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Enantioselective Hydrolysis of N-Acyl Amino Acid Esters by Tripeptide-type L-Histidine Derivative in a Bilayer Vesicular System

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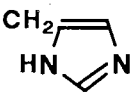
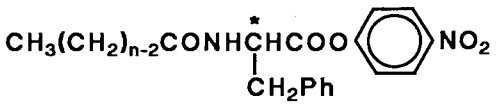
Peculiar enantioselective hydrolysis of N-acyl amino acid esters was found in the bilayer vesicular systems containing the tripeptide-type histidine derivative, Z-L-Leu-L-His-L-Leu. The enantioselectivity for the hydrolysis of long chain N-acyl phenylalanine *p*-nitrophenyl ester, C_{16}-Phe-PNP, appeared in the binding process and was governed by an entropy factor.

Molecular recognition by peptides has received considerable attention in connection with antibody-antigen, enzyme-substrate, and so on. It is required to understand interaction between amino acid residues for construction of antibody or enzyme model systems. Therefore, the investigation for the relation between structures and the functions (*e.g.* molecular recognition) of peptides seems to be important fundamentally. We have hitherto investigated the enantioselective hydrolyses of amino acid esters by N-protected peptides including L-histidine which acts as nucleophile in bilayer vesicular systems, and the interaction between the peptide derivative and the substrate.[1] In the course of our investigation, We found the peculiar enantioselective hydrolysis of C_{16}-Phe-PNP by Z-L-Leu-L-His-L-Leu (Z is a benzyl oxycarbonyl group).

$R_1 = R_3 = CH_2CH(CH_3)_2$ $R_2 =$ 

Z-L-Leu-L-His-L-Leu



C_n-L(D)-Phe-PNP (n = 2, 10, 16)

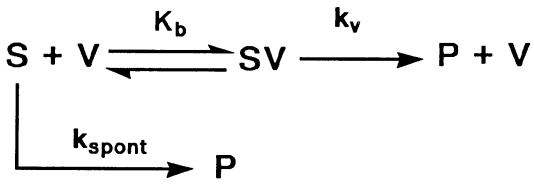
$(CH_3(CH_2)_{11})_2N^+(CH_3)_2Br^-$

$2C_{12}N2C_1$

Table 1. Rate constants, stereoselectivities, and kinetic parameters for hydrolysis of C_n-Phe-PNP (n = 2, 10, and 16) with Z-L-Leu-L-His-L-Leu and $2C_{12}N2C_1$ at 25 °C

	C_n-Phe-PNP								
	n = 2			n = 10			n = 16		
	L	D	L/D	L	D	L/D	L	D	L/D
k / mol^{-1} dm^3 s^{-1}	149	34	4.4	958	65	15	618	12	52
K_b N^{-1} / mol^{-1} dm^3	360	400	0.90	610	630	0.97	2560	230	11.1
$10^2 k_v$ / s^{-1}	6.1	1.5	4.1	36	5.7	6.3	10	2.4	4.2

Hydrolyses of C_n-Phe-PNP (n = 2, 10, and 16; 1.0 x 10^{-5} mol dm^{-3}) were carried out with the vesicular system of the histidine derivative, Z-L-Leu-L-His-L-Leu (1.0 x 10^{-4} mol dm^{-3}) and the surfactant, $2C_{12}N2C_1$ (1.0 x 10^{-3} mol dm^{-3}; critical micelle concentration 5 x 10^{-5} mol dm^{-3}) at 10 - 25 °C in Tris buffer (pH 7.68, μ = 0.15) in 3%(v/v) CH_3CN-H_2O. The rate constants for the hydrolysis with or without the histidine derivative (k_{obs} and k_{spont}, respectively) were obtained from good pseudo-first-order rate constants by determining *p*-nitrophenolate concentration spectrophotometrically (λ = 400 nm), and the second-order rate constants, $k = (k_{obs} - k_{spont})/[\text{Z-L-Leu-L-His-L-Leu}]$, were taken as the average value from more than three reactions repeated under identical conditions.



Scheme 1.

The hydrolyses of C_n-Phe-PNP (n = 2, 10, and 16) proceeded selectively for L isomer in all cases, and the largest enantioselectivity as 52 was observed in the C_{16}-substrate hydrolysis, as listed in Table 1. The kinetic analyses can be applied to the system as shown in Scheme 1, where S = substrate, V = vesicle, SV = vesicle-substrate complex, and P = *p*-nitrophenylate anion. The kinetic parameters, *viz.*, the binding constant K_b/N (N is the aggregation number) and the rate constant k_v, were obtained according to the literature.[2] The kinetic parameters reported so far[1,3] exhibited in all cases that the binding constant ratios for L and D substrates were small and the ratios of the reaction rates, k_v, were dominant for the enantioselectivities. In the C_2- and C_{10}-Phe-PNP hydrolysis, the binding constants for L isomers were smaller than these for D isomers, and the k_v ratios were relatively large as expected.

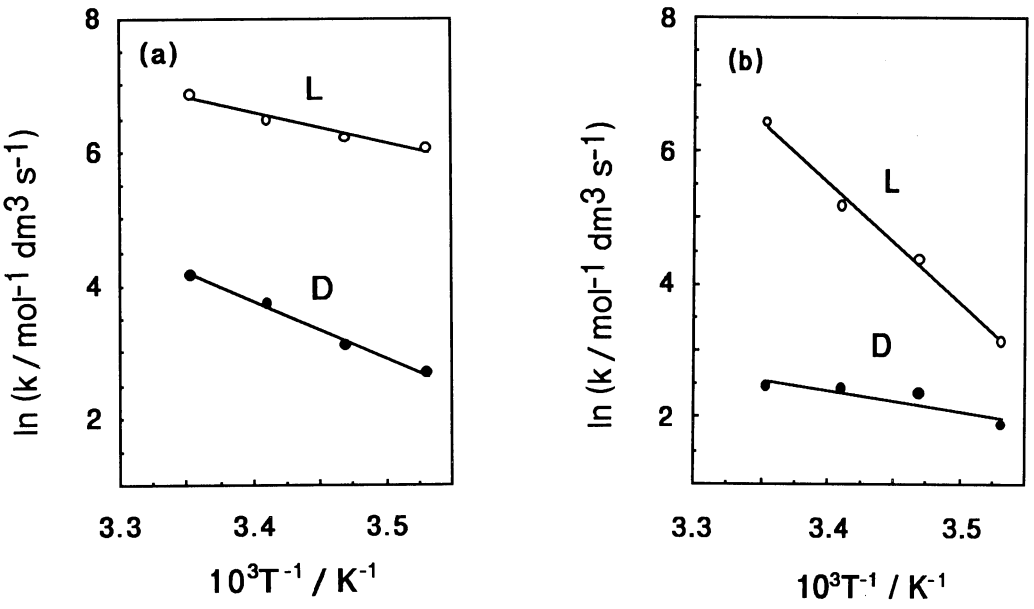


Fig. 1. Arrhenius plots of ln k vs. T^{-1} in the hydrolyses of (a) C_{10}-Phe-PNP and (b) C_{16}-Phe-PNP with Z-L-Leu-L-His-L-Leu and $2C_{12}N2C_1$.

However, the binding constants ratio in C_{16}-Phe-PNP hydrolyses was considerably large.

Such a drastic change in reactivities from C_2- and C_{10}- to C_{16}-substrate was also observed in temperature effects of these reactions. As shown in Figure 1a, the enantioselectivities for C_{10}-Phe-PNP increased with decreased reaction temperatures. On the other hand, the enantioselectivities for C_{16}-substrates increased with increased the temperatures as shown in Figure 1b. The activation parameters obtained from Figure 1 are listed in Table 2. In the C_{10}-Phe-PNP substrate, the $\Delta H^{\neq}$ value for L isomer is smaller than that of D isomer though $\Delta S^{\neq}$ value is negatively larger than that of D isomer. On the other hand, the L isomer of C_{16}-Phe-PNP has the positively large activation entropy factor though the reactivity of L isomer is advantageous

Table 2. Activation parameters for the hydrolyses of C_n-Phe-PNP (n = 10 and 16) with Z-L-Leu-L-His-L-Leu and $2C_{12}N2C_1$

	C_n-Phe-PNP			
	n = 10		n = 16	
	L	D	L	D
$\Delta H^{\neq}$ / kcal mol^{-1}	8.5	16.9	35.2	6.0
$\Delta S^{\neq}$ /cal mol^{-1} K^{-1}	-16.4	6.6	72.2	-33.5

in the enthalpy factor. Therefore, the differences in reactivities between L and D isomers are caused by enthalpy factor in C_{10}-Phe-PNP, and by entropy in C_{16}-Phe-PNP.

The large binding constants ratio suggests that the extent of the contact between nucleophile and L-substrate largely differ from D isomer and/or that there is difference between L and D isomer in the formation ratio of the complex which has a favorable conformation for the reaction. In the favorable complex for the reaction, the imidazolyl group in the nucleophile may be close to carboxylic carbon in the substrate. Therefore, the reaction *via*. such a complex may be considered to be advantageous entropically by a proximity effect. The interaction between nucleophile and substrate is proposed in the dipeptide derivative.[1b] Since such a peculiar enantioselectivity was not observed in the dipeptide, Z-L-Leu-L-His, the results in the study suggest the third amino acid residue plays an important role. Further investigation should be necessary to clarify the interaction between the tripeptide derivative and the phenylalanine substrate in a vesicular system.

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References

1) a) K. Ohkubo, M. Kawata, T. Orito, and H. Ishida, *J. Chem. Soc., Perkin Trans 1*, **1989**, 666; b) K. Ohkubo and S. Miyake, *J. Chem. Soc., Perkin Trans. 2*, **1987**, 995; c) K. Ohkubo, N. Matsumoto, M. Nagasaki, K. Yamaki, and H. Ogata, *Bull. Chem. Soc. Jpn.*, **57**, 214 (1984); d) K. Ohkubo, H. Ogata, K. Yamaki, and K. Yamashita, *Macromol. Chem.*, **185**, 891 (1984); e) K. Ohkubo and N. Matsumoto, *J. Mol. Cat.*, **17**, 23 (1982); f) K. Ohkubo, N. Matsumoto, and H. Ohta, *J. Chem. Soc., Chem. Commun.*, **1982**, 738; g) R. Ueoka, Y. Nonomiya, Y. Nakagawa, K. Inoue, and K. Ohkubo, *Chem. Lett.*, **1981**, 785.

2) C. A. Bunton, L. Robinson, L. Sepulveda, *J. Org. Chem.*, **35**, 108 (1970).

3) R. Ueoka, Y. Matsumoto, R. A. Moss, S. Swarup, A. Sugii, K. Harada, J. Kikuchi, and Y. Murakami, *J. Am. Chem. Soc.*, **110**, 1588 (1988); Y. Murakami, A. Nakano, H. Ikeda, T. Imori, and K. Akiyoshi, *Bull. Chem. Soc. Jpn.*, **58**, 172 (1985); Y. Kimura, M. Nango, Y. Ihara, and N. Kuroki, *Chem. Lett.*, **1984**, 429; Y. Kimura, S. Kanda, M. Nango, Y. Ihara, J. Koga, and N. Kuroki, *ibid.*, **1984**, 433; Y. Ihara, R. Hosako, M. Nango, and N. Kuroki, *J. Chem. Soc., Perkin Trans. 2*, **1983**, 5.; R. Ueoka and Y. Murakami, *ibid.*, **1983**, 219.

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