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Steric-Control for the Enantioselective Hydrolysis of Amino Acid Esters with Membrane-Bound Enzyme Models

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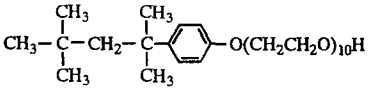
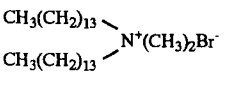
Abstract : The apparently complete stereoselectivity ($k^{L}_{a,obsd}/k^{D}_{a,obsd}=\infty$) for the hydrolysis of enantiomeric substrate (*p*-nitrophenyl *n*-dodecanoyl-D(L)-phenyalaninate; C_{12}-D(L)-Phe-PNP) catalyzed by active tripeptide (*N*-(benzyloxycarbonyl)-L-phenylalanyl -L-histidyl-L-leucine; Z-PheHisLeu) was attained by regulating the composition of coaggregates, ionic strength, and temperature, in coaggregate systems composed of vesicular and micellar surfactants. This can be related to the optimization of conformation in the Z-PheHisLeu catalyst to react with amino acid esters by changing of physical properties of coaggregates.

The membrane mimetic agents including micelles, vesicles, and hybrid membranes and so on have been utilized in the reactivity control of membrane-bound enzyme models for providing hydrophobic microenvironments. In our previous studies of enantioselective catalysis in coaggregate systems, the following interesting results were obtained: (1)Excellent correlations are observed between the stereoselectivity for the hydrolysis of the amino acid esters and the apparent mean hydrodynamic diameters of coaggregates. [1,2] (2)The enantioselectivity is markedly enhanced by employing peptide catalysts having specific amino acid residues including L-histidine.[3] (3)The origin of the high stereoselective hydrolysis can be simulated by computer modeling studies.[4]

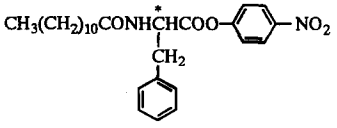
In this study, we report on the composition and temperature sensitive artificial hybrid-membranes composed of ditetradecyldimethylammonium bromide ($2C_{14}Br$) vesicles and α-[4-(1,1,3,3-tetramethylbutyl)phenyl]-ω-hydroxypoly(oxy-1,2-ethanediyl) (Triton X-100)



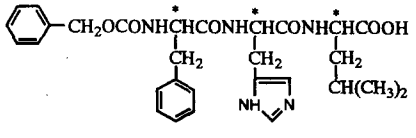
Triton X-100



$2C_{14}Br$



C_{12}-D(L)-Phe-PNP



Z-PheHisLeu

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micelles for the enantioselective hydrolysis of amino acid esters by the membrane-bound enzyme.

It is already known that the tripeptide Z-PheHisLeu is the most effective catalyst for the enhancement of enantioselectivity for the hydrolysis of C_{12}-D(L)-Phe-PNP.[2] So, first, we examined the composition effect of coaggregates and ionic strength(μ) on the enantioselective hydrolysis of C_{12}-D(L)-Phe-PNP catalyzed by Z-PheHisLeu.[5] The results are shown in Fig.1. It is noteworthy that the enantioselectivity was maximized at the Triton X-100 concentration of 40 mol% (the concentration of Triton X-100 is expressed as mol percent of total surfactant) in 0.05 M Tris buffer (μ =0.05). Furthermore, the apparently complete enantioselectivity ($k^L_{a,obsd}/k^D_{a,obsd}=\infty$) is attained at the Triton X-100 concentration of 40 - 50 mol% in 0.02M Tris buffer (μ =0.02).

The composition dependence of apparent hydrodynamic diameter (d_{hy})[6] in coaggregates and fluorescence polarization (P) of diphenylhexatriene (DPH) in the Triton X-100/$2C_{14}$Br coaggregates are shown in Fig. 2. The d_{hy} value was sharply enlarged in the range of 40 - 50 mol% Triton X-100 concentration, and, interestingly, the enantioselectivity for the hydrolysis of C_{12}-D(L)-Phe-PNP was also extremely enhanced in the same region of coaggregate composition at μ =0.02. On the other hand, the fluidity (which was reflected in the 1/P value) of the hydrophobic core of coaggregates was gradually decreased in the range of 0 - 50 mol% Triton X-100 concentration as the mixed ratio increased. These results suggest that the size of coaggregates and the fluidity of the hydrophobic core in coaggregates should change upon the addition of Triton X-100 and result in a large enhancement of enantioselectivity for the long-chain substrates (C_{12}-D(L)-Phe-PNP) at the Triton X-100 concentration of 40 - 50 mol%. Thus, the coaggregates having a large size and small fluidity would present an appropriate microenvironment for providing an ideal stereoselectivity.

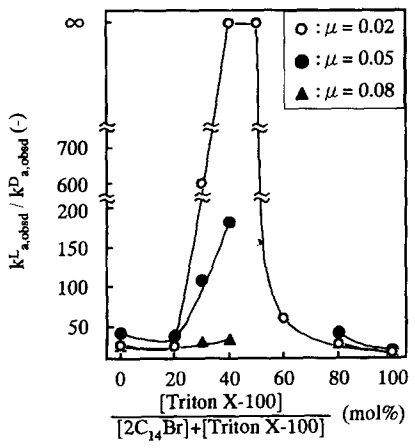

Fig. 1 Composition and ionic strength (μ) dependences of enantioselectivity ($k^L_{a,obsd}$ / $k^D_{a,obsd}$) for the hydrolysis of C12-D(L)-Phe-PNP catalyzed by Z-PheHisLeu in the Triton X-100 / 2C14Br coaggregates at 25°C and pH 7.6. [C12-D(L)-Phe-PNP] = 1.0×10^{-5} M, [Z-PheHisLeu] = 5.0×10^{-5} M.

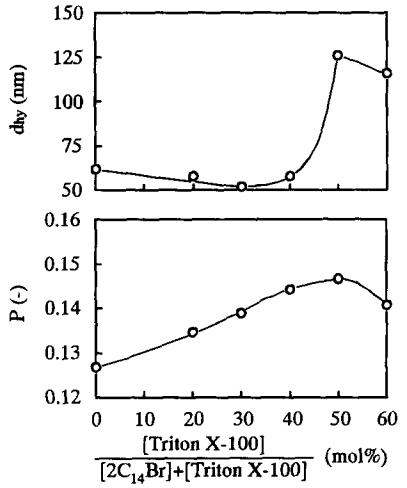

Fig. 2 Composition dependences of apparent hydrodynamic diameter (d_{hy}) and fluorescence polarization (P) of DPH in the Triton X-100 / 2C14Br coaggregates at 25°C and μ = 0.02.

Second, we examined CD spectra of Z-PheHisLeu in order to determine the relationship between the enantioselectivity and the conformation of Z-PheHisLeu in the coaggregates of $2C_{14}Br$ and Triton X-100.[7] The change of CD spectra was observed by changing the miceller concentrations of coaggregates as shown in Fig.3. The CD spectra for the pure vesicles of $2C_{14}Br$ and the coaggregates containing 20 mol% Triton X-100 were similar to the CD spectra in methanol (data not shown). Interestingly, it was found that the CD patterns were fairly changed by increasing the ratio of Triton X-100 in the coaggregate including 30 - 80 mol% Triton X-100. The change of CD patterns might be related to the conformational change of Z-PheHisLeu in the coaggregates. These results suggest that the enantioselectivity for the hydrolysis of C_{12}-D(L)-Phe-PNP in the hydrophobic coaggregates should respond to the conformational change of Z-PheHisLeu. Furthermore, the computer modeling study supports that a favorable molecular recognition between C_{12}-D(L)-Phe-PNP and Z-PheHisLeu through the effective hydrophobic interaction and hydrogen bond should be very important for the enhancement of enantioselectivity.[8]

Finally, the temperature effect on the enantioselective hydrolysis of C_{12}-D(L)-Phe-PNP with Z-PheHisLeu in the coaggregates composed of 70mol% $2C_{14}Br$ and 30mol% Triton X-100 in the condition of $\mu = 0.02$ were examined as shown in Fig.4 . The second order rate constant ($k_{a,obsd}$) for the L-S_{12} hydrolysis was sharply enhanced as the temperature increased. On the other hand, the $k_{a,obsd}$ value for the D-S_{12} hydrolysis was gradually decreased, and no catalysis ($k^{D}_{a,obsd} = 0$) was observed up to 30℃. As a result, the enantioselectivity ($k^{L}_{a,obsd}$/

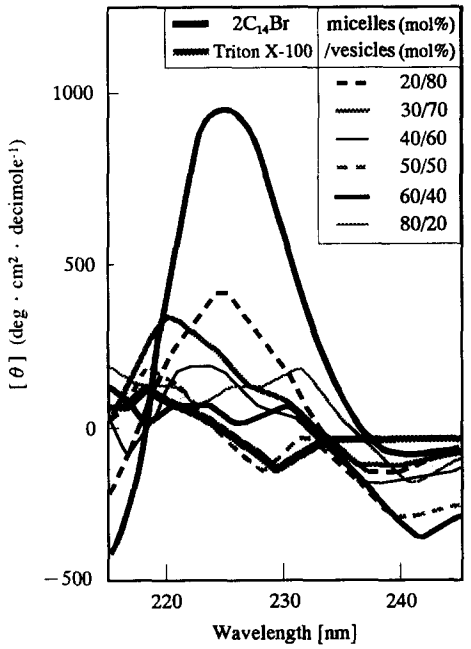

Fig. 3 CD Spectra of Z-PheHisLeu with 2C14Br vesicles, coaggregates containing 20, 30, 40, 50, 60, 80, and 100 mol% Triton X-100 micelles ($\mu = 0.02$).

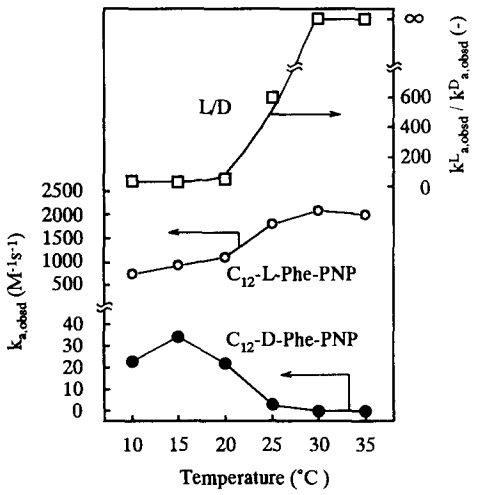

Fig. 4 Temperature dependence of rate constants ($k_{a,obsd}$) and enantioselectivity ($k^{L}_{a,obsd}/k^{D}_{a,obsd}$) for the hydrolysis of C12-D(L)-Phe-PNP catalyzed by Z-PheHisLeu in the 30mol% Triton X-100 / 70mol% 2C14Br coaggregates at pH 7.6 and $\mu = 0.02$.

$k^{D}_{a,obsd}$) was increased along with the elevation of temperature and the complete enantioselectivity ($k^{L}_{a,obsd}/k^{D}_{a,obsd} = \infty$) was attained in the temperature range of 30-35℃. We also examined the temperature dependence of CD spectra, and no remarkable change in CD spectra of Z-PheHisLeu was observed (data not shown). So, it is suggested that the conformation of the catalyst would be delicately adjusted along with elevation of temperature to enhance the enantioselectivity.

In conclusion, it is noteworthy that the apparently complete enantioselective hydrolysis of the long-chain enantiomer (C_{12}-D(L)-Phe-PNP) with the effective catalyst (Z-PheHisLeu) was attained by regulating the composition of coaggregates, ionic strength, and temperature. Our findings may well be related to the conformational change of Z-PheHisLeu like that of the "induced fit theory" through the optimization of the microenvironment of coaggregates.

Acknowledgments

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5 Rates of p-nitrophenol liberation from p-nitrophenyl ester were measured at 400 nm with a Hitachi 150-20 UV spectrophotometer. The reaction obeyed the usual pseudo-first-order rate law, and the apparent second-order rate constant ($k_{a,obsd}$) for the hydrolysis of an ester substrate was evaluated by the equation of $k_{a,obsd} = (k_t - k_s) / [cat]_0$ where k_t and k_s denote the first-order rate constants with and without a catalyst, respectively, and $[cat]_0$ indicates the initial catalyst concentration. However, special attention should be paid to consider no catalysis ($k_{a,obsd} = 0$) because k_t is extremely close to k_s. The clear stock solutions were prepared by dissolving both catalyst and surfactant in Tris-KCl buffer with the sonication (BRANSONIC Model B2200 apparatus, 80W) at 50℃ for 30 min.

6 The dynamic light-scattering measurements were performed with BROOKHAVEN BI-90 particle sizer. The hydrodynamic diameter (d_{hy}) was evaluated by the Stokes-Einstein equation of $d_{hy} = kT / (3\pi \eta D)$, where k is Boltzmann's constant, T is the absolute temperature, η is the solvent viscosity and D is the different coefficient.

7 The CD (Circular Dichroism) spectra of peptide catalysts (Z-PheHisLeu) in the coaggregates were obtained using a Jasco J-720WI recording spectropolarimeter (Xe lamp, 1.0cm cell) in the range of 215 - 260 nm at room temperature.

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