used in this experiment are *N*-decanoyl-L-histidine (**1a**) and *N*-benzyloxycarbonyl-L-phenylalanyl-L-histidine (**2a**), both having an imidazolyl group, and *N*-acyl-L-tyrosine (**1b,c**) and *N*-decanoyl-L-serine (**1d**), both having a hydroxyl group, or *N*-benzyloxycarbonyl-L-tyrosyl-L-histidine (**2b**) and *N*-benzyloxycarbonyl-L-histidyl-L-tyrosine (**2d**), both having imidazolyl and hydroxyl groups.

Experimental

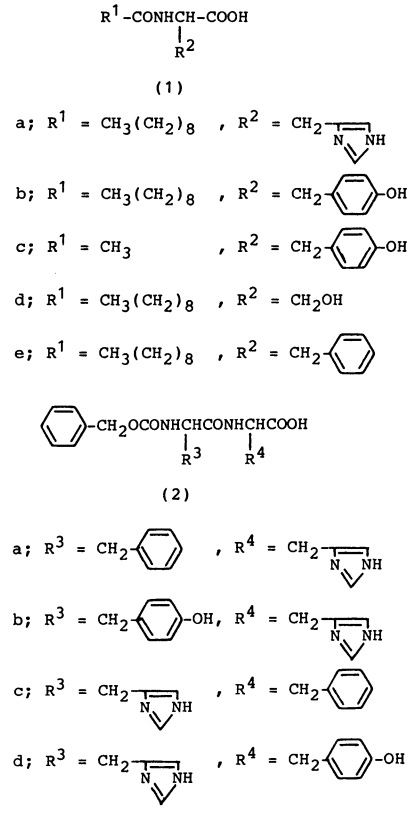
Materials. *N*-Decanoyl-L-tyrosine (**1b**), *N*-decanoyl-L-serine (**1d**), and *N*-decanoyl-L-phenylalanine (**1e**) were prepared and purified by standard methods.[7] *N*-Acetyl-L-tyrosine (**1c**), *N*-benzyloxycarbonyl-L-phenylalanyl-L-histidine (**2a**), *N*-benzyloxycarbonyl-L-tyrosyl-L-histidine (**2b**), *N*-benzyloxycarbonyl-L-histidyl-L-phenylalanine (**2c**), and *N*-benzyloxycarbonyl-L-histidyl-L-tyrosine (**2d**) were commercially available and were used without further purification. Other materials have been described elsewhere.[8-11]

Kinetic Measurements. Reactions were generally monitored on a Hitachi 200 spectrophotometer or a Shimadzu 140 spectrophotometer with a thermostated cell holder at 25 °C. In the general procedure, a solution (25 μl) of substrate in acetonitrile was added to a buffer solution (3.00 cm^3) containing the catalyst and surfactant at the desired concentrations. Pseudo-first-order rate constants were obtained from plots of $\log(A_\infty - A_t)$ versus time (t) by use of the least-squares method. Correlation coefficients were >0.999.

Results and Discussion

The catalytic process can be described by Eq. 1, where C_{Im} designates the imidazole catalyst, AcONp is the substrate, Ac-C_{Im} is the acylated intermediate, and k_a and k_d represent the rate constants for acylation and deacylation processes, respectively.

$$C_{Im} + AcONp \xrightarrow{k_a} Ac - C_{Im} \xrightarrow{k_d} C_{Im} + AcOH \quad (1)$$
$$+$$
$$p\text{-nitrophenol}$$



Kinetic studies are carried out at fixed concentration of the catalyst in the presence of CTAB. The hydrolysis of the substrates was examined under single turnover conditions, [surfactant]>[catalyst]>[ester] at pH 7.30, 0.02 mol dm^{-3} phosphate buffer and 25 °C. The pseudo-first-order rate constant k_ψ was determined by monitoring the release of *p*-nitrophenolate ion spectrophotometrically at 410 nm. The acylation rate constant (k_a) was calculated from the following equation, $k_a = k_\psi - k_{CTAB}$, where k_{CTAB} refers the rate con-

Table 1. Rate Constants for the Hydrolysis of Esters by **2a—d** in the Presence of CTAB[a)]

Ester	Catalytic Systems	$10^3 k_a/s^{-1}$	Rel. k_a	$10^4 k_d/s^{-1}$	Rel. k_d
PNPA	**2a**	3.89	1	6.89	1
	2b	3.85	0.99	21.0	3.05
	2c	3.64	1	8.05	1
	2d	3.34	0.92	13.3	1.65
PNPH	**2a**	3.31	1	2.73	1
	2b	3.22	0.97	5.76	2.11
	2c	3.07	1	4.73	1
	2d	3.36	1.09	6.77	1.43
PNPL	**2a**	3.12	1	2.28	1
	2b	2.80	0.90	4.48	1.96
	2c	2.55	1	4.47	1
	2d	2.88	1.13	6.69	1.50

a) At pH 7.30, 0.02 mol dm^{-3} phosphate buffer, and 25 °C, [CTAB]=1.00×10^{-2} mol dm^{-3}, [**2a,b,c,d**]=1.00×10^{-3} mol dm^{-3}, [Ester]=1.0×10^{-4} mol dm^{-3}. From three or more independent experiments, we estimate that the rate constants are reproducible to ±4%.

stant for spontaneous hydrolysis in the presence of CTAB. The deacylation rate constant (k_d) was directly measured spectrophotometrically by following the slow decrease in absorption at 245 nm or at near the isosbestic point[12] for the acylation reactions. In both cases, the kinetics were first order and good rate constants were obtained ($r>0.999$). The deacylation rate constants were calculated from absorbances at 245 nm, near the isosbestic point, and agree with the observed constants within experimental errors. We also carried out some experiments in CTAB under burst conditions, [substrate]≫[catalyst]. The kinetic treatment was that of Bender[13] and has been used by others.[4]

Table 1 summarizes the results for hydrolysis of three esters (PNPA, PNPH, and PNPL) by **2a—d** in the presence of CTAB micelles. The deacylation of the esters by **2b** and **d** which contain both the imidazolyl and hydroxyl functions in the catalysts is 1.4—3.1 times faster than that by **2a** and **c** which contain imidazolyl function alone although the acylation rates are essentially the same. This cooperative effect of the imidazolyl and hydroxyl functions in the catalysts **2b** and **d** for the deacylation process can be visualized in two mechanistic pathways[14] as illustrated in Scheme 1.

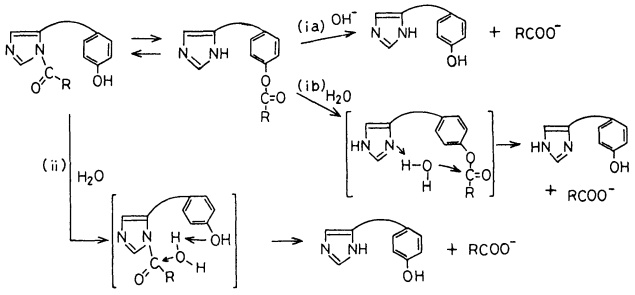
In order to obtain more information about the mechanism of deacylation process, the reactions of **2a** and **b** were examined using excess substrate (PNPA) so that there were rapid evolution of *p*-nitrophenol followed by a slow reaction as the acylated intermediates were hydrolyzed to generate the catalyst. The kinetic analysis under burst conditions shows that the deacylation rate enhancement by **2b** is almost similar to that obtained under the single turnover conditions (see Tables 1 and 2). This result suggests that acyl transfer from the imidazolyl to the hydroxyl groups (**ia** or **ib**) is less probable than the general base catalysis by the neighboring phenolic hydroxyl groups (**ii**) as a deacylation mechanism in the present catalytic system (Scheme 1).

The deacylation rate enhancement by **2b** was slightly larger than those by **2d** in all esters used (Table 1). These differences in deacylation process are due to the steric environment of the acylated intermediate in micelles. Thus, these results suggest that the deacylation involves geometrical interactions between the acyl intermediate and the hydroxyl group and that the acyl

Table 2. Kinetic Analysis under Burst Conditions[a)]

Catalytic Systems	k_a / mol^{-1} dm^3 s^{-1}	$10^4 k_d$ / s^{-1}	Rel. k_d
2a	2.38	4.2	1
2b	2.19	11.5	2.74

a) At pH 7.30, 0.02 mol dm^{-3} phosphate buffer, 25 °C, [CTAB]=1.00×10^{-2} mol dm^{-3}, [**2a** or **b**]=1.00—2.50×10^{-5} mol dm^{-3}, [PNPA]=2.50×10^{-3} mol dm^{-3}.



Scheme 1.

intermediate formed from **2b** and PNPA is optimally positioned for reaction by the hydroxyl function of **2b** in micellar phase and results in the largest deacylation rate (3.05).

Tables 3 and 4 summarize the rate constants for acylation and deacylation processes in the hydrolysis of the esters by **1a** and **2a** in four catalytic systems in the presence of micellar CTAB. From Tables 3 and 4, it is apparent that the deacylation is slower than the acylation in all cases. For the acylation process, the catalysis by **1a** was higher than that by **2a** in all esters used. On the other hand, **2a** is more reactive than **1a** in deacylation process. More importantly, PNPA is uniformly most reactive among the esters used in deacylation process. These results indicate that the deacylation process is sensitive to the structures of the catalysts and the esters.

The deacylation rate constants apparently increase in the presence of the catalysts **1b—d** which include the hydroxyl functions, although the acylation rate constants are almost independent of the catalytic systems used. The differences in deacylation rate enhancements suggest that the reaction depends on the catalytic activity of the hydroxyl functions in micelles. This probably reflects the steric environment and acidity of the hydroxyl groups. In our previous papers,[15] we also found the acyl transfer reaction in the hydrolysis of enantiomeric substrates in micelles.

Table 3. Rate Constants for the Hydrolysis of Esters in the Presence of Comicellar Catalytic Systems[a)]

Ester	Catalytic Systems	$10^3 k_a/s^{-1}$	Rel. k_a	$10^4 k_d/s^{-1}$	Rel. k_d
PNPA	**1a**	6.61	1	3.31	1
	1a/1b	6.67	1.01	7.08	2.14
	1a/1c	6.16	0.93	4.41	1.33
	1a/1d	6.28	0.95	4.56	1.38
	1a/1e	6.42	0.97	2.92	0.88
PNPH	**1a**	9.31	1	1.96	1
	1a/1b	9.24	0.99	3.41	1.74
	1a/1c	8.48	0.91	2.35	1.20
	1a/1d	8.38	0.90	2.48	1.27
	1a/1e	9.08	0.98	1.86	0.95
PNPL	**1a**	8.34	1	1.85	1
	1a/1b	8.11	0.97	2.66	1.44
	1a/1c	7.85	0.94	2.46	1.33
	1a/1d	7.33	0.88	2.08	1.12
	1a/1e	7.38	0.88	1.44	0.78

a) At pH 7.30, 0.02 mol dm^{-3} phosphate buffer, and 25°C, [CTAB]=1.00×10^{-2} mol dm^{-3}, [**1a**]=1.00×10^{-3} mol dm^{-3}, [**1b**–**e**]=1.00×10^{-3} mol dm^{-3}, [Ester]=1.0×10^{-4} mol dm^{-3}. From three or more independent experiments, we estimate that the rate constants are reproducible to ±4%.

Table 4. Rate Constants for the Hydrolysis of Esters in the Presence of Comicellar Catalytic Systems[a)]

Ester	Catalytic Systems	$10^3 k_a/s^{-1}$	Rel. k_a	$10^4 k_d/s^{-1}$	Rel. k_d
PNPA	**2a**	3.89	1	6.89	1
	2a/1b	3.49	0.90	8.80	1.28
	2a/1c	3.59	0.92	8.16	1.18
	2a/1d	3.39	0.87	7.24	1.05
	2a/1e	3.38	0.87	6.25	0.91
PNPH	**2a**	3.31	1	2.73	1
	2a/1b	3.32	1.00	4.11	1.51
	2a/1c	3.10	0.94	3.31	1.21
	2a/1d	3.03	0.92	3.56	1.30
	2a/1e	2.68	0.81	2.68	0.98
PNPL	**2a**	3.12	1	2.28	1
	2a/1b	2.68	0.86	5.26	2.31
	2a/1c	3.03	0.97	2.54	1.11
	2a/1d	2.65	0.85	3.00	1.32
	2a/1e	2.18	0.70	1.82	0.80

a) At pH 7.30, 0.02 mol dm^{-3} phosphate buffer, and 25°C, [CTAB]=1.00×10^{-2} mol dm^{-3}, [**2a**]=1.00×10^{-3} mol dm^{-3}, [**1b**–**e**]=1.00×10^{-3} mol dm^{-3}, [Ester]=1.0×10^{-4} mol dm^{-3}. From three or more independent experiments, we estimate that the rate constants are reproducible to ±4%.

Although deacylation enhancements in the present catalytic systems are relatively small, it is clear that these micellar systems cause acylation of the imidazolyl group, followed by the deacylation rate acceleration by the hydroxyl group of the catalyst.

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