Pergamon

Tetrahedron Letters, Vol. 37, No. 20, pp. 3461-3464, 1996

0040-4039/96 $15.00 + 0.00

S0040-4039(96)00590-4

A Remarkably Enhanced Diastereoselectivity for the Hydrolysis of Dipeptide Esters Responding to pH and Temperature in Buffer Solutions

Ryuichi Ueoka*, Yoko Matsumoto, Koichi Goto, Tetsuo Ito, Shigeyuki Mori, Yoshito Matsumoto, Akihiro Sakoguchi, Yasuji Ihara+ and Fumio Hirata++

Graduate Course of Applied Chemistry, Kumamoto Institute of Technology, Ikeda, Kumamoto 860, Japan

+Yamaguchi Women's University, Sakurabatake, Yamaguchi 753, Japan

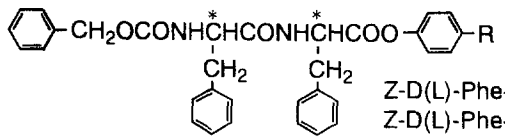
++Devision of Theoritical Studies, Institute for Molecular Science, Okazaki 444, Japan

Abstract: *The remarkable high diastereoselectivity for the hydrolysis of Z-D(L)-Phe-L-Phe-PMCP (DL/LL = 40) or Z-D(L)-Phe-L-Phe-PNP (DL/LL = 130) was attained by regulating pH and temperature in buffer solutions.* Copyright © 1996 Elsevier Science Ltd

Functional molecular assemblies composed of surfactants and reactive species have attracted much attention in recent years as enzyme models for the clarification of the catalytic specificities of native enzymes from the biomimetic view point.[1] In the course of our study on the stereoselective hydrolysis of amino acid esters in coaggregate systems, we emphasized that the stereochemical control is attained by regulating temperature[2,3] and ionic strength.[4,5] In particular, the authors observed almost complete L-enantiomer-selective catalysis, which was attained by controlling the reaction microenvironment.[6,7]

On the other hand, Moss and others examined the kinetic diastereoselectivity exercised in the cleavage of dipeptide esters in nucleophile-functionalized micellar,[8,9] vesicular,[10] and coaggregate systems.[11] Furthermore, Ueoka et al have succeeded in the high diastereoselective hydrolysis as mediated by the unmodified cyclodextrins.[12] Particularly, these authors emphasized that the diastereoselectivity must originate from supramolecular interaction of the substrate with the host assembly. However, little is known about the stereospecificity for the hydrolysis of dipeptide esters in buffer solutions.

In the present study, we report the first successful experiment resulting in marked diastereoselectivity in buffer solutions responding to pH and temperature. Kinetic and CD measurements were carried out at the solubilized concentration of substrate (5×10^{-6} M). Firstly, we examined the pH and temperature effects on the stereoselective hydrolysis of p-methoxycarbonylphenyl N-benzyloxycarbonyl-D(L)-phenylalanyl-L-phenylalaninates (Z-D(L)-Phe-L-Phe-PMCP)[13] without a catalyst in 0.02 M carbonate buffers (0.05 M KCl). The kinetic studies were carried out in the pH and temperature ranges of 9.0-10.5 and 17.5-45.0 °C, respectively.



Z-D(L)-Phe-L-Phe-PNP (R=NO_2)
Z-D(L)-Phe-L-Phe-PMCP (R=$COOCH_3$)

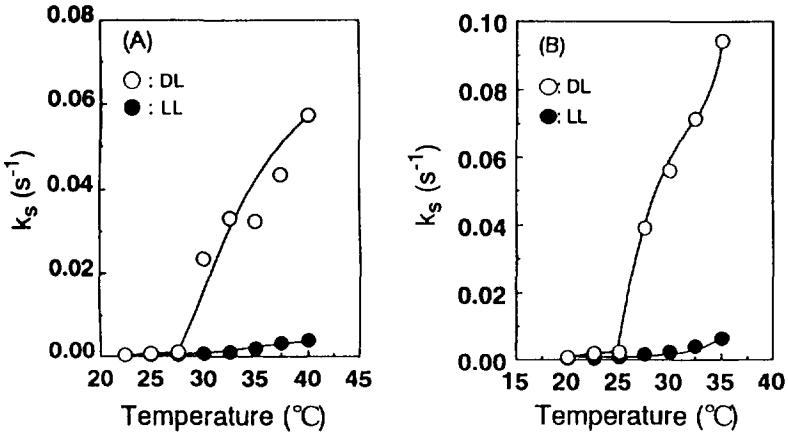


Figure 1. Temperature dependence of k_S for the hydrolysis of Z-D(L)-Phe-L-Phe-PMCP in buffer solutions at pH 9.5 (A) and pH 10.0 (B).

The pseudo-first-order rate constants (k_S) were evaluated by monitoring the release of p-methoxycarbonylphenolate ion at 297 nm. The temperature dependences of k_S for the hydrolysis of Z-D(L)-Phe-L-Phe-PMCP at pH 9.5 and 10.0 are presented in Figure 1(A) and (B), respectively. Moreover, the temperature and pH effects on the diastereoselectivity were summarized as shown in Figure 2.

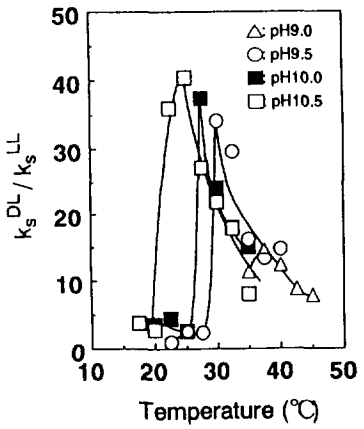


Figure 2. Temperature dependence of diastereoselectivity for the hydrolysis of Z-D(L)-Phe-L-Phe-PMCP in the pH range of pH 9.0-10.5.

The noteworthy aspects are as follows: a) The hydrolysis rates of Z-D-Phe-L-Phe-PMCP were sharply increased around 30 °C (at pH 9.5) and 27.5 °C (at pH10.0). On the other hand, such a rate enhancement was not observed for the hydrolysis of Z-L-Phe-L-Phe-PMCP around the same temperatures at respective pHs. b) The diastereoselectivity for the hydrolysis of Z-D(L)-Phe-L-Phe-PMCP was maximized at the optimum temperature in the pH range of 9.0-10.5 (that is, 37.5 °C at pH 9.0, 30 °C at pH 9.5, 27.5 °C at pH 10.0, and 25 °C at pH 10.5). c) The optimum temperatures were lowered as the pH value increased. As a result, d) The highest diastereoselectivity (DL/LL = 40) was attained for the hydrolysis of Z-D(L)-Phe-L-Phe-PMCP at pH10.5 and 25 °C.

It is already known that the specific CD spectra at 235 nm should occur through the intramolecular interaction between L(D)-Phe and L-Phe residues in the Z-D(L)-Phe-L-Phe-PMCP substrates.[14] Thus, the authors examined the pH and temperature dependences of CD intensity at 235 nm as shown in Figure 3. Interestingly, the CD intensity of Z-D-Phe-L-Phe-PMCP sharply changed around optimum temperatures, which closely correlated to the large rate enhancement for the hydrolysis of Z-D-Phe-L-Phe-PMCP and the maximized diastereoselectivity. This result suggests that the conformation of Z-D-Phe-L-Phe-PMCP should be changed and/or the aggregates-monomer transition of the same substrate might occur around these temperatures.[15]

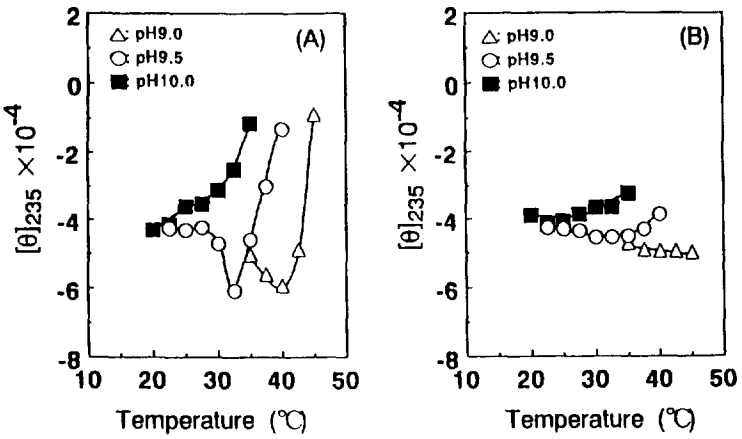


Figure 3. Temperature dependence of the CD intensity of Z-D-Phe-L-Phe-PMCP (A) and Z-L-Phe-L-Phe-PMCP (B) in the pH range of pH 9.0-10.0.

On the other hand, the CD intensity of Z-L-Phe-L-Phe-PMCP was almost constant in the temperature range of 20-45 °C at respective pHs.

Furthermore, the pH and temperature effects on the hydrolysis of Z-D(L)-Phe-L-Phe p-nitrophenyl esters (Z-D(L)-Phe-L-Phe-PNP) were also observed as shown in Figure 4. It is noteworthy that the dramatically high diastereoselectivity (DL/LL = 130) could be attained at pH 10 and 30 °C. The conformation of Z-D-Phe-L-Phe-PNP (Figure 5 (A)) is more favorable for the OH^- attack at the substrate's scissile carbonyl as compared with in the case of Z-L-Phe-L-Phe-PNP (Figure 5 (B)) on the basis of the computer calculation (the COSMO method[16] implemented into the MOPAC93).

In conclusion, it is noteworthy that the high diastereoselectivity (DL/LL = 130) was attained for the hydrolysis of dipeptide esters (DL and LL isomers) in buffer solutions. This could be attributed to the conformational change and/or aggregates-monomer transition in the DL-isomer substrate for the rate enhancement of hydrolysis by regulating pH and temperature.

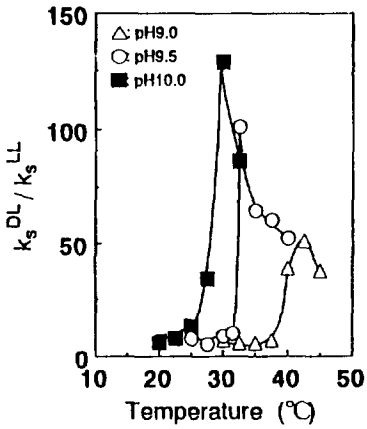


Figure 4. Temperature dependence of diastereoselectivity for the hydrolysis of Z-D(L)-Phe-L-Phe-PNP in the pH range of pH 9.0-10.0.

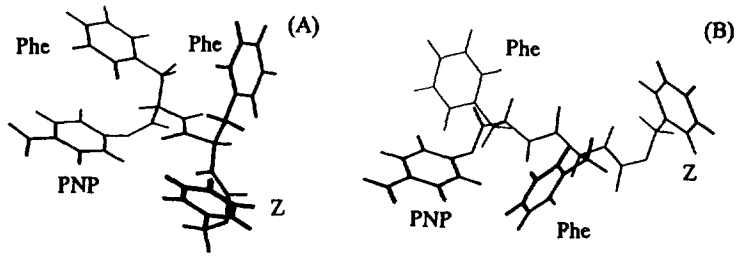


Figure 5. Optimized geometries of Z-D-Phe-L-Phe-PNP (A) and Z-L-Phe-L-Phe-PNP (B) by the COSMO method implemented into the MOPAC93.

Acknowledgements This work was supported in part by a Grant-in-Aid for Science Research from the Ministry of Education, Science, and Culture of Japan (No. 07305034 and 07650968), and was encouraged by the JRDC (Research Development Corporation of Japan) Pre-Research for New Fields Program, "Water and Biological Systems". We are grateful to Dr. Hatsuho Uedaira of National Institute of Bioscience and Human-Technology for helpful discussions.

References and Notes

1. Fendler, J. H. "*Membrane Mimetic Chemistry*," John Wiley & Sons, New York, **1982**.
2. Matsumoto, Y.; Ueoka, R. *J. Org. Chem.*, **1992**, *55*, 5797-5799.
3. Ueoka, R.; Yamada, E.; Matsumoto, Y.; Yamashita, O.; Kato, Y. *Tetrahedron Lett.*, **1991**, *32*, 6597-6600.
4. Ueoka, R.; Matsumoto, Y.; Yoshino, T.; Hirose, T.; Kikuchi, J.; Murakami, Y. *Chem. Lett.*, **1986**, 127-130.
5. Ueoka, R.; Cho, M.; Matsumoto, Y.; Goto, K.; Kato, Y.; Harada, K.; Sugii, A. *Tetrahedron Lett.*, **1990**, *31*, 5335-5338.
6. Ueoka, R.; Matsumoto, Y.; Yoshino, T.; Watanabe, N.; Omura, K.; Murakami, Y. *Chem. Lett.*, **1986**, 1743-1746.
7. Ihara, Y.; Igata, K.; Okubo, Y.; Nango, M. *J. Chem. Soc., Chem. Comm.*, **1989**, 1900-1902.
8. Moss, R. A.; Lee, Y. -S.; Lukas, T. J. *J. Am. Chem. Soc.*, **1979**, *101*, 2499-2503.
9. Moss, R. A.; Lee, Y. -S. *Tetrahedron Lett.*, **1981**, *22*, 2353-2356.
10. Moss, R. A.; Taguchi, T.; Bizzigotte, G. O. *Tetrahedron Lett.*, **1982**, *23*, 1985-1988.
11. Ueoka, R.; Matsumoto, Y.; Yoshino, T.; Hirose, T.; Moss, R. A.; Kim, K. Y.; Swarup, S. *Tetrahedron Lett.*, **1986**, *27*, 1183-1186.
12. Ueoka, R.; Matsumoto, Y.; Harada, K.; Akahoshi, H.; Ihara, Y.; Kato, Y. *J. Am. Chem. Soc.*, **1992**, *114*, 8339-8341.
13. The dipeptide esters were synthesized by mixed anhydride coupling of Z-phenylalanine and phenylalanine p-methoxycarbonyl ester. Satisfactory elemental analyses were obtained for Z-D(L)-Phe-L-Phe-PMCP. Z-D-Phe-L-Phe-PMCP: mp 183.9-184.8 °C. Anal. Calcd for $C_{34}H_{33}N_2O_7$: C, 70.36; H, 5.51; N, 4.83. Found: C, 70.55; H, 5.31; N, 4.94. Z-L-Phe-L-Phe-PMCP: mp 206.9-208.2 °C. Found: C, 70.50; H, 5.61; N, 5.01.
14. Ueoka, R.; Matsumoto, Y.; Moss, R. A.; Swarup, S.; Sugii, A.; Harada, K.; Kikuchi, J.; Murakami, Y. *J. Am. Chem. Soc.*, **1988**, *110*, 1588-1589.
15. We can consider these two possibilities by putting together CD measurements and kinetic results as described in Morii, H.; Ichimura, K.; Uedaira, H. *Chem. Lett.*, **1990**, 1987-1990.
16. Klamt, A.; Schuurmann, G. *J. Chem. Soc., Perkin Trans.2*, **1993**, 799-800.

(*Received in Japan* 5 *February* 1996; *revised* 22 *March* 1996; *accepted* 25 *March* 1996)