TEMPERATURE CONTROL FOR THE DIASTEREOSELECTIVE HYDROLYSIS OF DIPEPTIDE ESTERS IN THE BUFFER SOLUTION

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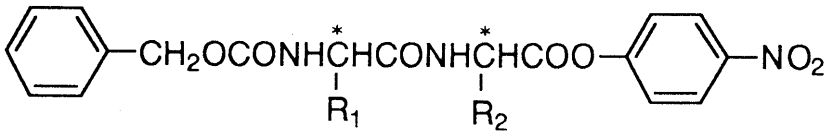
A remarkably high stereoselectivity was attained for the hydrolysis of dipeptide p-nitrophenyl esters (DL/LL=101 for Z-Phe-Phe-PNP and DL/LL=71 for Z-Phe-Leu-PNP) without a catalyst in pH 9.5, 0.02 M carbonate buffer at the optimum temperature (32.5 ℃).

KEYWORDS dipeptide ester; diastereoselective hydrolysis; conformational change; temperature control

Stereoselective cleavages of N-protected amino acid and peptide p-nitrophenyl esters in various surfactant aggregate systems have been used as models to probe the origins of stereoselectivity in the proteolytic enzymes. In the course of our study on the stereoselective hydrolysis of amino acid esters in the coaggregate systems, we emphasized that stereochemical control is attained by regulating temperature[1, 2] and ionic strength[3] and by changing the composition of the coaggregates.[4, 5] In particular, the almost complete L-enantioselective catalysis[6-8] can be attributed to optimization of the enzyme model conformation in the coaggregate systems.[9]

Most recently, a markedly high diastereoselectivity was observed for the deacylation of dipeptide esters catalyzed by unmodified cyclodextrins (CyD).[10] The CyD-dipeptide substrate complex could be one of the typical examples of the so-called supramolecular assemblies which demonstrated novel functions only after association of individual molecular components. However, there have been few reports concerning the diastereoselective hydrolysis of dipeptide esters without a catalyst, and the stereoselectivity observed was not so attractive.[11-13]

In this study we report for the first time on the dramatically enhanced diastereoselective hydrolysis of dipeptide esters (Z-Phe-Phe-PNP and Z-Phe-Leu-PNP) without a catalyst in pH 9.5, 0.02 M carbonate buffer at 32.5 ℃. The dipeptide p-nitrophenyl esters were synthesized by mixed anhydride coupling of Z-amino acid and amino acid p-nitrophenyl ester,[13] and pseudo-first-order rate constants (k_s) were evaluated by monitoring the release of p-nitrophenol at 400 nm.



Z-Phe-Phe-PNP (R_1=R_2=CH_2Ph) Z-Phe-Leu-PNP (R_1=CH_2Ph, R_2=$CH_2CH(CH_3)_2$)
Z-Leu-Phe-PNP (R_1=$CH_2CH(CH_3)_2$, R_2=CH_2Ph) Z-Leu-Leu-PNP(R_1=R_2=$CH_2CH(CH_3)_2$)

The temperature dependency of k_s for the hydrolysis of dipeptide esters (Z-D-Phe-L-Phe-PNP and Z-L-Phe-L-Phe-PNP) and the relations of the CD intensity and absorbance of Z-D-Phe-L-Phe-PMCP[14] with the temperature in the buffer solution are presented in Fig. 1(A) and (B), respectively.

The noteworthy aspects are as follows: With respect to the hydrolysis of Z-Phe-Phe-PNP and Z-Phe-Leu-PNP, (a) the rate enhancement of Z-D-Phe-L-Phe-PNP was sharply increased with the temperature elevation from 31.5 ℃ to 32.5 ℃, though that of Z-L-Phe-L-Phe-PNP was not observed around the same temperatures. Interestingly, both the CD intensity and the

absorbance of Z-D-Phe-L-Phe-PMCP change sharply around 30 ℃, and this is in good harmony with the extremely large rate-enhancement for the hydrolysis of Z-D-Phe-L-Phe-PNP. On the other hand, such changes were not observed in the case of Z-L-Phe-L-Phe-PMCP in concert with the gentle change for the hydrolysis of Z-L-Phe-L-Phe-PNP. As a result, (b) the diastereoselectivity for the hydrolysis of Z-Phe-Phe-PNP was maximized at 32.5 ℃ (DL/LL=101). A similar maximized diastereoselectivity was observed for the hydrolysis of Z-Phe-Leu-PNP. On the other hand, with respect to the hydrolysis of Z-Leu-Phe-PNP, (c) a sharp increment of rates occurred between 27.5 and 28.5 ℃ for the hydrolysis of Z-L-Leu-L-Phe-PNP, though such an increment was not observed for the diastereomeric Z-D-Leu-L-Phe-PNP around the same temperatures. However, interestingly, the rate enhancement of Z-D-Leu-L-Phe-PNP was fairly remarkable at temperatures above 32.5 ℃. As a necessary result, (d) the maximized diastereoselectivity (LL/DL=20) for the hydrolysis of Z-Leu-Phe-PNP was presented at 28.5 ℃. With respect to the hydrolysis of Z-Leu-Leu-PNP, temperature characteristics of crucial rate- enhancement and remarkably high stereoselectivity were not observed (LL/DL=1.8-2.3 in the temperature range of 15-40 ℃).

From the above-mentioned kinetic results, we can emphasize that the sequence of amino acids in peptide esters should be

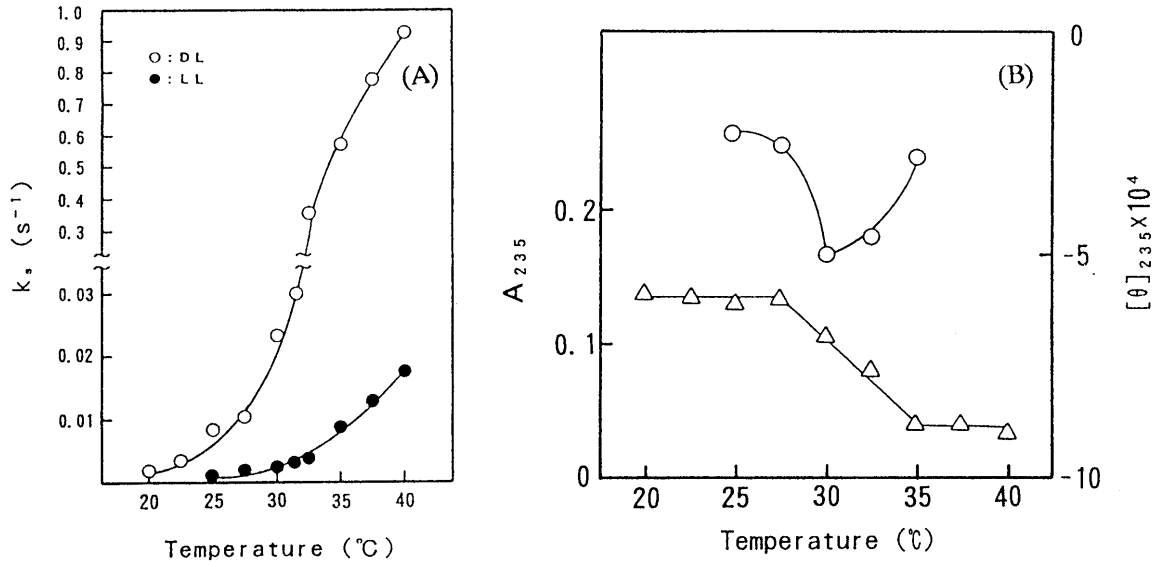


Fig. 1. Temperature Dependences of k_s for the Hydrolysis of Z-D-Phe-L-Phe-PNP and Z-L-Phe-L-Phe-PNP (A) and of the CD Intensity ($[\theta]_{235}$, ○) and Absorbance (A_{235}, △) of Z-D-Phe-L-Phe-PMCP (B)

Table I. Diastereoselectivity for the Hydrolysis of Dipeptide Esters Having Various Amino Acid Sequences in the Buffer Solution[a)]

Dipeptide esters	k_s (s^{-1}) DL	k_s (s^{-1}) LL	Diastereoselectivity	Temperature (℃)
Z-Phe-Phe-PNP	0.356	0.00351	DL/LL=101	32.5
Z-Phe-Leu-PNP	0.266	0.00372	DL/LL=71	32.5
Z-Leu-Phe-PNP	0.0194	0.389	LL/DL=20	28.5
Z-Leu-Leu-PNP	0.164	0.377	LL/DL=2.3	30.0

a) pH 9.5, 0.02M Carbonate buffer (0.05M KCl), 10%($^v/_v$) CH_3CN-H_20, [Sub]=5×10^{-6}M.

most important for the determination of DL- or LL- diastereoselectivity. That is, the dipeptide esters (Z-Phe-Phe-PNP and Z-Phe-Leu-PNP) having a Phe part attached directly to the Z-group commonly produced extremely large rate-enhancement for the hydrolysis of DL-isomer as compared with the LL-isomer. On the other hand, LL-diastereoselectivities were observed for the hydrolysis of Z-Leu-Phe-PNP and Z-Leu-Leu-PNP having a Leu part attached directly to the Z-group. It is also noteworthy that the highest diastereoselectivity was attained at an optimum temperature (32.5 ℃ for Z-Phe-Phe-PNP and Z-Phe-Leu-PNP or 28.5 ℃ for Z-Leu-Phe-PNP).

Hydrolysis of Z-AA-Pro-PNP (AA=Ala, Phe, Leu, Val, and Trp) in buffer was DL-diastereoselective. This reaction proceeds mainly via intramolecular cyclization to diketopiperazines and partly through intermolecular attack by OH^-.[11-13] Moreover, related reactions could occur with tripeptide Pro-PNP esters.[15] In these cases, the Pro-PNP part in the peptide substrates was essential for the intramolecular cyclization. In this study, however, we employed dipeptide esters without the Pro-PNP part and might neglect the path via cyclization.

It is concluded that the remarkably high diastereoselectivity could be attributed to the conformation of dipeptide esters related to the amino acid sequence and should depend on the difference in the facilitation of the OH^- attack on the carbonyl in diastereomeric substrates. Particularly, it is noteworthy that remarkably high diastereoselectivity (DL/LL=101) was attained for the hydrolysis of Z-Phe-Phe-PNP at 32.5 ℃; this should be related to the conformational change of Z-D-Phe-L-Phe-PNP in conjunction with the large rate-enhancement around the same temperature.

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14) The analogous and inactive compound (Z-D-Phe-L-Phe p-methoxycarbonylphenyl ester : Z-D-Phe-L-Phe- PMCP) instead of Z-D-Phe-L-Phe-PNP was employed for the CD and UV measurement. The specific CD spectra at 235 nm may occur through the intramolecular exciton chirality interaction between D-Phe and L-Phe units of Z-D-Phe-L-Phe-PMCP as described in N. Harada and K. Nakanishi, Circular Dichroic Spectroscopy Exciton Coupling in Organic and Bioorganic Chemistry, University Science Books, Mill Vally, CA, 1993, and ref. 5.
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