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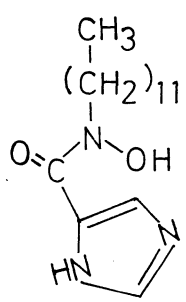
THE HYDROLYSIS OF P-NITROPHENYL ACETATE BY A MICELLAR BIFUNCTIONAL CATALYST

Toyoki KUNITAKE,* Yoshio OKAHATA, and Tetsuo SAKAMOTO
Department of Organic Synthesis, Faculty of Engineering,
Kyushu University, Fukuoka 812

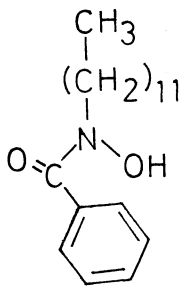
A surfactant molecule which contains the hydroxamic acid and imidazole groups was synthesized and its catalytic efficiency in the hydrolysis of p-nitrophenyl acetate was examined in a mixed micelle with CTAB. The presence of the complementary functions in cationic micelles led to much enhanced catalytic efficiency compared with micellar, monofunctional systems.

The high catalytic efficiency of serine proteolytic enzymes arises from the rapid formation and decomposition of the acyl enzyme intermediate.[1)]

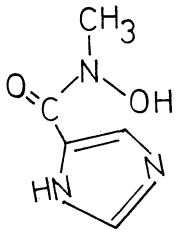
Recently, it has been reported that anionic nucleophiles such as thiolate,[2)] oximate,[3)] and hydroxamate[4)] anions show remarkably high reactivities toward p-nitrophenyl acetate(PNPA) in cationic micellar systems. The rate of acylation becomes comparable to that of enzyme α-chymotrypsin in some instances. On the other hand, we showed in non-micellar systems that the decomposition of the acyl hydroxamate intermediate could be accelerated by the intramolecular catalysis of imidazole.[5-7)] These two catalytic features may be combined if we use a surfactant molecule which possesses hydroxamic acid and imidazole functions. In this communication is described the hydrolysis of PNPA catalyzed by CTAB micelles containing a bifunctional nucleophile(LImHA), and the results are compared with those of a non-micellar, bifunctional catalyst(MImHA)[7)] and a micellar, monofunctional catalyst(LBHA).



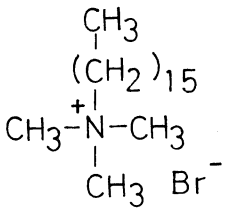
LImHA



LBHA



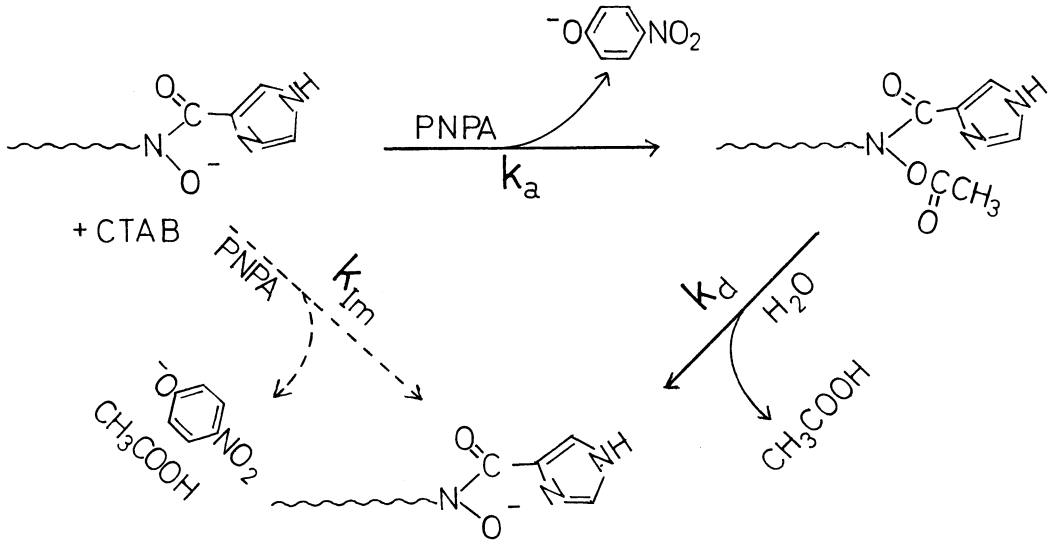
MImHA



CTAB

N-Laurylbenzohydroxamic acid(LBHA) was prepared by the reaction of O-benzylbenzohydroxamate and lauryl bromide in the presence of NaH and the subsequent hydrogenolysis over Pd-$SrCO_3$, mp 75-76°C. N-Lauryl-4-imidazolylcarbohydroxamic acid(LImHA) was prepared by the reaction of N-lauryl-O-benzylhydroxylamine and imidazole-4-carbonyl chloride and the subsequent hydrogenolysis, mp 181-4°C. The elemental analyses and spectroscopic data were satisfactory for both of these compounds.

The time course of reaction was followed by the absorbance at 401 nm of the p-nitrophenolate anion formed. The uncatalyzed hydrolysis of PNPA was corrected. When an excess of PNPA was reacted with LImHA in a CTAB micelle, typical burst kinetics were observed, indicating the rapid p-nitrophenol release due to acylation of the hydroxamate anion and the subsequent, slower hydrolysis of the acyl intermediate. From the kinetic analysis as already described[5] are obtained the apparent second-order rate constant of acylation of the hydroxamic acid ($k_{a,obs}$), the apparent second-order rate constant of hydrolysis of PNPA by the imidazole group(k_{Im}), and the apparent first-order rate constant of hydrolysis of the acyl hydroxamate formed($k_{d,obs}$).



(1)

On the other hand, the reaction of LBHA with excess PNPA obeyed the pseudo first-order kinetics up to at least 90% completion. This means that the acylated LBHA is quite stable under the condition employed. The $k_{d,obs}$ value for LBHA was separately determined by using acetyl N-laurylbenzohydroxamate, mp 41-45°C.

The apparent rate constants thus determined are summarized in Table 1. The k_{Im} value was much smaller than $k_{a,obs}$ and, in fact, negligible at pH 8.1. Therefore, the acylation of LImHA occurred mostly at the hydroxamic acid site. The pK_a value of the hydroxamic acid group determined by the use of the absorption at

Table 1. Apparent Rate Constants[a]

pH	Catalyst	$k_{a,obs}$ $M^{-1}sec^{-1}$	k_{Im} $M^{-1}sec^{-1}$	$k_{d,obs}$ $\times 10^{3}sec^{-1}$
8.10	LImHA + CTAB	159	<1	4 ± 1
	LBHA + CTAB	74.3		0.006[c]
	MImHA[b]	1.92		ca.0.4
8.80	LImHA + CTAB	800	14	3.2
	LBHA + CTAB	268		0.040[c]
	MImHA[b]	4.94	<0.1	0.70

a. 30°C, 3 v/v% EtOH-H_2O, μ = 0.5(KCl), 0.15 M Tris buffer. [CTAB] = 1.10×10^{-3}M, [PNPA] = 1.90×10^{-4}M, [LImHA] = 1.50×10^{-5}M, [LBHA] = 2.70×10^{-5}M.

b. from ref.7: 30°C, 28.9 v/v% EtOH-H_2O, μ = 0.1(KCl) 0.15 M Tris buffer.

c. obtained by extrapolation to the zero buffer concentration.

300 nm of the hydroxamate anion (30°C, 3 v/v% EtOH-H_2O, μ=0.5(KCl), 0.15 M Tris, 1.10×10^{-3}M CTAB) was 9.75 ± 0.05 for LImHA and 9.72 ± 0.05 for LBHA. Therefore, the presence of the neighboring imidazole group did not affect the dissociation behavior of LImHA. Interestingly, $k_{a,obs}$ for LImHA was 2-3 times greater than that of LBHA. The acylation rate constant of LImHA was enhanced about eight times by decreasing the ionic strength from 0.5 to 0.1(KCl).

The plots of $k_{a,obs}$ against α_{HA}(degree of dissociation of the hydroxamic acid group) give straight lines which pass the origin. The true second-order rate constant of acylation of the hydroxamate anion in LImHA was calculated from the slope to be 11700 $M^{-1}sec^{-1}$, 7700 $M^{-1}sec^{-1}$ and 3600 $M^{-1}sec^{-1}$ in phosphate, Tris and Barbital buffers(0.10 - 0.15 M), respectively. The corresponding k_a value for LBHA was 3100 $M^{-1}sec^{-1}$ in Tris buffer. These values are 300-1000 fold greater than that of MImHA(k_a = 12 $M^{-1}sec^{-1}$). The rate-enhancing effect of the CTAB micelle is truly remarkable, as already mentioned in other monofunctional systems.

As for the deacylation process, the data obtained may be summarized as follows: (1) In CTAB micelles the deacylation rate for LImHA is greater than that for LBHA by a factor of 80-700(Table 1), (2) $k_{d,obs}$ for micellar LImHA is fairly constant($3-4 \times 10^{-3}sec^{-1}$) over the pH range studied, whereas log $k_{d,obs}$ for micellar LBHA is proportional to pH with a slope of unity, (3) $k_{d,obs}$ for micellar LImHA is not sensitive to the change of the ionic strength, whereas $k_{d,obs}$ for micellar LBHA increases with decreasing ionic strengths(30 times rate

enhancement for a change of μ=0.5 to 0.01), and (4) $k_{d,obs}$ does not depend on the concentration of micellar LImHA, indicating that the contribution of the intermolecular imidazole catalysis is excluded.

From these results it is concluded that acetyl LBHA in CTAB micelles is hydrolyzed due to the action of OH^-. Its rate is controlled by the hydroxide concentration at the micellar surface which increases with increasing pH of the bulk phase and is diminished by increasing ionic strength. This has been observed generally in micellar systems.[8] In contrast, deacylation for micellar LImHA is concluded to arise from the intramolecular imidazole catalysis which is much more effective than the hydroxide ion catalysis. The $k_{d,obs}$ value observed in this study was only 3-4 times greater than that for MImHA(a related compound in a nonmicellar system). The micellar effect is not remarkable for the intramolecular imidazole catalysis of deacylation, in sharp contrast with the very large rate augmentation in the acylation process.

The apparent rate constant of turnover in the nucleophilic catalysis is given by

$$k_{cat} = \frac{k_a \cdot k_d}{k_a[PNPA] + k_d} \qquad (2)$$

At [PNPA] = 10^{-4}M and from $k_{a,obs}$ and $k_{d,obs}$ at pH 8.1, the k_{cat} value was calculated to be 32, 1.3, and 0.06 $M^{-1}sec^{-1}$ for LImHA - CTAB, MImHA and LBHA - CTAB systems, respectively. Thus, the catalytic efficiency increased by 500 fold by introducing the intramolecular imidazole catalysis in the micellar system. The efficiency of the monofunctional, micellar system(LBHA - CTAB) is limited due to its slow deacylation step and is only 1/20 of the bifunctional, nonmicellar system(MImHA), in spite of the enormous acceleration of the acylation rate in the former.

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