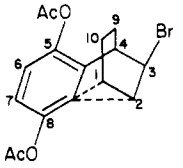
sterically least favored side of the π-system. Most reasonably the driving force for this mode of addition is supplied by the formation of an aryl-bridged intermediate **10**. The question of whether the transition state leading



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to this intermediate involves homoconjugation with the aryl π-system is a moot point. Attack of bromide on **10** at position 2 would lead to the formation of an energetically unfavorable cis vicinal dibromide. Consequently, the only product of the reaction is formed by attack at position 1. This interpretation is consitent with the observations of Tanida et al.[8] who reported the acetolysis of the benzobicyclo[2.2.2]octen-*anti*-2-yl brosylate to give a mixture of 83% unrearranged *anti*-2-acetate and 17% of benzo[6.7]bicyclo[3.2.1]octen-*anti*-2-yl acetate which they suggested was formed from a aryl-bridged intermediate.

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Registry No. 1, 63350-92-5; 9, 91632-19-8.

(8) Tanida, H.; Tori, K.; Kitahonoki, K. *J. Am. Chem. Soc.* **1967**, *89*, 3212.

Effect of Cholesterol on the Stereoselective Hydrolysis in Artificial Membrane Systems

Ryuichi Ueoka* and Yōko Matsumoto

Department of Industrial Chemistry, Kumamoto Institute of Technology, Kumamoto 860, Japan

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The catalytic efficiency and enantioselectivity for the hydrolysis of *p*-nitrophenyl *N*-dodecanoyl-D(L)-phenylalaninate (**2b**) in the catalytic system of *N*-tetradecanoyl-L-histidyl-L-leucine (**1b**) and didodecyldimethylammonium bromide (**3a**) were enhanced by addition of cholesterol at an optimum temperature (25 °C). Furthermore, it is suggested that the hydrophobic interaction between reactants in the bilayer membrane system (**3a**) might be reduced by adding cholesterol on the basis of the isokinetic temperature. It is concluded from these results that the fluidity of the artificial bilayer matrix (**3a**) varies upon addition of cholesterol.

It is widely known that enzyme catalyses are usually 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[2-5] 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 understanding the origins of stereoselectivity in the above-mentioned proteolytic enzymes.[1]

The stereoselective hydrolysis of N-protected amino acid *p*-nitrophenyl esters catalyzed by *N*-acyl-L-histidines has recently attracted considerable attention.[2] Relatively high stereoselectivity has been attained in the hydrolysis of diastereomeric dipeptide substrates with a thio functionalized surfactant,[3] in the hydrolysis of *N*-acyl amino acid esters with dipeptide L-histidine derivatives in the presence of cationic surfactants,[4] or in that with L-histidine derivatives in the presence of cationic surfactants,[4] or in that with L-histidine derivatives in bilayer systems.[5] On the other hand, the stereoselectivity of proteolytic metalloenzymes has been modeled by micellar systems.[6] Furthermore, we attempted to examine the kinetic origin of high enantioselectivity by measuring substrate-binding properties, activation parameters, and kinetic salt and organic cosolvent effects in the comicellar systems and emphasized the importance of hydrophobicity of the enantiomer substrate for the elevation of enantioselectivity.[7] However, there has been no report of the effect of fluidity of the reaction field on stereoselective hydrolysis in molecular assembly systems.

The purpose of this paper is to explore the cholesterol effect on the enantioselective hydrolytic cleavage (hydrolysis) of *p*-nitrophenyl *N*-dodecanoyl-D(L)-phenylalaninate (**2b**), which bears a long hydrophobic acyl chain, by the bilayer and micellar catalytic systems of L-histidine

(1) Ingles, D. W.; Knowles, J. R. *Biochem. J.* **1967**, *104*, 369.
(2) (a) Brown, J. M.; Bunton, C. A. *J. Chem. Soc., Chem. Commun.* **1974**, 969. (b) Ihara, Y. *J. Chem. Soc., Chem. Commun.* **1978**, 984. (c) Yamada, K.; Shosenji, H.; Ihara, H. *Chem. Lett.* **1979**, 491. (d) Ueoka, R.; Terao, T.; Ohkubo, K. *Nippon Kagaku Kaishi* **1980**, 462.
(3) (a) Moss, R. A.; Lee, Y.-S.; Lukas, T. J. *J. Am. Chem. Soc.* **1979**, *101*, 2499. (b) Moss, R. A.; Lee, Y.-S.; Alwis, K. W. *J. Am. Chem. Soc.* **1980**, *102*, 6646.
(4) (a) Ohkubo, K.; Sugahara, K.; Yoshinaga, K.; Ueoka, R. *J. Chem. Soc., Chem. Commun.* **1980**, 637. (b) Ihara, Y.; Kunikiyo, N.; Kunimasa, T.; Nango, M.; Kuroki, N. *Chem. Lett.* **1981**, 667.
(5) (a) Murakami, Y.; Nakano, A.; Yoshimatsu, A.; Fukuya, K. *J. Am. Chem. Soc.* **1981**, *103*, 728. (b) Ueoka, R.; Matsumoto, Y.; Ninomiya, Y.; Nakagawa, Y.; Inoue, K.; Ohkubo, K. *Chem. Lett.* **1981**, 785. (c) Ohkubo, K.; Matsumoto, N.; Ohta, H. *J. Chem. Soc., Chem. Commun.* **1982**, 738. (d) Matsumoto, Y.; Ueoka, R. *Nippon Kagaku Kaishi* **1983**, 901.
(6) Ogino, K.; Tomita, I.; Machiya, K.; Tagaki, W. *Chem. Lett.* **1982**, 1875.
(7) Ueoka, R.; Murakami, Y. *J. Chem. Soc., Perkin Trans. 2* **1983**, 219.

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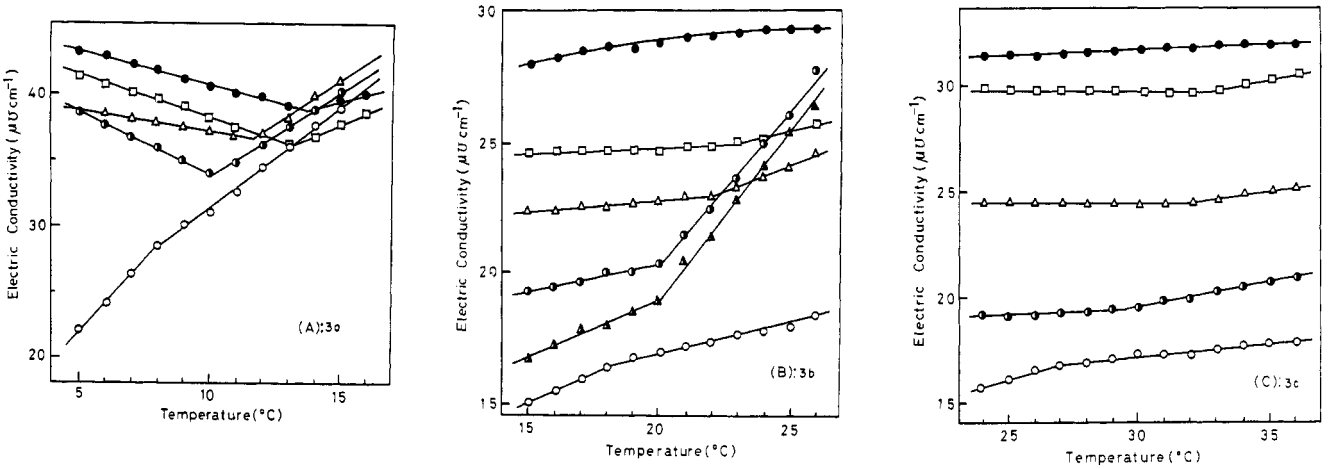
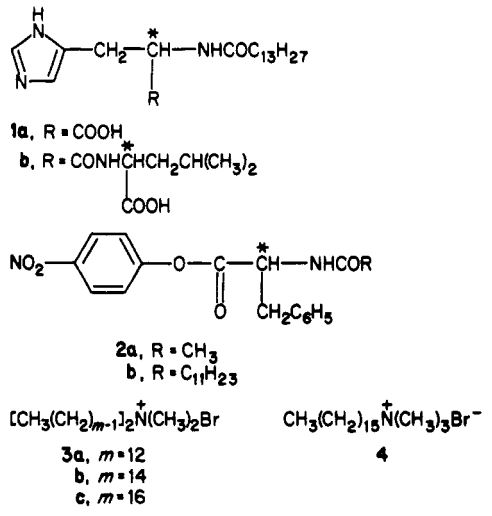


Figure 1. Parts A–C. Conductivity of aqueous **3** (1×10^{-3} M) membrane as a function of temperature. Concentration of cholesterol: (○) 0, (▲) 1.0 mol %, (◑) 4.8 mol %, (△) 9.1 mol %, (□) 16.7 mol %, (●) 20.0 mol %.

derivatives with didodecyldimethylammonium bromide (**3a**) and hexadecyltrimethylammonium bromide (**4**). Especially the connection between enantioselectivity and the fluidity of the artificial membrane matrix is emphasized.



Results and Discussion

Phase Transition Temperature (T_c). It is well-known that the crystalline gel–liquid crystalline-phase transition of various dialkylammonium bilayers takes place around room temperature.[8] The phase transition temperature (T_c) of aqueous dialkylammonium bilayers (1 wt %, ca. 10 mM) was estimated from the endothermic peak in the differential scanning calorimetry trace and the T_c values obtained are as follows: didodecyldimethylammonium bromide (**3a**); 5–10 °C,[9] ditetradecyldimethylammonium bromide (**3b**); 16 °C,[9] dihexadecyldimethylammonium bromide (**3c**); 28 °C.[9]

In the present paper we have attempted to estimate T_c of aqueous **3a–c** (ca. 1 mM) by the conductivity method. A sharp discontinuity in the conductivity changes of the bilayer surfactants upon elevation of temperature was observed as shown in Figure 1. The concentration of cholesterol is expressed as mol % of total lipid. The temperature dependence of the conductivity is represented by two patterns for all the bilayer surfactants employed

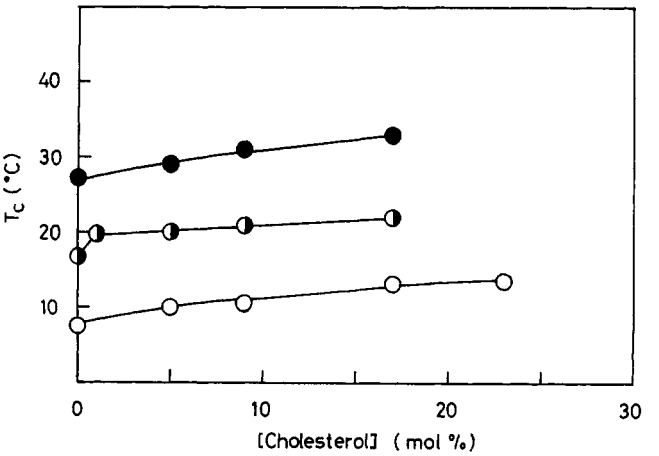


Figure 2. Phase transition temperature (T_c) of (○) **3a**, (◑) **3b**, and (●) **3c** as a function of cholesterol concentration (mol % of total lipid).

in this study: one is the maximum-type inflection point in the conductivity with temperature in the absence of cholesterol and the other is the minimum-type inflection point with temperature in the presence of cholesterol. The inflection point appears to be related to T_c of the bilayer matrix. The T_c values of the dialkylammonium surfactants without cholesterol estimated by the conductivity method (8, 18, and 27 °C for **3a**, **3b**, and **3c**, respectively) are in good harmony with those determined by the differential scanning calorimetry.[9] Therefore, it can be considered that, in addition to the DSC method, the conductivity method is available for estimating the T_c of aqueous dialkylammonium bilayers. Figure 2 shows the T_c values as a function of cholesterol concentration for **3a**, **3b**, and **3c**, determined by using the conductivity method. Interestingly, cholesterol gradually elevated the T_c value as its concentration increased in the range of 1.0–16.7 mol % for all the three bilayer surfactants, though the addition of cholesterol to phospholipid[10,11] and glycolipid[11] membranes in water lowered or abolished their T_c values. With respect to the cholesterol effect on the T_c value, it is noteworthy that no transition was observed at the high cholesterol concentration of 20.0 mol % for **3b** and **3c**, as shown in Figure 1 Parts B and C, respectively, and was confirmed by using the DSC method. This behavior of cholesterol is similar to its effect on the endothermic transition of

(8) (a) Okahata, Y.; Ando, R.; Kunitake, T. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 3647. (b) Kunitake, T.; Sakamoto, T. *Chem. Lett.* **1979**, 1059.
(9) Okahata, Y.; Tanamachi, S.; Kunitake, T. *Nippon Kagaku Kaishi* **1980**, 442.
(10) Ladbrooke, B. D.; Williams, R. M.; Chapman, D. *Biochim. Biophys. Acta* **1968**, *150*, 333.
(11) Oldfield, E.; Chapman, D. *FEBS Lett.* **1972**, *21*, 302.

Table I. Dependence of Rate Constant ($k_{a,obsd}/M^{-1}$ s^{-1}) and Enantioselectivity ($k^L_{a,obsd}/k^D_{a,obsd}$) on the Concentration of Cholesterol for the Hydrolysis of 2b by 1a plus 3a[a]

[cholesterol], mol %	substrate	15 °C		20 °C		25 °C		30 °C		35 °C	
0	L-**2b**	570	(3.6)	810	(3.2)	1500	(3.9)	1900	(3.1)	2500	(3.1)
	D-**2b**	160		250		380		620		800	
2.0	L-**2b**			920	(3.7)			2300	(3.4)	2600	(3.6)
	D-**2b**			250				670		730	
7.4	L-**2b**	930	(4.4)	1400	(4.4)	1600	(4.1)	2300	(4.1)	2500	(4.0)
	D-**2b**	210		320		390		560		630	
13.0	L-**2b**	1100	(5.5)	1500	(5.0)	2100	(5.1)	2300	(3.7)	2700	(3.5)
	D-**2b**	200		300		410		630		770	
18.0	L-**2b**	1000	(5.0)	1500	(4.7)	1700	(4.1)	2200	(3.5)	2800	(3.4)
	D-**2b**	200		320		410		630		830	

[a] Values in the parentheses are enantioselective parameters which include the experimental error within ±0.2, because the $k_{a,obsd}$ value has the maximum error of ±3.5%. Conditions: pH 7.6, 0.083 M Tris buffer (0.083 M KCl), 3% (v/v) CH_3CN–H_2O, [**1a**] = 5×10^{-5} M, [**2b**] = 1×10^{-5} M, [**3a**] = 1×10^{-3} M.

1,2-dihexadecanoyl-L-lecithin, which gradually disappeared as the concentration of cholesterol increased above 20 mol %.[10] By using calorimetric and X-ray techniques, Chapman et al. concluded that the cholesterol controls the fluidity of the hydrocarbon chains of the phospholipid by disruption of the crystalline chain lattice of the gel phase. On the other hand, the morphological change of the artificial membranes resulting from cholesterol addition was observed.[12] Although it cannot be definitely concluded, it is assumed that the cholesterol, when present in high concentration, would control the fluidity of the hydrocarbon chains of dialkylammonium bilayers (**3**) in this experiment.

Temperature Dependence of Catalytic Efficiency and Stereoselectivity. It is obvious that the hydrophobicity of nucleophiles and substrates should be important to enhance the catalytic efficiency and stereoselectivity in micellar[2d] and bilayer[5b] systems. Therefore, we have employed reactants bearing a hydrophobic acyl chain.

The temperature dependence of second-order rate constant ($k_{a,obsd}$) and enantioselectivity (reflected in $k^L_{a,obsd}/k^D_{a,obsd}$) for the hydrolysis of *p*-nitrophenyl *N*-acyl-D(L)-phenylalaninate (D- and L-**2**) catalyzed by *N*-tetradecanoyl-L-histidine (**1a**) with **3a** in the concentration range of 0–18.0 mol % cholesterol was examined at temperatures above T_c of the dialkylammonium surfactant (**3a**) as described in Table I. The rate constants for L and D isomer substrates increase almost linearly with the elevation of temperature, though their magnitudes are widely different. On the other hand, some interesting temperature dependences of enantioselectivity were observed as follows: (a) The temperature dependence of enantioselectivity is bell shaped with a maximum at 25 °C in the absence of cholesterol. (b) An enantioselectivity parameter ($k^L_{a,obsd}/k^D_{a,obsd}$) remains almost constant over the temperature range of 20–35 °C in the lower cholesterol concentration range from 2.0 mol % to 7.4 mol %. (c) The $k^L_{a,obsd}/k^D_{a,obsd}$ values decrease gradually as temperature is raised over the experimental temperature range in the higher cholesterol concentration range from 13.0 mol % to 18.0 mol %. Here, it is noteworthy that the temperature dependence of enantioselectivity in the system of the dipeptide nucleophile (*N*-tetradecanoyl-L-histidyl-L-leucine, **1b**) and **3a** was fairly bell shaped with a maximum ($k^L_{a,obsd}/k^D_{a,obsd}$ = 5.2–5.4) at 15–25 °C.[13] A similar tendency with a maximum (3.9) at 25 °C was observed in the system of **1a** plus **3a** as mentioned above. This difference of magnitude in the maximum enantioselectivity between **1b** plus **3a** and **1a** plus **3a** suggests that the leucine residue in the dipeptide (**1b**) has an important role in enhancement of the enantioselectivity at an appropriate temperature (15–25 °C).

Cholesterol Effect on the Catalytic Efficiency and Stereoselectivity. It is known that the cholesterol controls the fluidity of the native membrane matrix.[10,11,14] This behavior of cholesterol in biological membrane might be closely related to the catalytic activity of the membrane protein. Therefore, we have examined the cholesterol effect on the catalytic efficiency and stereoselectivity for the hydrolysis of the amino acid esters (L(D)-**2**) catalyzed by the enzyme models (**1a** and **1b**) in artificial membrane systems (**3a**). Table I shows the temperature effect along with the cholesterol effect on the rate constant and enantioselectivity for the hydrolysis of **2b** by **1a** plus **3a** in the temperature range of 15–35 °C. The catalytic efficiency of **1a** for the hydrolysis of L-**2b** has a tendency to increase with increasing cholesterol concentration in the range of 0–13.0 mol % over the temperature range of this study. On the other hand, the cholesterol effect on the catalytic efficiency of **1a** for the D-**2b** hydrolysis was divided roughly into the following two trends: (a) the rate constant increases with increasing cholesterol concentration from 0% to 7.4 mol % and is constant above 7.4 mol % of cholesterol at 15–25 °C, (b) the minimum $k_{a,obsd}$ value is at 7.4 mol % cholesterol at a temperature above 30 °C. Interestingly, the above-mentioned different trends of the cholesterol effect on the catalytic efficiency for the D-**2b** hydrolysis resulted in different maximum points of enantioselectivity in different temperature ranges; i.e., the highest enantioselectivity, when the cholesterol concentration is 13.0 mol %, occurs over the temperature range 15–25 °C ($k^L_{a,obsd}/k^D_{a,obsd}$ = 5.5–5.1) and, when the cholesterol concentration is 7.4 mol %, occurs at 30–35 °C (4.1–4.0), though the highest $k^L_{a,obsd}/k^D_{a,obsd}$ value decreases from 5.5 to 4.0 with elevation of temperature.

The cholesterol effect on the rate constant and enantioselectivity for the hydrolysis of **2b** by **1b** plus **3a** at 25 °C is shown in Figure 3. The experimental temperature (25 °C) is that of optimum for the elevation of enantioselectivity in the system of **1b** plus **3a** but without cholesterol.[13] The stereoselectivity parameter increases linearly with increasing concentration of cholesterol in the range of 0–18.0 mol %. The highest enantiomer rate ratio ($k^L_{a,obsd}/k^D_{a,obsd}$ = 7.5) in 18.0 mol % cholesterol is 1.4-fold higher than the ratio (5.2) in the absence of cholesterol. We have not examined the cholesterol effect above 18.0 mol % because of the turbidity of the reaction solution.

(12) (a) Okahata, Y.; Kunitake, T. *J. Am. Chem. Soc.* **1979**, *101*, 5231. (b) Okahata, Y.; Kunitake, T. *Ber. Bunsenges. Phys. Chem.* **1980**, *84*, 550.
(13) Matsumoto, Y.; Ueoka, R. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 3370.
(14) Papahadjopoulos, D.; Jacobson, K.; Nir, S.; Isac, T. *Biochim. Biophys. Acta* **1973**, *311*, 330.

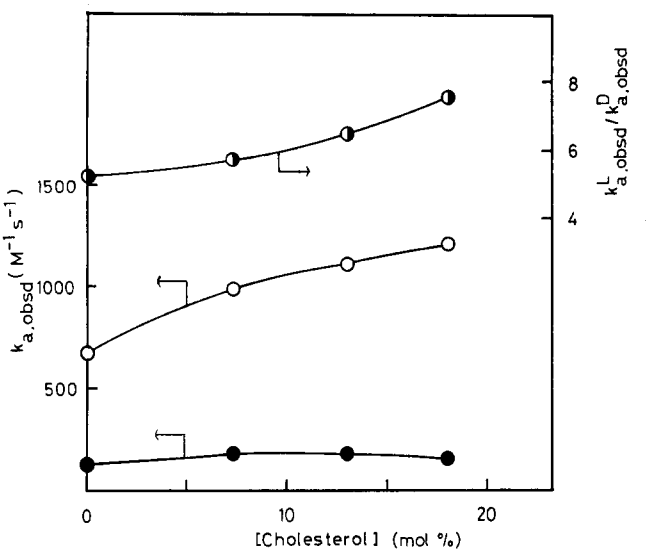


Figure 3. Cholesterol effect on the rate constant and enantioselectivity in the hydrolysis of (○) L-**2b** and (●) D-**2b** catalyzed by **1b** with **3a** at 25 °C and pH 7.6 (0.083 M Tris-KCl buffer) in 3% (v/v) CH_3CN–H_2O. [**1b**] = 3×10^{-5} M, [**2b**] = 1×10^{-5} M, [**3a**] = 1×10^{-3} M.

Table II. Temperature Dependence of Rate Constant ($k_{a,obsd}/M^{-1}\ s^{-1}$) and Enantioselectivity ($k^L_{a,obsd}/k^D_{a,obsd}$) for the Hydrolysis of 2 by 1a plus 4 with and without Cholesterol[a]

substrate	15 °C		25 °C		35 °C	40 °C
L-**2a**	81	(62)	140	(130)	140	(300)
D-**2a**	33	(23)	79	(70)	99	(210)
L-**2a**/D-**2a**[b]	2.5	(2.7)	1.8	(1.9)	1.4	(1.4)
L-**2b**	450	(450)	750	(740)	810	(1800)
D-**2b**	84	(81)	170	(190)	280	(500)
L-**2b**/D-**2b**[b]	5.4	(5.6)	4.4	(3.9)	2.9	(3.6)

[a] Values in the parentheses are those for the hydrolysis of **2** by **1a** plus **4** without cholesterol. Conditions: pH 7.6, 0.083 M Tris buffer (0.083 M KCl), 3% (v/v) CH_3CN–H_2O, [**1a**] = 5×10^{-5} M, [**2**] = 1×10^{-5} M, [**4**] = 3×10^{-3} M, [cholesterol] = 13.0 mol %. [b] These values are enantioselective parameters which include the experimental error within ±5%.

Although the kinetic effect of cholesterol has already been discussed in the nucleophile cleavage of phosphate triesters in dialkylammonium bilayer membranes,[15] the above-mentioned kinetic results on the enantioselective hydrolysis would be related to changes of fluidity of the artificial membrane matrix resulting from addition of cholesterol.

On the other hand, the rate constant and enantioselectivity for the hydrolysis of **2** in the micellar catalytic system of **1a** plus **4** with and without cholesterol are summarized in Table II. The lack of any effect of cholesterol on the stereoselective hydrolysis suggests that the micellar matrix does vary upon addition of cholesterol.

Isokinetic Temperature (β). The enantioselective hydrolysis of **2** in the presence of cholesterol was carried out at pH 7.6 in the temperature range of 15–35 °C in order to obtain activation parameters as described in Table I; $\Delta G^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta H^{\ddagger}$ were calculated according to eq 1.

$$\Delta G^{\ddagger} = 2.303RT \log (kT/hk_{a,obsd}) = \Delta H^{\ddagger} - T\Delta S^{\ddagger} \quad (1)$$

Here, R is the gas constant, T is the absolute temperature, and k and h stand for Boltzmann's and Plank's constants, respectively.

(15) Okahata, Y. Ihara, H.; Kunitake, T. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 2072.

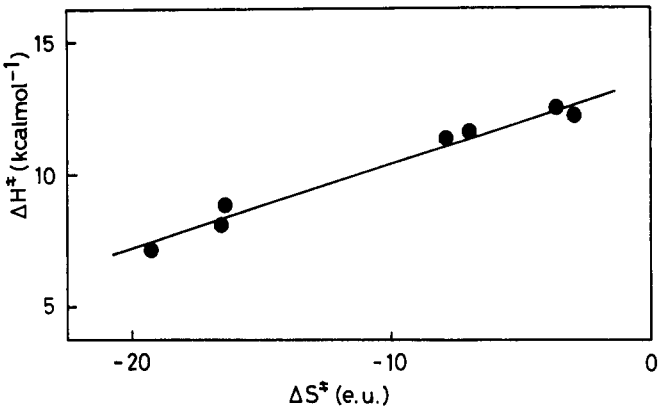


Figure 4. Isokinetic relationship for the hydrolysis of **2b** by **1a** plus **3a** with cholesterol at pH 7.6 (0.083 M Tris–KCl buffer) in 3% (v/v) CH_3CN–H_2O. [**1a**] = 5×10^{-5} M, [**2b**] = 1×10^{-5} M, [**3a**] = 1×10^{-3} M, [cholesterol] = 2.0 – 18.0 mol %.

Table III. Isokinetic Temperature (β) for the Hydrolysis of 2 by 1a in the Bilayer and Micellar Systems with and without Cholesterol[a]

system	cholesterol	β, K[b]	$\bar{T}$, K	r[c]
bilayer	without	269 ± 8	296	0.99
(**3a**)	with	332 ± 8	298	0.98
micellar	without	363 ± 14	300	0.99
(**4**)	with	325 ± 12	298	0.99

[a] Conditions: pH 7.6, 0.083 M Tris buffer (0.083 M KCl), 3% (v/v) CH_3CN–H_2O, [**1a**] = 5×10^{-5} M, [**2**] = 1×10^{-5} M, [**3a**] = 1×10^{-3} M, [**4**] = 3×10^{-3} M, [cholesterol] = 2.0–18.0 mol %. [b] The error limits were obtained by the standard error treatment. [c] Coefficient of correlation.

Isokinetic relationships appear to hold for the hydrolysis of **2** catalyzed by **1a** in the bilayer (**3a**) and micellar (**4**) systems. For example, Figure 4 shows the linear relationship between $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ in the catalytic system of **1a** and **3a** with cholesterol. The isokinetic temperature (β) was evaluated on the basis of eq 2.[16]

$$\Delta H^{\ddagger} = \Delta H^{\ddagger}_0 + \beta\Delta S^{\ddagger} \quad (2)$$

where $\Delta H^{\ddagger}_0$ is simply the intercept of $\Delta H^{\ddagger}$ corresponding to $\Delta S^{\ddagger} = 0$. The β values obtained are summarized in Table III.

Recently, the authors have attempted to classify into three catalytic systems (micellar, bilayer, or macromolecular system) on the basis of the β value and average the value of the experimental temperature ($\bar{T}$) for the hydrolysis of phenyl esters, which have a various hydrophobic acyl chain, catalyzed by the long-chain L-histidine derivatives and hydroxamic acids, and telomeric hydroxamic acids.[13,17] The correlations between β and $\bar{T}$ were as follows: (a) $\beta > \bar{T}$ for the micellar system. (b) $\beta < \bar{T}$ for the bilayer system. (c) $\beta >> \bar{T}$ for the macromolecular system.

It is known that hydrophobic interactions are mainly entropy driven while lyophobic ones are mainly enthalpy driven.[18,19] On the basis of the β value in connection with $\bar{T}$, it is acceptable that the bilayer (liquid crystalline)[20]

(16) Leffler, J. E. *J. Org. Chem.* **1955**, *20*, 1202.
(17) Ueoka, R.; Matsumoto, Y.; Furuya, Y.; Shiraishi, M. *Nippon Kagaku Kaishi* **1983**, 1412.
(18) Némethy, G. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 195; *Ann. N.Y. Acad. Sci.* **1969**, *155*, 492.
(19) Overberger, C. G.; Glowaky, R. C.; Vandewyer, P.-H. *J. Am. Chem. Soc.* **1973**, *95*, 6008.
(20) The reactions in the bilayer catalytic system were carried out above the phase transition temperature for the **3a** aqueous solution (see ref 12(b)).

catalytic system of **1a** and **3a** tends to be governed by the entropy of activation, that is, $\bar{T}$ (296 K) exceeded β (269 K), though the comicellar catalytic system of **1a** and **4** tends to be governed by the enthalpy of activation ($\beta - \bar{T}$ = 63 K). These results suggest that the stereoselective hydrolysis in the liquid-crystalline bilayer system would proceed through a stronger hydrophobic interaction between reactants.[21]

Interesting to note, however, is that the β value in the bilayer system of **1a** and **3a** is elevated markedly by addition of cholesterol. The enhancement of catalytic efficiency and stereoselectivity, which is shown in Table I and Figure 3, would be attributed to the change of membrane fluidity resulting from addition of cholesterol, because the catalytic system of **1a** plus **3a** with cholesterol is changed to be enthalpy driven ($\beta - \bar{T}$ = 34 K). On the other hand, the comicellar catalytic system of **1a** and **4** is enthalpy driven in both the absence and presence of cholesterol, though the β value in slightly reduced by addition of cholesterol. No cholesterol effect on the catalytic efficiency and stereoselectivity in the comicellar system would be related to the above-mentioned extrathermodynamic result.

Conclusion

A summary of the unique results in this study is as follows: (a) Cholesterol elevated gradually the T_c value of the artificial bilayer matrix (**3a–c**) as its concentration increased and no transition was observed at high cholesterol concentrations for **3b** and **3c**. (b) The catalytic efficiency and stereoselectivity in the bilayer catalytic systems are enhanced by addition of cholesterol. (c) The hydrophobic interaction between the enantiomer substrates (**2b**) and the mixed nucleophile (**1a** plus **3a**) may be reduced by addition of cholesterol on the basis of the iskinetic temperature value (β). These results suggest that the fluidity of the artificial bilayer matrix (**3**) varies upon addition of cholesterol. Especially, the cholesterol effect is marked for the enhancement of the enantioselectivity in the hydrolysis of **2b** by **1b** plus **3a** and the highest $k^{L}_{a,obsd}/k^{D}_{a,obsd}$ value (7.5) is attained in the presence of 18.0 mol % cholesterol.

Experimental Section

Materials. *p*-Nitrophenyl *N*-Acetyl-D(L)-phenylalaninate (D(L)-2a) and *p*-Nitrophenyl *N*-Dodecanoyl-D(L)-phenylalaninate (D(L)-2b). The enantiomer substrates (D(L)-**2a-b**) were prepared from *N*-benzyloxycarbonyl-D(L)-phenylalaninate by the esterification of the COOH group using *p*-nitrophenol and dicyclohexylcarbodiimide,[22] followed by hydrobromination of the NH_2 group,[23] and then acylation of the NH_2·HBr group using acetic anhydride or dodecanoic anhydride.[22] Satisfactory results of elemental analyses and specific rotations were obtained for D(L)-**2a** and D(L)-**2b**. D-**2a**: mp 133.5–134.0 °C (lit.[23] 135–137 °C); $[\alpha]^{25}_{D}$ +18.15° (*c* 2, $CHCl_3$) (lit.[23] $[\alpha]^{20}_{D}$ +17.4° (*c* 2, $CHCl_3$)). Anal. Calcd for $C_{17}H_{16}N_2O_5$: C, 62.19; H, 4.91; N, 8.55. Found: C, 62.08; H, 4.83; N, 8.49. L-**2a**: mp 137.5–138 °C (lit.[23] 140.0–140.5 °C); $[\alpha]^{25}_{D}$ −17.57° (*c* 2, $CHCl_3$) (lit.[23] $[\alpha]^{20}_{D}$ −18.6° (*c* 2, $CHCl_3$)). Anal. Found: C, 62.08; H, 4.80; N, 8.48. D-**2b**: mp 107.5–108.5 °C; $[\alpha]^{25}_{D}$ +11.91° (*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, 69.17; H, 7.76; N, 5.89. L-**2b**: mp 107.0–107.5 °C; $[\alpha]^{25}_{D}$ −11.44° (*c* 2, $CHCl_3$). Anal. Found: C, 69.15; H, 7.76; N, 5.91.

***N*-Tetradecanoyl-L-histidine (1a) and *N*-Tetradecanoyl-L-histidyl-L-leucine (1b).** The compounds **1a–b** were prepared from the acylation of L-histidine (Wako Chemicals) and L-histidyl-L-leucine (Protein Research Foundation) using tetradecanoyl chloride in a way similar to the previous method.[24] Satisfactory elemental analyses were obtained. **1a**: mp 196 °C dec (lit.[24] mp 192 °C dec). Anal. Calcd for $C_{20}H_{35}N_3O_3$: C, 65.75; H, 9.59; N, 11.51. Found: C, 65.87; H, 9.61; N, 11.30. **1b**: mp 212–214 °C. Anal. Calcd for $C_{26}H_{46}N_4O_4$: C, 65.24; H, 9.69; N, 11.71. Found: C, 65.03; H, 9.63; N, 11.60.

Didodecyldimethylammonium Bromide (3a), Ditetradecyldimethylammonium Bromide (3b), and Dihexadecyldimethylammonium Bromide (3c). The surfactants of **3a–c** were prepared by reaction of *N,N*-dimethylalkylamine and the corresponding alkyl bromide in refluxing ethanol in the presence of sodium carbonate and purified by recrystallizations from ethyl acetate as described previously.[8a] Satisfactory elemental analyses were obtained for **3**. **3a**: Anal. Calcd for $C_{26}H_{56}NBr$: C, 67.50; H, 12.20; N, 3.03. Found: C, 67.77; H, 12.20; N, 2.98. **3b**: Anal. Calcd for $C_{30}H_{64}NBr$: C, 69.44; H, 12.46; N, 2.70. Found: C, 69.18; H, 12.52; N, 2.68. **3c**: Anal. Calcd for $C_{34}H_{72}NBr$: C, 71.02; H, 12.65; N, 2.44. Found: C, 70.46; H, 12.49; N, 2.52.

Cholesterol was obtained from Wako Chemicals and used without further purification.

Kinetic Measurements. Rates of *p*-nitrophenol liberation from *p*-nitrophenyl esters were measured at 400 nm with a Shimadzu UV-200 spectrophotometer. Each run was initiated by adding an acetonitrile solution (0.10 mL) of a substrate ester to a reaction medium of tris(hydroxymethyl)aminomethane (Tris) buffer (3.40 mL) containing the nucleophile and surfactant. 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 eq 3 on the basis of triplicate runs.

$$k_{a,obsd} = (k_t - k_s)/[\text{nucleophile}]_0 \quad (3)$$

where k_t and k_s refer, respectively, to the observed first-order rate constants for the hydrolytic cleavage (hydrolysis) of D(L)-**2a–b** with and without a nucleophile, and $[\text{nucleophile}]_0$ indicates the initial nucleophile concentration.

The clear stock solutions were prepared by dissolving both nucleophile and surfactant in Tris–KCl buffer sonication (Bransonic 12, Yamato Scientific Co.) at 50 °C for 1 h and presented for the kinetic measurements.

Conductivity Measurements. The solution conductivity of the bilayer surfactants was measured using a Kyoto Electronics conductivity meter (CM-05) with a temperature compensative compartment.

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Registry No. **1a**, 16804-63-0; **1b**, 89092-59-1; D-**2a**, 14009-95-1; L-**2a**, 14009-94-0; D-**2b**, 75531-12-3; L-**2b**, 75531-11-2; **3a**, 3282-73-3; **3b**, 68105-02-2; **3c**, 70755-47-4; **4**, 57-09-0; L-histidine, 71-00-1; L-histidyl-L-leucine, 7763-65-7.

(21) Ueoka, R.; Matsumoto, Y.; Nagamatsu, T.; Hirohata, S. *Tetrahedron Lett.* **1984**, *25*, 1363.
(22) Bodanszky, M.; Vigneaud, V. du *J. Am. Chem. Soc.* **1959**, *81*, 6072.
(23) Ingles, D. W.; Knowles, J. R. *Biochem. J.* **1967**, *104*, 369.
(24) Gitler, C.; Ochoa-Solano, A. *J. Am. Chem. Soc.* **1968**, *90*, 5004.