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MARKED CHANGES IN THE CIRCULAR DICHROISM OF THE TRIPEPTIDE CATALYST RELATED TO
ENHANCED ENANTIOSELECTIVE HYDROLYSIS IN MICELLAR SYSTEMS

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The enantioselectivity for the hydrolytic cleavage of p-nitro-phenyl N-dodecanoyl-D(L)-phenylalaninates in the micellar systems was augmented by using the active tripeptide (Z-L-Phe-L-His-L-Leu), which showed an α -helix-like CD curve, combined with a marked change of the CD intensity of the tripeptide above the cmc.

KEYWORDS — enantioselectivity; amino acid ester; peptide catalyst; hydrolysis; circular dichroism; micellar catalysis

The sequence of amino acids is fairly important in providing the rigid conformation of peptides and may also be closely related to the biological activity of peptides. With respect to the enhancement of the stereoselective hydrolytic cleavage of p-nitrophenyl esters derived from N-protected amino acids,¹⁾ the importance of the amino acid sequence of peptide catalysts²⁾ has been emphasized. It is also important to control the reaction field by temperature regulation³⁾ or by changing the composition of vesicular and micellar surfactants.⁴⁾

However, there have been only a few reports of the relation between stereoselectivity and the conformation of peptide catalysts for the enantioselective hydrolysis of amino acid esters,⁵⁾ though the helical structure of polypeptides and cyclopeptides has been discussed in detail on the basis of the circular dichroism (CD) experiments.⁶⁾

In this paper, we report the dramatic changes of the CD curves and CD intensities of peptide catalysts in relation to the enhancement of enantioselective hydrolysis of p-nitrophenyl N-dodecanoyl-D(L)-phenylalaninates [C_{12} -D(L)-Phe-PNP] in the micellar systems.

We have examined the hydrolytic cleavage of long-chain enantiomers [C_{12} -D(L)-Phe-PNP] by peptide catalysts including the L-histidine part in the micellar systems (benzylhexadecyldimethylammonium chloride:CBzAC, hexadecyltrimethylammonium chloride:CTAC)⁷⁾ at room temperature (25 °C) and pH 7.6 in 0.08 M Tris-KCl buffer [3% (v/v) CH_3CN-H_2O]. The results are summarized in Table I. The noteworthy aspects are as follows: (a) The tripeptide Z-L-Phe-L-His-L-Leu and the dipeptide Z-L-Phe-L-His catalysts, having in common an L-Phe-L-His unit, greatly enhance

enantioselectivity (reflected in $k_{a,obsd}(L)/k_{a,obsd}(D)$)⁸⁾ compared with the other peptides (Z-L-His, Z-L-His-L-Phe, and Bz-Gly-L-His-L-Leu) in the presence of micelles (CBzAC and CTAC). (b) The CBzAC micelles having a benzyl group more greatly enhanced both the catalytic efficiency (reflected in $k_{a,obsd}$) and the enantioselectivity for the hydrolysis of C_{12} -D(L)-Phe-PNP when Z-L-Phe-L-His-L-Leu was used as a catalyst, and the highest enantiomer rate ratio (L/D = 35) was attained. This suggests that the L-Phe-L-His unit in Z-L-Phe-L-His-L-Leu and Z-L-Phe-L-His, which was attained directly to the Z-group, and the hydrophobicity in the C_{12} -L-Phe-PNP substrate with an L-Phe unit are of great importance in enhancing the rate and enantioselectivity in the micellar systems.

The CD spectra of L-histidine derivatives (Z-L-His, Z-L-His-L-Phe, Z-L-Phe-L-His, Bz-Gly-L-His-L-Leu, and Z-L-Phe-L-His-L-Leu) with CTAC micelles were measured as shown in Fig. 1.⁹⁾ It is noteworthy that the specific CD spectra at ca. 235 nm occurred commonly in the active peptides (Z-L-Phe-L-His-L-Leu and Z-L-Phe-L-His) with CTAC micelles, which promoted the enantioselectivity for the hydrolysis of the long-chain enantiomers (C_{12} -D(L)-Phe-PNP). This implies that these specific CD patterns of Z-L-Phe-L-His-L-Leu and Z-L-Phe-L-His may support the rigid conformation of the common unit of L-Phe-L-His. The Phe, His, and Leu units in Z-L-Phe-L-His-L-Leu are well known for forming a stable α -helix structure. And interestingly, the CD curve of this tripeptide in the presence of micelles (CBzAC or CTAC) closely resembles those of α -helical peptides.¹⁰⁾

The authors also examined the dependence of the CD intensity of the active tripeptide (Z-L-Phe-L-His-L-Leu) at ca. 235 nm on the concentration of CBzAC micelles and compared the kinetic results as shown in Fig. 2. The enantioselectivity for the hydrolysis of the long-chain enantiomers (C_{12} -D(L)-Phe-PNP) sharply increased at the cmc⁷⁾ of CBzAC and the high enantioselectivity remained almost constant above the cmc. It is of interest also (Fig. 2) that the same relationship exists between the CD intensity and the concentration of CBzAC, though the CD intensity was kept constant above the somewhat higher concentration than the cmc. These results suggest that the rigid conformation of Z-L-Phe-L-His-L-Leu (like an α -helical arrangement) should be stable in the hydrophobic micellar system and may be related to the enhancement of the enantioselective hydrolysis when above the cmc.

Table I. Rate Constants ($k_{a,obsd}$, $M^{-1}s^{-1}$) and Enantioselectivity ($k_{a,obsd}(L)/k_{a,obsd}(D)$) in the CBzAC or CTAC Micellar Catalytic System^{a)}

Catalyst	C_{12} -L-Phe-PNP		C_{12} -D-Phe-PNP		L/D	
Z-L-His	28	(30)	7.0	(15)	4.0	(2.0)
Z-L-His-L-Phe	50	(30)	28	(26)	1.8	(1.2)
Z-L-Phe-L-His	2800	(1600)	190	(110)	15	(15)
Bz-Gly-L-His-L-Leu	9.5	(3.4)	3.9	(2.2)	2.4	(1.5)
Z-L-Phe-L-His-L-Leu	1900	(410)	55	(19)	<u>35</u>	(22)

Values in the parentheses were obtained in the CTAC micellar system.¹¹⁾

a) pH 7.6, 25°C, 0.08 M Tris buffer (0.08 M KCl), 3% (v/v) CH_3CN-H_2O , [Sub] = 1×10^{-5} M, [Cat] = 1×10^{-4} M, [CBzAC] = 1×10^{-3} M, [CTAC] = 3×10^{-3} M.

Fig. 1. CD Spectra of peptide Catalysts with CTAC Micelles in CH_3OH-H_2O (5:95 v/v): ---, Z-L-His; ---, Z-L-His-L-Phe; ····, Z-L-Phe-L-His; - · - ·, Bz-Gly-L-His-L-Leu; —, Z-L-Phe-L-His-L-Leu. [cat] = 2.5×10^{-4} M, [CTAC] = 3×10^{-3} M

Fig. 2. Concentration Dependence of CBzAC Micelles on CD Intensity ($[\theta]$) of Z-L-Phe-L-His-L-Leu and Enantioselectivity (L/D) for the Hydrolysis of C_{12} -D(L)-Phe-PNP Catalyzed by Z-L-Phe-L-His-L-Leu:

○, $[\theta]$ ([cat] = 2.5×10^{-4} M);
●, L/D ([cat] = 1×10^{-4} M).

In conclusion, it should be emphasized that the enantioselectivity for the hydrolysis of the long-chain enantiomers (C_{12} -D(L)-Phe-PNP) in the CBzAC or CTAC micellar system was augmented by using the active tripeptide (Z-L-Phe-L-His-L-Leu), which showed an α -helix-like CD curve. Also a drastic change of the CD intensity of the tripeptide above the cmc and amplified enantioselectivity ($L/D = 38$) was attained in the micelles of CBzAC having a benzyl head group.

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- 7) The critical micelle concentration (cmc) values of CBzAC and CTAC were determined to be 5.0×10^{-4} M and 1.7×10^{-3} M, respectively, by using the conductivity method. The kinetic experiments were carried out above the cmc values.
- 8) The second-order rate constants ($k_{a,obsd}$) were calculated from $(k_t - k_s)/[cat]_0$, where k_t and k_s denote the first-order rate constants with and without catalyst, respectively, and $[cat]_0$ stands for the initial concentration of the catalyst.
- 9) The CD spectra were recorded with a Jasco J-50A recording spectropolarimeter (Xe lamp, 1.0 cm cell) at room temperature and were expressed as a molar ellipticity ($[\theta]$, $\text{deg} \cdot \text{cm}^2 \cdot \text{d mol}^{-1}$).
- 10) For example, physiologically active peptide mastparan composed of 14 amino acid residues is well known to form a typical helix structure.
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