General-Base Catalysis of the Hydrolysis of *p*-Nitrophenyl Carboxylates by Micellar Surfactants Involving a Histidyl Residue

Yukito Murakami,* Akio Nakano, Akira Yoshimatsu, and Kiyoshi Matsumoto

Contribution from the Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Fukuoka 812, Japan. *Received October 6, 1980*

Abstract: In order to obtain a clue in understanding enzymatic hydrolysis in which the Asp-His-Ser triad of serine proteases is involved, we prepared cationic peptide–surfactants bearing a histidyl residue ($N^+C_5HisC_{12}$) and both histidyl and aspartyl residues ($N^+C_5HisAspC_{12}$). Their catalytic activities toward the hydrolysis of *p*-nitrophenyl carboxylates were investigated in comicellar phases formed with $N^+C_5AlaC_{12}$ in dioxane–ethanol–water (1:1:98 v/v) at 30.0 ± 0.1 °C. The hydrolysis of *p*-nitrophenyl hexanoate (PNPH) in $N^+C_5HisC_{12}/N^+C_5AlaC_{12}$ and $N^+C_5HisAspC_{12}/N^+C_5AlaC_{12}$ comicelles predominantly undergoes general-base catalysis by the imidazolyl group, even though its normal nucleophilic catalysis is exercised. Relatively large solvent deuterium isotope effects [$k_m(H_2O)/k_m(D_2O)$ at μ = 0.80 (KCl): 2.6 for $N^+C_5HisC_{12}/N^+C_5AlaC_{12}$, and 2.1 for $N^+C_5HisAspC_{12}/N^+C_5AlaC_{12}$] are consistent with general-base catalysis. The general-base mechanism was also confirmed by the kinetic conversion rate: the release of *p*-nitrophenol occurred continuously beyond the equimolar conversion range with respect to the active imidazolyl group in the presence of excess PNPH. The overall reaction scheme consists of both general-base and nucleophilic mechanisms in the present comicellar systems with the contribution of the former mechanism of ca. 65%. The normal nucleophilic catalysis by an imidazolyl group in the hydrolysis of *p*-nitrophenyl carboxylates was suppressed to a marked extent in the appreciably tight micelles of peptide surfactants. It must be pointed out that both catalyst and substrate cannot approach to make intimate contact in such tight micelles due to steric reasons and that the reaction mechanism can be manipulated by changing the aggregation properties of micellar phase consequently. The reaction mechanism proposed for the hydrolysis of PNPH as catalyzed by $N^+C_5HisAspC_{12}/N^+C_5AlaC_{12}$ comicelles bears a close resemblance to that predicted for the hydrolysis catalyzed by the triad system of serine proteases which is operative in the deacylation step and presumably the acylation step.

The hydrolysis catalyzed by α-chymotrypsin, one of the serine proteases, has been predicted to involve general-base catalysis by the imidazolyl group of His-57 in the deacylation step which is enhanced by the carboxyl group of Asp-102 through proton abstraction from His-57.[1] Recently, much attention has been focused on reality and confirmation of the so-called charge-relay mechanism involved in reactions with the serine proteases and their related model systems. Bender et al.[2] reported that the benzoate anion remarkably accelerated the intermolecular general-base-catalyzed hydrolysis of *endo*-5-[4(5)-imidazolyl]bicyclo[2.2.1]-hept-*endo*-2-yl *trans*-cinnamate in dioxane–water as the dioxane content was increased. The proposed mechanism involves proton abstraction by the benzoate anion from the imidazolyl group, followed by proton abstraction by the imidazolyl group from water. On the other hand, Bruice et al.[3] did not observe such a charge-relay pathway with their model compound, 2-(2-acetoxyphenyl)-4(5)-methyl-5(4)-(2,2-dimethylacetic acid)-imidazolium chloride. Markley[4] and Roberts[5] suspected the previously predicted mechanism of charge-relay triad in the serine proteases on the basis of pK_a values of the carboxyl and imidazolyl groups involved in the catalytic triad of α-lytic protease.

In order to obtain a clue in understanding reaction mechanisms involved in the hydrolysis of ester substrates as catalyzed by the serine proteases, there have been developed extensively micellar surfactants bearing an imidazolyl group as enzyme models.[6] Judging from their molecular structures, we must place the imidazolyl group in the cationic Stern layer of micelles, and the nucleophilic catalysis is operative. The acylimidazole moiety is subsequently cleaved by concentrated hydroxide ions or transferred to the hydroxyl group placed in the same Stern layer of comicellar systems. The surfactants by Brown et al.[7] and Bunton et al.[8] are somewhat structurally related to one of our present peptide surfactants ($N^+C_5HisC_{12}$). An imidazolyl group of their surfactants, however, is attached on a side chain substituted nearly at the end of the primary molecular chain and seems to be placed in the cationic Stern layer where the nucleophilic reaction pathway is favored.

We reported previously[9] that cationic surfactants involving an amino acid residue separated from the cationic head by several methylene groups may form much tighter aggregates than ordinary micelles, with anisotropic immobilization. In order to characterize the novel reactivity of a neutral imidazolyl group in such tight aggregates and to solve the controversy involved in the serine protease catalysis (charge-relay mechanism), we prepared cationic peptide–surfactants having a histidyl residue ($N^+C_5HisC_{12}$) and also having an additional aspartyl residue ($N^+C_5HisAspC_{12}$) and investigated their catalytic functions in the hydrolysis of carboxylic esters. Bunton's work[8] on the hydrolysis of diphenyl *p*-nitrophenyl phosphate, in which he claims the reaction proceeds through the general-base mechanism, is interesting. However, phosphate esters are generally more susceptible to general-base catalysis, and an almost identical surfactant did not catalyze the decomposition of carboxylic esters by the general-base mechanism.[7] It seems, therefore, general-base catalysis became effective by using tight peptide–surfactant micelles for the first time in our present work.

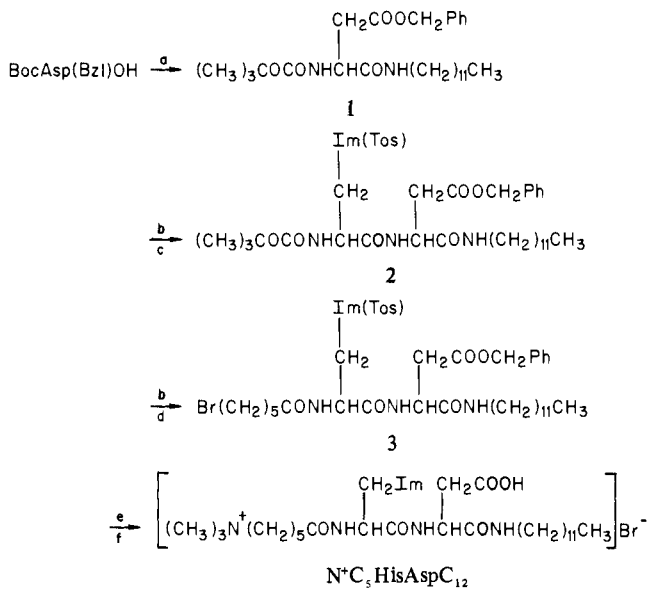
Experimental Section

Spectroscopic data were taken on a JASCO DS-403G grating IR spectrophotometer, a Varian A-60 NMR spectrometer, and a Union Giken SM-401 high-sensitive spectrophotometer. pH measurements were carried out with a Beckman expandomatic SS-2 pH meter equipped with

(1) Blow, D. M.; Birktoft, J. J.; Hartley, B. S. *Nature (London)* **1969**, *221*, 337.
(2) Komiyama, M.; Bender, M. L.; Utaka, M.; Takeda, A. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 2634.
(3) Rogers, G. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1974**, *96*, 2473.
(4) Markley, J. L.; Porubcan, M. A. *J. Mol. Biol.* **1976**, *102*, 487.
(5) Bachovchin, W. W.; Roberts, J. D. *J. Am. Chem. Soc.* **1978**, *100*, 8041.
(6) For example: (a) Tagaki, W.; Fukushima, D.; Eiki, T.; Yano, Y. *J. Org. Chem.* **1979**, *44*, 555. (b) Tonellato, U. *J. Chem. Soc., Perkin Trans. 2* **1976**, 771. (c) Moss, R. A.; Nahas, R. C.; Ramaswami, S. *J. Am. Chem. Soc.* **1977**, *99*, 627. (d) Brown, J. M.; Bunton, C. A. *J. Chem. Soc., Chem. Commun.* **1974**, 969. (e) Guthrie, J. P.; Ueda, Y. *Can. J. Chem.* **1976**, *54*, 2745. (f) Okahata, Y.; Ando, R.; Kunitake, T. *J. Am. Chem. Soc.* **1977**, *99*, 3067.
(7) Brown, J. M.; Chaloner, P. A.; Colens, A. *J. Chem. Soc., Perkin Trans. 2* **1979**, 71.
(8) Brown, J. M.; Bunton, C. A.; Diaz, S. *J. Chem. Soc., Chem. Commun.* **1974**, 971.
(9) (a) Murakami, Y.; Nakano, A.; Iwamoto, K.; Yoshimatsu, A. *Chem. Lett.* **1979**, 951. (b) Murakami, Y.; Nakano, A.; Iwamoto, K.; Yoshimatsu, A. *J. Chem. Soc., Perkin Trans. 2* **1980**, 1809.

0002-7863/81/1503-2750$01.25/0

Scheme I[a, b]



[a] Reagents: a, $CH_3(CH_2)_{11}NH_2$ and DCC; b, CF_3COOH; c, BocHis(Tos)OH and DCC; d, $Br(CH_2)_5COCl$; e, $N(CH_3)_3$; f, $N(CH_3)_3$/{H_2O, CH_3COCH_3, benzene}. [b] Abbreviations: Boc, *tert*-butoxycarbonyl; Bzl, benzyl; Ph, phenyl; Im, imidazolyl; Tos, tosyl.

a Metrohm EA-125 combined electrode after calibration with a combination of appropriate aqueous standard buffers.

Octadecyltrimethylammonium chloride (STAC) of Ishizu Pharmaceutical Co. was recrystallized from aqueous ethanol; it has no definite melting point, softens at 80 °C, and decomposes above 180 °C. *p*-Nitrophenyl carboxylates were prepared by the reaction of the corresponding carbonyl chlorides with *p*-nitrophenol and identified by spectroscopic methods and elemental analyses. Synthetic procedure for $N^+C_5AspC_{12}$ has already been reported elsewhere.[9b] Both $N^+C_5HisC_{12}$ and $N^+C_5AlaC_{12}$ were prepared according to the procedure similar to those used for the syntheses of peptide surfactants containing a cysteinyl residue.[10]

***N*-Dodecyl-N^α-(6-(trimethylammonio)hexanoyl)-L-histidinamide Bromide, $N^+C_5HisC_{12}$.** Recrystallization from chloroform–dichloromethane gave a hygroscopic powder: mp 128–130 °C, $[\alpha]^{25}_D$ +4.2° (*c* 1.0, ethanol), Dragendorff positive, Pauly positive; IR (KBr disk) 3280 (NH str), 2920 and 2850 (CH str), 1650 (C=O str), 1560 cm^{-1} (NH def); NMR (methanol-d_4, Me_4Si) δ 0.89 (3 H, br t, $CH_3(CH_2)_{11}$), 1.26 (20 H, s, $CH_3(CH_2)_{10}$), ~2.00 (6 H, m, $N^+CH_2(CH_2)_3CH_2$), 2.26 (2H, t, CH_2CO), 2.85–3.35 (6 H, m, $CONHCH_2$, $(CH_3)_3N^+CH_2$, and $CH(CH_2Im)$), 3.15 (9 H, s, $(CH_3)_3N^+$), 4.50 (1 H, m, CH), 6.93 (1 H, s, Im-5H), 7.70 (1 H, s, Im-2H). Anal. Calcd for $C_{27}H_{52}BrN_5O_2 \cdot H_2O$: C, 56.23; H, 9.44; N, 12.14. Found: C, 55.84; H, 9.36; N, 12.06.

***N*-Dodecyl-N^α-(6-(trimethylammonio)hexanoyl)-L-alaninamide Bromide, $N^+C_5AlaC_{12}$.** Recrystallization from ethyl acetate gave a hygroscopic powder: mp 94-97 °C, $[\alpha]^{20}_D$ −16.8° (*c* 18.5, ethanol), Dragendorff positive; IR (KBr disk) 3260 (NH str), 2980 and 2840 (CH str), 1630 (C=O str), 1545 cm^{-1} (NH def); NMR (methanol-d_4, Me_4Si) δ 0.88 (3 H, s, $CH_3(CH_2)_{11}$), 1.23 (20 H, s, $CH_3(CH_2)_{10}$), ~2.00 (6 H, m, $N^+CH_2(CH_2)_3CH_2$), 1.35 (3 H, s, $CH(CH_3)$), 2.23 (2 H, t, CH_2CO), 2.95–3.40 (4 H, m, $CONHCH_2$ and N^+CH_2), 3.27 (9 H, s, $(CH_3)_3N^+$), 4.80 (1 H, m, CH). Anal. Calcd for $C_{24}H_{50}BrN_3O_2 \cdot 0.5H_2O$: C, 57.47; H, 10.24; N, 8.37. Found: C, 57.25; H, 10.14; N, 8.31.

N^α-Hexadecanoyl-L-histidine, $HisC_{16}$. This was prepared in a manner similar to that reported by Gitler et al.[11] for the preparation of N^α-tetradecanoylhistidine, mp 124–127 °C. Anal. Calcd for $C_{22}H_{39}N_3O_3$: C, 67.14; H, 9.99; N, 10.68. Found: C, 67.04; H, 10.03; N, 10.80.

The synthetic procedure for N^α_{adp}-[N^α_{his}-(6-(trimethylammonio)hexanoyl)-L-histidyl]-*N*-dodecyl-L-aspartamide bromide, $N^+C_5HisAspC_{12}$, is outlined in Scheme I.

***N*-Dodecyl-N^α-(*tert*-butoxycarbonyl)-*O*-benzyl-L-aspartamide (1).** Dicyclohexylcarbodiimide (6.4 g, 0.03 mol) was added to a dichloromethane solution (40 mL) of *N*-(*tert*-butoxycarbonyl)-*O*-benzyl-L-aspartic acid[12] at 0 °C with stirring. After the mixture was stirred for 10 min at 0 °C, 1-aminododecane (5.4 g, 0.03 mol) dissolved in dichloromethane (40 mL) was added to it. The resulting reaction mixture was stirred for 3 h at 0 °C and for 12 h at room temperature and evaporated off in vacuo, and the residue was suspended in chilled ethyl acetate. A precipitated white solid was removed by filtration, and the filtrate was washed with saturated aqueous sodium chloride (2 × 50 mL) 10% aqueous citric acid (2 × 50 mL), saturated aqueous sodium chloride (2 × 50 mL), 4% aqueous sodium hydrogencarbonate (2 × 50 mL), and finally saturated aqueous sodium chloride (2 × 50 mL) and then dried (Na_2SO_4). The solvent was removed in vacuo, and the residual oil was solidified on trituration in hexane. Recrystallization from hexane gave a white powder: yield 13.0 g (86%), mp 52.5–55.0 °C; IR (KBr disk) 3300 (NH str), 2950 and 2680 (CH str), 1660 cm^{-1} (C=O str); NMR ($CDCl_3$, Me_4Si) δ 0.88 (3 H, br t, $CH_3(CH_2)_{11}$), 1.25 (20 H, s, $CH_3(CH_2)_{10}CH_2$), 1.45 (9 H, s, $(CH_3)_3C$), 2.88 (2 H, d, CH_2COOCH_2Ph), 3.21 (2 H, t, $NHCH_2(CH_2)_{10}CH_3$), 4.75 (1 H, br t, $CH(CH_2COOCH_2Ph)$), 5.15 (2 H, s, $COOCH_2Ph$), 7.37 (5 H, s, phenyl H's).

N^α-(*tert*-Butoxycarbonyl)-N^{im}-tosyl-L-histidyl-*N*-dodecyl-*O*-benzyl-L-aspartamide (2). Trifluoroacetic acid (25.0 g) was added to a dry dichloromethane solution (17 mL) of 1 (6.0 g, 12.2 mmol), and the mixture was stirred at room temperature for 1 h. Evaporation of excess trifluoroacetic acid in vacuo gave a colorless oil (A). Elimination of the *tert*-butoxycarbonyl group was confirmed by means of NMR spectroscopy. Amine component A and the dicyclohexylammonium salt of *N*-(*tert*-butoxycarbonyl)-N^{im}-tosyl-L-histidine (7.2 g, 12.2 mmol) were dissolved in dry dichloromethane (20 mL) and cooled down to 0 °C. Dicyclohexylcarbodiimide (3.0 g, 14.5 mmol) was added to the solution at this temperature, and the mixture was stirred for 3 h at 0 °C and overnight at room temperature. The subsequent workup in a manner as described for 1 followed by recrystallization from hexane–ethyl acetate gave the product: yield 6.4 g (80%), mp 82–86 °C; IR (KBr disk) 3320 (NH str), 2960 and 2880 (CH str), 1740 and 1670 cm^{-1} (C=O str); NMR ($CDCl_3$, Me_4Si) δ 0.90 (3 H, br t, $CH_3(CH_2)_{11}$), 1.28 (20 H, s, $CH_3(CH_2)_{10}CH_2$), 1.47 (9 H, s, $(CH_3)_3C$), 2.45 (3 H, s, SO_2PhCH_3), 2.80–3.42 (6 H, m, $NHCH_2(CH_2)_{10}CH_3$, CH_2COOCH_2Ph, and $CH_2Im(Tos)$), 4.55–5.05 (2 H, m, $CHCH_2COOCH_2Ph$ and $CHCH_2Im(Tos)$), 5.55 (2 H, s, $COOCH_2Ph$), 7.20 (1 H, s, Im-5H), 7.40 (5 H, s, phenyl H's), 7.82 (1 H, s, Im-2H), 7.15 and 7.78 (4 H, AB q, tosyl H's).

N^α_{asp}-[N^α_{his}-(6-bromohexanoyl)-N^{im}-tosyl-L-histidyl]-*N*-dodecyl-*O*-benzyl-L-aspartamide (3). The *tert*-butoxycarbonyl group was removed in a manner as described above. The obtained amine component was dissolved in dry dichloromethane, and triethylamine (1.3 g, 8.1 mmol) was added to the solution. The mixture was cooled down to 0 °C, and 6-bromohexanoyl chloride (2.0 g, 9.7 mmol) was added at this temperature with stirring. The solution was stirred at 0 °C for 3 h and overnight at room temperature and evaporated off in vacuo below 40 °C. Ethyl acetate was added to the residue, and the precipitated white solid was removed by filtration. The filtrate was washed with saturated aqueous sodium chloride (2 × 50 mL), 10% aqueous citric acid (2 × 50 mL), saturated aqueous sodium chloride (2 × 50 mL), 4% aqueous sodium hydrogencarbonate (2 × 50 mL), and saturated aqueous sodium chloride (2 × 50 mL) in this sequence. After being dried over anhydrous sodium sulfate, the purified filtrate was evaporated off in vacuo to give a viscous oil: yield 4.4 g (63%); IR (neat) 3260 (NH str), 2900 and 2840 (CH str), 1740 and 1650 cm^{-1} (C=O str); NMR ($CDCl_3$, Me_4Si) δ 0.88 (3 H, br t, $CH_3(CH_2)_{10}CH_2$), 1.27 (20 H, s, $CH_3(CH_2)_{10}$), ~2.00, (6 H, m, $BrCH_2(CH_2)_3CH_2$), 2.24 (2 H, t, $BrCH_2(CH_2)_3CH_2CO$), 2.45 (3 H, s, SO_2PhCH_3), 2.75–3.30 (6 H, m, $CH(CH_2Im(Tos))$, $CH(CH_2COOCH_2Ph)$, and $NHCH_2(CH_2)_{10}CH_3$), 3.95 (2 H, br t, $BrCH_2$), 4.40–4.80 (2 H, m, $CH(CH_2Im(Tos))$ and $CH(CH_2COOCH_2Ph)$), 5.12 (2 H, s, CH_2Ph), 6.95 (1 H, s, Im-5H), 7.30 and 7.90 (4 H, AB q, tosyl H's), 7.36 (5 H, s, phenyl H's), 8.12 (1 H, s, Im-2H).

N^α_{asp}-[N^α_{his}-(6-(trimethylammonio)hexanoyl)-L-histidyl]-*N*-dodecyl-L-aspartamide Bromide, $N^+C_5HisAspC_{12}$. Dry trimethylamine gas was introduced into a benzene solution (30 mL) of 3 (3.0 g, 3.5 mmol) for 3 h, and the solution was stirred at room temperature overnight. Then, aqueous trimethylamine (40%, 10 mL) and acetone (10 mL) were added to the benzene solution to eliminate the protecting groups for both imidazolyl and carboxyl. The mixture was stirred at room temperature for 6 h and evaporated to dryness. The crude product was purified by gel filtration chromatography (Sephadex LH-20, methanol as eluant) to afford a pale brown powder: yield 0.85 g (25%); mp 158–161 °C, $[\alpha]^{23}_D$ −74.1° (*c* 0.3, ethanol); IR (KBr disk) 3340 (NH str), 2900 and 2840 (CH str), 1702 and 1650 cm^{-1} (C=O str); NMR (methanol-d_4, Me_4Si) δ 0.89 (3 H, br t, $CH_3(CH_2)_{10}CH_2$), 1.27 (20 H, s, $CH_3(CH_2)_{10}CH_2$), ~2.00 (6 H, m, $N^+CH_2(CH_2)_3CH_2$), 2.34 (2 H, t, N^+CH_2-

(10) Murakami, Y.; Nakano, A.; Matsumoto, K.; Iwamoto, K. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 3573.

(11) Gitler, C.; Ochoa-Solano, A. *J. Am. Chem. Soc.* **1968**, *90*, 5004.

(12) Bayer, E.; Jung, G.; Hagenmaier, H. *Tetrahedron* **1968**, *24*, 4853.

Table I. Kinetic Parameters for the Hydrolysis of PNPA and PNPH as Catalyzed by Peptide Surfactant Comicellar Systems[a]

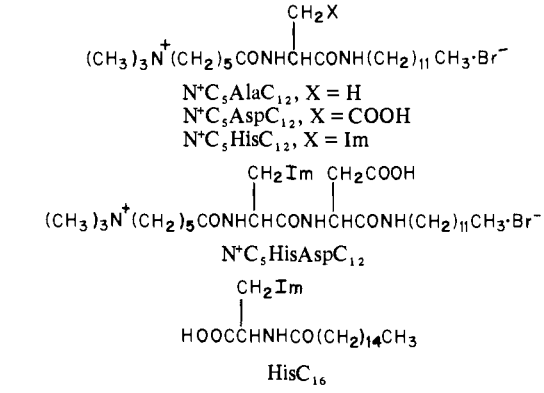
catalyst[b]	PNPA			PNPH		
	$10^4 k_{obsd}$,[e] s^{-1}	$10^3 k_m$, s^{-1}	K_b/N, M^{-1}	$10^4 k_{obsd}$,[e] s^{-1}	$10^4 k_m$, s^{-1}	K_b/N, M^{-1}
none	0.12			0.10		
$N^+C_5AlaC_{12}$ (3.77×10^{-3} M)	0.12			0.10		
$N^+C_5AspC_{12}/N^+C_5AlaC_{12}$[c] ($3.77 \times 10^{-3}$ M)	0.20			0.18		
$N^+C_5HisC_{12}/N^+C_5AlaC_{12}$[c] ($1.25 \times 10^{-4}$–$3.78 \times 10^{-3}$ M)	6.30	1.22	1250	2.77	3.05	1880
$N^+C_5HisAspC_{12}/N^+C_5AlaC_{12}$[c] ($1.25 \times 10^{-4}$–$3.14 \times 10^{-3}$ M)	8.60	3.14	1050	4.10	5.22	2620
$N^+C_5HisC_{12}/N^+C_5AspC_{12}/N^+C_5AlaC_{12}$[d] ($2.51 \times 10^{-4}$–$3.77 \times 10^{-3}$ M)	5.40	0.87	970	2.80	2.88	1880
$N^+C_5HisAspC_{12}$/STAC[c] (2.62×10^{-4}–3.20×10^{-3} M)	6.68	2.25	1120	3.38	3.67	1140
$HisC_{16}/N^+C_5AlaC_{12}$[c] ($1.25 \times 10^{-4}$–$3.78 \times 10^{-3}$ M)	12.8	5.87	140	18.5	28.1	650

[a] In ethanol–dioxane–water (1:1:98 v/v) at 30.0 ± 0.1 °C, pH 7.30, and μ = 0.80 (KCl). Initial concentrations: PNPA, 1.00×10^{-5} M; PNPH, 1.01×10^{-5} M. [b] Concentration of a comicellar system (C_D) is given in parentheses; C_D = [surfactant] + [$N^+C_5AlaC_{12}$ or STAC]. CMC of $N^+C_5AlaC_{12}$ by surface tnesion method (Wilhelmy principle), 8.0×10^{-5} M at pH 7.85, μ = 0.80 (KCl), and room temperature. [c] [surfactant]/[$N^+C_5AlaC_{12}$ or STAC] = 1/15. [d] [$N^+C_5HisC_{12}$]/[$N^+C_5AspC_{12}$]/[$N^+C_5AlaC_{12}$] = 1/1/15. [e] First-order rate constant observed at highest concentration of each catalyst system.

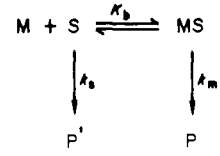
$(CH_2)_3CH_2CO$), 2.75–3.60 (8 H, m, $CH(CH_2Im)$, $CH(CH_2COOH)$, $N^+CH_2(CH_2)_3CH_2$, and $N^+CH_2(CH_2)_{10}$), 3.14 (9 H, s, $(CH_3)_3N^+$), 4.30–4.60 (2 H, m, $CH(CH_2Im)$ and $CH(CH_2COOH)$), 7.40 (1 H, s, Im-5H), 8.70 (1 H, s, Im-2H). Anal. Calcd for $C_{31}H_{57}BrN_6O_5 \cdot 0.5H_2O$: C, 54.53; H, 8.56; N, 12.31. Found: C, 54.70; H, 8.29; N, 12.40. Amino acid analysis: His, 1.1 (1); Asp, 1.0 (1).

Kinetic Measurements. Rates of *p*-nitrophenol liberation from *p*-nitrophenyl esters were measured at 317 nm (pH <6) and 400 nm (pH >6) with a Union Giken SM-401 high-sensitive spectrophotometer. Each run was initiated by adding a dry dioxane solution (30 μL) of a substrate ester to a mixture of a reaction medium (3.0 mL) and a dry ethanol solution of a catalyst, which was preequilibrated at 30.0 ± 0.1 °C in a thermostated cell set in the spectrophotometer. All the aqueous buffers were prepared by using deionized and distilled water, and the ionic strength of sample solutions was adjusted at μ = 0.80 (KCl) unless otherwise stated. The aqueous buffer solutions used are as follows: 1/10 M potassium succinate–1/20 M sodium borate for pH 4–6; 1/10 M potassium dihydrogenphosphate–1/20 M sodium borate for pH 6–9; and 1/20 M sodium borate–1/20 M sodium carbonate for pH 9–11. A pH value of each reaction mixture was measured before and after the reaction, and no pH change was detected throughout each kinetic run.

Isolation of Acylated $N^+C_5HisC_{12}$, *N*-Dodecyl-N^{α}-(6-(trimethylammonio)hexanoyl)-N^{im}-hexanoyl-L-histidinamide Bromide. A solution of hexanoyl chloride (240 mg, 1 mmol) in dichloromethane (2 mL) was added dropwise to a suspension of $N^+C_5HisC_{12}$ (200 mg, 0.34 mmol) in dichloromethane (3 mL) containing triethylamine (110 mg, 1 mmol) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and for another 2 h at room temperature. Then, the solvent was removed in vacuo, and the residual oil was applied on the top of a column of aluminum oxide (Ishizu Pharmaceutical Co., 1.5 × 30 cm). The product on the column was washed with chloroform first and then eluted with chloroform–ethanol (2:1 v/v). The main fraction was concentrated in vacuo, and the residue was recrystallized from ethyl acetate–methanol: yield 110 mg (49%); mp 143.0–144.5 °C, $[\alpha]^{25}_D$ +12° (*c* 1.0, ethanol); Pauly negative, Dragendorff positive; R_f 0.76 (with methanol) and 0.24 (with 1-butanol–water–acetic acid at 4:2:1 v/v); NMR (methanol-d_4, Me_4Si) δ 0.89 (6 H, br t, $CH_3(CH_2)_{11}$ and $CH_3(CH_2)_5$), 1.25 (20 H, s, $CH_3(CH_2)_{10}CH_2$), ~2.0 (14 H, m, $(CH_2)_3$ and $CH_3(CH_2)_4CH_2$), 2.10–2.52 (4 H, m, CH_2CONH and CH_2COIm), 2.85–3.40 (6 H, m, $(CH_3)_3N^+CH_2$, $CONHCH_2$, and $CHCH_2Im$), 3.12 (9 H, s, $(CH_3)_3N^+$), 3.80–4.20 (1 H, br t, CH), 7.18 (1 H, s, Im-5H), 8.31 (1 H, s, Im-2H). Anal. Calcd for $C_{33}H_{62}BrN_5O_3 \cdot H_2O$: C, 58.73; H, 9.56; N, 10.38. Found: C, 58.71; H, 9.48; N, 10.30.



Scheme II



Results and Discussion

Catalytic Efficiency of Mixed Micellar Systems. Degradation of *p*-nitrophenyl acetate (PNPA) and hexanoate (PNPH), as catalyzed by comicellar systems formed with histidine-containing surfactants and $N^+C_5AlaC_{12}$ (generally shown as surfactant/$N^+C_5AlaC_{12}$), has been studied in ethanol–dioxane–water (1:1:98 v/v) at pH 7.30, μ = 0.80 (KCl), and 30.0 ± 0.1 °C. Apparent first-order rate constants (k_{obsd}) were obtained by measuring the amounts of liberated *p*-nitrophenol. The first-order kinetics was found to hold up to 90% conversion of the substrate for each kinetic run under conditions of [E] > [S]; S and E stand for substrate and catalyst species, respectively. Rate (k_{obsd}) – concentration profiles (Figure 1) for the deacylation of PNPH as catalyzed by the comicellar systems of surfactant/$N^+C_5AlaC_{12}$ are typical for micellar catalysis (saturation kinetics). The kinetic data were analyzed according to the micellar reaction pathway given by Scheme II, where S denotes an ester substrate (PNPA or PNPH), M denotes a micelle constructed by $N^+C_5AlaC_{12}$ and a histidine-containing surfactant ($N^+C_5HisC_{12}$, $N^+C_5HisAspC_{12}$, or $HisC_{16}$), MS denotes a complex formed with a micelle and a substrate, and P and P′ stand for reaction products, k_s and k_m refer to rate constants for the product formation in bulk phase and in comicellar phase, respectively, and K_b is a binding constant for the formation of a micelle–substrate complex. Under the present experimental conditions, the catalytic activity of $N^+C_5AlaC_{12}$ micelle alone was negligibly small compared with those of the comicellar systems of surfactant/$N^+C_5AlaC_{12}$, and, therefore, the alkaline hydrolysis by hydroxide ions concentrated in the Stern layer of cationic micelles was neglected. An apparent first-order rate constant (k_{obsd}) and other related kinetic parameters appeared in Scheme II are interrelated by eq 1,[13] where C_D refers

$$\frac{1}{k_{obsd} - k_s} = \frac{1}{k_m - k_s} + \frac{N}{(k_m - k_s)K_bC_D} \quad (1)$$

to the sum of initial concentrations of $N^+C_5AlaC_{12}$ and a histidine-containing surfactant, and N is the aggregation number. The kinetic parameters thus evaluated are summarized in Table I. The comicelle $N^+C_5AspC_{12}/N^+C_5AlaC_{12}$ showed only a little larger activity than the $N^+C_5AlaC_{12}$ micelle in the hydrolysis of *p*-nitrophenyl esters. On the other hand, all comicellar systems formed with histidine-containing surfactants and $N^+C_5AlaC_{12}$ enhanced the reaction to much greater extents. These results

(13) Fendler, J. H.; Fendler, E. J. "Catalysis in Micellar and Macromolecular Systems"; Academic Press: New York, 1975; p 83.

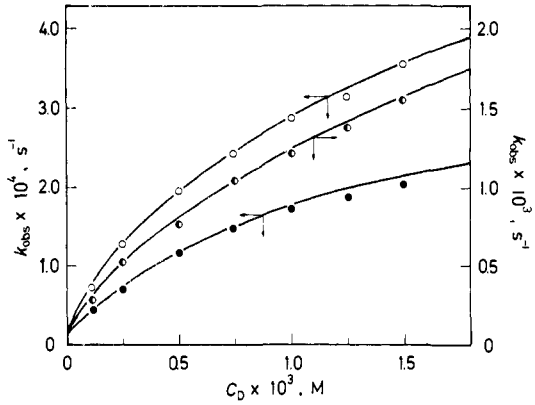


Figure 1. Saturation-type kinetics for the hydrolysis of PNPH as catalyzed by comicelles of $N^+C_5HisC_{12}/N^+C_5AlaC_{12}$ (●), $N^+C_5HisAspC_{12}/N^+C_5AlaC_{12}$ (○), and $HisC_{16}/N^+C_5AlaC_{12}$ (◐) in ethanol–dioxane–water (1:1:98 v/v) at pH 7.30, μ = 0.80 (KCl), and 30.0 ± 0.1 °C: C_D = [surfactant] + [$N^+C_5AlaC_{12}$]; [surfactant]/[$N^+C_5AlaC_{12}$] = 1/15; initial concentration of PNPH = 1.01×10^{-5} M. Solid lines are theoretical curves calculated by using parameters listed in Table I.

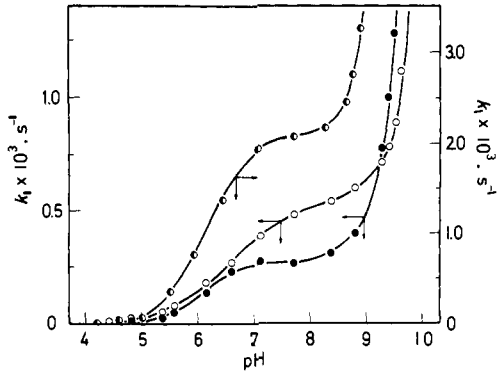


Figure 2. pH–rate profiles for the hydrolysis of PNPH as catalyzed by comicelles of $N^+C_5HisC_{12}/N^+C_5AlaC_{12}$ (●), $N^+C_5HisAspC_{12}/N^+C_5AlaC_{12}$ (○), and $HisC_{16}/N^+C_5AlaC_{12}$ (◐) in ethanol–dioxane–water (1:1:98 v/v) at μ = 0.80 (KCl) and 30.0 ± 0.1 °C; C_D = 3.77×10^{-3} M; [surfactant]/[$N^+C_5AlaC_{12}$] = 1/15; initial concentration of PNPH = 1.01×10^{-5} M; $k_1 = k_{obsd} - k(N^+C_5AlaC_{12})$. $k(N^+C_5AlaC_{12})$ is the rate constant for hydrolysis catalyzed by $N^+C_5AlaC_{12}$ micelle alone.

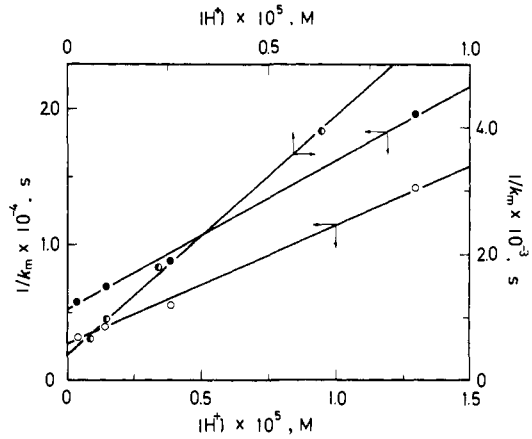


Figure 3. Plots of $1/k_m$ vs. $[H^+]$ (eq 4) for the hydrolysis of PNPH as catalyzed by comicelles of $N^+C_5HisC_{12}/N^+C_5AlaC_{12}$ (●), $N^+C_5HisAspC_{12}/N^+C_5AlaC_{12}$ (○), and $HisC_{16}/N^+C_5AlaC_{12}$ (◐) in ethanol–dioxane–water (1:1:98 v/v) at μ = 0.80 (KCl) and 30.0 ± 0.1 °C: initial concentration of PNPH = 1.01×10^{-5} M; [surfactant]/[$N^+C_5AlaC_{12}$] = 1/15.

strongly indicate that an imidazolyl group acts as the catalytic center. $HisC_{16}$ is the most efficient catalyst for the hydrolysis of both PNPA and PNPH among the surfactant/$N^+C_5AlaC_{12}$ comicellar systems. The rate constant evaluated for the hydrolysis of PNPH as catalyzed by the $HisC_{16}/N^+C_5AlaC_{12}$ comicelle is nearly identical with that for the hydrolysis of *p*-nitrophenyl butyrate as catalyzed by the $HisC_{14}$/CTAB comicelle.[11] Meanwhile, $N^+C_5HisAspC_{12}$ is more efficient than $N^+C_5HisC_{12}$ for the hydrolysis of *p*-nitrophenyl carboxylates in the comicellar phase. The larger efficiency of the former cannot be attributed to the catalysis exercised by the imidazolyl and aspartyl residues independently since the catalytic activity of $N^+C_5AspC_{12}/N^+C_5AlaC_{12}$ comicelle is almost identical with that of $N^+C_5AlaC_{12}$ micelle under the present conditions. In $N^+C_5HisAspC_{12}/N^+C_5AlaC_{12}$ comicelles, an intramolecular assistance in the reactivity of the imidazolyl group by the carboxyl group seems to be responsible for the rate enhancement since the possible participation of intermolecular interaction between two kinds of the functional groups must be ruled out according to the following facts. The active surfactant was diluted with $N^+C_5AlaC_{12}$ so that the intermolecular interaction may not operate. In addition, the comicelle composed of $N^+C_5HisC_{12}$, $N^+C_5AspC_{12}$, and $N^+C_5AlaC_{12}$ did not enhance the reaction rate beyond the extent exerted by $N^+C_5HisC_{12}/N^+C_5AlaC_{12}$ (Table I). A cooperative effect exercised by the imidazolyl and carboxyl groups has been claimed to polymer systems effective in esterolytic reactions.[14] These results lead us to conclude that the carboxyl group may intramolecularly (directly or indirectly) enhance the reactivity of the imidazolyl group of $N^+C_5HisAspC_{12}$ toward the deacylation of PNPH.

Catalytic Activity of Neutral Imidazolyl Group in Micellar Phase. Correlations between pH and the apparent first-order rate constant for the deacylation of PNPH as catalyzed by comicelles formed with the histidine-containing surfactants and $N^+C_5AlaC_{12}$ are shown in Figure 2. They apparently show that there are two kinds of catalytic species in the present reactions. These must be the anionic and neutral imidazolyl groups because the carboxyl group itself did not catalyze the reaction: pK_a of $N^+C_5AspC_{12}$ in $N^+C_5AlaC_{12}$ micelles in water = 5.10 at 25.0 ± 0.1 °C by potentiometric titration.[15] Although the anionic imidazolyl group shows a pronounced catalytic activity for the deacylation of *p*-nitrophenyl carboxylates above pH 9, we are interested in the reactivity of the neutral imidazolyl group in connection with enzyme catalysis. In order to evaluate the catalytic activity of the neutral imidazolyl group of each surfactant, we determined true first-order rate constants (k_m*). The pH–rate profiles indicate that the reactive species is the neutral imidazolyl group in the micellar phase below pH 8 as shown by Scheme III, where M-(ImH$^+$) and M(Im) denote surfactants bearing protonated and neutral imidazolyl groups, respectively, in the mixed micellar systems. The following relations are established.

Scheme III

$$S\cdot M(ImH^+) \underset{}{\overset{K_a}{\rightleftharpoons}} S\cdot M(Im) \xrightarrow{k_m^*} P$$

$$k_m\{[S\cdot M(ImH^+)] + [S\cdot M(Im)]\} = k_m^*[S\cdot M(Im)] \quad (2)$$

$$K_a = \frac{[S\cdot M(Im)][H^+]}{[S\cdot M(ImH^+)]} \quad (3)$$

Combination and rearrangement of eq 2 and 3 give eq 4.

$$\frac{1}{k_m} = \frac{1}{k_m^*} + \frac{[H^+]}{k_m^* K_a} \quad (4)$$

Plots of $1/k_m$ against $[H^+]$ for the deacylation of PNPH as catalyzed by $N^+C_5HisC_{12}/N^+C_5AlaC_{12}$, $N^+C_5HisAspC_{12}/N^+C_5AlaC_{12}$, and $HisC_{16}/N^+C_5AlaC_{12}$ comicelles (Figure 3)

(14) For example: (a) Overberger, C. G.; Maki, H. *Macromolecules* **1970**, *3*, 220. (b) Shimidzu, T.; Furuta, A.; Nakamoto, Y. *Macromolecules* **1974**, *7*, 160.

(15) The pK_a value of the carboxyl group of $N^+C_5AspC_{12}$ in the $N^+C_5AlaC_{12}$ micelle ($N^+C_5AspC_{12}$, 1.00×10^{-3} M; $N^+C_5AlaC_{12}$, 1.00×10^{-2} M) was determined in water by potentiometric titration: 5.10 at 25.0 ± 0.1 °C under nitrogen (our unpublished result).

Table II. True Rate Constants of Neutral Imidazolyl Group (k_m*) and Acid Dissociation Constants of Imidazolium Group (pK_a^{app}) in Some Comicelles[a]

comicellar system	$10^3 k_m$*, s^{-1}	pK_a^{app}
$N^+C_5HisC_{12}/N^+C_5AlaC_{12}$[b]	1.78	6.35
$N^+C_5HisAspC_{12}/N^+C_5AlaC_{12}$[c]	4.08	6.50
$HisC_{16}/N^+C_5AlaC_{12}$[c]	28.26	6.16

[a] In ethanol–dioxane–water (1:1:98 v/v) at 30.0 ± 0.1 °C and μ = 0.80 (KCl); initial concentration of PNPH = 1.01×10^{-5} M and [surfactant]/[$N^+C_5AlaC_{12}$] = 1/15. [b] C_D, 6.28×10^{-4}–3.77×10^{-3} M. [c] C_D, 2.51×10^{-4}–3.77×10^{-3} M.

provide the corresponding k_m* and pK_a values as listed in Table II; the neutral imidazolyl group of $HisC_{16}/N^+C_5AlaC_{12}$ acts most effectively among those of the present comicellar systems. The pK_a values for various imidazolyl groups suggest that the active imidazolyl group involved in $HisC_{16}$ is placed in the effective cationic region of mixed micelles and there is no apparent linear relationship between pK_a and k_m* among the three catalytic systems. This seems to indicate that the catalytic reaction mechanism involved in the deacylation of PNPH varies as the catalyst surfactant changes from one to another. Furthermore, the catalytic activity of the $N^+C_5HisAspC_{12}/N^+C_5AlaC_{12}$ comicelle is greater than that of $N^+C_5HisC_{12}/N^+C_5AlaC_{12}$ by 2.3-fold. This is undoubtedly due to the intramolecular assistance provided by the carboxyl group next to the active imidazolyl group in $N^+C_5HisAspC_{12}$.

Mechanism for the Hydrolysis of PNPH. In order to clarify the catalytic mechanism involved in the deacylation of PNPH as catalyzed by these comicellar systems, we examined the solvent deuterium isotope effects at pH(D) 7.40 and 30.0 ± 0.1 °C as summarized in Table III. The isotope effects on substrate binding (K_b/N) are consistent with the preferential solubility of hydrophobic compounds in deuterium oxide.[16] Tagaki and his coworkers[6a] showed that $HisC_{14}$ is an effective nucleophile for the deacylation of *p*-nitrophenyl esters as confirmed by the accumulation of acylimidazoles during the reaction. The deuterium isotope effect for the catalysis by $HisC_{16}$/STAC comicelle is consistent with the nucleophilic process as shown in Table III. On the other hand, larger values (2.6, 2.1, and 1.6 at μ = 0.80) were obtained when catalyzed by the mixed micellar systems containing a histidine-bearing surfactant. This seems to indicate that the reaction proceeds through general-base mechanism. The influence of ionic strength on the solvent deuterium isotope effects was studied since it is generally known that structure[17] and catalytic activity[18] of micelles are subjected to change by variation of ionic strength. The isotope effects were, therefore, investigated at μ = 0.10 and 0.80 (KCl) for all the comicellar systems and without added salt for $N^+C_5HisC_{12}/N^+C_5AlaC_{12}$ as listed in Table III; no significant ionic strength effect was observed. This fact shows that the reaction occurs in the hydrophobic micellar core since the catalytic activity would be affected by ionic strength if the reaction occurred at the Stern layer. Tightness of the hydrophobic core of peptide–surfactant comicelles may not be influenced by ionic strength in bulk phase since the micelles are already appreciably tight as confirmed by the spin-probe method.[9]

General-base mechanism was also confirmed by kinetic conversion data: the release of *p*-nitrophenol occurred continuously beyond the equimolar conversion range with respect to the active imidazolyl group in the presence of excess PNPH as shown in Figure 4. In order to make the situation clear, we carried out an experiment to detect the formation of the acylimidazole component. The acylated $N^+C_5HisC_{12}$ (abbreviated as acyl-$N^+C_5HisC_{12}$) was prepared independently from $N^+C_5HisC_{12}$ and

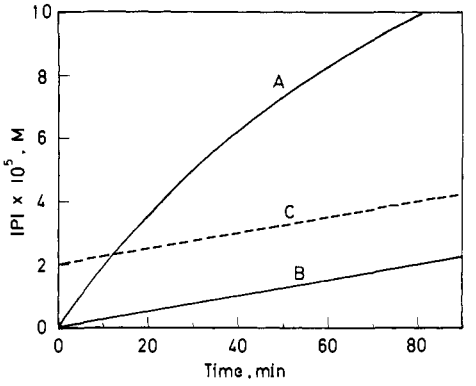


Figure 4. Time courses for liberation of *p*-nitrophenol (P) in the deacylation of PNPH as catalyzed by $N^+C_5HisC_{12}/N^+C_5AlaC_{12}$ comicelles (A: $N^+C_5HisC_{12}$, 2.00×10^{-5} M; $N^+C_5AlaC_{12}$, 8.45×10^{-4} M) and micellar $N^+C_5AlaC_{12}$ (B: 8.45×10^{-4} M) at pH 7.66, μ = 0.80 (KCl), and 44.3 ± 0.1 °C: initial concentration of PNPH = 2.02×10^{-4} M; line C is a hypothetical one along which the liberation of *p*-nitrophenol takes place after completion of the nucleophilic reaction.

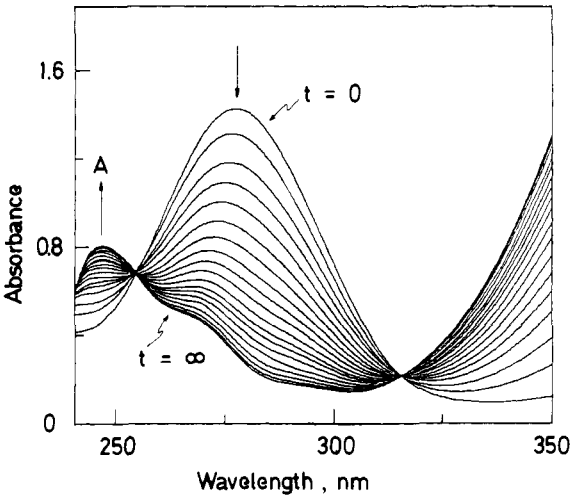


Figure 5. Spectral change for the deacylation of PNPH as catalyzed by $N^+C_5HisC_{12}/N^+C_5AlaC_{12}$ comicelles in ethanol–dioxane–water (1:1:98 v/v) at pH 7.50, μ = 0.80 (KCl), and 30.0 ± 0.1 °C: $C_D = 1.00 \times 10^{-2}$ M; [$N^+C_5HisC_{12}$]/[$N^+C_5AlaC_{12}$] = 1/15; initial concentration of PNPH = 1.01×10^{-4} M; *A* = absorption maximum for the acylated imidazolyl group (245 nm). A solution of $N^+C_5HisC_{12}$–$N^+C_5AlaC_{12}$ of identical component concentrations was placed in both reference and sample cells.

hexanoyl chloride and identified by means of IR, NMR, TLC, and elemental analysis (see Experimental Section): UV_{max} 245 nm (ε 9350) under the same conditions as employed for kinetic runs. Acyl-$N^+C_5HisC_{12}$ in the micellar state did not undergo deacylation to any detectable extent under the present kinetic conditions and did so only at higher pHs in the presence of $N^+C_5AlaC_{12}$ micelles: $k_{obsd} = 1.50 \times 10^{-5}$ s^{-1} at pH 8.50 and 5.61×10^{-5} s^{-1} at pH 9.20, at 30.0 ± 0.1 °C in ethanol–dioxane–water (1:1:98 v/v); [acyl-$N^+C_5HisC_{12}$] + [$N^+C_5AlaC_{12}$] = 3.78×10^{-3} M; [acyl-$N^+C_5HisC_{12}$]/[$N^+C_5AlaC_{12}$] = 1/15. In reference to the spectral change for the comicelle-catalyzed hydrolysis of PNPH (Figure 5), some 35–40% mole fraction of PNPH was accumulated as the acylimidazole at the end of reaction. Some 65–60% of the reaction proceeds through the general-base mechanism and the remaining fraction is referred to normal nucleophilic process (Table IV), judging from the results. Thus, general base (k_m^G) and nucleophilic (k_m^N) catalysis contribute to the overall reaction rate in micellar phase (k_m) as given by eq 5. Apparent first-order rate constants for the nucleophilic reaction, k_m^N, were determined by monitoring the absorbance change at 245 nm (due to appearance of the acylimidazole moiety) in the light of eq 1. The acylimidazole was accumulated progressively

$$k_m = k_m^G + k_m^N \quad (5)$$

(16) Kresheck, G. C.; Schneider, H.; Scheraga, H. A. *J. Phys. Chem.* **1965**, *69*, 3132.
(17) Kalyanasundaram, K.; Grätzel, M.; Thomas, J. K. *J. Am. Chem. Soc.* **1975**, *97*, 3915.
(18) Bunton, C. A. In "Application of Biochemical Systems in Organic Chemistry"; Part 2; Jones, J. B., Sih, C. J., Perlman, D., Eds.; Wiley: New York, 1976; Chapter IV.

Table III. Kinetic Solvent Deuterium Isotope Effects for the Hydrolysis of PNPH as Catalyzed by $N^+C_5HisC_{12}$, $N^+C_5HisAspC_{12}$, and $HisC_{16}$ Comicellar Systems[a]

catalyst[b]	KCl, M	H_2O 10^4k_m, s^{-1}	H_2O K_b/N, M^{-1}	D_2O 10^4k_m, s^{-1}	D_2O K_b/N, M^{-1}	$k_m(H_2O)/k_m(D_2O)$
$N^+C_5HisC_{12}/N^+C_5AlaC_{12}$ (2.51×10^{-4}–3.77×10^{-3} M)	0.80	3.18	1940	1.21	1450	2.6
	0.10	3.28	2010	1.30	1480	2.5
	0	3.38	1850	1.33	1460	2.5
$N^+C_5HisAspC_{12}/N^+C_5AlaC_{12}$ (6.28×10^{-4}–2.51×10^{-3} M)	0.80	5.62	2850	2.73	1810	2.1
	0.10	6.21	2320	2.88	1740	2.2
$N^+C_5HisC_{12}$/STAC (5.50×10^{-4}–3.01×10^{-3} M)	0.80	3.24	1140	2.03	810	1.6
	0.10	3.14	1480	2.12	1340	1.5
$HisC_{16}$/STAC (2.60×10^{-4}–3.77×10^{-3} M)	0.80	28.80	650	26.01	420	1.1
	0.10	30.88	630	26.81	450	1.2

[a] In ethanol–dioxane–water (1:1:98 v/v) at 30.0 ± 0.1 °C and pH(D) 7.40. [b] Concentration (C_D) range of a comicellar system is given in parentheses; C_D = [surfactant] + [$N^+C_5AlaC_{12}$ or STAC] and [surfactant]/[$N^+C_5AlaC_{12}$ or STAC] = 1/15.

Table IV. Fractions of General-Base Catalysis (F_G) in the Hydrolysis of PNPH (Evaluated from the Product Analysis)[a]

catalyst	F_N	F_G
$N^+C_5HisC_{12}/N^+C_5AlaC_{12}$	0.35	0.65
$N^+C_5HisAspC_{12}/N^+C_5AlaC_{12}$	0.38	0.62
$HisC_{16}$/STAC	1.0	0

[a] In ethanol–dioxane–water (1:1:98 v/v) at pH 7.50, μ = 0.80 (KCl), and 30.0 ± 0.1 °C; initial concentration of PNPH = 1.01×10^{-4} M, $C_D = 1.00 \times 10^{-2}$ M, and [surfactant]/[$N^+C_5AlaC_{12}$ or STAC] = 1/15; F_N = fraction of nucleophilic catalysis and $F_G = 1 - F_N$.

Table V. Fractions of General-Base Catalysis in the Hydrolysis of PNPH (Evaluated from Kinetic Analysis)[a]

catalyst[b]	10^4-k_m,[c] s^{-1}	10^4-k_m^N,[d] s^{-1}	10^4-k_m^G,[e] s^{-1}	F_G[f]
$N^+C_5HisC_{12}/N^+C_5AlaC_{12}$ (3.76×10^{-4}–3.77×10^{-3} M)	3.18	1.06	2.12	0.67
$N^+C_5HisAspC_{12}/N^+C_5AlaC_{12}$ (6.28×10^{-4}–2.51×10^{-3} M)	5.62	2.11	3.51	0.62
$HisC_{16}$/STAC (6.28×10^{-4}–2.51×10^{-3} M)	28.80	28.40		

[a] In ethanol–dioxane–water (1:1:98 v/v) at 30.0 ± 0.1 °C and μ = 0.80 (KCl); initial concentration of PNPH = 1.01×10^{-5} M. [b] Concentration (C_D) range of a comicellar system is given in parentheses; C_D = [surfactant] + [$N^+C_5AlaC_{12}$ or STAC]; [surfactant]/[$N^+C_5AlaC_{12}$ or STAC] = 1/15. [c] pH 7.40. [d] pH 7.50. [e] $k_m^G = k_m - k_m^N$. [f] F_G values are somewhat underestimated since pHs employed for obtaining k_m and k_m^N were not exactly identical though the pH values are in the plateau region of each pH–rate profile.

without further reaction. The fractions of general-base catalysis, k_m^G/k_m, in the comicelle-catalyzed hydrolysis of PNPH are identical with those obtained from the spectral changes (Tables IV and V). The comicelle $HisC_{16}$/STAC deacylated PNPH with the neutral imidazolyl group of $HisC_{16}$ exclusively via the nucleophilic pathway. The nucleophilic reactivity (k_m^N) of $HisC_{16}$/STAC comicelles is much larger than those of $N^+C_5HisC_{12}/N^+C_5AlaC_{12}$ and $N^+C_5HisAspC_{12}/N^+C_5AlaC_{12}$ by 27- and 13-fold, respectively. The reactivity difference is primarily due to the microenvironmental effect; a charge-separated transition state in the nucleophilic reaction of the neutral imidazolyl group with a carboxylic ester must be stabilized in polar environments. In $HisC_{16}$/STAC comicelles, the active imidazolyl group is placed in the electrostatic Stern layer, judging from the molecular structure of $HisC_{16}$. On the other hand, the imidazolyl groups of $N^+C_5HisC_{12}$ and $N^+C_5HisAspC_{12}$ are located inevitably in the hydrophobic micellar core in a manner as reported for micelles of a cysteine-containing surfactant.[10] Under such circumstances, generation of the charge-separated transition state would not be favored. Table V shows that the neutral imidazolyl groups act as an effective general-base catalyst in $N^+C_5HisC_{12}/N^+C_5AlaC_{12}$ and $N^+C_5HisAspC_{12}/N^+C_5AlaC_{12}$ comicelles. It should be noted that the imidazole-catalyzed hydrolysis of *p*-nitrophenyl carboxylates generally proceeds via the nucleophilic mechanism because *p*-nitrophenol is a good leaving group. Even in enzymatic reactions, an imidazolyl group frequently acts as a nucleophile in the hydrolysis of a nonspecific ester substrate, such as in the hydrolysis of *p*-nitrophenyl benzoates by α-chymotrypsin.[19] On the other hand, an imidazolyl group hydrolyzes carboxylic esters with a poor leaving group via the general-base mechanism.[20] *p*-Nitrophenol would become a poor leaving group in peptide–surfactant micelles. Indeed, we found out that the reaction center in micellar phase is highly hydrophobic; an absorption maximum for the imidazolyl group of monomeric $N^+C_5HisC_{12}$ observed at 208 nm underwent a bathochromic shift by 14 nm upon micelle formation.[21] Nevertheless, the pK_a value of the imidazolyl group in micellar phase is not changed to any appreciable extent among the present systems as shown in Table II regardless of change in reaction mechanism.

Consequently, another explanation is needed for the change in reaction mechanism. The general-base hydrolysis of PNPA as catalyzed by 2-substituted imidazole[22] and that of diphenyl *p*-nitrophenyl phosphate[8] as catalyzed by imidazole-containing surfactants have been reported. In the former case, the mechanism is transformed from nucleophilic into general base as a consequence to avoid the formation of a sterically crowded nucleophilic intermediate. In the latter case, the substrate itself is quite susceptible to the general-base hydrolysis in addition to the fact that the surfactant employed is one of the peptide–surfactants bearing a histidyl residue. As we have already found out, peptide–surfactants tend to form much tighter aggregates than ordinary micelles, and, consequently, both catalyst and substrate become more or less immobilized in aggregated state.[9] Such reaction fields provided by peptide–surfactant micelles are unfavorable for reactions which require effective collision between two reactants such as nucleophilic reactions. In fact, as the extent of immobilization of the comicellar system increases, the solvent deuterium isotope effect is raised: 1.6 for $N^+C_5HisC_{12}$/STAC and 2.6 for $N^+C_5HisC_{12}/N^+C_5AlaC_{12}$, both at μ = 0.80 (Table III). In conclusion, normal nucleophilic catalysis by an imidazolyl group in the hydrolysis of *p*-nitrophenyl carboxylates was suppressed to a marked extent in peptide–surfactant comicelles. It must be pointed out that both catalyst and substrate cannot approach to make intimate contact due to steric reasons and the reaction mechanism can be manipulated by changing aggregation properties of the micellar phase consequently.

(19) Hubbard, C. D.; Kirsch, J. F. *Biochemistry* **1972**, *11*, 2483.
(20) Kirsch, J. F.; Jencks, W. P. *J. Am. Chem. Soc.* **1964**, *86*, 833.
(21) The absorption maximum for imidazole was measured in the following solvents: water, *tert*-butyl alcohol, 214; acetonitrile, 220 nm.
(22) Akiyama, M.; Hara, Y.; Tanabe, M. *J. Chem. Soc., Perkin Trans. 2* **1978**, 288.

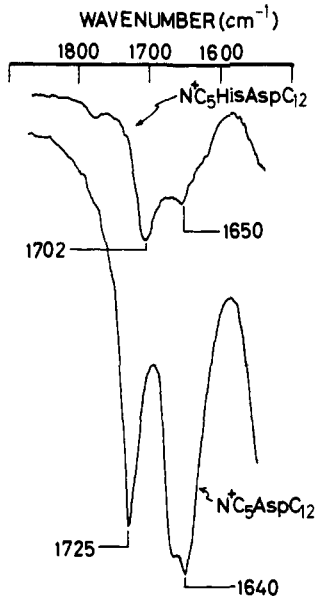


Figure 6. IR spectra of peptide–surfactants (KBr disk, 1600–1800 cm^{-1}).

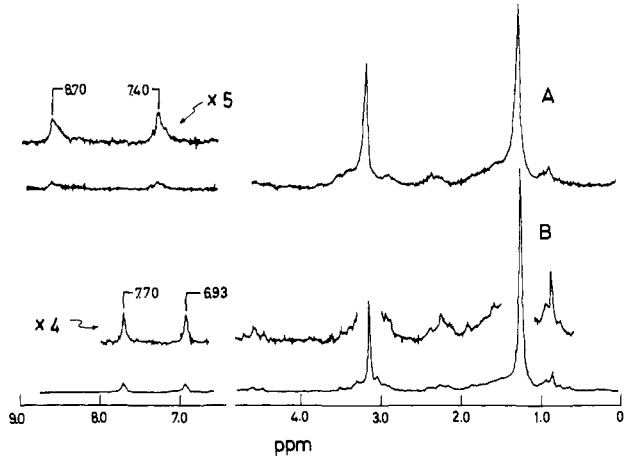
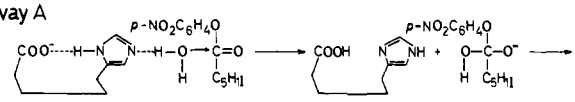


Figure 7. ^1H NMR spectra of peptide–surfactants in methanol-d_4 with Me_4Si as an internal reference: A, $N^+C_5HisAspC_{12}$; B, $N^+C_5HisC_{12}$.

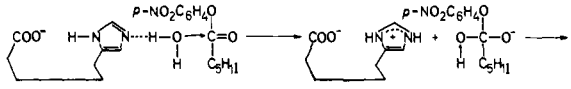
Mechanistic Resemblance to the Triad of Serine Proteases. We showed above that the imidazolyl groups of $N^+C_5HisAspC_{12}$ and $N^+C_5HisC_{12}$ act predominantly as general bases for the hydrolysis of PNPH in comicellar systems formed with $N^+C_5AlaC_{12}$ and

Scheme IV

Pathway A



Pathway B



that the carboxyl group of $N^+C_5HisAspC_{12}$ must intramolecularly enhance the catalytic activity of the imidazolyl group. The CPK molecular model of $N^+C_5HisAspC_{12}$ suggests that the carboxyl group of the aspartyl residue and the imidazolyl group of the histidyl residue are placed at the juxtaposition within hydrogen-bonding distance, and, in fact, the spectral data indicate that both groups are hydrogen bonded in the solid state and in methanol. The carbonyl stretching frequencies for $N^+C_5AspC_{12}$ and $N^+C_5HisAspC_{12}$ (Figure 6) indicate the presence of a free carbonyl group (1725 cm^{-1}) and a hydrogen-bonded one (1702 cm^{-1}), respectively. In addition, proton signals for the imidazole ring of $N^+C_5HisAspC_{12}$ appear in a lower field relative to those of $N^+C_5HisC_{12}$, as shown in Figure 7, due to hydrogen-bonding interaction between the neighboring histidyl and aspartyl residues in the former surfactant.

The pH–rate profile for the hydrolysis of PNPH as catalyzed by the $N^+C_5HisAspC_{12}/N^+C_5AlaC_{12}$ comicelle indicates that only the neutral imidazolyl group acts as an active species in the pH region 5–9. In addition, the pK_a value of the aspartyl–carboxyl group in peptide–surfactant micelles is about 5.10,[15] and, therefore, it has no primary concern with catalytic activity. All the results show that the anionic carboxylate group of $N^+C_5HisAspC_{12}$ intramolecularly assists the imidazole-catalyzed hydrolysis of PNPH, and, therefore, the overall reaction mechanism may be given by Scheme IV. As for pathway A in Scheme IV, two protons migrate among the so-called catalytic triad which is referred to the charge-relay system. On the other hand, only one proton migrates from water to the imidazolyl group as for pathway B. In the latter case, the anionic carboxylate group acts not only to stabilize the cationic imidazolium group formed at the intermediate state but also to increase pK_a of the imidazolyl group by the electrostatic effect. The reaction mechanism proposed for the hydrolysis of PNPH (either pathway A or B in Scheme IV) as catalyzed by the $N^+C_5HisAspC_{12}/N^+C_5AlaC_{12}$ comicelle bears a close resemblance to that predicted for the hydrolytic reaction catalyzed by the triad system of serine proteases which is operative in the deacylation step.