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ENZYME-LIKE ENANTIOSELECTIVE CATALYSIS IN THE SPECIFIC COAGGREGATE SYSTEM OF VESICULAR AND MICELLAR SURFACTANTS

Ryuichi UEOKA,* Yōko MATSUMOTO, Takashige YOSHINO, Takashi HIROSE, Jun-ichi KIKUCHI,† and Yukito MURAKAMI*†

Department of Industrial Chemistry, Faculty of Engineering, Kumamoto Institute of Technology, Ikeda, Kumamoto 860
† Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812

The enzyme-like enantioselective catalysis ($k^{L}_{a,obsd}$ = 1300 $dm^3\ mol^{-1}\ s^{-1}$ and $k^{D}_{a,obsd}$ = 0) was attained for the hydrolytic cleavage of p-nitrophenyl N-dodecanoyl-D(L)-phenylalaninate by N-benzyloxycarbonyl-L-phenylalanyl-L-histidyl-L-leucine in the large and stable vesicular system composed of 59 mol% ditetradecyldimethylammonium bromide and 41 mol% hexadecyltrimethylammonium bromide at 30 °C in 0.02 mol dm^{-3} Tris/0.02 mol dm^{-3} KCl buffer.

It is widely known that enzyme catalyses are generally stereospecific. For example, the α-chymotrypsin-catalyzed hydrolysis of N-acetyl-D(L)-amino acid p-nitrophenyl esters demonstrates the interrelationship between substrate specificity and stereoselectivity.[1] Enzyme-model studies have been the subject of continued interest in such areas as the development of stereoselective reaction sites for the hydrolysis of enantiomeric esters and in aiding the understanding of the origins of stereoselectivity in the proteolytic enzymes.

Very recently, the authors have emphasized that the reaction field provided by bilayer systems is modified by the addition of cholesterol[2,3] and micelles[2,4] and by temperature-regulation[5] as well as by the amino acid sequence of peptide catalysts.[4] These modifications are very important for enhancing the enantioselective hydrolysis by using the long-chain enantiomeric substrates (D(L)-S_{12}).[6] It is also noteworthy that the enantioselectivity for the hydrolytic cleavage of D(L)-S_{12} by the tripeptide (Z-PheHisLeu) was in good harmony with the apparent mean hydrodynamic diameter (d_{hy}) of coaggregates which are composed of the double-chain surfactant ($2C_{14}$) and single-chain one, hexadecyltrimethylammonium bromide (CTAB); an enantiomer rate ratio ($k^{L}_{a,obsd}/k^{D}_{a,obsd}$) of 71 was observed in the coaggregate system composed of 67 mol% CTAB and 33 mol% $2C_{14}$.[7] Furthermore, it was concluded that the highly enantioselectivity-supportive coaggregates composed of 67 mol% CTAB and 33 mol% $2C_{14}$, which was close to phase boundaries between bilayer and micelles, was found to be rod-like by variable-angle dynamic light scattering (dls) and electron microscopy.[8] However, the time dependence of d_{hy} for the coaggregates indicated that the rod-like aggregates were stable in shape for about one week, although the amphiphile system composed of 41 mol% CTAB and 59 mol% $2C_{14}$, which

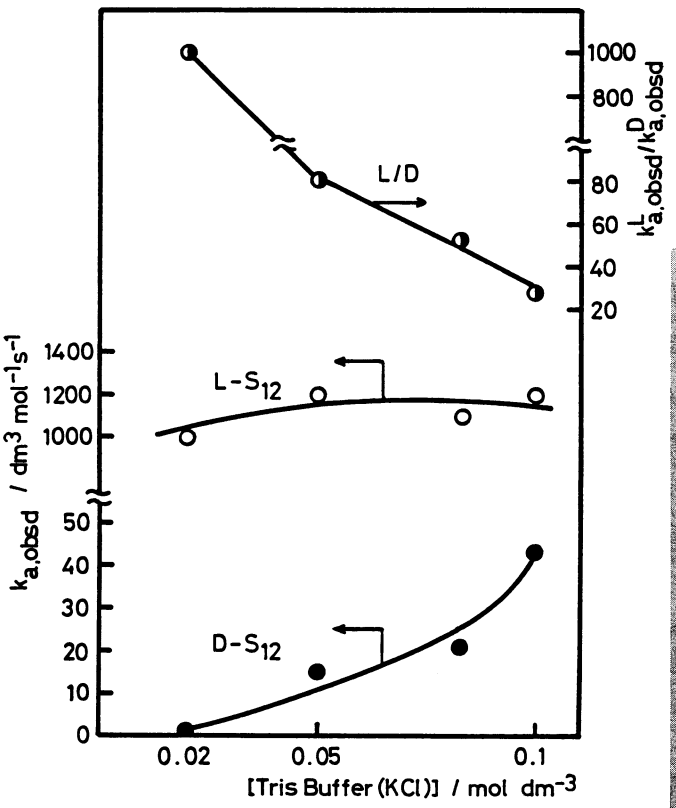
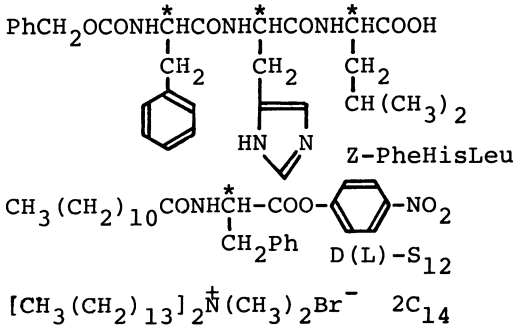


Fig. 1. Effects of buffer concentration on enantioselectivity ($k^{L}_{a,obsd}/k^{D}_{a,obsd}$) and rate constants ($k_{a,obsd}$) for the hydrolysis of the long-chain enantiomers (D(L)-S_{12}) by Z-PheHisLeu in the coaggregate system composed of 41 mol% CTAB and 59 mol% $2C_{14}$ under identical concentrations of Tris and KCl: [Z-PheHisLeu]=5x10^{-5} mol dm^{-3}, [D(L)-S_{12}]=1x10^{-5} mol dm^{-3}.



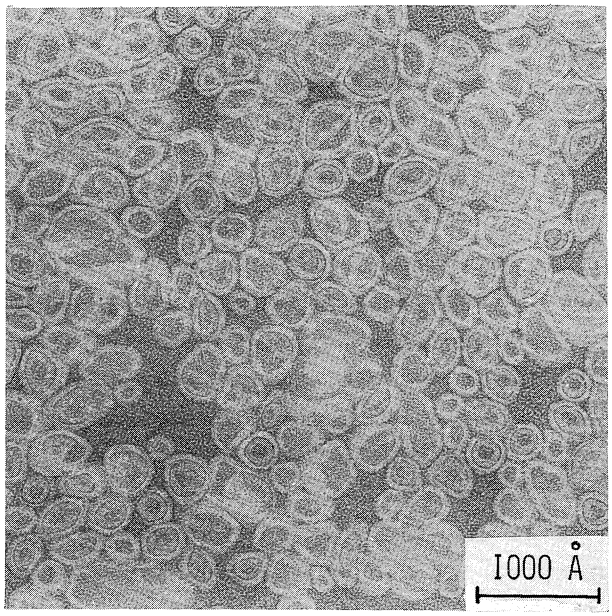


Fig. 2. Electron micrograph for the coaggregates composed of 41 mol% CTAB (0.7 mmol dm^{-3}) and 59 mol% $2C_{14}$ (1.0 mmol dm^{-3}); negatively stained with 0.5 wt% uranyl acetate; 0.02 mol dm^{-3} Tris (KCl) buffer solution sonicated with a Bransonic 12 apparatus at 80 W and 50 °C for 1 h.

showed a relatively high enantioselectivity ($k^{L}_{a,obsd}/k^{D}_{a,obsd}$ = 52), retained the aggregate morphology of spherical particle for over two weeks.

In this work, we investigated the stereoselective hydrolysis in the coaggregate system composed of 41 mol% CTAB and 59 mol% $2C_{14}$ by changing the concentration of the Tris-KCl buffer and temperature. First, we examined the hydrolytic cleavage of the long-chain enantiomers (D(L)-S_{12}) as catalyzed by Z-PheHisLeu in the coaggregate system composed of 0.7 mmol dm^{-3} CTAB (41 mol%) and 1.0 mmol dm^{-3} $2C_{14}$ (59 mol%) in the range of 0.02-0.10 mol dm^{-3} Tris (KCl) buffer at 25 °C and pH 7.6 in CH_3CN-H_2O (3:97 v/v). The stock solutions were prepared by dissolving both nucleophile and surfactant in Tris-KCl buffer upon sonication with a Bransonic sonicator (bath-type) at 80 W and 50 °C for 1 h.

As shown in Fig. 1, the apparent second-order rate constants ($k_{a,obsd}$)[9] for the hydrolytic cleavage of L-S_{12} were almost constant in the concentration range

of 0.02-0.10 mol dm^{-3} Tris (KCl) buffer, though the $k_{a,obsd}$ value for the D-S_{12} hydrolysis increased gradually as the Tris (KCl) concentration was raised. As a result, the enantioselectivity ($k^{L}_{a,obsd}/k^{D}_{a,obsd}$) increases drastically as the Tris (KCl) concentration is lowered and the dramatically high enantiomer rate ratio ($k^{L}_{a,obsd}/k^{D}_{a,obsd}$ = 1000) is attained in 0.02 mol dm^{-3} Tris-KCl buffer at 25 °C. With respect to the morphology of the coaggregates composed of 41 mol% CTAB and 59 mol% $2C_{14}$, the electron micrograph showed the presence of single- and double-walled vesicles in the diameter range of 200-600 Å (Fig. 2). The maximum diameter value (600 Å) by electron micrography is in agreement with the hydrodynamic diameter (d_{hy}) 640 ± 10 Å by dls. The aggregate morphology remained unchanged for at least two weeks. We also found that morphological stability of these co-aggregates was larger than that of single-walled vesicles composed of $2C_{14}$ alone by means of dls and electron microscopy. Thus, thermodynamic stability of the vesicle having large curvatures seems to be increased by mixing CTAB with $2C_{14}$ due to favorable molecular packing with two kinds of amphiphiles which are different from each other in packing geometry.

Second, we examined the temperature effect on the enantioselective hydrolysis of D(L)-S_{12} with Z-PheHisLeu in the vesicular system composed of CTAB (41 mol%) and $2C_{14}$ (59 mol%) in 0.02 mol dm^{-3} Tris/0.02 mol dm^{-3} KCl buffer as shown in Fig. 3. The rate constant ($k_{a,obsd}$) for the D-S_{12} hydrolysis decreased gradually as the temperature was raised and no catalysis ($k^{D}_{a,obsd}$ = 0) was observed up to 30 °C, although the L-S_{12} hydrolysis was enhanced sharply as the temperature was raised. Thus, we successfully established the enzyme-like enantioselective catalysis for the hydrolytic cleavage of the long-chain enantiomers (D(L)-S_{12}) as catalyzed by Z-PheHisLeu in the stable vesicular system composed of 41 mol% CTAB and 59 mol% $2C_{14}$ at 30 °C ($k^{L}_{a,obsd}$ = 1300 dm^3 mol^{-1} s^{-1} and $k^{D}_{a,obsd}$ = 0) and 35 °C ($k^{L}_{a,obsd}$ = 1800 dm^3 mol^{-1} s^{-1} and $k^{D}_{a,obsd}$ = 0) in 0.02 mol dm^{-3} Tris/0.02 mol dm^{-3} KCl buffer (pH 7.6). In the vesicular system composed of 41 mol% CTAB and 59 mol% $2C_{14}$, the fluorescence intensity of originated from 1-[(4-trimethylammonio)phenyl]-6-phenyl-1,3,5-hexatriene iodide (tma-DPH) placed in the pseudo-hydrophobic domain near the membrane surface,[10]

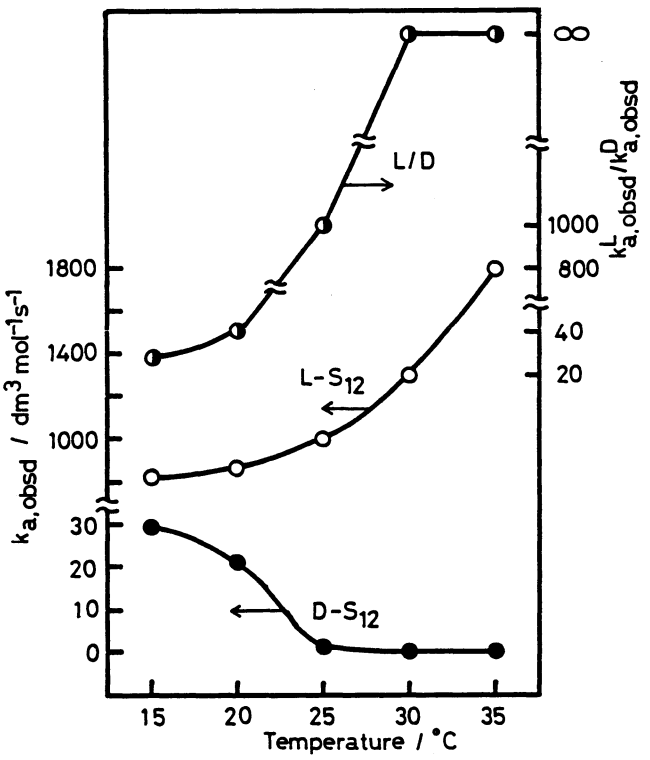


Fig. 3. Temperature dependence of enantioselectivity and rate constants for the hydrolysis of D(L)-S_{12} with Z-PheHisLeu in the system composed of 41 mol% CTAB (0.7 mmol dm^{-3}) and 59 mol% $2C_{14}$ (1.0 mmol dm^{-3}) at pH 7.6 in 0.02 mol dm^{-3} Tris (0.02 mol dm^{-3} KCl) buffer prepared with CH_3CN-H_2O (3:97 v/v).

where the hydrolysis presumably takes place, decreased to 60% of the original one as the buffer concentration was lowered from 0.08 to 0.02 mol dm^{-3} at 25 °C. Such a change in the microenvironmental property may come from an increase in repulsive interaction among the cationic head moieties of the amphiphiles as the ionic strength is decreased. On the other hand, the steady-state fluorescence anisotropies of tma-DPH and 1,6-diphenyl-1,3,5-hexatriene, a probe placed in the inner hydrophobic membrane domain,[11] were not influenced by the buffer concentration, but monotonously decreased as the temperature was raised in a range of 10-30 °C. In the light of the results shown in Figs. 1 and 3, it seems that the loosely oriented and hydrophobic reaction field, where matrix amphiphile molecules do not disturb the molecular recognition of the substrate by the catalyst, is in favor of exhibiting high enantioselectivity.

In conclusion, it is noteworthy that the high enantioselectivity ($k^{L}_{a,obsd}/k^{D}_{a,obsd}$ = 52) for the hydrolysis of the long-chain enantiomers (D(L)-S_{12}) with the efficient catalyst (Z-PheHisLeu) in the stable vesicular system composed of the vesicular surfactant ($2C_{14}$; 59 mol%) and the micellar one (CTAB; 41 mol%) in 0.08 mol dm^{-3} Tris/0.08 mol dm^{-3} KCl buffer was augmented to the enantiomer rate ratio ($k^{L}_{a,obsd}/k^{D}_{a,obsd}$) = 1000 by lowering the buffer concentration (0.02 mol dm^{-3} Tris/0.02 mol dm^{-3} KCl buffer) at room temperature (25 °C). Moreover, with regard to the same catalytic system in 0.02 mol dm^{-3} Tris/0.02 mol dm^{-3} KCl, the enzyme-like enantioselectivity ($k^{L}_{a,obsd}$ = 1300 dm^3 mol^{-1} s^{-1} and $k^{D}_{a,obsd}$ = 0 at 30 °C, $k^{L}_{a,obsd}$ = 1800 dm^3 mol^{-1} s^{-1} and $k^{D}_{a,obsd}$ = 0 at 35 °C) was attained along with elevation of temperature.

References

1) D. W. Ingles and J. R. Knowles, Biochem. J., 104, 369(1967).
2) R. Ueoka, Y. Matsumoto, T. Nagamatsu, and S. Hirohata, Tetrahedron Lett., 1984, 1363.
3) R. Ueoka and Y. Matsumoto, J. Org. Chem., 49, 3774(1984).
4) R. Ueoka, Y. Matsumoto, and Y. Ihara, Chem. Lett., 1984, 1807.
5) R. Ueoka, Y. Matsumoto, T. Nagamatsu, and S. Hirohata, Chem. Lett., 1984, 583.
6) R. Ueoka, Y. Matsumoto, Y. Ninomiya, Y. Nakagawa, K. Inoue, and K. Ohkubo, Chem. Lett., 1981, 785.
7) R. Ueoka, R. A. Moss, S. Swarup, Y. Matsumoto, G. Strauss, and Y. Murakami, J. Am. Chem. Soc., 107, 2185(1985).
8) R. Ueoka, Y. Matsumoto, R. A. Moss, S. Swarup, J. Kikuchi, and Y. Murakami, J. Am. Chem. Soc., to be submitted.
9) The $k_{a,obsd}$ values were evaluated from $(k_t - k_s)/[\text{nucleophile}]_0$, where k_t and k_s denote the first-order rate constants with and without nucleophile, respectively.
10) F. G. Prendergast, R. P. Haugland, and P. J. Callahan, Biochemistry, 20, 7333 (1981).
11) Y. Murakami, J. Kikuchi, T. Takaki, K. Uchitomi, and A. Nakano, J. Am. Chem. Soc., 107, 2161(1985); Y. Murakami, J. Kikuchi, and K. Akiyoshi, Chem. Lett., 1984, 1185.

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