CHEMISTRY LETTERS, pp. 2081-2084, 1994.

Remarkable Salt Effects in the Highly Enhanced Enantioselective Hydrolysis of Amino Acid Esters with the Active Tripeptide in the Vesicular System

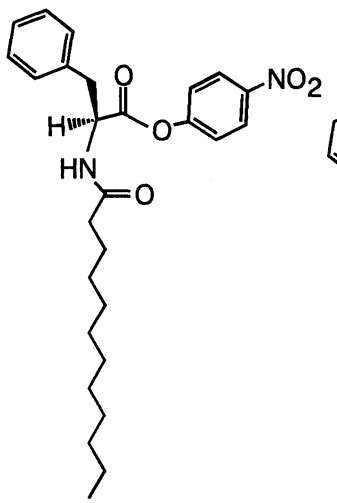
Kōichi GOTO, Chikara IMAMURA, Shinichi YAMAMOTO, Yōko MATSUMOTO, and Ryuichi UEOKA*
Department of Industrial Chemistry, Kumamoto Institute of Technology, Ikeda, Kumamoto 860

The remarkably high enantioselectivity ($k^{L}_{a,obsd} / k^{D}_{a,obsd} = 67$) was attained along with the large rate-enhancement of the L-form substrate for the hydrolytic cleavage of N-dodecanoyl-D(L)-Phe-PNP by controlling the ionic strength ([KCl] = 0.03 M, 1M = 1 mol dm^{-3}) in the vesicular system.

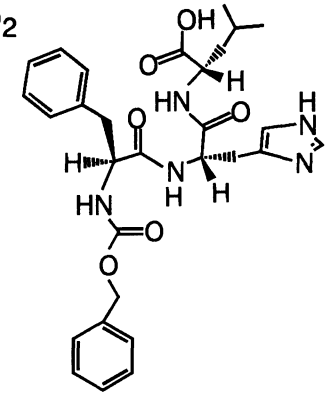
The stereoselective cleavages of p-nitrophenyl esters derived from N-protected amino acids in surfactant aggregate systems have been attracting considerable attention in order to understand the origins of stereoselectivity in the proteolytic enzymes. Studies of stereoselective hydrolysis have emphasized the structural effects of peptide catalysts,[1,2] the role of the composition of the aggregates,[2,3] and the effects of temperature.[4,5] As regards the peptide catalysts, the large enhanced enantioselectivity obtained for the hydrolysis of the long-chain substrate, p-nitrophenyl N-dodecanoyl-D(L)-phenylalaninate (C_{12}-D(L)-Phe-PNP) with LLL-tripeptide N-(benzyloxycarbonyl)-L-phenylalanyl-L-histidyl-L-leucine (Z-L-Phe-L-His-L-Leu) in vesicular systems[2] is of vital importance. However, there have been only a few reports of the relation between remarkably enhanced enantioselectivity and the microenvironment of reaction field for the hydrolysis of amino acid esters in vesicular systems.

In this study, we report on the remarkable salt (ionic strength) effects on the enantioselective hydrolysis of C_{12}-D(L)-Phe-PNP by the tripeptide Z-L-Phe-L-His-L-Leu in the ditetradecyldimethyl-ammonium bromide ($2C_{14}Br$) vesicles at pH 7.6 and 25 ℃. The relation between the enantio-selectivity and the microenvironment of the reaction fields is also discussed.

The kinetic results[6] are summerized in Table 1. The noteworthy aspects are as follows : (a) The rate constants ($k_{a,obsd}$) for the hydrolysis of C_{12}-L-Phe-PNP as a function of ionic strength was bell-shaped



C_{12}-L-Phe-PNP



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

Table 1. Ionic strength dependence of rate constants ($k_{a,obsd}$) and enantioselectivity ($k^L_{a,obsd}/k^D_{a,obsd}$) for the hydrolysis of C_{12}-D(L)-Phe-PNP catalyzed by Z-L-Phe-L-His-L-Leu in $2C_{14}Br$[a)]

[Tris-KCl] (M)	$k_{a,obsd}$ ($M^{-1}s^{-1}$)		L/D
	L-isomer	D-isomer	
0.01	2200	910	2.4
0.015	2300	73	30
0.02	2800	55	51
0.03	3300	49	67
0.04	2400	40	60
0.06	2100	39	54
0.08	1500	51	29
0.20	1400	53	26

a) 25 ℃, pH 7.6, 3 %(v/v) CH_3CN-H_2O, [Z-L-Phe-L-His-L-Leu]=1.0×10^{-4} M, [C_{12}-D(L)-Phe-PNP]= 1.0×10^{-5} M, [$2C_{14}B$]=1.0×10^{-3} M.

with a maximum ($k^L_{a,obsd}$ = 3300 $M^{-1}s^{-1}$) at the condition of 0.03 M Tris-KCl (μ = 0.03) buffer, although the $k_{a,obsd}$ value for C_{12}-D-Phe-PNP was almost constant except at the condition of μ = 0.01. Moreover, (b) the high enantioselectivities ($k^L_{a,obsd}/k^D_{a,obsd}$ = 51 - 67) were obtained in the ionic strength range of μ = 0.02 - 0.06. Particularly, it is worthy to note that the highest enantioselectivity ($k^L_{a,obsd}/k^D_{a,obsd}$ = 67) was attained along with an ideal great rate-enhancement of the L-isomer at μ = 0.03. On the other hand, the hydrodynamic diameter (d_{hy}),[7] phase transition parameter (enthalpy change, $\triangle$H)[8] and fluorescence polarization (P)[9] of the $2C_{14}Br$ vesicles are shown in Fig. 1. Interestingly, d_{hy} abruptly changed from μ = 0.01 to μ = 0.03, although the $\triangle$H values were gradually increasing in the range of μ = 0.01 - 0.03 and were almost constant in the range of μ = 0.03 - 0.2. Thus, the packing of vesicular surfactants and hydrophobicity of $2C_{14}Br$ vesicles seem to be changed at μ = 0.03. The fluidity (which was reflected in the 1/P value) of the hydrophobic region in vesicles decreased by increasing the ionic strength. These results suggest that the enantioselectivity was enhanced around a kind of chaos (so called fluctuation) between unstable and stable regions, because the specific point of ionic strength (μ = 0.03) along with the highest enantioselectivity should be related to the inflection point of d_{hy} and $\triangle$H values.

Furthermore, we examined the ionic strength dependence of fluorescence intensity originating from 1-[(4-trimethylammonio)phenyl]-6-phenyl-1,3,5-hexatriene iodide (tma-DPH) placed in the pseudo-hydrophobic domain near the membrane surface[10] and 1,6-diphenyl-1,3,5-hexatriene (DPH) placed in the inner hydrophobic membrane domein as shown in Fig. 2. The fluorescence intensity originating from tma-DPH decreased to 63 % of the original one as the ionic strength (μ) was lowered from 0.08 to 0.02, while those from DPH was kept constant in the range of μ = 0.01 - 0.08. This result suggests that the hydrophobic microenvironment should be quite changed near the membrane surface where the hydrolysis presumably might take place.[5]

In conclusion, it is noteworthy that the remarkably high enantioselectivity along with the great enhancement for the hydrolysis of the L-isomer substrate should be produced around a kind of chaos between unstable and stable membrane matrices. This study is the first report to attain an attractively enhanced enantioselectivity by controlling the ionic strength in the pure vesicular systems and verify the relation between the kinetic specificity and the microenvironment of the reaction field.

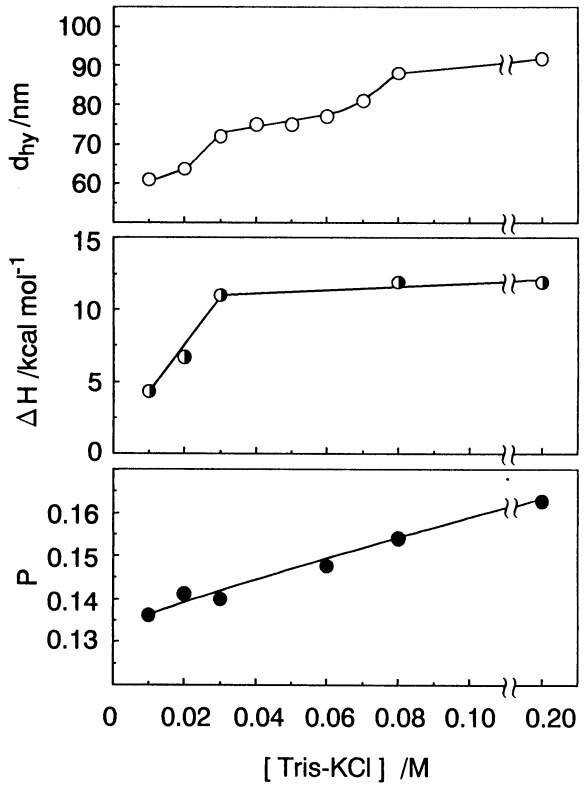


Fig. 1. Ionic strength dependence of hydrodynamic diameter (d_{hy}), phase transition enthalpy ($\triangle$H), and fluorescence polarization (P) of DPH in $2C_{14}$Br vesicles.

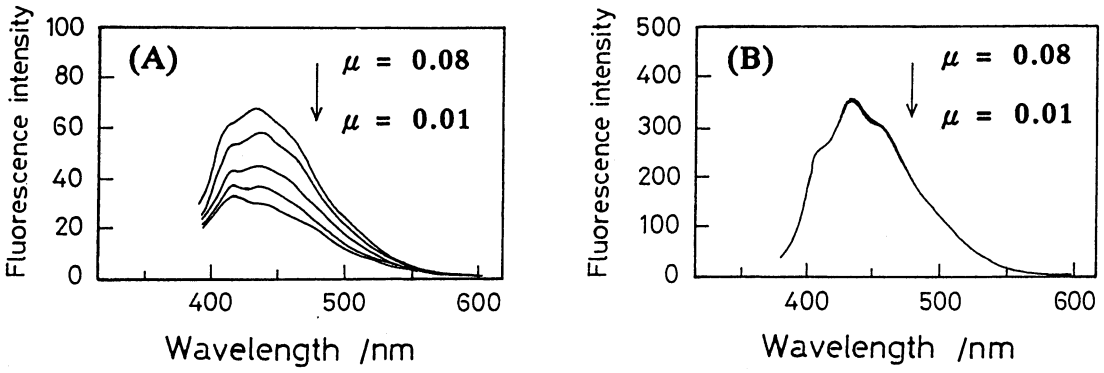


Fig. 2. Fluorescence spectra of tma-DPH (A) and DPH (B) in $2C_{14}$Br vesicles at the various ionic strength between μ = 0.08 and 0.01.

References

1) R. Ueoka, Y. Matsumoto, and Y. Ihara, *Chem. Lett.*, **1984**, 1807.
2) 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).
3) R. Ueoka, E. Yamada, O. Yamashita, Y. Matsumoto, and Y. Kato, *Tetrahedron Lett.*, **32**, 6597 (1991).
4) R. Ueoka, Y. Matsumoto, T. Nagamatsu, and S. Hirohara, *Chem. Lett.*, **1984**, 583.
5) Y. Matsumoto and R. Ueoka, *J. Org. Chem.*, **55**, 5797 (1990).
6) Rates of p-nitrophenol liberation from p-nitrophenyl ester were measured at 400 nm with a Hitachi 150-20 UV spectrophotometer. The reaction obeyed the usual pseudo-first-order rate law, and the apparent second-order rate constant ($k_{a,obsd}$) for the hydrolysis of an ester substrate was evaluated by Eq. 1,

$$k_{a,obsd} = (k_t - k_s) / [Cat]_0 \qquad (1)$$

where k_t and k_s denote the first-order rate constants with and without catalyst, respectively, $[Cat]_0$ indicates the initial catalyst concentration. The clear stock solutions were prepared by dissolving both catalyst and surfactant in Tris-KCl buffer with the sonication (BRANSONIC Model B2200 apparatus, 80W) at 50 ℃ for 60 min.
7) The dynamic light-scattering measurements were performed with BROOKHAVEN BI-90 particle sizer. The hydrodynamic diameter (d_{hy}) was evaluated by the Stokes-Einstein relation, Eq. 2,

$$d_{hy} = kT / 3\pi \eta D \qquad (2)$$

where k is Boltzmann's constant, T is the absolute temperature, η is the solvent viscosity and D is the diffusion coefficient. d_{hy} has maximum errors of $\pm 5\%$.
8) The $\triangle$H values were measured by using differential scanning calorimeter (SEIKO SSC 520). It has been already known that $\triangle$H values should be related to the hydrophobicity of membrane matrix.
9) Steady-state fluorescence spectra from DPH or tma-DPH in surfactant solution were measured on a Hitachi F-2000 spectrophotometer. The fluorescence polarization (P) of DPH was calculated by Eq. 3,

$$P = (I_{vv} - C_f I_{vv}) / (I_{vv} + C_f I_{vv}) \qquad (3)$$

where I is the fluorescence intensity (emission at 432 nm) and the subscripts v and h refer to the orientations, vertical and horizontal, respectively : e.g., I_{vh} indicates the fluorescence intensity measured with a vertical excitation polarizer and a horizontal analyzer polarizer. C_f is the grating correction factor, given by I_{hv} / I_{hh}.
10) F. G. Prendergast, R. P.Haugland, and P. J. Callahan, *Biochemistry*, **20**, 7333 (1981).

(Received July 4, 1994)