The Catalytic Action of a Mixed Micelle of Lauroylamino Acid and a Detergent

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The catalytic effect of the mixed micelles of lauroylamino acids (LauAm) and detergents on the hydrolysis of *p*-nitrophenyl acetate has been studied. The amino acids used for the synthesis of LauAm were glycine, serine, histidine, tyrosine, lysine, and arginine. Cetyltrimethylammonium bromide (CTAB), polyoxyethylene dodecyl ether ($C_{12}E_6$) and sodium dodecyl sulfate (SDS) were used for the preparation of the mixed micelles with LauAm. Among the detergents used, only CTAB remarkably enhanced the catalytic activity of LauAm. A reaction scheme was proposed, and the rate constant for the catalytic activity of LauAm was estimated from the kinetic data. The effect of pH was also studied. Discussions were given of the mechanism of the catalytic action on the basis of the present study.

In general, it is thought that the detergent micelle is roughly spherical and that the hydrophilic groups occupy the micellar surface and are exposed to the solvent phase, while the hydrophobic groups occupy the interior and form the hydrophobic region. In this respect, micelles resemble globular proteins, which have a structure in which the nonpolar amino acid residues are inside and the polar ones are outside. On the other hand, it has been known that several organic reactions are accelerated or inhibited when they occur in solutions containing detergent micelles and that this catalytic action shows a pattern similar to that of the Michaelis–Menten type. For these reasons, the study of the reaction rates in micellar system is useful as a model of enzymatic reaction, and recently many investigations in this area have been carried out.[1,2]

Gitler *et al.* found that the mixed micelle of N^{α}-myristoyl-L-histidine and cetyltrimethylammonium bromide (CTAB) remarkably enhanced the rate of the liberation of *p*-nitrophenol by the hydrolysis of *p*-nitrophenyl acetate (PNPA).[3] Heitman observed a similar enhancement of the reaction rate in the presence of sodium dodecanoylcysteinate in the micelle.[4] It has been regarded that the acylation reaction accompanies the liberation of *p*-nitrophenol in these systems.

When amino-acid side chains are in the vicinity of the micellar surface or the boundary between apolar and polar regions, their properties might be different from those in an aqueous environment. Thus, it is interesting to investigate the catalytic action of amino-acid residues in micelles in comparison with that of the enzyme. Thus, the effect of the mixed micelle of *N*-long chain acylamino acid and a detergent on the rate of the hydrolysis of PNPA has been studied.

Experimental

Materials. N^{α}-lauroylamino acid (LauAm) was prepared by the method of Lapidot *et al.*[5] The amino acids used for the synthesis of LauAm were glycine, serine, histidine, tyrosine, lysine, and arginine. To a solution of amino acid (20 mmol) and sodium hydrogencarbonate (20 mmol) in 200 ml of water we added a solution of the *N*-hydroxysuccimid ester of lauric acid (20 mmol) in 200 ml of tetrahydrofuran. The mixture was stirred for 16 h at room temperature and then acidified with 1 M HCl to pH 2—3, and the organic solvent was removed *in vacuo*. After the addition of 100 ml of water, the precipitate was filtered and recrystallized from the methanol–water mixture three times. In the cases of N^{α}-lauroyllysine (LauLys) and N^{α}-lauroylarginine (LauArg), two protective groups were removed by the catalytic hydrogenation of palladium black after the coupling of ε-benzyloxycarbonyl-L-lysine (or N^{ω}-nitro-L-arginine) with lauric acid. The products were recrystallized from the methanol–ether mixture or ethanol. Each compound moved as a single spot on a thin-layer chromatogram. The results of the elementary analysis of LauAm are given in Table 1.

The cetyltrimethylammonium bromide (CTAB), *p*-nitrophenyl acetate (PNPA), tris(hydroxymethyl)aminomethane (Tris), 2-amino-2-methyl-1,3-propanediol, potassium hydrogenphosphate, and disodium hydrogenphosphate were of a guaranteed grade and were used without further purification. A purified specimen of polyoxyethylene dodecyl ether, $C_{12}H_{25}O(CH_2CH_2O)_6H$, ($C_{12}E_6$), was obtained from the Kao Soap Co., Ltd. The sodium dodecyl sulfate (SDS) was purified by recrystallization from 95% ethanol.

The experiments were carried out at detergent concentrations well above the critical micelle concentration (CMC) of the detergent. Gitler *et al.* reported that N^{α}-myristoyl-L-histidine and CTAB formed a mixed micelle.[3] Although LauAm is insoluble in water at a neutral pH, it is easily solubilized by detergent micelles. In this study, sample solutions were prepared by solubilizing LauAm into the detergents in a buffer solution where the molar ratio of LauAm to detergents was less than 1/5. The micellar solutions were transparent for a long time at room temperature. The pH of the sample solution was adjusted with a 1/20 M Tris buffer unless otherwise stated.

Kinetic Measurements. An absorption cell was filled with 3.0 ml of a sample solution containing a mixed micelle of LauAm and a detergent, and was then placed in the thermostated chamber (25 °C) of a HITACHI 124 spectrophotometer. 15 min later, 20 μl of an acetonitrile solution of PNPA was added to the sample solution, and the two were mixed by stirring. The initial concentration of PNPA in the system was maintained at 10^{-5} M throughout. Then, the increase with time in the absorbance at 400 nm (at pH 6 and above) or 317 nm (below pH 6) due to the liberated *p*-nitrophenol was recorded. The pseudo-first-order kinetics were

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Table 1. Results of elementary analysis

Compound Molecular Formula (mol wt)	Calculated C	H	N	Found C	H	N	Mp °C
	percentage						
LauGly $C_{14}H_{27}O_3N$ (257.2)	65.31	10.58	5.44	65.16	10.50	5.45	117—119 (117)[a]
LauSer $C_{15}H_{29}O_4N$ (287.2)	62.69	10.17	4.87	62.33	10.21	4.66	89—90 (87)[a]
LauHis $C_{18}H_{31}O_3N_3$ (337.5)	64.07	9.26	12.45	63.94	9.35	12.41	160—161 (158—159)[b]
LauTyr $C_{21}H_{33}O_4N$ (363.3)	69.39	9.15	3.85	68.97	9.20	3.80	126—127 (127—128)[b]
LauLys $C_{18}H_{36}O_3N_2$ (328.5)	65.81	11.05	8.53	65.54	11.11	8.65	
LauArg $C_{18}H_{36}O_3N_4$ (356.5)	60.64	10.18	15.72	60.37	10.17	15.85	

a) Ref. 5). b) Ref. 6).

observed under the present experimental condition: [detergent]>[LauAm]≫[PNPA]. The pseudo-first-order rate constants, k_ϕ, were calculated from $(1/t)\cdot\ln a/(a-x)$, where a and x are the absorbances at an infinite time (about ten half-lives) and at time t respectively. The duplicate values of k_ϕ agreed within 4%.

Results and Discussion

Effect of Detergent on the Rate of Hydrolysis of PNPA. The rate constant, k_ϕ, was measured with detergent solutions at pH 8.7, 25 °C. SDS and $C_{12}E_6$ did not appreciably alter k_ϕ as compared with the non-micellar system, whereas in the presence of CTAB greater values of k_ϕ were observed.

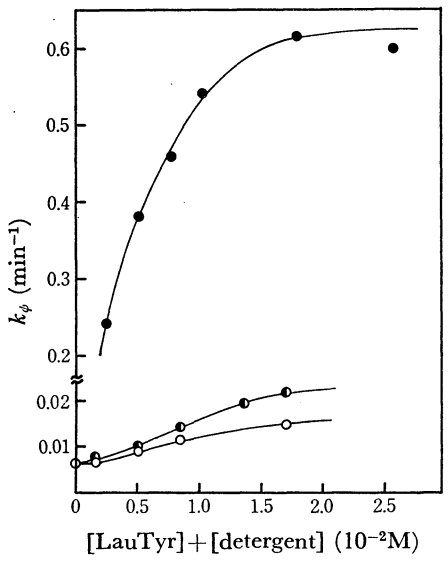

Fig. 1. Catalytic action of LauTyr.
●: CTAB, ◐: SDS, ○: $C_{12}E_6$. The molar ratio of LauTyr to detergent is 1:16. $[PNPA]_0=10^{-5}$ M.

A series of experiments were carried out with a constant molar ratio of LauAm to the detergent. The results for LauTyr are shown in Fig. 1. This figure indicates that CTAB remarkably accelerates the rate of the hydrolysis of PNPA and that SDS causes a small increase in the reaction rate. However, in the presence of SDS the reaction rate is somewhat lower than that in the absence of SDS or in the presence of LauTyr alone. This indicates that SDS suppresses the reaction rate to some extent. Similar effects of CTAB and SDS were observed with LauHis, LauLys, LauSer, and LauArg. It may be noted that the acceleration by CTAB is small for mixed micelles with LauSer and LauArg. $C_{12}E_6$ tends to decrease the catalytic activity of LauAm except in the case of LauHis.

On the other hand, the mixed micelle of LauGly and a detergent showed a smaller catalytic activity than the detergent solution itself.

Change in the Reaction Rate with the Detergent Concentration. The catalytic effect of the mixed micelles of CTAB and LauAm has been studied as a function of the concentration with a fixed molar ratio of LauAm to CTAB. It may be seen, *e.g.*, in Fig. 1, that the rate constant, k_ϕ, increases with the concentration and approaches a saturation value. The concentration dependence showed a similar pattern regardless of the composition of the mixed micelle. However, the saturation value becomes greater as the fraction of LauTyr becomes larger. This can be attributed to the increase in the number of the active sites of a mixed micelle. Similar results were obtained with LauHis, LauLys, LauArg, and LauSer.

Kinetics. The micellar concentration dependence of the rate constant can be explained by assuming the following reaction scheme:

$$S \xrightarrow{k_b} P$$

$$S + M \underset{}{\overset{K}{\rightleftharpoons}} MS \xrightarrow{k} P \qquad (1)$$

where S denotes the substrate (PNPA); M, the micelle; MS, the micelle–substrate complex and P, the product (*p*-nitrophenol); k_b and k are the rate constants of the product formation in the bulk and at the micellar surface respectively; K represents the dissociation constant of the micelle–substrate complex.

This scheme differs from that proposed by Gitler *et al.*[3] in the catalytic action of the mixed micelle of N^{α}-myristoyl-L-histidine and CTAB. The application of their theory to the results of present study showed that the dissociation constant of the inactive micelle-substrate complex changed appreciably with the kind of LauAm in the mixed micelle. This disagrees with their assumption that the dissociation constant is independent of the kind of LauAm.

In the present study, the experiments were carried out under these conditions: [detergent]≫CMC, and [detergent]>[LauAm]≫[PNPA]. According to Scheme (1), the pseudo-first-order rate constant, k_ϕ, is given by:

$$k_\phi = \frac{k_b + k \cdot \frac{[M]}{K}}{1 + \frac{[M]}{K}} \qquad (2)$$

where [M] denotes the sum of the initial concentrations of detergent and LauAm. By rearranging Eq. (2) we obtain:

$$\frac{1}{k_\phi - k_b} = \frac{K}{(k-k_b)[M]} + \frac{1}{k-k_b} \qquad (3)$$

Thus, $(k-k_b)$ and K can be evaluated from the plot according to Eq. (3); an example is shown in Fig. 2. For estimating k and K, the value of k_b is necessary. It was found that k_b increased with the pH. The values of k_b at pH's, 7.5, 8.7, and 9.7 were found to be 7.02×10^{-4}, 6.55×10^{-3}, and 2.23×10^{-2} min^{-1} respectively.

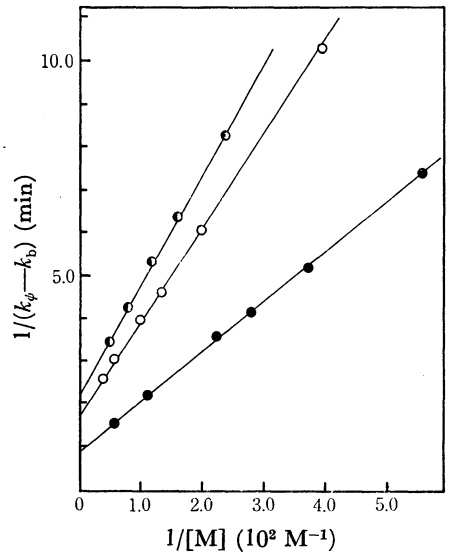

Fig. 2. $1/(k_\phi-k_b)$ *vs.* 1/[M]. The molar ratios of LauHis to CTAB are 1:8 (●), 1:16 (○), and 1:20 (◐), respectively. [M]=[LauHis]+[CTAB].

Table 2. Kinetic data for the hydrolysis of PNPA catalyzed by mixed micelles (25 °C)

System	pH	[LauAm]/[CTAB]	K (10^{-2}M)	k (min^{-1})	k_a (min^{-1})	k_c (min^{-1})
CTAB	7.5[a]	0/1	0.26	5.8×10^{-3}		5.8×10^{-3}
	8.7	0/1	0.45	2.7×10^{-2}		2.7×10^{-2}
	9.7[b]	0/1	0.45	2.3×10^{-1}		2.3×10^{-1}
LauHis -CTAB	8.7	1/8	1.30	1.12	10.1	
		1/16	1.24	0.572	9.72	
		1/20	1.15	0.451	9.47	
		av.	1.23		9.76	
LauTyr -CTAB	8.7	1/8	0.724	1.73	15.6	
		1/16	0.693	0.884	15.0	
		1/20	0.659	0.748	15.7	
		av.	0.692		15.4	
LauLys -CTAB	7.5[a]	1/5	0.882	0.292	1.75	
		3/20	0.984	0.271	1.81	
		1/10	1.09	0.187	2.05	
		1/20	0.981	0.0975	2.05	
		av.	0.984		1.92	
LauArg -CTAB	9.7[b]	1/8	0.483	0.361	1.39	
		1/10	0.418	0.333	1.39	
		3/40	0.383	0.309	1.39	
		1/20	0.375	0.291	1.39	

a) in phosphate buffer. b) in 2-amino-2-methyl-1,3-propanediol–HCl buffer.

The k and K values for mixed micelles are summarized in Table 2, together with those for CTAB micelle. It may be seen in this table that the mixed micelles consisting of the same components yield almost a constant K, regardless of the molar ratio of LauAm to CTAB. This may imply that the micelle–substrate complex formation takes place without electrical processes, since changes in the molar ratio of LauAm to CTAB should alter the micellar charge. This may be attributed to the fact that the substrate, PNPA, is electrically neutral.

It may also be seen in this table that the K value is a characteristic constant for each LauAm. If the micelle-substrate complex formation is regarded as a solubilization of PNPA into the interior of the micellar hydrocarbon phase, the K value should be a constant, regardless of the kind of LauAm in the mixed micelle. Thus, it is likely that the complex formation takes place near the micellar surface or the palisade layer of the micelle.

The treatment given in the present paper assumes that the molar ratio of the micellar substance to the substrate is 1:1. When n molecules bind with a substrate molecule, K in Eqs. (2) and (3) should be replaced by nK. However, no information on n could be obtained from the present data.

On the other hand, k increases as the fraction of LauAm increases, as is shown in Table 2. Since the surface layer of mixed micelles consists of CTAB and LauAm, the rate constant, k, may be expressed as:

$$k = k_a\frac{[\text{LauAm}]}{[M]} + k_c\frac{[\text{CTAB}]}{[M]} \qquad (4)$$

or:

$$k = (k_a-k_c)\frac{[\text{LauAm}]}{[M]} + k_c$$

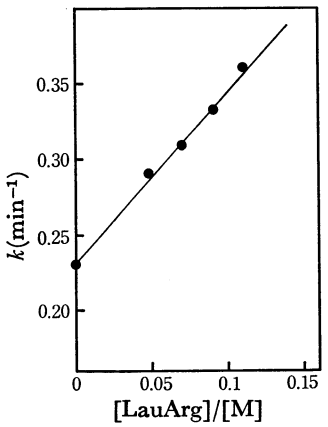

Fig. 3. Plot of k *vs.* [LauArg]/[M]. [M]=[LauArg]+[CTAB]

where k_a denotes the rate constant for the active site (LauAm), and k_c, that for the nonspecific site (CTAB).

For the LauArg–CTAB system, the variation in k with the molar ratio of LauArg to CTAB obeys Eq. (4), as is shown in Fig. 3. The intercept of the ordinate agrees well with the value of k for the CTAB micelle. It may be seen in Table 2 that the k's are much greater in mixed micelles than in the CTAB micelle and that k_c may be ignored as compared with k except in the case of LauArg. Thus, the k_a values for the other systems were estimated according to this relation:

$$k_a = k[\mathrm{M}]/[\mathrm{LauAm}]$$

which is found to be almost independent of the composition of the mixed micelles, as is shown in Table 2.

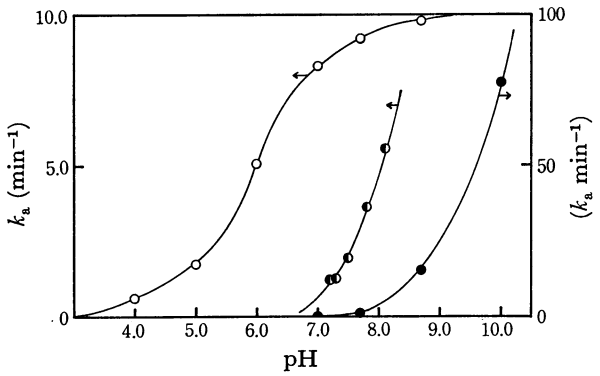

Fig. 4. pH dependence of k_a.
○: LauHis, ◐: LauLys, ●: LauTyr.
Buffer; 1/30 M phosphate (for LauLys), 1/20 M acetate pH 4—6 (for LauHis), 1/20 M Tris pH 7—8.7 (for LauHis and LauTyr), 1/20 M sodium carbonate–sodium hydrogencarbonate (for LauTyr).

The pH Dependence of k_a. The pH dependence of the catalytic activity has been measured; the results are shown in Fig. 4. The values of k_a varied with the pH to a great extent, in contrast to the small change in K. In mixed micelles of LauHis and CTAB, a sigmoidal curve was obtained. This suggests that k_a is proportional to the concentration of the dissociated form of the group with a pK_a value of *ca.* 6.0. Since the pK_a value of the imidazolium group of histidine in protein is 5.6—7.0,[7] the catalytic activity may be ascribed to the imidazolium group of LauHis.

For the mixed micelle of LauTyr and CTAB, it can be predicted from Fig. 4 that phenolate ions, the dissociated form of the side group of tyrosine, possess catalytic activity. The UV spectra of LauTyr were observed in order to find out evidence of dissociation. Two absorptions are found around 245 nm and 300 nm at pH 8.7; they are more enhanced at pH 10.0, while at pH 7.7 they disappear. These two absorptions correspond to those of tyrosine in an alkaline solution.[8] Therefore, it can be concluded that the dissociation of the *p*-hydroxyphenyl group of tyrosine is small at pH 7.7 and that it becomes appreciable at pH 8.7. Thus, it is evident that the catalytic activity of LauTyr is ascribable to the dissociated form of the *p*-hydroxyphenyl group. However, in the mixed micelles of LauTyr and SDS or $C_{12}E_6$ as well as the LauTyr solution without a detergent, the catalytic effect was very small, even at pH 8.7, where the absorption spectra associated with the phenolate ion were not observed. The dissociation of the *p*-hydroxyphenyl group of LauTyr incorporated into the micelle may be promoted by a positive charge at the surface of the CTAB micelle. Probably, the actual pH is higher in the vicinity of the surface of the mixed micelle than in the bulk phase because of an accumulation of hydroxide ions, which are responsible for the dissociation of the *p*-hydroxyphenyl group.

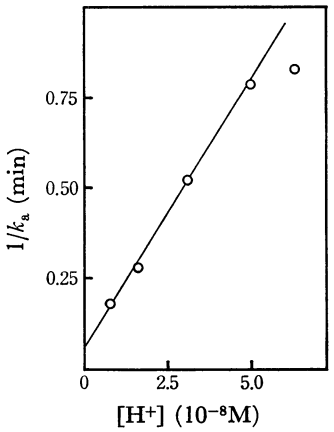

Fig. 5. $1/k_a$ *vs.* [H$^+$] for the mixed micelle of LauLys and CTAB.

From Fig. 4, it can be presumed that the dissociated form of the ε-amino group of N^{α}-lauroyllysine (LauLys) possesses catalytic activity. On this assumption, k_a is expressed by:

$$k_a = k_a^* \cdot \frac{K_a}{[\mathrm{H}^+]+K_a} \tag{6}$$

where k_a^* denotes the rate constant, which is independent of the pH, and K_a, the apparent dissociation constant of the functional group in the mixed micelle. By rearranging Eq. (6) we obtain:

$$\frac{1}{k_a} = \frac{[\mathrm{H}^+]}{k_a^* \cdot K_a} + \frac{1}{k_a^*} \tag{7}$$

The results for the mixed micelle of LauLys and CTAB obey Eq. (7), as is shown in Fig. 5. From the slope and

intercept of the plot, pK_a and k_a* were found to be 8.8 and 37 (min^{-1}) respectively. The value of pK_a determined from the kinetic data is somewhat smaller than that of the ε-amino group of lysine in an aqueous solution.[9] However, it may be thought that this smaller value of pK_a is caused by electrostatic interaction with a positive charge at the surface of the mixed micelle. Such an effect has already been shown in the mixed micellar system of N^{α}-myristoyl-L-histidine and CTAB.[3] A detailed study of the effect of the pH will be required in order to elucidate the mechanism underlying the catalytic action of the functional groups in mixed micelles.

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