6. E. H. Mercer and L. Wolpert, *Exp. Cell Res.* **14**, 629 (1958).
7. R. S. Weinstein and R. R. Hebert, *J. Cell Biol.* **23**, 101A (1964); R. S. Weinstein, *J. Appl. Physics* **36**, 2621 (1965).
8. L. Wolpert, *Exp. Cell Res.* **41**, 385 (1966).
9. Reviewed in L. Wolpert, *Int. Rev. Cytol.* **10**, 163 (1960); H. S. Roberts, *Quart. Rev. Biol.* **36**, 155 (1961).
10. H. Sakai, *Gen. Physiol.* **45**, 411 (1962); *J. Gen. Physiol.* **45**, 427 (1962).
11. H. E. Huxley in *The Cell*, J. Brachet and A. E. Mirsky, Eds. (Academic, New York, 1960), vol. 4, p. 365.
12. H. Portzehl, *Z. Naturforsch.* **66**, 355 (1951).
13. Y. Hiramoto, *Exp. Cell Res.* **32**, 59 (1963).
14. This investigation was supported by NSF grant GB4054 and was carried out at the Misaki Marine Biological Station of Tokyo University. I thank Prof. I. Tomiyama, station director, K. Dan, and Y. Hiramoto for many kindnesses extended during my stay. M. Kojima of the Sugashima Marine Biological Station gave invaluable assistance in obtaining references.

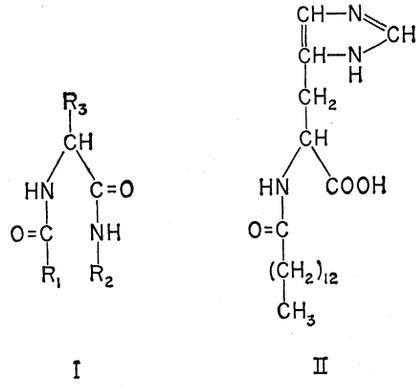
12 April 1967

Catalysis of Ester Hydrolysis by Mixed Micelles containing N-α-Myristoyl-L-Histidine

Abstract. *Model compounds are described for the study of the properties of amino acid side chains in the surface of micelles. Mixed micelles of* N-α-*myristoyl*-L-*histidine and cetyltrimethylammonium bromide catalyze the hydrolyses of* p-*nitrophenyl acetate and* p-*nitrophenyl caprylate at much higher rates than imidazole or histidine do. The reaction shows a kinetic behavior similar to that of surface-catalyzed reactions.*

Kauzmann (*1*) has emphasized the importance of hydrophobic bonding in stabilizing the native conformation of proteins. This postulate has received support of Kendrew's (*2*) and Perutz's (*3*) x-ray data which implies that in many regions the orientation of the side chains in proteins is similar to that occurring in micelles of amphipathic molecules (*1*, *3*, *4*). That is, the nonpolar amino acid side chains are directed away from the water in close van der Waals contact, whereas the polar side chains are directed so that they have maximum contact with water. This orientation would place many functional side-chain groups adjacent to or within hydrophobic regions, giving them properties which might differ from those expected if these same groups were in an aqueous environment (*5*).

We report an initial example of a model system (*6*) which permits study of the properties of amino acid side chains when they are present at the lipid-water interphase. For this purpose, compounds with the general formula (I) may be synthesized; R_1 and R_2 are hydrocarbon chains whose length may be varied depending on the hydrophilic nature of the amino acid functional group R_3. In this manner, the proper hydrophilic-to-hydrophobic ratio could be maintained to yield the desired solubility in water and the desired micelle-forming character (*4*). These micelles would have the hydrocarbon chains directed away from the water, while the amino acid side chain would lie in the micellar surface at the lipid-water interphase. Both R_1 and R_2 need not be present simultaneously, because only one hydrocarbon chain of proper length is necessary for micelle formation.



I II

The imidazole group of histidine is apparently involved in the active center of enzymes (*7*). Also, histidine-containing peptides (*8*) and imidazole *per se* (*9*, *10*) can catalyze the hydrolysis of esters. We therefore have made tests to ascertain whether histidine, when located in a micellar surface, can catalyze the hydrolysis either of *p*-nitrophenyl acetate (NPA) or of *p*-nitrophenyl caprylate (NPC). For this purpose *N*-α-myristoyl-L-histidine (II) was synthesized (*11*). Initial findings indicated that aqueous solutions of (II) at *p*H 7.2 did not accelerate the hydrolysis of NPA. These solutions, although clear when initially prepared, became opalescent on standing and showed strong flow birefringence, indicating that marked association occurred. Also, it seemed likely that the micelles formed by (II) would have the ionized carboxyl groups on the surface, decreasing any possible interaction of the ester with the imidazole group. In order to overcome these difficulties, mixed micelles of II and cetyltrimethylammonium bromide (III) were studied. The rate of NPA hydrolysis increased markedly as the ratio of III to II was increased, a maximum rate being attained at a ratio of 20:1. In the case of NPC, the maximum rate of hydrolysis occurred at a ratio of 2:1 and decreased sharply at higher ratios. All solutions at ratios of (III) to (II) greater than one were clear and showed no opalescence on standing.

When the rate of formation of *p*-nitrophenolate ion was studied under conditions where the concentration of (II) was greater than that of the ester, satisfactory *pseudo*-first-order kinetics were observed. In view of the variation in the extinction coefficient of the *p*-nitrophenol in the presence of varying concentrations of detergent, the specific rate constants were calculated from the amount of *p*-nitrophenol liberated at infinite time (20 to 40 hours) determined for each experimental point. Typical curves from which the specific *pseudo*-first-order rate constants, k_{obs}, were obtained are shown in Fig. 1. These rate constants were corrected by subtracting the rate constants obtained in the presence of (III) alone (less than 3 percent in all cases studied) (*12*). At low concentrations of

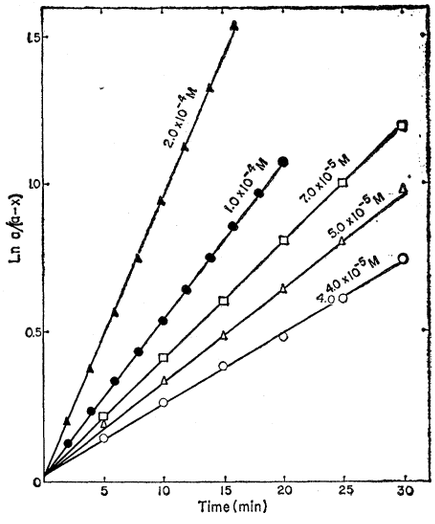


Fig. 1. *Pseudo*-first-order rate constants for the hydrolysis of *p*-nitrophenyl acetate by mixed micelles of *N*-α-myristoyl-L-histidine (II)-cetyltrimethylammonium bromide (III). In addition to the concentrations of II shown in the figure, the assay system contained a 20-fold greater concentration of III, both dissolved in 50 m*M* tris-HCl buffer, *p*H 7.2. To this was added 10 μl of the NPA (3.45 to 6.91 × 10^{-6}*M*) dissolved in acetonitrile. The rate of *p*-nitrophenol liberation was measured by following the change in optical density at 400 mμ at 25 ± 0.5°C in a Cary 15 recording spectrophotometer. *a* is the optical density of the *p*-nitrophenol liberated at infinite time. *x* is the optical density of the *p*-nitrophenol liberated at the times indicated.

Table 1. Specific rate constants for the hydrolysis of *p*-nitrophenyl esters by mixed micelles of *N*-α-myristoyl-L-histidine (NMH) and cetyltrimethylammonium bromide (CTAB). Conditions are the same as in Fig. 1. At all concentrations of II and III studied, the surface tension of the solutions gave values of 34 to 37 dyne/cm.

NMH ($10^{-5}M$)	CTAB ($10^{-4}M$)	k_{obs}* (min^{-1})	k_c† (liter $mole^{-1}$ min^{-1})
p-*Nitrophenyl acetate (3.45 to 6.91* × *10⁻⁶*M)			
4	8	2.41×10^{-2}	602
5	10	3.02×10^{-2}	604
6	12	3.42×10^{-2}	570
7	14	3.94×10^{-2}	563
8	16	4.49×10^{-2}	561
10	20	5.17×10^{-2}	517
20	40	9.12×10^{-2}	456
30	60	12.81×10^{-2}	427
40	80	15.34×10^{-2}	384
50	100	18.94×10^{-2}	379
p-*Nitrophenyl caprylate (9.0* × *10⁻⁶*M)			
4	0.8	1.26	315×10^2
5	1.0	1.36	272×10^2
6	1.2	1.51	252×10^2
7	1.4	1.63	233×10^2
8	1.6	1.64	205×10^2
10	2.0	1.78	178×10^2

* k_{obs} was corrected for the rate of hydrolysis observed in the presence of cetyltrimethylammonium bromide alone. † $k_c = k_{obs}$/concentration of *N*-α-myristoyl-L-histidine (*9*).

catalyst, when NPA is used as substrate, the rate of ester hydrolysis as the concentration of II is increased appears to be proportional to the catalyst concentration; at higher concentrations, the rate is independent of catalyst (Table 1). These results can be explained if it is assumed that the initial step in the reaction sequence involves the formation of an adsorption complex between the ester and the mixed micelle. Then under conditions where [III] ≫ [ester] (square brackets indicating concentration), the substrate should become saturated with respect to catalyst (*13*). In the case of the NPC hydrolysis, this ester appears to have a higher affinity for the mixed micellar catalyst so that the limiting value is reached at lower catalyst concentrations. This would be expected because of the longer, more hydrophobic acyl chain present.

If the reciprocal of k_{obs} is plotted against the reciprocal of the concentration of the catalyst from the values shown in Table 1, a straight line is obtained for both esters, a further indication that the kinetics of the reactions studied show saturation phenomena. The k_c values (Table 1) for NPA hydrolysis are higher than those reported (*8*) for histidine hydrochloride ($k_c = 6$), imidazole ($k_c = 20$) or histidyl-peptides ($k_c = 15$ to 147).

The liberation of *p*-nitrophenol is apparently accompanied by the simultaneous formation of an *N*-imidazole-acyl- *N*-α-myristoyl-L-histidine intermediate, and the subsequent hydrolysis of this intermediate is rate-limiting in the overall hydrolysis of the esters. Under conditions where the [ester] ≫ [II], approximately 1 mole of *p*-nitrophenol per mole of II is liberated instantaneously (*14*).

The kinetic behavior of our model makes it quite similar to enzyme-catalyzed reactions.

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References and Notes

1. W. Kauzmann, *Advances Protein Chem.* **14**, 1 (1959).
2. J. C. Kendrew, *Brookhaven Symp. Biol.* **15**, 216 (1962).
3. M. F. Perutz, *J. Mol. Biol.* **13**, 646 (1965).
4. K. Shinoda, T. Nakagawa, B. Tamamushi, T. Isemura, in *Colloidal Surfactants: Some Physicochemical Properties* (Academic Press, New York, 1963), chap. 1.
5. D. B. Wetlaufer, J. T. Edsall, B. R. Hollingworth, *J. Biol. Chem.* **233**, 1421 (1958); S. Yanari and F. A. Bovey, *ibid.* 235, 2818 (1960).
6. Reactions in the surface of micelles have been previously studied. See, for example, E. F. J. Duynstee and E. Grunwald, *J. Amer. Chem. Soc.* **81**, 4540, 4547 (1959); D. G. Herries, W. Bishop, F. M. Richards, *J. Phys. Chem.* **68**, 1842 (1964); L. J. Winters and E. Grunwald, *J. Amer. Chem. Soc.* **87**, 4608 (1965); M. T. A. Behme, J. G. Fullington, R. Noel, E. H. Cordes, *ibid.*, p. 266.
7. L. Weil and A. R. Buchert, *Fed. Proc.* **11**, 307 (1952); A. J. Cohen, R. A. Oösterbaan, H. S. Jansz, F. Behrends, *J. Cell. Comp. Physiol.* **54**, Suppl. 1, 231 (1959); E. A. Barnard and W. D. Stein, *Advances Enzymol.* **20**, 51 (1958); B. S. Hartley, in *Structure and Activity of Enzymes*, T. W. Goodwin, J. I. Harris, B. S. Hartley, Eds. (Academic Press, London, 1964), p. 47.
8. J. C. Sheehan, G. B. Bennett, J. A. Schneider, *J. Amer. Chem. Soc.* **88**, 3455 (1966).
9. M. L. Bender and B. W. Turnquest, *ibid.* **79**, 1952 (1957).
10. T. C. Bruice and G. L. Schmir, *ibid.* p. 1663; W. P. Jencks, *Biochim. Biophys. Acta* **24**, 227 (1957).
11. Prepared by reacting myristoyl chloride with L-histidine in a basic aqueous medium (m.p. solid $\xrightarrow{158°C}$ liquid cryst. I, $\xrightarrow{182°C}$ liquid cryst. II $\xrightarrow{192°C}$ decomp.). Analyses: calculated for $C_{20}H_{35}O_3N_3$: C, 65.75; H, 9.59; O, 13.15; N, 11.51. Found: C, 65.84; H, 9.60; O, 13.20; N, 11.36. (Analyses by Mikroanalytische Laboratorium, Bonn, Germany.)
12. The rate of ester hydrolysis in 50 m*M* tris-HCl buffer *p*H 7.2 decreased markedly in the presence of micelles of cetyltrimethylammonium bromide. With NPA, the rate decreased about 50 percent, while with NPC the decrease was in the order of 90 percent. These results agree with those of S. Riegelman, *J. Amer. Pharm. Assoc. Sci. Ed.* **49**, 339 (1960), and A. G. Mitchell, *J. Pharm. Pharmacol.* **14**, 172 (1962).
13. K. J. Laidler, *Chemical Kinetics* (McGraw-Hill, New York, 1950), p. 280; F. J. Kézdy and M. L. Bender, *Biochemistry* **1**, 1097 (1962).
14. B. S. Hartley and B. A. Kilby, *Biochem. J.* **56**, 288 (1954).
15. Supported in part by NIH grant AM-07928 from NIAMD.
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Lactones as Inhibitors of the Fibrinolytic System

Abstract. *Caprolactone, valerolactone, and butyrolactone inhibit proteolytic and fibrinolytic activities of human plasmin. In very low concentrations, they also inhibit activation of plasminogen through plasmin-streptokinase activator and human urokinase. The degree of inhibitory potency depends upon the number of carbon atoms in the lactone.*

Aliphatic aminocarboxylic acids act as inhibitors of the fibrinolytic system. Their effect upon the activation process of plasminogen is stronger than their action upon plasmin itself. The activity of these compounds is maximum, when there are six carbon atoms in the chain and the amino group is located in terminal position (*1*). Lactams also inhibit fibrinolysis competitively, and caprolactam exerts the strongest inhibitory action (*2*).

We studied lactones to determine whether the simultaneous presence of carboxylic and amino groups in both the aliphatic and cyclic compounds causes inhibition, or whether the specific polarity of the cyclic arrangement alone is of importance for the inhibitory effect. Lactones are the internal esters of carboxylic acids, which correspond to lactams in their cyclic configuration, but which do not have the amino group; they also exert inhibitory effects in other biological systems (*3*).

By means of the casein test (*4*), we investigated the influence of caprolactone, valerolactone, and butyrolactone

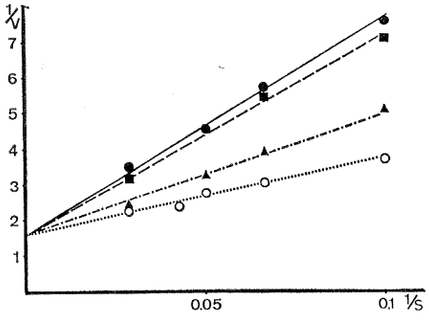

Fig. 1. Competitive inhibition of human plasmin in the casein test by 0.5*M* caprolactone (———), valerolactone (– – –), and butyrolactone (– · – · –). Control or action of plasmin without lactone (. . . . .). S, concentration of substrate in milligrams of casein per milliliter; V, extinction at 280 mμ of supernatant, that is, the fraction soluble in chloroacetic acid.