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A MIXED MICELLE CATALYST OF EXTREMELY GREAT ACTIVITY FOR HYDROLYSIS OF P-NITROPHENYL ESTERS

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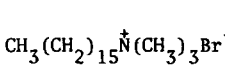
Related to the hydrolytic enzymes of well established structures and functions, such as chymotrypsin, a large number of simple catalysts for ester hydrolyses have been investigated by many organic and bioorganic chemists[1]. These catalysts for the model of chymotrypsin (especially model of the chymotrypsin function) should have a good oxygen nucleophile whose pKa is relatively small in order to achieve the effective acylation just as Ser-195 does in chymotrypsin. Hydroxamic acid was shown to satisfy these requirements by Bender et al.[2], though its catalytic activity for the ester hydrolysis is not yet so high as chymotrypsin itself.

Recently, we have found that the catalyst composed of cetyltrimethylammonium bromide I and N-lauroyl-N-methylhydroxamic acid II showed activity as high as chymotrypsin for the hydrolysis of p-nitrophenyl acetate[3]. However, the rate constant observed for p-nitrophenyl acetate and the micelle catalyst was still much smaller than that for p-nitrophenyl ester of tryptophan III and chymotrypsin.



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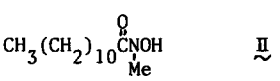

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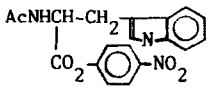

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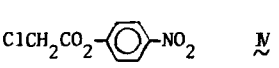

IV

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Now we wish to report our latest achievement to develop a catalytic activity using the mixed micelle catalyst for some p-nitrophenyl esters where the acylation rate constant of catalyst $\underset{\sim}{II}$ by p-nitrophenyl chloroacetate $\underset{\sim}{M}$ reaches to 10^6 $sec^{-1}M^{-1}$ (see Table 1), which is in comparable order of magnitude to that of chymotrypsin for the tryptophan derivative.

Thus, the rate of the hydrolysis of p-nitrophenyl chloroacetate or p-nitrophenyl ester of N-acetyltryptophan catalyzed by chymotrypsin or mixed micelle catalyst $\underset{\sim}{I}$ + $\underset{\sim}{II}$ was investigated. The results obtained were summarized in Table 1.

Table 1 Rate constants of p-nitrophenol release from $p\text{-}NO_2PhOX$[a]

	k_{cat} $(sec^{-1}M^{-1})$[b]	
cat \ X	N-Ac-Try-	$ClCH_2CO$
$\underset{\sim}{I}$	7.22×10^2	3.58×10^2
$\underset{\sim}{II}$	2.44×10^2	2.71×10^3
$\underset{\sim}{I}$ + $\underset{\sim}{II}$	3.31×10^4	1.29×10^6
α-chymotrypsin	1.5×10^7[c]	1.03×10^6[d]

a. 22°C, in aqueous borate buffer (pH 9.99), containing 5% of acetonitrile (v/v), 2×10^{-5} M of an ester, 1.0×10^{-3} M of $\underset{\sim}{I}$ and 1.68×10^{-4} M of $\underset{\sim}{II}$, unless otherwise noted ; the rate was followed by the spectrophotometric determination of p-nitrophenolate (400nm) produced by means of a stopped flow apparatus (Union Giken Co.,type RA 1300).

b. Calculated from the observed pseudo first order rate constant according to $[k_{obs}^{\underset{\sim}{I}(or\underset{\sim}{II})} - k_{obs}^{OH^-}] / [cat]$ for $\underset{\sim}{I}$ or $\underset{\sim}{II}$ or $[k_{obs}^{\underset{\sim}{I}+\underset{\sim}{II}} - k_{obs}^{\underset{\sim}{I}}]/[\underset{\sim}{II}]$ for $\underset{\sim}{I}$ + $\underset{\sim}{II}$.

c. k_2/K_s at pH 7.00. B. Zerner, R.P.M. Bond and M.L. Bender, J. Amer. Chem. Soc., 86, 3674(1964).

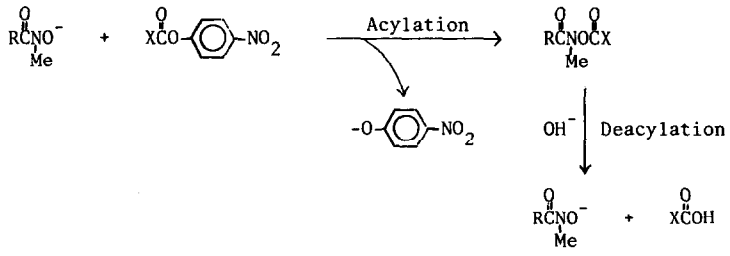
d. 20°C in aqueous phosphate buffer (pH 7.85) containing 5%

acetonitrile (v/v), 2×10^{-6} M of an ester and 2×10^{-5} M of chymotrypsin were employed. k_{cat} was calculated similar to other catalysts. In the same condition, k_{cat} for p-nitrophenyl acetate was 2220 $sec^{-1}M^{-1}$.

As shown in the Table, the catalytic constant of chymotrypsin for the tryptophan ester was ca. 500 times as large as the mixed micelle. However, for the chloroacetate, the mixed micelle was more effective than chymotrypsin. Especially worthy to note was that the observed optimum acylation rate constant (k_{cat}) of the micelle catalyst by the chloroacetate, 1.29×10^{6} $sec^{-1}M^{-1}$, was in very close order of magnitude to that (k_2/K_s)[4] of chymotrypsin by the tryptophan ester, the largest acylation rate constant ever reported. Taking the hydrophobic acceleration into account, one can reasonably assume that more hydrophobic chlorocarboxylates are hydrolyzed by the micelle catalyst as effective as the tryptophan ester by chymotrypsin.

Furthermore, for chloroacetate, the turnover of the catalyst was found very effective[2] and a large excess of the ester was readily hydrolyzed by the present catalyst at pH 8.96, where the first order rate constant for the deacylation of chloroacetyl-$\underset{\sim}{\text{II}}$ was 0.6 sec^{-1} (see Scheme 1). That the rate is, in spite of the absence of imidazole assist, considerably large, (comparable to 2.62 sec^{-1} for the deacylation of chloroacetyl chymotrypsin[5]) is also worthy to note.

Scheme 1



It may be seen from the Table that every catalytic system shows its own unique specificity.

Thus, a conclusion may be drawn that the catalytic system composed of the hydrophobic

hydroxamic acid and the ammonium surfactant behaves just like chymotrypsin in the sense that (i) the largest activity toward the corresponding specific substrate is in the same order of magnitude for the artificial system and for the enzyme, (ii) the mechanism is similar (nucleophilic attack of an oxide moiety followed by the relatively slow hydrolysis of acylenzyme) and (iii) unique selectivity or specificity for the substrate is observed[6].

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4. The comparison of k_2/K_s for chymotrypsin with k_{cat} for the micelle catalyst results in underestimation for the micelle catalyst, because k_2/K_s is the ideal maximum of the second order rate constant ($k_2/K_s \geq k_{cat}$).

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6. Further work on this last point is now in progress.