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THE NUCLEOPHILIC ACTIVITY OF THE IMIDAZOLE GROUP IN MICELLES

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Abstract—The acetylation of a long-chain imidazole derivative, N$^{\alpha}$-dodecanoyl-L-histidine, by *p*-nitrophenyl acetate is accelerated by the presence of positively or negatively charged micelles. The effects observed with the anionic micelles can be readily understood on the basis of an enrichment of the reactants in the micellar phase. In cationic micelles, however, at pH values above 8 the acceleration is additionally caused by a strong facilitation of a second reaction pathway. Very likely this pathway consists in the nucleophilic attack of the imidazolyl anion on the carboxylic acid ester group.

Catalysis of chemical reactions by detergent micelles has drawn considerable interest in recent years.[1] One motivation for such studies is the fact that micelles correspond to globular protein molecules in size and in the general arrangement of hydrophobic and hydrophilic groups.[2] For this reason they may serve as a model for enzymes. For instance micelles have been shown to reflect the differences in the reactivity of sulfhydryl groups in proteins.[3] Here we report the results of studies dealing with the nucleophilic attack of the imidazolyl moiety on the carboxylic acid ester group in the presence of negatively and positively charged micelles. A long-chain histidine derivative, N$^{\alpha}$-dodecanoyl-L-histidine, (DoHis) is used as nucleophilic reactant. The ester was *p*-nitrophenyl acetate (NPA).

RESULTS

Anionic micelles. Fig 1 shows the rates of the liberation of the *p*-nitrophenoxide ion from NPA as a function of the concentration of DoHis (sodium salt). Two straight lines are observed, which intersect at a DoHis concentration of 6 mM. This value corresponds to the critical micelle concentration of DoHis at the conditions of the experiment, which was determined to be 6·6 mM with the dye method.[4] The greater slope of the second line can be readily interpreted by the assumption that the reaction between DoHis and NPA proceeds faster in the micellar phase (by a factor of 3·5) than in the bulk phase.

This conclusion is supported by the data plotted in Fig 2. Here the concentration of DoHis is kept constant at 2 mM, which is below the critical micelle concentration, while that of a second micelle-forming detergent, sodium dodecanoyl-glycinate (DoGly), is increased progressively. Beginning at a DoGly concentration of about 3 mM there occurs an increase in the reaction rate, which approaches a maximal value 2·5 times greater than the rate in the micelle-free solution at a DoGly concentration of about 15 mM. This observation is in agreement with the assumption that mixed micelles consisting of DoGly and DoHis are formed. The shape of the curve reflects the increasing exhaustion of the bulk phase from DoHis with raising DoGly concentration.

Fig 3 indicates the influence of a neutral salt, sodium chloride, on the rate of the reaction between DoHis and NPA. The addition of salt is known to reduce the critical micelle concentration.[2] At a DoHis concentration of 3·0 mM (○), which is far below its critical micelle concentration in the absence of salts (11·6 mM), no salt effect can be detected up to a sodium chloride concentration of about 150 mM. Above this concentration the reaction rate increases with an increase of the sodium chloride concentration tending to a limiting value at higher concentrations. Very likely the increase of the reaction rate is caused by the formation of micelles. This conclusion is confirmed by experiments performed with detergent concentrations corresponding approximately to the critical micelle concentration in the absence of salt. With 12 mM DoHis only (●) as well as with a mixture of 2 mM DoHis and 15 mM DoGly (▲; The critical micelle concentration of DoGly amounts to 14 mM in the absence of salt.[4]) The reaction rates increase by a factor of 2·5 and 1·6, respectively, when the sodium chloride concentration is raised from 0 to 100 mM.

Cationic micelles. Fig 4 indicates, how the pseudo-first-order rate constant of the reaction between DoHis and NPA depends on the concentration of DoHis in the absence (○) and in the presence (●) of a cationic detergent, hexadecyltrimethylammonium bromide (CTAB). In both cases there exists the expected linear relation between the rate and the concentration of the imidazole derivative. In the presence of CTAB, however, the slope is much larger than in the CTAB-free solution. The concentration of CTAB is much higher than its critical micelle concentration, which is about 1 mM.[5] Therefore one may assume that mixed micelles are formed, which consist of a small portion of the anionic detergent DoHis with the excess cationic detergent CTAB. From the two slopes it can be calculated that the reaction rate in the micelles is 15 times higher than in the micelle-free solution.

The acceleration effect caused by the cationic detergent decreases with increasing counterion concentration (Fig 5). For an explanation of this result one has to take into account the fact that the hydroxide ion concentration in the surface region of a cationic micelle is higher than in the bulk phase.[6] The difference between both values

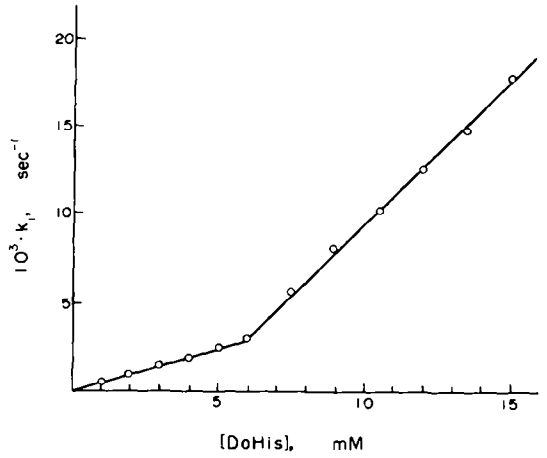

Fig 1. First-order rate constants k_1 for the liberation of the *p*-nitrophenoxide ion from NPA at pH 9·50 plotted as a function of the concentration of DoHis. [Na^+] = 50 mM (addition of NaCl).

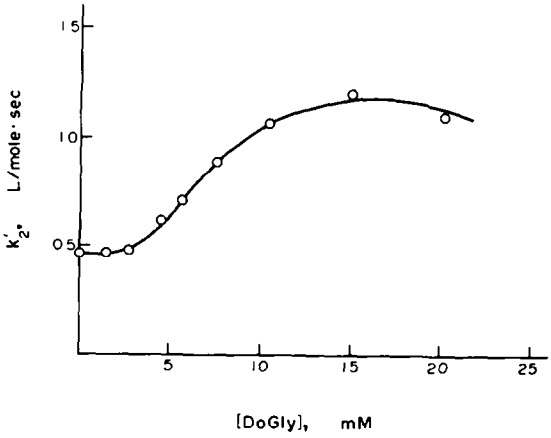

Fig 2. Apparent second-order rate constants k'_2 for the liberation of the *p*-nitrophenoxide ion from NPA at pH 9·50 plotted as a function of the concentration of DoGly. [DoHis] = 2 mM, [Na^+] = 50 mM (addition of NaCl).

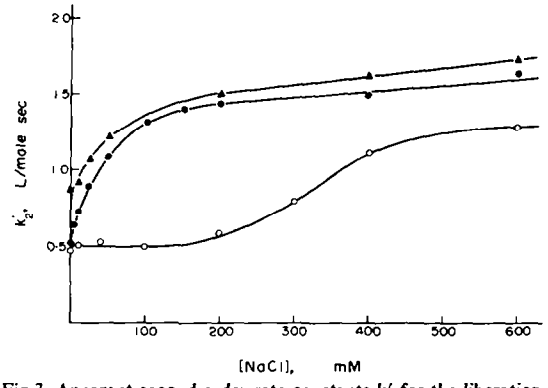

Fig 3. Apparent second-order rate constants k'_2 for the liberation of the *p*-nitrophenoxide ion from NPA at pH 9·50 plotted as a function of the concentration of sodium chloride. ○, [DoHis] = 3·0 mM; ●, [DoHis] = 12 mM; ▲, [DoHis] = 2·0 mM, [DoGly] = 15 mM.

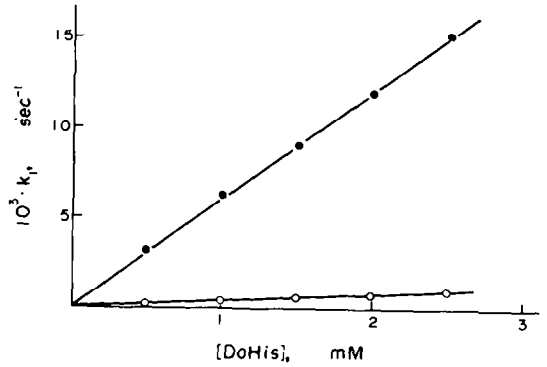

Fig 4. First-order rate constants k_1 for the liberation of the *p*-nitrophenoxide ion from NPA at pH 8·50 plotted as a function of the concentration of DoHis, ○, no additives; ●, in the presence of 12·5 mM CTAB.

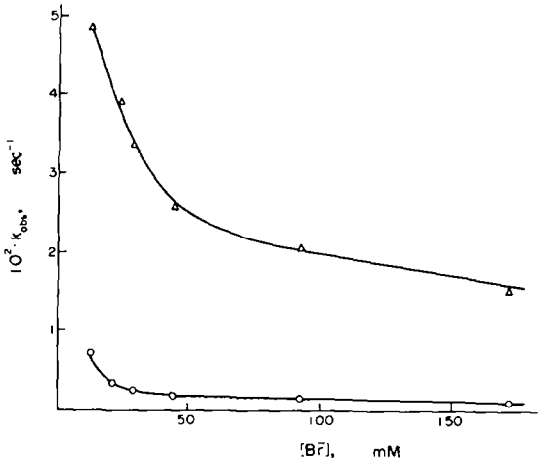

Fig 5. Rate constants k_{obs} for the liberation of the *p*-nitrophenoxide ion from NPA at pH 10·00 in the presence of 12·5 mM CTAB plotted as function of the total concentration of bromide, [Br^-] = [CTAB] + [NaBr]. ○, without DoHis; △, [DoHis] = 1·5 mM.

depends on the electrolyte concentration of the medium. It tends to lower values with increased salt concentrations.[7] Both reactions, the attack of NPA by DoHis as well as the solvolysis of the ester in the absence of DoHis depend strongly on the concentration of the hydroxide ion concentrations even above pH 9.

pH Dependence. The influence of an increasing activity of hydroxide ions on the liberation of the *p*-nitrophenoxide ion from NPA is presented in Fig 6. In the absence of micelles (□) or in the presence of anionic micelles (△, ■) the reaction rate depends only little on the hydroxide ion activity above pH 9. The slopes of the linear sections of the curves obtained in the presence of DoHis have the same value and are identical with the slope obtained for the solvolysis of NPA in the absence of any additives (●), which is caused by an attack of hydroxide ions.[8] Therefore, under these conditions the velocity of the reaction of DoHis and NPA is independent of the pH.

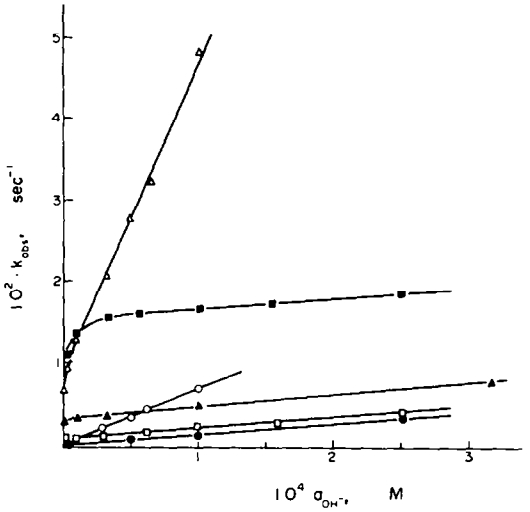

Fig 6. Rate constants k_{obs} for the liberation of the *p*-nitrophenoxide ion from NPA plotted as function of the activity of the hydroxide ions in the bulk phase. ●, no additives; ○, [CTAB] = 12·5 mM; □, [DoHis] = 1·5 mM; ■, [DoHis] = 13·8 mM, [NaCl] = 50 mM; ▲, [DoHis] = 2·0 mM, [DoGly] = 15 mM, [NaCl] = 400 mM; △, [DoHis] = 1·5 mM, [CTAB] = 12·5 mM.

In the presence of cationic micelles, however, the reaction rate increases strongly when the activity of the hydroxide ions is raised. This is true for the liberation of the *p*-nitrophenoxide ion in the presence of DoHis (△) as well as for the solvolytic reaction alone (○). But the slopes of the linear sections of the curves are not identical. Obviously, in the presence of CTAB the velocity of the reaction between DoHis and NPA depends strongly on the OH^- concentration even at high pH values.

DISCUSSION

The pseudo-first-order rate constants for the liberation of the *p*-phenoxide ion from *p*-nitrophenyl acetate in the presence of imidazole k_{obs} follow the equation

$$k_{obs} = k_w + k_2[Im]$$

where k_w represents the solvolytic constant and [Im] the concentration of the unionized imidazole. The reaction proceeds via a nucleophilic attack of the base on the carboxylic acid ester group yielding an N-acetyl imidazole derivative. In a second step this compound is hydrolyzed to imidazole and the acetate ion.[9] The same mechanism has been found to be valid for the reaction of NPA with long-chain N^{α}-acyl-histidine derivatives.[10] In the present study the occurrence of an intermediate is recognized by the fact that the proton release follows another time course than the liberation of the phenoxide ion, which obeys strictly pseudo-first-order kinetics.

The presence of micelles, regardless whether they are of the negatively or positively charged type, causes an enhancement of the reaction rates. The influence of the micelles on the reaction rates are explicable by differences in the reactivity of the reactants and by their distribution between the micellar and the bulk phase (concentration effect). From data given in the literature[10,11] it can be calculated that under the conditions of the present experiments the amount of ester incorporated into the CTAB micelles constitutes approximately 25% of its total amount. It is reasonable to assume that the negatively charged DoHis is completely incorporated into the cationic micelles. On this basis an acceleration factor of about 60 is expected to be produced by the concentration effect in the presence of CTAB. This value is to be compared with the acceleration factor of 15 obtained at pH 8·5. The result shows that in the low pH range the concentration effect sufficiently explains the rate accelerations caused by cationic micelles. From the fact that the actual reaction rate is somewhat lower (by a factor of about 4) than the expected one it may be concluded that the true second-order rate constant is smaller in the micellar phase than in the bulk phase probably due to steric or/and medium effects. A similar decrease of reactivity has been observed for the acylation of aryl oximes.[12] It is assumed to be due to an unfavourable orientation of the reactants in the micellar phase. An analogous estimate of the acceleration factor expected for the reaction in anionic micelles leads to the conclusion that in this case also the concentration effect is sufficient to explain the observed rate accelerations.

In contrast to anionic micelles or to the micelle-free solution in cationic micelles the reaction rate grows strongly with an increase of the concentration of hydroxide ions even in the pH range of 8·5 to 10·0, where practically all the imidazole moieties are expected to exist in the unionized form[1]. Very likely this effect is caused by a facilitation of the nucleophilic attack of the imidazolyl anion on the carboxylic acid ester group. The possibility of such an attack has been demonstrated in the absence of micelles for the reaction of imidazole with carboxylic acid esters other than NPA[13] as well as for the reaction of NPA with imidazole derivatives more acidic than imidazole itself.[14] In the case of the reaction between imidazole and NPA the imidazolyl anion attack is "masked" due to the unfavourable ratio of the rates of the attack by the anion and by the hydroxide ion. One may expect that cationic micelles favour the attack of the imidazolyl anion owing to a decrease of the value of the corresponding apparent pK_a,* which is 14·5 for imidazole in the absence of micelles.[15] An argument for this view is the fact that the magnitude of the acceleration caused by cationic micelles decreases strongly with increasing salt concentration (see Fig 5). Furthermore, a concentration effect like that discussed for the reaction of the uncharged imidazolyl moiety and an electrostatical stabilization of the transient state[1,3] have to be taken into consideration as causes of the acceleration effect observed at high pH values. A participation of an imidazolyl anion has also been postulated in the reaction of poly-4(5)-vinylimidazole and NPA.[16]

*The apparent pK_a of the imidazolyl residue of long-chain N-acylhistidines incorporated into cationic micelles has been determined to be 6·1.[10]

EXPERIMENTAL

Materials. N^{α}-Dodecanoyl-L-histidine (DoHis) was synthesized according to the mixed carboxylic-carbonic acid anhydride method.[17] A soln of 8·0 g (0·04 mole) of lauric acid (Fluka AG, Buchs; purity 99·5%) and 5·6 ml (0·04 mole) of triethylamine in 75 ml of anhyd THF was cooled to about −10° and was treated with 3·8 ml (0·04 mole) of ethylchloroformate. After cooling to −10° an ice-cold soln of 8·4 g (0·04 mole) of L-histidine monohydrochloride monohydrate in 40 ml of 2N NaOH was added with vigorous stirring. After standing for ½ h without cooling the mixture was poured into about 300 ml of ice-cold dilute HCl. After bringing the pH to about 5 the ppt was collected, dried (yield of the crude product: 80%), and recrystallized 6 times from EtOH. The yield of the pure product amounted to 24%, mp (corr.) 162–164° (158–159° reported in[18]), α_D^{20} 22·1° (MeOH, $c = 1$). (Found: C, 64·02; H, 9·15; N, 12·65. Calcd. for $C_{18}H_{31}N_3$ (M 337·47): C, 64·06; H, 9·26; N, 12·45%).

Hexadecyltrimethylammonium-bromide (CTAB), sodium dodecanoyl-glycinate (DoGly) and *p*-nitrophenyl acetate (NPA) were obtained as described previously.[3]

Kinetic measurements. The reaction of DoHis with NPA was followed in aqueous medium at 25 ± 0·1° by recording the rate of appearence of the *p*-nitrophenoxide ion spectrophotometrically at 402 nm keeping the pH constant by means of the pH-stat technique.[3] The reaction was started with 0·1 ml of a stock solution of *p*-nitrophenyl acetate in dioxane. The concentration of the *p*-nitrophenyl acetate in the test soln, the final volume of which amounted to 20 ml, was 0·125 mM. The liberation of the *p*-nitrophenoxide ion was followed to more than 99% completion. Straight lines were obtained by plotting of $\log_{10} A_\infty/(A_\infty - A)$ against t, where A_∞ is the final absorbance and A the absorbance at the time t. From the slope of these lines the rate constants k_{ob}, were calculated.

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