Diastereoselective Specificity for the Hydrolysis of Dipeptide Esters in Aqueous Media

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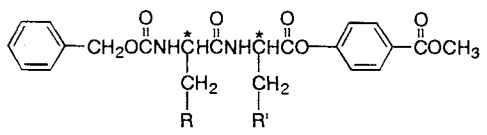
The diastereoselectivity for the hydrolysis of *p*-methoxycarbonylphenyl *N*-(benzyloxycarbonyl)-D(L)-phenylalanyl-L-leucinate in aqueous solution was inverted by changing concentrations of the substrates or the reaction temperature. The monomer-aggregate transition operated on the LL-substrate, which was suggested by the spectroscopic measurements, seems to be responsible for such behavior.

The stereochemical control has been recognized to be very important subject and attracted considerable attention in connection with, for example, understanding enzyme functions and creating the corresponding artificial enzymes. In the course of our study on the enantioselective hydrolysis of amino acid esters catalyzed by active peptides in micellar, vesicular, and coaggregate systems, we emphasized that the stereochemical control could be established by changing amino acid residues covalently introduced into substrates and catalysts (reactants), and composition of coaggregates (reaction fields), and by regulating temperature and ionic strength of reaction media.[1]

On the other hand, a markedly high stereoselectivity was also attained for the diastereoselective hydrolysis of dipeptide esters in aggregate systems[2,3] and cyclodextrins.[4] Furthermore, we observed a high DL-diastereoselectivity for the hydrolysis of dipeptide esters having two phenylalanines (Phe) without any catalyst in aqueous solution.[5-7]

In order to gain further insight into the diastereoselective specificity for the hydrolysis of dipeptide esters, we examined the effects of amino acid sequence, concentration of substrates, and reaction temperature on the diastereoselective hydrolysis of dipeptide *p*-methoxycarbonylphenyl esters (Z-D(L)-Leu-L-Phe-PMCP and Z-D(L)-Phe-L-Leu-PMCP) having different amino acid sequences (Phe and leucine : Leu) in aqueous media.[8]



R=$CH(CH_3)_2$, R'=Ph : Z-D(L)-Leu-L-Phe-PMCP
R=Ph, R'=$CH(CH_3)_2$: Z-D(L)-Phe-L-Leu-PMCP

The concentration dependent hydrolysis of Z-D(L)-Leu-L-Phe-PMCP and Z-D(L)-Phe-L-Leu-PMCP in an aqueous medium (20 vol% CH_3CN) at 25 °C are shown in Figure 1. The rate constants (k_s) for the hydrolysis of Z-D(L)-Leu-L-Phe-PMCP remained apparently constant, though those for the LL-substrate were slightly larger than those for the DL-substrate over the whole concentration range examined. On the other hand, in the hydrolysis of Z-D(L)-Phe-L-Leu-PMCP, the rate constant for the

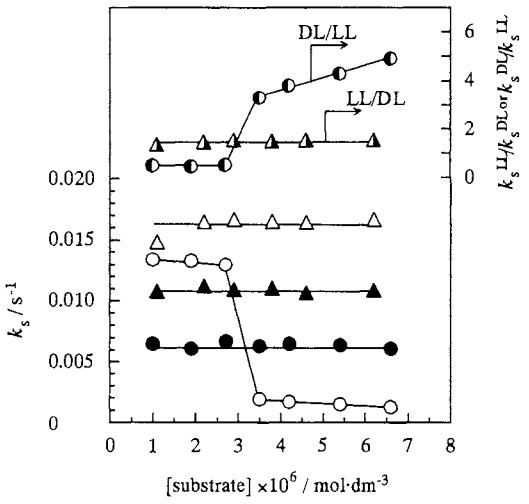


Figure 1. Concentration effects of diastereomeric substrates on rate constant (k_s) and diastereoselectivity (k_s^{LL}/k_s^{DL} or k_s^{DL}/k_s^{LL}) in their hydrolysis studied in 20 vol% CH_3CN-H_2O (0.02 mol·dm^{-3} carbonate buffer (pH 9.5)) at 25 °C. Z-D(L)-Leu-L-Phe-PMCP ; k_s^{DL}:▲, k_s^{LL}:△, k_s^{LL}/k_s^{DL}:◭, Z-D(L)-Phe-L-Leu-PMCP ; k_s^{DL}:●, k_s^{LL}:○, k_s^{DL}/k_s^{LL}:◐.

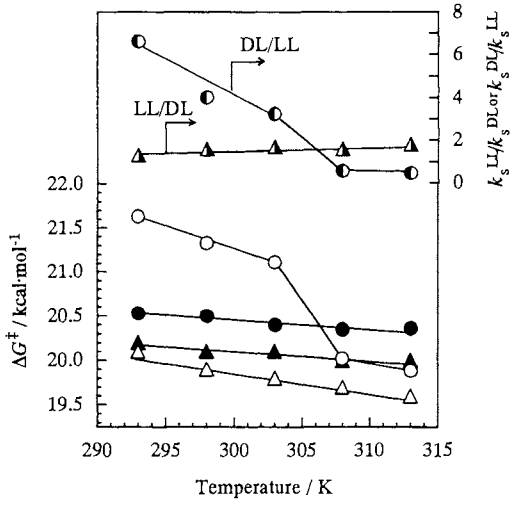


Figure 2. Temperature effects on free energy of activation ($\Delta G^{\ddagger}$) and diastereoselectivity (k_s^{LL}/k_s^{DL} or k_s^{DL}/k_s^{LL}) for the hydrolysis diastereomeric substrates of in 20 vol% CH_3CN-H_2O (0.02 mol·dm^{-3} carbonate buffer (pH 9.5)). [substrate]=5.0×10^{-6} mol·dm^{-3}. Z-D(L)-Leu-L-Phe-PMCP ; k_s^{DL}:▲, k_s^{LL}:△, k_s^{LL}/k_s^{DL}:◭, Z-D(L)-Phe-L-Leu-PMCP ; k_s^{DL}:●, k_s^{LL}:○, k_s^{DL}/k_s^{LL}:◐.

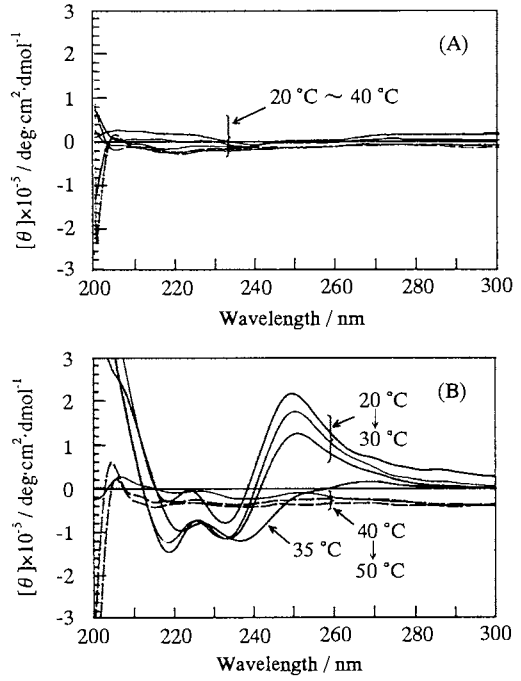


Figure 3. CD spectra of (A) Z-D-Phe-L-Leu-PMCP and (B) Z-L-Phe-L-Leu-PMCP in 20 vol% CH_3CN-H_2O (0.02 mol·dm^{-3} phosphate buffer (pH 7.4)). [Z-D(L)-Phe-L-Leu-PMCP]=5.0×10^{-6} mol·dm^{-3}.

LL-substrate decreased drastically in the concentration range from 2.7×10^{-6} to 3.5×10^{-6} mol·dm^{-3}, though that for the DL-substrate remained nearly. As a result, the diastereoselectivity became favorable for the DL-substrate over the LL-substrate ($k_s^{LL}<k_s^{DL}$) in the concentration range above 3.5×10^{-6} mol·dm^{-3}. The result suggests that the amino acid sequence in dipeptide substrates must play an important role in governing the diastereospecificity.

The temperature effect on the hydrolysis of Z-D(L)-Leu-L-Phe-PMCP and Z-D(L)-Phe-L-Leu-PMCP ([substrate]=5.0×10^{-6} mol·dm^{-3}) in an aqueous medium is shown in Figure 2. As for the hydrolysis of Z-D(L)-Phe-L-Leu-PMCP, the inflection range for the plot of free energy of activation ($\Delta G^{\ddagger}$) versus reaction temperature for the LL-substrate was observed between 30.0 °C and 35.0 °C, though a linear relationship between $\Delta G^{\ddagger}$ and temperature was observed for the DL-substrate. Thus, the rate constant for the hydrolysis of LL-substrate sharply increased and the diastereoselectivity became favorable for the DL-substrate over the LL-substrate above 35.0 °C.

In addition, we carried out spectroscopic measurements to explore the origin of diastereospecificity and obtained interesting results. In UV measurements on Z-D(L)-Phe-L-Leu-PMCP in an aqueous medium, an inflection for the plot of absorbance versus concentration of the LL-substrate was observed in the concentration range from 3.0×10^{-6} to 3.5×10^{-6} mol·dm^{-3}.[9] This may be attributed to the aggregation of LL-substrates in aqueous solution in a concentration range above 3.5×10^{-6} mol·dm^{-3}. As for CD measurements on Z-D(L)-Phe-L-Leu-PMCP (5.0×10^{-6} mol·dm^{-3}) in aqueous solution (Figure 3), the CD intensity for the LL-substrate sharply changed at 35 °C, though such a specific change was not observed for the DL-substrate. This is in good conformity with the temperature dependent behavior of free energy of activation for the hydrolysis of Z-D(L)-Phe-L-Leu-PMCP in aqueous solution. Plausibly, the diastereoseletivity in the hydrolysis of Z-D(L)-Phe-L-Leu-PMCP must be inverted by the monomer-aggregate transition for the LL-substrate by controlling concentration of the substrate and the reaction temperature.

In conclusion, the diastereoselectivity was observed for the hydrolysis of Z-D(L)-Phe-L-Leu-PMCP without any catalyst in aqueous media by controlling concentration of the substrate and the reaction temperature. The spectroscopic measurments seems to indicate that this stereospecificity is originated from the monomer–aggregate transition for the LL-substrate.

References and Notes

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8 The dipeptide esters were synthesized by mixed anhydride coupling of Z-amino acid and L-amino acid ester as described in Ref. 2. Satisfactory analytical data were obtained for Z-D(L)-Leu-L-Phe-PMCP and Z-D(L)-Phe-L-Leu-PMCP. Z-D-Leu-L-Phe-PMCP: mp 181.0-183.0 °C, $[\alpha]_D^{25}$-14.9° (*c* 1, 1,4-dioxane). Found: C, 68.04; H, 6.33; N, 5.21 %. Anal. Calcd for $C_{31}H_{34}N_2$: C, 68.11; H, 6.27; N, 5.13 %. Z-L-Leu-L-Phe-PMCP: mp 164.0-165.0 °C, $[\alpha]_D^{25}$-27.8° (*c* 1, 1,4-dioxane). Anal. Found: C, 68.16; H, 6.33; N, 5.16 %. Z-D-Phe-L-Leu-PMCP: mp 162.0-164.0 °C, $[\alpha]_D^{25}$-20.9° (*c* 1, 1,4-dioxane). Found: C, 67.83; H, 6.34; N, 5.15 %. Anal. Calcd for $C_{31}H_{34}N_2$: C, 68.11; H, 6.27; N, 5.13 %. Z-L-Phe-L-Leu-PMCP: mp 173.0-179.0 °C, $[\alpha]_D^{25}$-17.7° (*c* 1, 1,4-dioxane). Anal. Found: C, 68.16; H, 6.38; N, 5.20 %.

Rates of *p*-methoxycarbonylphenolate ion liberation from dipeptide esters were measured at 297 nm with a Hitachi U-200A spectrophotometer. The reactions were evaluated with the usual pseudo-first-order rate law, and the rate constant (k_s) and free energy of activation ($\Delta G^{\ddagger}$) for the hydrolysis were calculated by Eqs. 1 and 2,

$$\log(A_\infty - A_t) = -k_s t/2.303 + \log A_\infty \quad (1)$$

$$\Delta G^{\ddagger} = 2.303RT\log(k_B T/hk_s) \quad (2)$$

where A_∞ and A_t denote the absorbances of *p*-methoxycarbonylphenolate ion at time t and infinite time, respectively.

9 R. Ueoka, K. Goto, O. Tanoue, A. Miki, S. Yoshimitsu, and Y. Murakami, unpublished observations.