The substrates were D-desulfopantetheine 4′-phosphate (4 μmol) and dephosphodesulfo-CoA (2 μmol). When dephosphodesulfo-CoA was incubated with inorganic pyrophosphate (40 μmol), ATP was omitted. The reactions were stopped by placing the incubation tubes in a boiling water bath for 1 min and removing the precipitated protein by centrifugation. The supernatant solutions were diluted to a total volume of 25 ml and applied to a 1.3 × 50 cm DEAE-cellulose (chloride form) column. The column was washed with 0.003 *N* HCl. The adsorbed compounds were eluted with an acidic lithium chloride linear gradient, 350-ml reservoir of 0.12 *N* LiCl in 0.003 *N* HCl and 350-ml mixing vessel of 0.003 *N* HCl. Fractions (5 ml) were collected at a flow rate of 0.5 ml/min. Each ultraviolet-absorbing peak (260 mμ) was pooled, the pH adjusted to 4.5 with dilute LiOH, and solvent removed *in vacuo*. Lithium chloride was removed by repeated extractions with small volumes of methyl alcohol–acetone (1:15). After drying over P_2O_5 *in vacuo* at room temperature overnight, the residual material was dissolved in 1 ml of water and characterized by paper chromatography.

The concentration of each peak was calculated from the optical density units at 260 mμ, assuming an extinction coefficient of 15×10^3 for the adenosine moiety.

Nonpolar Contributions to the Rate of Nucleophilic Displacements of *p*-Nitrophenyl Esters in Micelles[1]

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Contribution from the Department of Biochemistry, Centro de Investigacion y de Estudios Avanzados del Instituto Poletecnico Nacional, Mexico 14, D. F., Mexico. *Received March 11, 1968*

Abstract: A remarkably large rate enhancement in the hydrolysis of straight-chain acyl esters of *p*-nitrophenol was observed in the presence of mixed micelles of N^{α}-myristoyl-L-histidine and cetyltrimethylammonium bromide (CTABr), as contrasted with the corresponding reaction with N^{α}-acetyl-L-histidine with or without CTABr. The micellar reaction was resolved into three steps: (1) the nonproductive rapid equilibrium binding of the esters to the CTABr regions of the micelle; (2) the reaction which leads to the acylation of the imidazolyl moiety of the N^{α}-myristoyl-L-histidine in or on the surface of the micelle, which follows second-order kinetics; and (3) the deacylation step. Both the logarithm of the apparent binding constant (step 1) and the logarithm of the second-order rate constant (step 2) increase linearly with the number of carbons in the acyl group of the *p*-nitrophenyl esters from acetate to hexanoate. A value of −630 cal/mol for the transfer of each methylene group of the acyl chain from the bulk solution to the CTABr micellar phase was calculated from the change in the apparent binding constants. In a similar manner, the observed differences in the second-order constant for the acylation reaction are compatible with a change in free energy of activation of 442 cal/mol for each methylene added to the acyl chain. These results suggest that in the micellar reaction the hydrophobic bond energy contributed by the acyl chain is used to decrease the activation energy of the catalyzed reaction.

The majority of the chemical reactions in biological systems occur in or on the vicinity of boundaries between apolar and polar regions. Thus, biological membranes are composed of phospholipid molecules associated to form lamellar or micellar aggregates. In addition, the participation of hydrophobic bonding in the stabilization of the native conformation of proteins[3] implies that in many regions the orientation of the side chains in proteins is similar to that occurring in micelles of amphipathic molecules. 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 the same groups were in an aqueous environment.[4]

It is important therefore, to ascertain how the behavior of a chemical reaction is altered when it occurs at such hydrocarbon–water interfaces. The most common approach to this problem has involved the study of reactivity in the presence of detergent micelles.[5] Recently, we reported a model system[6] which permits the study of the properties of amino acid side chains when present in a hydrocarbon–water interface. It is based on the fact that N^{α} long-chain acylamino acids in aqueous solutions form micelles with the hydrocarbon chains directed away from the water, while the amino acid side chains would lie in the micellar surface at the hydrocarbon–water interface. As an initial example of the model we showed that mixed micelles of N^{α}-myristoyl-L-histidine (MirHis) and cetyltrimethylammonium bromide (CTABr) catalyze the hydrolysis of *p*-nitrophenyl esters at significantly higher rates than those reported for histidine, imidazole, or histidine-containing peptides or polymers.[6] These results indicated the potential use of such micelles to create catalytic surfaces

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containing, in addition to the usual charged groups, nucleophilic or other reactive groups. Bruice, *et al.*,[7] simultaneously proposed a similar micellar model based on their findings of the reaction of N-nonyl-*p*-trimethylammonium benzylamines and 4-acetyloxy-3-nitrobenzenesulfonates of varying chain length. Subsequent similar results have been reported by Wagner, *et al.*[8] The present study is a detailed kinetic analysis of the hydrolysis of a series of *p*-nitrophenyl esters with increasing number of carbon atoms in the acyl moiety, catalyzed by mixed micelles of MirHis and CTABr. The results indicate that lengthening of the acyl group results in additional hydrophobic bond energy which is used in the micellar surface to lower the free energy of activation of the catalyzed reaction.

Experimental Section

Materials. N^{α}-Myristoyl-L-histidine. To 1.55 g (10 mmol) of L-histidine free base (Sigma) and 0.62 g (11 mmol) of potassium hydroxide in 400 ml of water at room temperature (22°) was added 2.47 g (10 mmol) of myristoyl chloride (British Drug House Ltd.) over a period of 1 hr. The solution was vigorously shaken during the addition and for a further 3-hr period. It was then adjusted to pH 4 with 1 *N* HCl and the precipitate filtered and washed with an excess of water. It was dissolved in methanol and precipitated with water. This step was repeated two more times. The final precipitate was dissolved in methanol containing 1% of concentrated formic acid and a trace amount of [1-^{14}C]myristic acid. This solution was extracted with *n*-hexane until no radioactivity was detected in the methanolic solution. Water was added, and the N^{α}-myristoyl-L-histidine was obtained as an amorphous powder (2.0 g, 55% yield); solid melts at 153° to form liquid crystal I which melts at 182° to form liquid crystal II which decomposes at 192°. *Anal.* Calcd 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. The product gave a single spot on thin layer chromatography which was ninhydrin negative and Pauly positive.

N^{α}-Acetyl-L-histidine·H_2O, mp 164–166° (Cyclo Chemical Co.), was used without further purification. Cetyltrimethylammonium bromide was a high-purity lot obtained from the British Drug House Ltd. It gave a critical micelle concentration (CMC) determined by surface tension of 1.02×10^{-3} *M* in aqueous solution (lit.[9] CMC value of 1.0×10^{-3} *M*) and of 5.0×10^{-5} *M* in Tris buffer 0.05 *M*, pH 7.2.

p-Nitrophenyl acetate (PNPA) was synthesized by the procedure of Chattaway[10] and recrystallized from ether; mp 77–78° (lit.[11] mp 77.3°). *p*-Nitrophenyl esters of propionate (PNPP), butyrate (PNPB), valerate (PNPV), and hexanoate (PNPH) obtained from Sigma Chemical Co. were used without further purification. Quartz-distilled water was used in making up all the solutions.

Kinetic Measurements. The general procedure used to measure the rates of ester hydrolysis in the presence of the mixed micelles consisted of adding 10 to 25 μl of a solution of the *p*-nitrophenyl ester in acetonitrile to 2.0 ml of a 0.05 *M* Tris buffer, pH 7.2 solution containing MirHis and CTABr at the desired concentrations. The rate of *p*-nitrophenol liberation at 25 ± 0.2° was measured by following the change in absorbance at 400 mμ (at pH 6 and above) and at 317 mμ (below pH 6) with a Cary 15 recording spectrophotometer.

Under conditions [CTABr] > [PNPX] >> [MirHis] the pseudo-first-order rate constants, k_b', for the presteady-state acylation reaction were calculated using as the absorbance at infinite time that obtained at the point where the steady-state rate extrapolates back to the presteady state as suggested by Kezdy and Bender.[12] A correction was also made for the rate observed in the presence of CTABr alone.

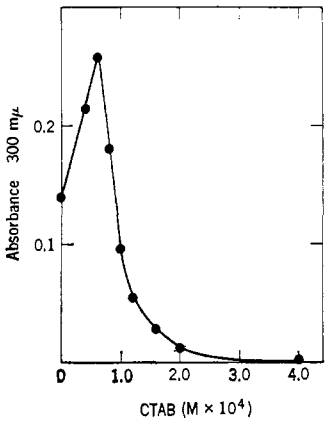

Figure 1. Turbidity of solutions containing N^{α}-myristoylhistidine (6.0×10^{-5} *M* in 0.05 *M* Tris buffer, pH 7.2) plotted as a function of increasing concentrations of cetyltrimethylammonium bromide.

Under conditions [CTABr] > [MirHis] >> [PNPX] the pseudo-first-order rate constants were calculated using the absorbance at infinite time (more than ten half-lives) determined for each experimental point as reported previously.[6] The rate constants were measured in the presence of the mixed micelles and in the presence of CTABr alone. The net rate due to the MirHis in the mixed micelles was thus obtained from ($k_{obsd}' - k_{CTAB}'$) = k_a'. The rate k_{CTAB}' was between 1 and 3% that of k_{obsd}' for PNPA and less than 1% for the other *p*-nitrophenyl esters.

In some cases the rate constants were also calculated using the method of Guggenheim;[13] essentially equal results were obtained with either method. The kinetic parameters k_2 and K_i and their standard errors were evaluated with a CDC 6400 computer using a program (HYPER) described by Cleland.[14,15] These calculations were also obtained using a nonlinear least-squares regression program (NONLIN).[16]

Results

Formation of Mixed Micelles. MirHis is quite insoluble in 0.05 *M* Tris buffer, pH 7.2. As shown in Figure 1 a solution 6×10^{-5} *M* of MirHis shows appreciable turbidity at 300 mμ. Addition of CTABr to this solution leads to an increase in the turbidity which reaches a maximum at a CTABr to MirHis ratio of 1:1. Further increase in CTABr leads to a fall in the turbidity and at ratios of 2:1 or above the solutions show negligible turbidity even after standing for prolonged periods. At pH 7.2 MirHis has a net negative charge due to the presence of the ionized carboxylic acid. Upon addition of CTABr, charge neutralization results which leads to the increased turbidity until complete neutralization occurs at a 1:1 ratio. Further addition of CTABr now leads to micelles with a net positive charge and, since the turbidity falls below that of MirHis alone, this is evidence that the MirHis is incorporated into CTABr micelles.[17] That micelles are actually present can be judged from the fact that the CMC of CTABr in 0.05 *M* Tris buffer, pH 7.2, is 5.0×10^{-5} *M* and that of the mixed micelles at a CTABr to MirHis ratio of 2 is depressed to a value of 2.5×10^{-5} *M*. All kinetic studies were performed at CTABr concentrations of 2.0×10^{-4} *M* or higher, well above the CMC.

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(16) Program NONLIN was designed by Dr. R. M. Baer of the University of California Computer Center.
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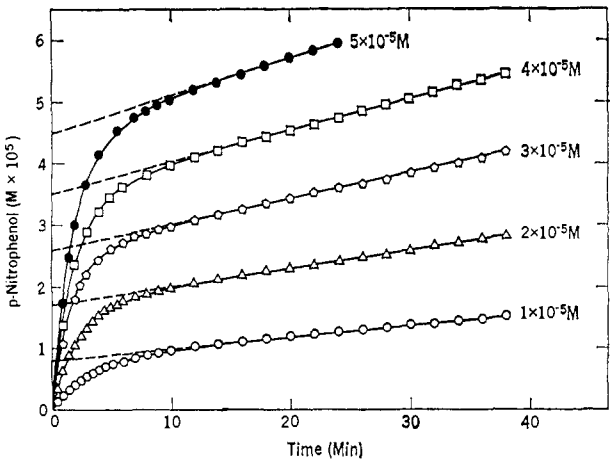

Figure 2. Time-dependent liberation of *p*-nitrophenol in the reaction of *p*-nitrophenyl acetate with increasing concentrations of N^{α}-myristoylhistidine (at a cetyltrimethylammonium bromide to N^{α}-myristoylhistidine ratio of 20). PNPA concentration varied from 0.4 to 2.0 × 10^{-3} *M*.

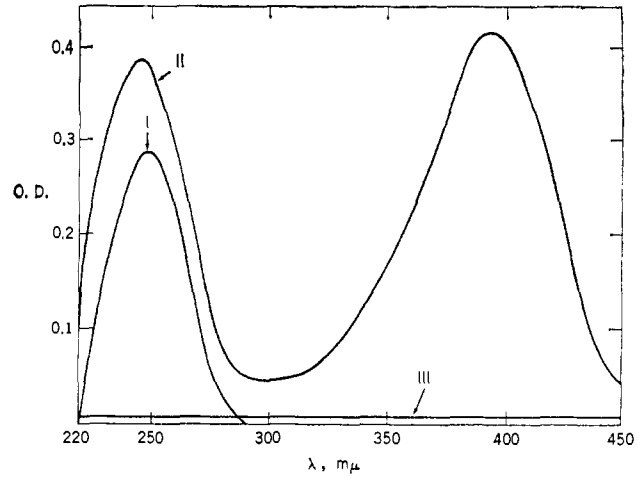

Figure 3. Spectrophotometric identification of the acetylated intermediate. A DEAE-Sephadex column was equilibrated with a solution 4.0 × 10^{-4} *M* N^{α}-myristoylhistidine and 8.0 × 10^{-3} *M* cetyltrimethylammonium bromide in 0.05 *M* Tris, pH 7.2. To 5.0 ml of this solution was added 20 μl of 0.33 *M* *p*-nitrophenyl acetate in acetonitrile. Two milliliters of this reaction mixture was applied to the column and eluted with the solution used for equilibration; 1.5-ml fractions were collected. The plot shows the difference spectra between the equilibrating solution and (I) that appearing at the void volume, and (II) that appearing at the void plus internal volumes; also, solution I adjusted to pH 11 (III).

Rate of Ester Hydrolysis in the Presence of Mixed Micelles. The rate of hydrolysis of PNPA in the presence of MirHis dissolved in Tris buffer was difficult to measure due to the insolubility of the MirHis. The measurements made indicated that the rate was essentially the same as that of Tris alone. Addition of an anionic detergent, sodium lauryl sulfate, to the MirHis solutions resulted in rates of hydrolysis slightly below those of the MirHis alone. On the other hand, mixed micelles of CTABr and MirHis showed markedly enhanced rates of hydrolysis of PNPA. In order to study the kinetic parameters of this reaction, two sets of conditions were tested. In the first set of conditions, [CTABr] > [PNPX] >> [MirHis], attempts were made to saturate the micelles with respect to substrate. In the second, [CTABr] > [MirHis] >> [PNPX], low concentrations of the esters were used in the presence of an excess of the mixed micelles. The second set of conditions had the advantage that the low concentrations of substrate and of products formed would not greatly alter the nature of the micelles studied. Also, if an acylated intermediate should be formed, the presence of an excess of MirHis would make the observed rates independent of the deacylation rate.[12]

Conditions [CTABr] > [PNPX] >> [MirHis]. The appearance of the *p*-nitrophenolate ion as a function of time for different concentrations of MirHis and PNPA is shown in Figure 2. There is an initial rapid liberation which is followed by a slow linear phase. By determining the concentration of *p*-nitrophenol at the point where the linear portion of the curve extrapolates back to the rapid initial phase, it was calculated that 1.06 ± 0.02 mol of *p*-nitrophenol is liberated per mol of MirHis during the rapid initial phase. The rate of *p*-nitrophenolate ion formation in the linear phase gave a value of 0.0136 mol of *p*-nitrophenol liberated per min per mol of MirHis.

The rapid portion of the reaction was determined to follow pseudo-first-order kinetics. The specific rate constant, k_b', has a linear dependence on PNPA concentration. Higher concentrations of PNPA than that of CTABr could not be tested since turbidity developed. In the workable range of substrate concentrations, no saturation kinetics were observed. The second-order rate constant obtained from $k_2 = k_b'/[\text{ester}]$ was 360 ± 9 M^{-1} min^{-1}.

Identification of the Intermediate Formed. It seemed reasonable to suspect that the initial rapid liberation of *p*-nitrophenol resulted from the formation of N^{α}-myristoylhistidine (Im-acetyl). However, difficulties were encountered in obtaining the spectrum of the intermediate during the course of the reaction due to the high absorbance of the *p*-nitrophenolate ion. Since the rate of breakdown of the intermediate was known to be quite slow, attempts were made to separate the reaction products from the reacted micelle. Figure 3 shows that this was accomplished by column chromatography on DEAE-Sephadex. The difference spectrum of the unreacted micelles *vs.* those that reacted with *p*-nitrophenyl acetate shows a peak at 248 mμ. The material in the 248-mμ peak is labile to base and has a spectrum after hydrolysis which is identical with that of the unreacted micelles.

Conditions [CTABr] > [MirHis] >> [PNPX]. The specific pseudo-first-order rate constant, k_a', for the hydrolysis of the *p*-nitrophenyl esters depends on the concentration of mixed micelles of CTABr and MirHis as shown in Figure 4. Each set of data was obtained at a constant CTABr to MirHis ratio. In the case of PNPA, there is a linear dependence of k_a' on MirHis only at the lowest ratios. At higher ratios the rates decrease and show a hyperbolic dependence on MirHis. This tendency becomes more apparent as the length of the acyl chain of the esters is increased. These results demonstrated that the observed rate constants decreased as the relative concentration of CTABr was increased.

As reported previously,[6] the rate of hydrolysis of the *p*-nitrophenyl esters catalyzed by Tris decreases markedly in the presence of CTABr. The decrease in rate varied from 2-fold for PNPA, 20-fold for PNPP, and 50-fold or greater for PNPB, PNPV, and PNPH. This

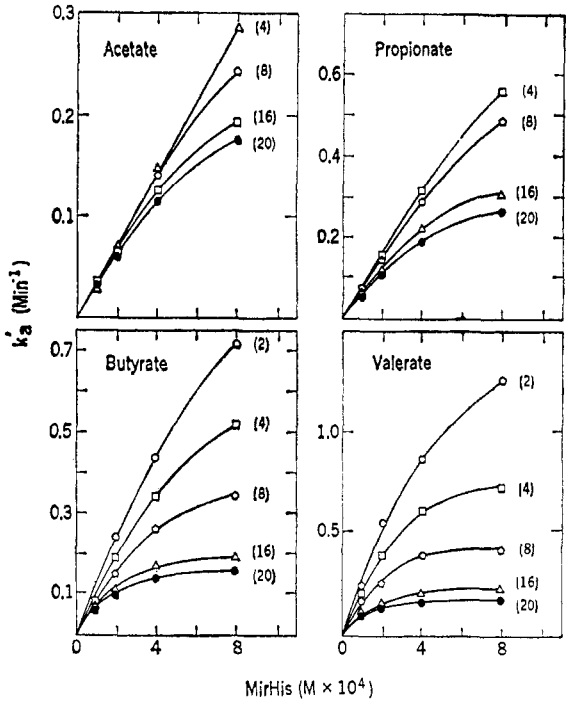

Figure 4. Pseudo-first-order rate constants for the liberation of *p*-nitrophenol in the reaction of mixed micelles of N^{α}-myristoylhistidine and cetyltrimethylammonium bromide (at the ratio indicated in parentheses) with *p*-nitrophenyl acetate (PNPA), propionate (PNPP), butyrate (PNPB), and valerate (PNPV).

indicates that the esters were incorporated into the CTABr micelles and thus protected from the Tris-catalyzed hydrolysis. In the presence of mixed micelles of CTABr and MirHis, the CTABr not in the vicinity of MirHis (Mi) should lead to nonproductive binding of substrate and cause a decrease in the observed reaction rates when studied under those conditions where the rate is dependent upon substrate concentration. For this situation the reaction sequence could be represented by

$$S + Mi \underset{}{\overset{K_i}{\rightleftharpoons}} S\cdot Mi \qquad (1)$$

$$S + Ma \xrightarrow{k_2} X\text{-}Ma + P_1 \qquad (2)$$

$$X\text{-}Ma + H_2O \xrightarrow{k_3} Ma + P_2 \qquad (3)$$

where S is the *p*-nitrophenyl ester; Ma and Mi are respectively the active and inactive regions of the mixed micelle; S·Mi is the rapidly formed inactive adsorption complex; X–Ma is N^{α}-myristoylhistidine (Im-acyl); P_1 is *p*-nitrophenol and P_2 is the acid of the corresponding acyl ester. The evidence presented under conditions [CTABr] > [PNPX] >> [MirHis] supports steps 2 and 3 of the proposed kinetic scheme. During the rapid initial reaction, 1 mol of *p*-nitrophenolate ion is liberated per mol of MirHis and the resultant intermediate has an absorption maximum of 248 mμ which is almost identical with that of acetylimidazole (245 mμ) reported by Stadtman.[18] The observed shift in adsorption maxima might be attributable to the fact that the acylated imidazolyl group of the MirHis is buried in a hydrophobic region of the micelle.[4] This could also explain its unusual stability. The presence of a steady-state linear portion also indicates that k_2

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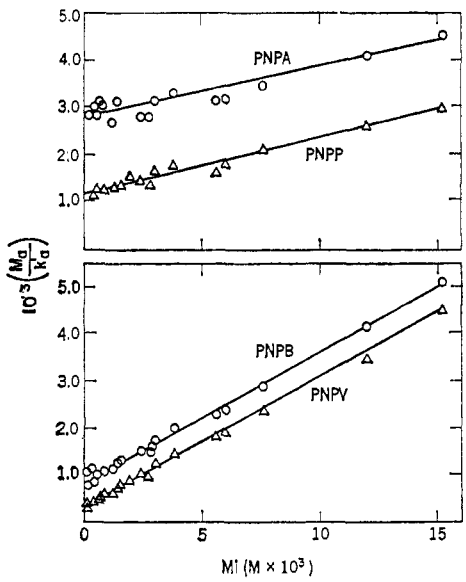

Figure 5. The reciprocal of the kinetic second-order rate constants as a function of increasing Mi concentration. Mi = [CTABr] − [MirHis].

>> k_3 in the proposed reaction sequence. Under conditions [CTABr] > [MirHis] >> [S], using the kinetic scheme of eq 1–3, it may be shown that

$$\frac{d[P_1]}{dt} = \frac{k_2[Ma]}{1 + ([Mi]/K_i)}([S_0] - [P_1]) \qquad (4)$$

so that the pseudo-first-order experimental rate constant k_a' is given by

$$k_a' = \frac{k_2[Ma]}{1 + ([Mi]/K_i)} \qquad (5)$$

We are assuming in deriving eq 4 and 5 that the rate of diffusion of substrate into the Mi region to form S·Mi is very fast compared with the rate of chemical reaction (eq 2). From eq 5 a plot of $[Ma]/k_a'$ *vs.* [Mi] should give a straight line with slope and intercept of $1/k_2K_i$ and $1/k_2$, respectively. Figure 5 shows that the data of Figure 4 when plotted in this manner gives straight-line relationships. In this plot, Mi has been equated with that portion of the CTABr not forming a salt with the MirHis. Since Figure 1 shows that a 1:1 salt is formed between MirHis and CTABr, [Mi] = [CTABr] − [MirHis]. The value k_2 is then the second-order rate constant for the 1:1 complex or Ma. Much greater scattering of the data is observed if the plots of Figure 5 are made *vs.* total CTABr or [CTABr] − 2[MirHis].[19]

(19) It should be emphasized that k_2 and K_i are apparent experimental constants. It is conceivable that the process depicted in eq 2 could proceed in two steps

$$S + Ma \underset{}{\overset{K_a}{\rightleftharpoons}} S\cdot Ma \qquad (2a)$$

$$S\cdot Ma \xrightarrow{k_m} X\text{-}Ma + P_1 \qquad (2b)$$

where S·Ma is the rapidly formed adsorption complex at the Ma region. Based on the same considerations as before, it can be shown that the over-all reaction would still be experimentally second order with a rate constant of k_m/K_a when [MirHis] < K_a. This scheme would further predict that under conditions where [MirHis] ≥ K_a, saturation kinetics should be observed. Since no saturation could be detected, no basis for choosing between either scheme presently exists.

In the expression for Mi = [CTABr] − [MirHis] the concentration of nonmicellar CTABr molecules has been neglected since it represents a minor correction and it is difficult to estimate accurately in the presence of added substrates.

Table I. Kinetic Data for the Hydrolysis of *p*-Nitrophenyl Esters Catalyzed by N^{α}-Acetylhistidine and by Mixed Micelles of MirHis and CTABr.[a] Conditions [CTABr] > [MirHis] >> [PNPX]

p-Nitrophenyl ester[b]	N-AcHis,[c] k_2,[f] M^{-1} min^{-1}	N-AcHis[d] + CTABr, k_2,[f] M^{-1} min^{-1}	Micelles of MirHis, CTABr[e] k_2,[g] M^{-1} min^{-1}	K_i, $M \times 10^3$
Acetate	12.6 ± 0.50	9.6 ± 0.42	377 ± 8.0	33.2 ± 6.5
Propionate	16.2 ± 0.62	9.6 ± 0.72	871 ± 3.4	9.3 ± 1.0
Butyrate	9.8 ± 0.60	4.0 ± 0.24	1350 ± 32	2.9 ± 0.19
Valerate	11.6 ± 0.36	3.0 ± 0.16	3025 ± 110	1.3 ± 0.13
Hexanoate	9.0 ± 0.24	2.2 ± 0.04	7310 ± 290	0.5 ± 0.04

[a] All solutions were made in Tris buffer 0.05 *M*, pH 7.2. [b] The ester concentrations varied from 0.34 to 1.0 × 10^{-5} *M*. [c] N-AcHis concentrations varied from 0.02 to 0.20 *M*; ester 0.5 to 1.0 × 10^{-4} *M*. [d] The N-AcHis concentrations as in footnote *c*. CTABr added to give a CTABr to N-AcHis ratio of 2. [e] MirHis concentration 0.1–0.8 × 10^{-3} *M*; CTABr concentration 0.2 to 15.2 × 10^{-3} *M*. Values derived from eq 5 using the programs HYPER or NONLIN. [f] Corrected for the basic form of the imidazolyl group. k_2/[N-AcHis, imidazolyl base form]. [g] Corrected for the basic form of the imidazole group. k_2/[MirHis, imidazolyl base form].

Table I shows the values derived from eq 5 for the kinetic second-order rate constants, k_2, and the dissociation constants, K_i, for different *p*-nitrophenyl esters. Table I also shows the second-order rate constants for the hydrolysis of the esters catalyzed by N^{α}-acetylhistidine alone and in the presence of CTABr.

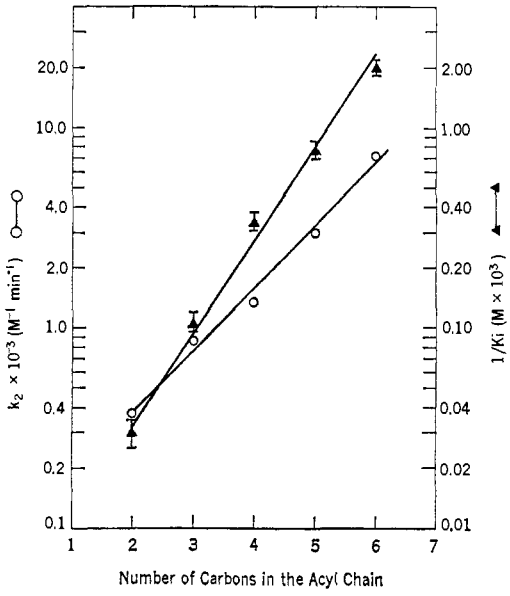

Figure 6. Variation in the logarithm of the kinetic second-order rate constants, k_2, and the logarithm of the reciprocal of the dissociation constant, K_i as a function of the number of carbons in the acyl group of the *p*-nitrophenyl esters.

Increasing the chain length of the acyl group of the esters decreases the rate of hydrolysis catalyzed by N-acetylhistidine alone or in the presence of CTABr. With the mixed micellar catalyst, a marked acceleration in the rates results as the acyl chain is lengthened. Figure 6 shows that there is a linear increase in log k_2 and in log $1/K_i$, the apparent binding constant in the micelle-catalyzed reaction, as the number of carbons in the acyl group of the ester is increased.

Effect of pH. Values of k_2 and K_i were determined as a function of pH. The results indicated that K_i was not affected by changes in the pH while the k_2 values increased in the manner shown in Figure 7. The sigmoidal nature of the curve indicates that the value of k_2 is proportional to the concentration of the dissociated form of a group with a pK_{app} of 6.2. This value is compatible with the assignment of the free imidazolyl moiety of MirHis in an environment where the imidazole experiences a large field effect from the highly positively charged micelle surface. The imidazole groups in polyhistidine (lit.[20] pK_{app} = 6.15) experience a similar field effect.

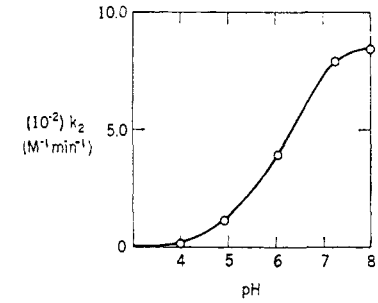

Figure 7. Kinetic second-order rate constants for the liberation of *p*-nitrophenol from *p*-nitrophenyl acetate in the presence of mixed micelles of N^{α}-myristoylhistidine and cetyltrimethylammonium bromide as a function of pH at 25° and ionic strength 0.05; acetate, phosphate, and carbonate buffers employed in the appropriate pH regions.

Discussion

The evidence presented indicates that the reaction studied is the hydrolysis of esters catalyzed by MirHis present in the mixed micelles. Participation of nonmicellar MirHis is unlikely since free MirHis did not increase the observed rate over that of Tris alone. In addition, all kinetic studies were performed above the CMC of CTABr or of the mixed micelles of CTABr and MirHis at a ratio of 2. As interaction of MirHis and CTABr involves both hydrophobic forces and charge neutralization, it is unlikely that an appreciable fraction of the MirHis could become desorbed from the CTABr micelles.

As mentioned, the Tris-catalyzed rate of ester hydrolysis is markedly reduced in the presence of CTABr. Apparently the esters are incorporated into or on the surface of the CTABr micelles and are not susceptible to Tris-catalyzed hydrolysis.[21] Since the mixed micelles

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(21) The effect of CTABr on ester hydrolysis depends greatly on the buffer used. A rate increase in the presence of CTABr is observed if no buffer or an anionic buffer (*e.g.*, phosphate) are used since the high charge density in the micellar surface will lead to a surface excess concentration of hydroxyl and or anionic buffer molecules (see Behme, *et al.*, and Menger and Portnoy[5]). In the present studies, Tris buffer was used and since the buffer molecules are either cationic or neutral they should not be concentrated at a cationic surface.

of CTABr and MirHis always contained an excess of CTABr, the micellar surface must consist of regions containing the histidyl moiety and its nearest neighbor CTABr (Ma) and regions where only CTABr molecules are present (Mi). When the CTABr concentration is increased relative to MirHis, more of the micellar surface contains Mi regions.[22] Since ester incorporated into mixed micelles in the Mi region would not be hydrolyzed, such nonproductive binding would be manifest as a decrease in the observed rate constant for conditions of limiting substrate. This is demonstrated by the data of Figure 4.

The kinetic scheme presented considers such nonproductive binding and assumes that a bimolecular reaction occurs between the ester and the Ma region. The finding that the k_2 values obtained from eq 5 show little variation though an 8-fold change in MirHis and a 75-fold change in CTABr concentration supports the validity of the suggested scheme.

Equation 5 allows determination of an apparent dissociation constant for the adsorption of the esters to the Mi region. The linear relation between log $1/K_i$ and the number of carbons in the acyl chain of the ester indicates that pK_i approaches a true binding constant. From this relationship, the free energy for the transfer of an acyl methylene group of the esters from the aqueous environment to the CTABr micelles can be calculated from the expression[23]

$$\Delta(\Delta F) = -RT \ln K_{i_2}/K_{i_1}$$

The value of −630 cal/mol obtained is in close agreement with the standard free energy change per methylene group (−650 cal/mol) calculated for the transfer of molecules of surface active agents from the bulk phase to the micellar state.[24] It is also within the range (360–900 cal/mol) suggested for interactions through dispersion forces[25] and those calculated by Nemethy and Scheraga[26] for hydrophobic interactions.

The most striking observation in this study was the marked acceleration in the rate of ester hydrolysis in the presence of mixed micelles as compared to the rate due to N^{α}-acetylhistidine. The apparent pK of 6.2 for the micellar histidyl group is lower than that of N^{α}-acetylhistidine (lit.[27] $pK_{app} = 7.04$), but this can not account for the differences in reactivity since the data of Table I have been corrected to represent the rate constants for the basic form of both histidyl derivatives.

Figure 6 demonstrates that the logarithm of the second-order rate constant, k_2, for the micellar reaction increases linearly with the chain length of the ester. This permits calculation of the standard free energy change required per methylene group to account for the increase in the second-order rate constant.[23] The calculated value of 442 cal/mol is again within the range of values expected for interactions through dispersion forces. This indicates that lengthening of the acyl group results in additional hydrophobic bond energy which is used in the micellar surface to lower the free energy of activation of the catalyzed reaction.

Duynstee and Grunwald[5] have discussed the factors which may account for the modification in reaction rates observed in the presence of detergent micelles. These include approximation, electrostatic, and medium effects. Similar factors have been invoked in enzyme-catalyzed reactions by Koshland,[28] Westheimer,[29] Jencks,[30] and Bruice.[31] The rate enhancement observed in the present experiments could be due to an approximation of the reactants at the micellar surface, the overall activation free energy being lowered due to a less negative proximity component of $\Delta^{\mp}$.[32] This would imply the presence of an intermediate adsorption complex in the Ma region similar to that found for Mi. No evidence for this step was found since, after correction for nonproductive binding, the rate of nitrophenol liberation with all the esters increased linearly with the concentration of MirHis.

An alternative explanation to the noted rate increase would be that the additional hydrophobic binding energy is involved at the micellar surface in the stabilization of the activated complex.[23,33] In this respect, the requirement noted to CTABr molecules in the vicinity of the MirHis could indicate that the positive charge contributed by these molecules is required for the stabilization of the transition state.

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