Steric Control for the Enantioselective Hydrolysis of Amino Acid Esters in Coaggregates Composed of Phosphatidylcholine and Triton X-100

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Remarkably high enantioselectivity (L/D = 19) along with marked enhancement of the hydrolytic cleavage of the L-form enantiomer (L-S_{12}) catalyzed by a tripeptide (Z–L-Phe–L-His–L-Leu) was attained by the use of specific coaggregates of 20 mol% dilauroylphosphatidylcholine and 80 mol% Triton X-100.

Keywords steric control; enantioselectivity; hydrolysis; amino acid ester; phospho-lipid membrane; coaggregate; vesicular catalysis

The stereoselective hydrolysis of amino acid esters has recently attracted considerable attention in connection with understanding the origins of the stereoselectivity observed with proteolytic enzymes.[1-3]

In the course of our study on enantioselective catalysis in vesicular systems[4] and organized assemblies of micellar and vesicular surfactants,[5] we emphasized that stereochemical control could be established by temperature regulation[6] and by changing the composition of the coaggregates (reaction field).[7]

We have recently found appropriate systems to control the stereoselectivity of hydrolysis. That is, the enantioselectivity for the hydrolytic cleavage of long-chain substrates (D(L)-S_{12}) by a tripeptide catalyst (Z–L-Phe–L-His–L-Leu) was well correlated with the apparent mean hydrodynamic diameter of coaggregates composed of synthesized double-chained surfactants (dialkyldimethylammonium halides) and single-chained ones (hexadecyltrimethylammonium halides).[8,9] Now, we can extend the correlation between the stereoselectivity and the diameter (radius) of coaggregates to native lipid membrane systems composed of L-α-dilauroylphosphatidylcholine (DLPC) and polyoxyethylene(10)p-1,1,3,3-tetramethylbutylphenol (Triton X-100).

The kinetic results are summarized in Table I. Furthermore, we investigated the apparent hydrodynamic radius (R) of coaggregates monitored by dynamic light-scattering (DLS) in order to consider the relation of R to the enantioselectivity. The R data were collected at a 90° scattering angle and are also shown in Table I. The noteworthy aspects are as follows. (a) We first developed a unique method to prepare uniform liposomes (lipid membrane) composed of DLPC and Triton X-100 by the usual sonication. For example, an electron micrograph of coaggregates composed of 65 mol% DLPC and 35 mol% Triton X-100 showed the presence of spherical particles with radii of 500—700Å in good agreement with hydrodynamic radii (700 Å) found by DLS. (b) With respect to the reaction of D(L)-S_{12} with Z–L-Phe–L-His–L-Leu, excellent correlations were observed between the stereoselectivity and the apparent mean hydrodynamic radii of the DLPC/Triton X-100 coaggregates containing 10—35 mol% Triton X-100. This behavior was similar to the system of coaggregates composed of synthetic double-chained surfactants.[9] On the other hand, it is also of interest that remarkably high enantioselectivity (L/D = 19) along with marked enhancement of the hydrolytic cleavage of L-S_{12} was obtained with specific coaggregates of 20 mol% DLPC and 80 mol% Triton X-100.[10]

In conclusion, it is very important that stereochemical

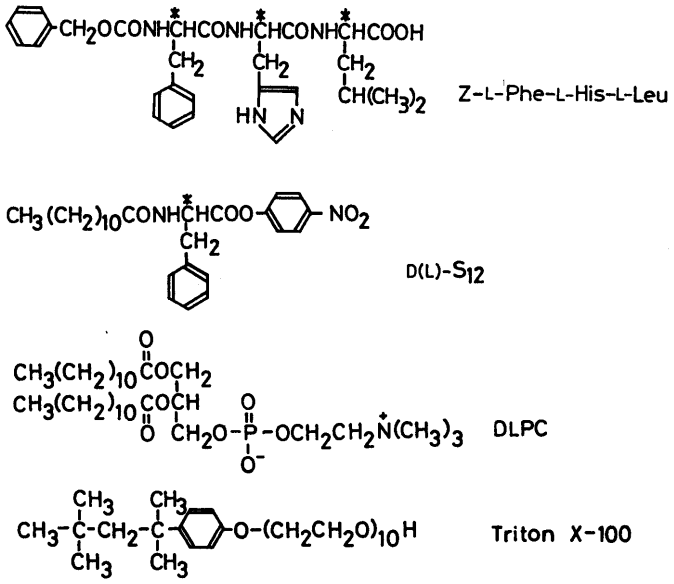

TABLE I. Rate Constants ($k_{a,obsd}$, $M^{-1}s^{-1}$) and Enantioselectivity ($k^L_{a,obsd}/k^D_{a,obsd}$) for the Hydrolysis of D(L)-S_{12} Catalyzed by Z–L-Phe–L-His–L-Leu in Coaggregates Composed of DLPC and Triton X-100 along with the Radii (R, Å)[a]

[Triton X-100] / [DLPC]+[Triton X-100] (mol%)	L	D	L/D	R (Å)[b]
10	30	3.1	9.7	360—600 (400)
21	26	3.4	7.6	290—450 (330)
35	48	3.4	14	700—850 (800)
40	43	3.5	12	—
57	62	4.0	16	150—250 (150)
68	66	5.0	13	110—360 (110)
80	78	4.1	19	—
90	56	3.0	19	—
100[c]	41	2.9	14	—

a) At 25 °C, pH 7.4, 0.01 M phosphate buffer (0.01 M KCl), 3% (v/v) CH_3CN–H_2O, [sub] = 1×10^{-5} M, [cat] = 1×10^{-4} M, [DLPC] = 1×10^{-3} M, [Triton X-100] = $(0.1-9.0) \times 10^{-3}$ M. The rate constants have maximum errors of ±3.4%. *b*) The R values are those immediately or from 1 d to one week after preparing the solution. Values in parentheses are those after standing for 1 d. *c*) [Triton X-100] = 9×10^{-3} M.

control of the enantioselective hydrolysis of amino acid esters could be established by changing the composition of coaggregates in native lipid membrane systems as well as with synthetic surfactants.

Experimental

Materials. ***p*-Nitrophenyl *N*-Dodecanoyl-D(L)-phenylalaninate (D(L)-S_{12})** The enantiomeric substrates (D(L)-S_{12}) were prepared from *N*-(benzyloxycarbonyl)-D(L)-phenylalaninate by the esterification of the COOH group with *p*-nitrophenol and dicyclohexylcarbodiimide,[11] followed by hydrobromination of the NH_2 group,[12] and then acylation of the $NH_2 \cdot HBr$ group with dodecanoic anhydride.[11] Satisfactory results of elemental analyses and specific rotations were obtained for D(L)-S_{12}. D-S_{12}: mp 108.0—108.2 °C, $[\alpha]_D^{23}$ +10.8° ($c=2$, $CHCl_3$). *Anal.* Calcd for $C_{27}H_{36}N_2O_5$: C, 69.21; H, 7.74; N, 5.98. Found: C, 69.32; H, 7.94; N, 5.98. L-S_{12}: mp 107.0—107.5 °C, $[\alpha]_D^{23}$ −10.8° ($c=2$, $CHCl_3$). *Anal.* Found: C, 69.15; H, 7.76; N, 5.91.

Commercially available *N*-(benzyloxycarbonyl)-L-phenylalanyl-L-histidyl-L-leucine (Z-L-Phe-L-His-L-Leu, Bachem), DLPC and Triton X-100 were used without further purification.

Kinetic Measurements 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 using Eq. 1:

$$k_{a,obsd}=(k_1-k_s)/[cat]_0 \quad (1)$$

where k_1 and k_s denote the first-order rate constants with and without a catalyst, respectively, and $[cat]_0$ indicates the initial catalyst concentration.

Clear stock solutions were prepared by dissolving both Z-L-Phe-L-His-L-Leu and coaggregates composed of DLPC and Triton X-100 in 0.01 M phosphate buffer ($\mu=0.01$ with KCl) with sonication (Braun Sonic Model B 3200 apparatus, 90 W) at 40 °C for 30 min. The stock solutions were employed for kinetic experiments after standing for 1 d at room temperature.

Dynamic Light-Scattering Measurements The dynamic light-scattering measurements were performed with a clipping-type photon correlator, a laser light source He-Ne(632.8 nm), and a microcomputer that used the cumulant program and directly afforded R values of the aggregates as described previously.[13] The particle hydrodynamic radius (R) is given by the Stokes–Einstein relation, Eq. 2:

$$R=kT/6\pi\eta D \quad (2)$$

where k is Boltzmann's constant, T is the temperature, η is the solvent viscosity and D is the diffusion coefficient.

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References and Notes

1) J. M. Brown and C. A. Bunton, *J. Chem. Soc., Chem. Commun.*, **1974**, 969.
2) Y. Ihara, *J. Chem. Soc., Chem. Commun.*, **1978**, 984.
3) J. H. Fendler, "Membrane Mimetic Chemistry," John Wiley & Sons, New York, 1982.
4) R. Ueoka, Y. Matsumoto, Y. Ninomiya, Y. Nakagawa, K. Inoue, and K. Ohkubo, *Chem. Lett.*, **1981**, 785.
5) R. Ueoka, Y. Matsumoto, T. Nagamatsu, and S. Hirohata, *Tetrahedron Lett.*, **1984**, 1363.
6) R. Ueoka, Y. Matsumoto, T. Nagamatsu, and S. Hirohata, *Chem. Lett.*, **1984**, 583.
7) R. Ueoka, Y. Matsumoto, and Y. Ihara, *Chem. Lett.*, **1984**, 1807.
8) R. Ueoka, Y. Matsumoto, T. Yoshino, T. Hirose, R. A. Moss, K. Y. Kim, and S. Swarup, *Tetrahedron Lett.*, **1986**, 1183.
9) R. Ueoka, Y. Matsumoto, R. A. Moss, S. Swarup, A. Sugii, K. Harada, J. Kikuchi, and Y. Murakami, *J. Am. Chem. Soc.*, **110**, 1588 (1988).
10) The hydrodynamic radii of the specific coaggregates are suggested to be smaller than 200 Å from the DLS results.
11) M. Bodanzky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 6072 (1959).
12) S. W. Ingles and J. R. Knowles, *Biochem. J.*, **104**, 369 (1967).
13) R. A. Moss, Y.-C. Chiang, and Y. Hui, *J. Am. Chem. Soc.*, **106**, 7506 (1984).