Highly Efficient Enantioselective Hydrolysis of Short Chain *N*-Acetyl Amino Acid *p*-Nitrophenyl Esters Catalysed by Esterase Models

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An enantioselectivity (L/D) of 167 ± 21 (pH 7.68; ionic strength = 0.01; 298 K) has been achieved for the hydrolysis of the short chain substrate, *N*-acetylphenylalanine *p*-nitrophenyl ester in the catalytic vesicular system of N^{α}-(*N*-benzyloxycarbonyl-L-leucyl)-L-histidine (Z-L-Leu-L-His) and *N,N*-ditetradecyl-*N,N*-dimethylammonium bromide.

In enzyme model reactions, it is of interest and significance to enhance the stereoselectivity of hydrolysis of enantiomeric amino acid esters with chiral model biological membranes.[1] Remarkably high stereoselectivity was established in the hydrolysis of long chain substrates such as *N*-dodecanoyl-L(D)-phenylalalnine *p*-nitrophenyl ester with the hydrophobic peptide-type histidine catalysts.[2] However, the enantioselective hydrolysis of short chain substrates is so difficult that the reported enantioselectivity for hydrolysis of *N*-acetyl-L(D)-phenylalanine *p*-nitrophenyl ester (Phe-C_2) has so far been small [for example, k_L/k_D = 6.5 (298 K) or 23.7 (277 K) in the hydrolysis of Phe-C_2 by the system of N^{α}-(*N*-dodecanoyl-L-phenylalanyl)-L-histidine and *N,N*-dididecyl-*N,N*-dimethylammonium bromide].[3] We report here the highly enantioselective hydrolysis of short chain substrates, the *N*-acetyl-L(D)-amino acid *p*-nitropenyl esters (Phe-C_2, Leu-C_2, and Ala-C_2),† in the catalytic system containing histidine-type catalysts (Z-L-His, Z-L-Leu-L-His, and Z-L-Leu-L-His-L-Leu) and the surfactant, *N,N*-ditetradecyl-*N,N*-dimethylammonium bromide ($2C_{14}N2C_1$).

The stereoselective hydrolysis of short chain *p*-nitrophenyl *N*-acetylamino acid esters (1.0×10^{-5} mol dm^{-3}) with the catalysts (5.0×10^{-5} mol dm^{-3}) and the surfactant, $2C_{14}N2C_1$ (1.0×10^{-3} mol dm^{-3}) was carried out at 298 K (pH 7.68) in Tris buffer (1.00×10^{-2}—8.00×10^{-2} mol dm^{-3}) containing KCl (2.73×10^{-3}—8.00×10^{-2} mol dm^{-3}) in 3% (v/v) acetonitrile–water; the stock solutions were prepared by dissolving the catalyst and the surfactant in Tris-KCl buffer by sonication at 318 K for 1 h. The enantiomer rate ratios (L/D) of the second-order catalytic rate constants (k), which were evaluated with the probable error analysis of good pseudo-first-order rate constants, were obtained by the spectrophotomeric determination (λ = 400 nm) of *p*-nitrophenolate concentration in more than nine reactions repeated under identical conditions with or without the histidine catalyst.

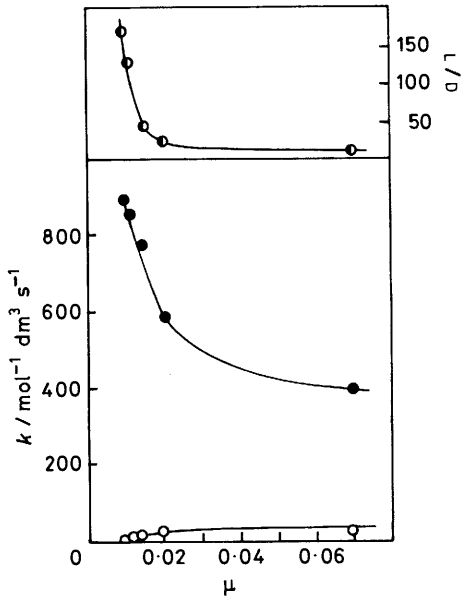

Figure. Ionic strength (μ) dependence of k_L (●) and k_D (○), and enantioselectivity (L/D) (◐) for the hydrolysis of Phe-C_2 with the Z-L-Leu-L-His and $2C_{14}N2C_1$ system at 298 K

The rate constants (k_L, k_D) and the rate ratio (L/D) of hydrolysis of Phe-C_2 with the various catalysts at an ionic strength (μ) of 0.15 are listed in Table 1. No efficient

† $MeCONHCH(R)CO_2C_6H_4NO_2$-*p* [R = $PhCH_2$ (Phe-C_2), Pr^iCH_2 (Leu-C_2), and Me (Ala-C_2)].

Table 1. The rate constants (k/mol^{-1} dm^3 s^{-1}) and the selectivity (L/D) in the hydrolysis of Phe-C_2 with various catalysts in bilayer system ($2C_{14}N2C_1$) at $\mu = 0.15$

Catalyst	k/mol^{-1} dm^3 s^{-1}		Selectivity
	L	D	L/D
Z-L-His	3.03 ± 0.2	3.80 ± 0.1	0.80 ± 0.03
Z-L-Leu-L-His	216.5 ± 0.3	14.0 ± 0.2	15.5 ± 0.2
Z-L-Leu-L-His-L-Leu	57.6 ± 0.2	10.0 ± 0.09	5.76 ± 0.03

Table 2. The rate constants (k/mol^{-1} dm^3 s^{-1}) and the selectivity (L/D) in the hydrolysis of short chain substrates catalysed by Z-L-Leu-L-His in the bilayer system ($2C_{14}N2C_1$)

Ionic strength	Substrate (k/mol^{-1} dm^3 s^{-1})								
	Phe-C_2			Leu-C_2			Ala-C_2		
	L	D	L/D	L	D	L/D	L	D	L/D
0.01	897 ± 5	5.4 ± 0.7	167 ± 21	619 ± 2	17.7 ± 0.3	35.0 ± 0.5	31.6 ± 0.2	4.5 ± 0.2	7.0 ± 0.2
0.15	216.5 ± 0.3	14.0 ± 0.2	15.5 ± 0.2				8.9 ± 0.1	6.4 ± 0.4	1.40 ± 0.07

enantioselection was observed with Z-L-His as a catalyst and the k value for hydrolysis was small. However, Z-L-Leu-L-His and Z-L-Leu-L-His-L-Leu catalysed the enantioselective hydrolysis of Phe-C_2. The best rate ratio for hydrolysis, 15.5 ± 0.2, was obtained at an ionic strength (μ) of 0.15 in the stated Tris buffer solution. However, lowering the ionic strength resulted in an increase and a decrease in the hydrolysis rate of the L and D enantiomer, respectively, as shown in the Figure. The maximum enantioselectivity achieved was 167 ± 21 at the low ionic strength (μ) of 0.01 for the hydrolysis of Phe-C_2.

This efficient enantioselectivity for a short chain substrate may result from a proximity effect; in the slightly loosened bilayer vesicle at low ionic strength, the short chain substrate might penetrate the hydrophobic vesicular core and/or the incorporated catalyst might become more mobile in the bilayer vesicle. Consequently, the proximity of the catalyst and the substrate may result in an intensified hydrophobic interaction and lead to enantioselective hydrolysis in the bilayer vesicular system at the low ionic strength. On the other hand, in the hydrolysis of long chain substrates, a large rate constant (k_L) of 1 046.5 ± 0.9 for the hydrolysis of L-Phe-C_6 was obtained even at $\mu = 0.15$ compared with the rate constant (k_L) of L-Phe-C_2 with 216.5 ± 0.3 under the same conditions. Hence, the catalyst and the long chain substrate are suitably located near each other for the efficient hydrolysis.

The efficient hydrolysis of short chain esters may also be explained by another effect on the hydrophobic interaction; a weakly hydrophobic environment around the catalyst and the substrate caused by influx of water into the vesicular system at the lower ionic strength would intensify the hydrophobic interaction between the benzyl group in Phe-C_2 and the imidazolyl (and/or leucine) part in the catalyst. The importance of such an hydrophobic interaction is supported by the fact that the enantioselectivities of hydrolysis of the Phe-C_2, Leu-C_2, and Ala-C_2 decreased in order of decreasing hydrophobicity of the R substituent (R = $PhCH_2$, Pr^iCH_2, and Me) as shown in Table 2. Therefore, the present, efficiently enantioselective hydrolysis of short chain esters has been attained by improving two important factors, proximity and hydrophobic interactions between the catalyst and the substrate.

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