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DRAMATICALLY ENHANCED ENANTIOSELECTIVE HYDROLYSIS OF AMINO ACID ESTERS WITH TRIPEPTIDE NUCLEOPHILE BY CONTROLLING THE REACTION FIELD AT ROOM TEMPERATURE

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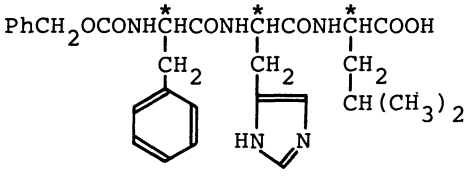
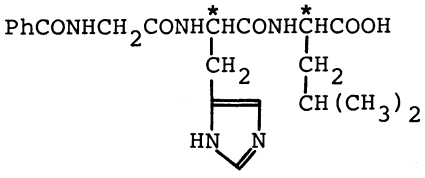
The high enantioselective hydrolysis ($k^{L}_{a,obsd}/k^{D}_{a,obsd}$ = 27) of p-nitrophenyl N-dodecanoyl-D(L)-phenylalaninate with N-benzyloxycarbonyl-L-phenylalanyl-L-histidyl-L-leucine in the vesicular system (ditetradecyldimethylammonium bromide) was enhanced dramatically by the addition of micelles (hexadecyltrimethylammonium bromide) and attained to the enantiomer rate ratio ($k^{L}_{a,obsd}/k^{D}_{a,obsd}$) = 71 at room temperature (25 °C).

The stereoselective hydrolyses of N-protected amino acid p-nitrophenyl esters have been lately attracted considerable attention in aiding the understanding the origins of stereoselectivity in the proteolytic enzymes. Very recently, many interesting results were obtained in the micellar,[1] vesicular,[2] cyclic peptide,[3] and macromolecular systems.[4] The authors have emphasized that the hydrophobic interactions between catalyst and substrate,[5] the introduction of two asymmetric centers into the catalyst,[1c, 6] and the controlling the reaction field of artificial membrane systems by the addition of cholesterol[2d,7] and micelles[2d] or by the temperature-regulation[8] were very important for enhancing the stereoselective hydrolysis. In this paper, we wish to demonstrate the importance of the amino acid sequence in the peptide catalyst (dipeptide and tripeptide) and the controlling of the vesicular catalytic field for the enhancement of enantioselective hydrolysis at room temperature (25 °C).

Recently, we reported that the temperature dependence of enantioselectivity (reflected in $k^{L}_{a,obsd}/k^{D}_{a,obsd}$)[9] for the long-chain substrate (S_{12}) was bell-shaped with a maximum ($k^{L}_{a,obsd}/k^{D}_{a,obsd}$ = 11) at the optimum temperature (25 °C) in the vesicular system of N-tetradecanoyl-L-histidyl-L-leucine (MyrHisLeu) + ditetradecyldimethylammonium bromide ($2C_{14}$).[8b] Furthermore, one of the authors



Z-PheHisLeu

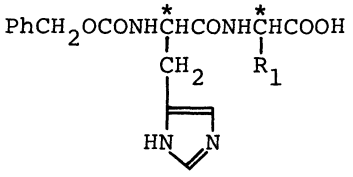


Bz-GlyHisLeu

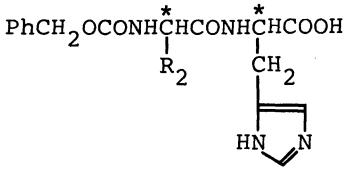
Table 1. Rate Constants ($k_{a,obsd}$, $s^{-1}M^{-1}$; 1 M=1 mol dm^{-3}) and Stereoselectivity ($k^{L}_{a,obsd}/k^{D}_{a,obsd}$) at 25 °C[a)]

Catalytic system	L-ZS	D-ZS	L/D	L-S_{12}	D-S_{12}	L/D
Z-His[b)] + $2C_{14}$	10	9	1.1	46	31	1.5
Z-HisLeu + $2C_{14}$	14	10	1.4	47	47	1.0
Z-HisPhe + $2C_{14}$	56	28	2.0	92	41	2.2
Z-LeuHis + $2C_{14}$	210	46	4.6	1400	63	22
Z-PheHis + $2C_{14}$	480	110	4.4	4300	170	25
Bz-GlyHisLeu + $2C_{14}$	2	2	1.0	45	34	1.3
Z-PheHisLeu + $2C_{14}$	260	62	4.2	1700	63	27
Z-LeuHis + $2C_{14}$ + CTAB	160	42	3.9	870	42	21
Z-PheHis + $2C_{14}$ + CTAB	150	35	4.2	2500	120	21
Z-PheHisLeu + $2C_{14}$ + CTAB	86	8	11	780	11	71

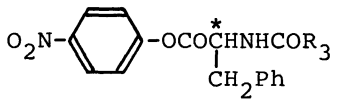
a) pH 7.6, 0.083 M Tris Buffer (0.083 M KCl), 3% (v/v) CH_3CN-H_2O, [cat]=5×10^{-5} M, [sub]=1×10^{-3} M, [$2C_{14}$]=1×10^{-3} M, [CTAB]=2×10^{-3} M. All of amino acids in the catalyst are L-form.
b) N-Benzyloxycarbonyl-L-histidine.



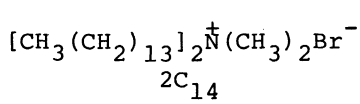
Z-HisLeu ($R_1=(CH_3)_2CHCH_2$)
Z-HisPhe ($R_1=PhCH_2$)



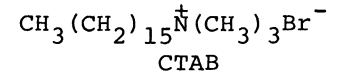
Z-LeuHis ($R_2=(CH_3)_2CHCH_2$)
Z-PheHis ($R_2=PhCH_2$)



D(L)-ZS ($R_3=PhCH_2O$)
D(L)-S_{12} ($R_3=CH_3(CH_2)_{10}$)



$2C_{14}$



CTAB

emphasized previously[1c)] that the hydrogen-bonding and hydrophobic interactions between substrates and catalysts are important to enhance the enantioselective hydrolysis with Z(N-Benzyloxycarbonyl)- type dipeptide catalysts including a histidine residue. Therefore, we have examined the hydrolytic cleavage of p-nitrophenyl N-benzyloxycarbonyl-D(L)-phenylalaninate (D(L)-ZS) and N-dodecanoyl--D(L)-phenylalaninate (D(L)-S_{12}) catalyzed by L-histidine derivatives (Z-His, Z-HisLeu, Z-HisPhe, Z-LeuHis, Z-PheHis, Bz-GlyHisLeu, and Z-PheHisLeu) in the vesicular system ($2C_{14}$) at room temperature (25 °C) and the results are summarized in Table 1. The noteworthy aspects are as follows: With respect to the position of a histidine (His) unit, (a) the dipeptide catalysts (Z-HisLeu and Z-HisPhe) having a His unit, which was directly attached to Z-group, were not always more efficient for the enhancement of catalytic efficiency (reflected in $k_{a,obsd}$) and stereoselectivity when compared with Z-His in both the hydrolyses of

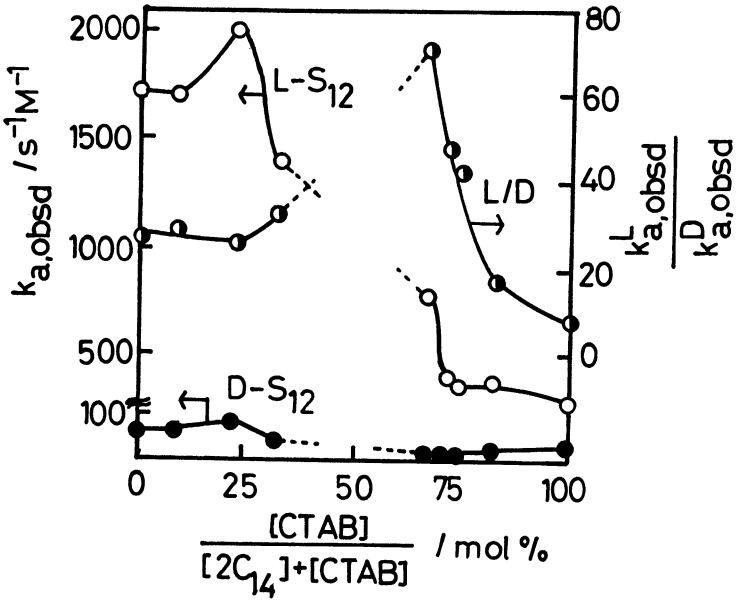


Fig. 1. Enantioselectivity and rate constants for the hydrolysis of enantiomers catalyzed by Z-PheHisLeu in the vesicular ($2C_{14}$) and/or micellar (CTAB) systems. $[2C_{14}]=1\times10^{-3}$ M

ZS and S_{12}; (b) the dipeptides (Z-LeuHis and Z-PheHis) having a His unit attached to their C-terminal enhanced dramatically the rate and enantioselectivity for the hydrolysis of the long-chain enantiomer substrate(S_{12}); (c) the tripeptide (Z-PheHis-Leu) having a His in its middle position was most efficient for the enhancement of enantioselectivity among all the catalysts employed in this study, but Bz-GlyHisLeu was not efficient; With respect to the position of a phenylalanine (Phe) unit, (d) the Phe unit in Z-PheHis and Z-PheHisLeu, which was attached directly to Z-group, played an important role to enhance the rate and enantioselectivity for the hydrolysis of S_{12}. The above-mentioned results suggest that the position of His and Phe units in the catalyst and the hydrophobicity in the S_{12} substrate having a Phe unit is of great importance for the enhancement of rate and enantioselectivity.

We also examined the effect of controlling the reaction field for the enantioselective hydrolysis of S_{12} catalyzed by Z-PheHisLeu in the vesicular and/or micellar systems as shown in Fig. 1. It is of interest that the rate for the hydrolysis of D(L)-S_{12} was almost bell-shaped with a maximum in the CTAB concentration of 23 mol% (the concentration of micelles is expressed mol% of total of vesicular and micellar moles) and, surprisingly, this tendency was fairly similar to the bell-shaped fluorescence polarization by using 1,6-diphenyl-1,3,5-hexatriene (DPH) as a fluorescence probe[10] under the same conditions as shown in Fig. 2, though no clear solution was obtained in the CTAB concentration range from 33 mol% to 67 mol%. This suggests that the fluidity of the hydrophobic region of $2C_{14}$ changed with the addition of micelles (CTAB)[11] and resulted in the large rate enhancement for the long-chain substrates at the CTAB concentration of 23 mol%. Furthermore, it is very noteworthy that the enantioselectivity ($k^L_{a,obsd}/k^D_{a,obsd}$ = 27 and 4.2 for the hydrolysis of S_{12} and ZS, respectively) in the pure

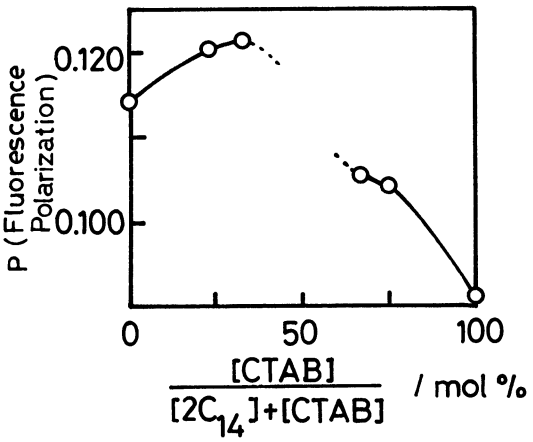


Fig. 2. Fluorescence polarization of DPH in the vesicular ($2C_{14}$) and/or micellar (CTAB) systems.

vesicular system of $2C_{14}$ was dramatically enhanced by adding micelles and attained to the enantiomer rate ratio = 71 (S_{12}) and 11 (ZS) at the CTAB concentration of 67 mol%, though the maximum of fluorescence polarization was presented at the CTAB concentration of 33 mol%. This large enhancement of enantioselectivity was attributed to the more reduced rate for the hydrolysis of D-isomers when compared with L-isomers. The effect of CTAB addition to the vesicular systems, however, was effective only for the tripeptide catalyst (Z-PheHisLeu) and no effect was observed for the dipeptide catalysts (Z-LeuHis and Z-PheHis) as described in Table 1. This unique reaction field of the mixed system of vesicles and micelles was estimated to be not too strong and not too weak hydrophobic environment for the greater enhancement of enantioselectivity on the basis of the isokinetic temperature (β) determined previously.[12)]

In conclusion, we wish to emphasize that the sequence of amino acids in the tripeptide catalyst (Z-PheHisLeu) is most important to enhance the enantioselectivity for the hydrolysis of the long-chain substrate (D(L)-S_{12}) having a Phe unit through the difference of L-S_{12} and D-S_{12} in hydrophobic interactions, hydrogen bonds, steric effect and so on, and, especially, that the PheHis unit in higher peptides might be important biologically.

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