Studies on Thermodynamics for the Hydrolysis. II. The Temperature Effect on the Stereoselective Deacylation of Amino Acid Esters in Bilayer and Micellar Systems

Yōko Matsumoto and Ryuichi Ueoka*

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

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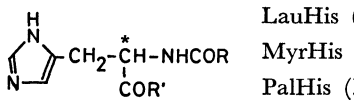
Stereoselective deacylations of *p*-nitrophenyl *N*-acyl-D(or L)-phenylalaninates(D(or L)-S_n; $n=2$, 12, and 16) and *N*-benzyloxycarbonyl-D(L)-phenylalaninates(D(L)-ZS) by the bilayer and micellar catalytic systems of L-histidine derivatives (LauHis, MyrHis, PalHis, LauHisLeu, and MyrHisLeu) and surfactants ($2C_mN2C_1Br$ with $m=12$, 14, and 16, CTAB, OTAC, and (*R*)-(+)-SUR_{16}) were performed. It is noteworthy that the temperature dependence of enantioselectivity for the deacylation of S_{12} having a long acyl chain is bell-shaped with maxima at 25 °C and 15—25 °C in the bilayer systems of MyrHis+$2C_{12}N2C_1Br$ and MyrHisLeu+$2C_{12}N2C_1Br$, respectively. On the other hand, the enantioselectivity for the S_{12} deacylation catalyzed by LauHisLeu in the presence of the CTAB micelles increases as temperature is lowered (the highest enantiomer rate ratio ($k^L_{a,obsd}/k^D_{a,obsd}=12$) is attained at 20 °C). The enantioselectivity for the deacylation of S_2 having a short acyl chain, however, is almost constant and considerably lowered in comparison with that for the deacylation of S_{12} in both the bilayer and micellar systems. According to the isokinetic temperature (β), the deacylation of S_n ($n=2$ and 12) and ZS in the bilayer systems might be entropy-driven, whereas that in the micellar systems might be enthalpy-driven. The stronger hydrophobic microenvironment of the bilayer systems in the ester-deacylation would probably be connected with the entropy-driven nature.

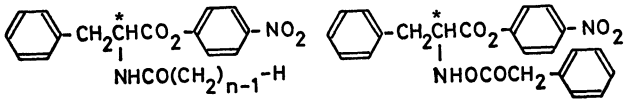
The stereoselective deacylation of *N*-protected amino acid *p*-nitrophenyl esters has recently received considerable attention,[1] and a relatively high stereoselectivity was attaind in the deacylation of diastereomeric dipeptide[2] and of *N*-acylamino acid esters with the dipeptide-type L-histidine derivative in the presence of cationic surfactants[3] or in bilayer systems.[4] However, there has been no report on thermodynamic specificity for the enantioselectivity in the deacylation of amino acid esters.

This study deals with the temperature dependence of catalytic efficiency and enantioselectivity for the stereoselective deacylation of *p*-nitrophenyl *N*-acyl-D(or L)-phenylalaninates (D(or L)-S_n; $n=2$, 12, and 16) and *N*-benzyloxycarbonyl-D(L)-phenylalaninates (D(L)-ZS) by the bilayer and micellar catalytic systems of L-histidine derivatives (LauHis, MyrHis, PalHis, LauHisLeu, and MyrHisLeu) and surfactants ($2C_mN2C_1Br$ with $m=12$, 14, and 16, CTAB, OTAC, and (*R*)-(+)-SUR_{16}) and has been performed in an attempt to discuss on the hydrophobic microenvironment of aggregates on the basis of isokinetic temperature (β).[5]

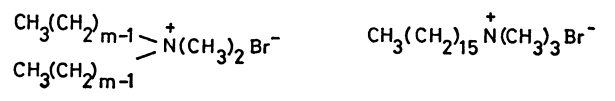


LauHis (R=$C_{11}H_{23}$, R′=OH)
MyrHis (R=$C_{13}H_{27}$, R′=OH)
PalHis (R=$C_{15}H_{31}$, R′=OH)

LauHisLeu (R=$C_{11}H_{23}$, R′=NH*CH(COOH)$CH_2CH(CH_3)_2$)
MyrHisLeu (R=$C_{13}H_{27}$, R′=NH*CH(COOH)$CH_2CH(CH_3)_2$)



D(L)-S_n ($n=2$, 12, 16) D(L)-ZS



$2C_mN2C_1Br$ ($m=12$, 14, 16) CTAB

OTAC (*R*)-(+)-SUR_{16}

Experimental

Materials. *p*-Nitrophenyl *N*-acyl-D(L)-phenylalaninates (D(L)-S_n; $n=2$, 12, and 16) were prepared by the previous method.[6,7]

p-Nitrophenyl *N*-Cbz-D(L)-phenylalaninates (D(L)-ZS) were prepared from *N*-benzyloxycarbonyl-D(L)-phenylalanine by the esterification of the CO_2H group with *p*-nitrophenol and dicyclohexylcarbodiimide in a way similar to the previous method.[8] Satisfactory results of elemental analysis were obtained for D(L)-ZS. D-ZS: mp 121.5—123.5 °C (lit,[8] mp 126—126.5 °C). Found: C, 65.88; H, 4.70; N, 6.67%. Calcd for $C_{23}H_{20}N_2O_6$: C, 65.70; H, 4.78; N, 6.66%. L-ZS: mp 119—120 °C (lit,[8] mp 126—126.5 °C). Found: C, 65.65; H, 4.73; N, 6.59%. Calcd for $C_{23}H_{20}N_2O_6$: C, 65.70; H, 4.78; N, 6.66%.

N-Dodecanoyl-L-histidine (LauHis), *N*-tetradecanoyl-L-histidine (MyrHis), and *N*-hexadecanoyl-L-histidine (PalHis) were prepared by reaction of L-histidine and the corresponding acid chlorides.[9] Satisfactory results of elemental analysis were obtained for LauHis,[7] MyrHis, and PalHis.[7] MyrHis: dp 196 °C (lit,[9] dp 192 °C). Found: C, 65.87; H, 9.61; N, 11.30%. Calcd for $C_{20}H_{35}N_3O_3$: C, 65.75; H, 9.59; N, 11.51%.

N-Dodecanoyl-L-histidyl-L-leucine (LauHisLeu) and *N*-tetradecanoyl-L-histidyl-L-leucine (MyrHisLeu) were prepared by reaction of L-histidyl-L-leucine with anhydrous dodecanoic acid and tetradecanoyl chloride, respectively. Satisfactory results of elemental analysis were obtained for LauHisLeu and MyrHisLeu. LauHisLeu: mp 194—196 °C. Found: C, 63.81; H, 9.17; N, 12.33%. Calcd for $C_{24}H_{42}N_4O_4$: C, 63.97; H, 9.39; N, 12.43%. MyrHisLeu: mp 212—214 °C. Found: C, 65.03; H, 9.63; N, 11.19%. Calcd for $C_{26}H_{46}N_4O_4$: C, 65.24; H, 9.69; N, 11.71%.

Didodecyldimethylammonium bromide ($2C_{12}N2C_1Br$), ditetradecyldimethylammonium bromide ($2C_{14}N2C_1Br$), and

dihexadecyldimethylammonium bromide ($2C_{16}N2C_1Br$) were prepared by reaction of *N,N*-dimethylalkylamine and the corresponding alkyl bromides in refluxing ethanol in the presence of sodium carbonate, and purified by repeated recrystallizations from ethyl acetate according to the previous method.[10] Satisfactory results of elemental analysis were obtained for $2C_{12}N2C_1Br$, $2C_{14}N2C_1Br$, and $2C_{16}N2C_1Br$. $2C_{12}N2C_1Br$: Found: C, 67.77; H, 12.20; N, 2.98%. Calcd for $C_{26}H_{56}NBr$: C, 67.50; H, 12.20; N, 3.03%. $2C_{14}N2C_1Br$: Found: C, 69.18; H, 12.52; N, 2.68%. Calcd for $C_{30}H_{64}NBr$: C, 69.44; H, 12.46; N, 2.70%. $2C_{16}N2C_1Br$: Found: C, 70.46; H, 12.49; N, 2.52%. Calcd for $C_{34}H_{72}NBr$: C, 71.02; H, 12.65; N, 2.44%.

Commercially available hexadecyltrimethylammonium bromide (CTAB) and octadecyltrimethylammonium chloride (OTAC) were recrystallized from an anhydrous ethanol–ether mixture.

(+)-Hexadecyldimethyl[(*R*)-α-methylbenzyl]ammonium bromide ((*R*)-(+)-SUR_{16}) was prepared by reaction of (*R*)-(+)-*N,N,*α-trimethylbenzylamine and hexadecyl bromide in accordance with the previous method.[11] Satisfactory results of elemental analysis were obtained.[7]

Determination of Rate Constants. Rates of *p*-nitrophenol liberation from *p*-nitrophenyl esters were measured at 400 nm with a Shimadzu UV-200 spectrophotometer. Each run was usually initiated by adding an acetonitrile solution (0.1 ml) of a substrate ester to a reaction medium of tris(hydroxymethyl)methanamine (Tris) buffer (3.4 ml) containing both 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 deacylation of ester substrate was evaluated by the following equation on the basis of triplicate runs:

$$k_{a,obsd} = (k_t - k_s)/[\text{nucleophile}]_0, \quad (1)$$

where k_t and k_s refer to the observed first-order rate constant for the deacylation of S_n with and without a nucleophile, respectively, and $[\text{nucleophile}]_0$ denotes the initial nucleophile concentration.

In the case of the bilayer catalytic systems, clear stock solutions were prepared by dissolving the nucleophile and the surfactant in Tris–KCl buffer by sonication (Bransonic 12, Yamato Scientific Co.) at 50 °C for 1 h, and used for kinetic measurements.

Results and Discussion

Since the reaction rate of D(L)-S_n (1×10^{-5} M; 1 M=1 mol dm^{-3}) deacylation with PalHis (5×10^{-5} M) attains a maximum in the presence of *ca.* 1×10^{-3} M (critical micelle concentration (cmc) $=5\times10^{-5}$ M)[12] $2C_{12}N2C_1Br$,[4b] the relative activity of nucleophiles will be discussed with the stereoselective deacylation of D(L)-S_n (n=2 and 12) and D(L)-ZS with the above concentration of the nucleophiles (PalHis, MyrHis, and MyrHisLeu) and the bilayer surfactants ($2C_mN2C_1Br$; m=12, 14, and 16). On the other hand, the concentrations of the micellar surfactants (CTAB, OTAC, and (*R*)-(+)-SUR_{16}) were set above their cmc's.

Catalytic Efficiency. It is obvious that the hydrophobicity of nucleophiles and substrates play an important role in enhancing the catalytic efficiency and stereoselectivity for the deacylation of amino acid esters in micellar[1d] and bilayer[4d] systems. Thus, in this study, we have employed the reactants having a hydrophobic segment.

Rate constants ($k_{a,obsd}$) as a function of temperature for the deacylation of D(L)-S_n (n=2 and 12) and D(L)-ZS catalyzed by the bilayer catalytic systems of MyrHis+$2C_{12}N2C_1Br$ and MyrHisLeu+$2C_{12}N2C_1Br$

Table 1. Temperature dependence of rate constant ($k_{a,obsd}$/M^{-1} s^{-1}) for the deacylation of S_n catalyzed by MyrHis and MyrHisLeu in the presence of $2C_{12}N2C_1Br$[a,b]

Substrate	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C
L-S_2	100 (39)	140 (61)	150 (82)	340 (—)	—(140)	380 (200)
D-S_2	41 (31)	48 (43)	60 (59)	130 (—)	— (72)	140 (100)
L-S_{12}	390(270)	570(420)	810(590)	1500(670)	1900 (—)	2500(1200)
D-S_{12}	88 (71)	160 (80)	250(110)	380(130)	620 (—)	800 (430)
L-ZS	330(120)	490(180)	650(250)	970(300)	1300(470)	1400 (700)
D-ZS	87 (46)	140 (70)	220(100)	370(130)	500(200)	620 (310)

a) pH 7.6, 3%(v/v) CH_3CN-H_2O, 0.083 M Tris buffer (0.083 M KCl), [MyrHis]=5×10^{-5} M, [MyrHisLeu]=3×10^{-5} M, [Sub]=1×10^{-5} M, and $[2C_{12}N2C_1Br]=1\times10^{-3}$ M. b) Values in the parentheses are those for the deacylation of S_n catalyzed by MyrHisLeu in the presence of $2C_{12}N2C_1Br$.

Table 2. Temperature dependence of rate constant ($k_{a,obsd}$/M^{-1} s^{-1}) and enantioselectivity ($k^L_{a,obsd}/k^D_{a,obsd}$) for the deacylation of S_n catalyzed by LauHis and LauHisLeu in the presence of CTAB[a,b]

	20 °C	25 °C	30 °C	35 °C	40 °C
L-S_2	100 (15)	130 (26)	220 (52)	340(120)	500(150)
D-S_2	56 (15)	78 (26)	120 (47)	200(110)	300(120)
L-S_2/D-S_2[c]	1.8(1.0)	1.7(1.0)	1.8(1.1)	1.7(1.1)	1.7(1.3)
L-S_{12}	470(140)	530(210)	770(300)	1000(400)	1200(580)
D-S_{12}	120 (12)	150 (21)	180 (36)	280 (71)	300(150)
L-S_{12}/D-S_{12}[c]	3.9 (12)	3.5 (10)	4.3(8.3)	3.6(5.6)	4.0(3.9)

a) pH 7.6, 3%(v/v) CH_3CN-H_2O, 0.083 M Tris buffer (0.083 M KCl), [LauHis]=[LauHisLeu]=5×10^{-5} M, [Sub]=1×10^{-5} M, and [CTAB]=3×10^{-3} M. b) Values in the parentheses are those for the deacylation of S_n catalyzed by LauHisLeu in the presence of CTAB. c) These values include the experimental error within ±6%.

are summarized in Table 1. The magnitude of rate enhancement increases in the orders L-S_{12}>L-ZS≫L-S_2 and D-S_{12}≳D-ZS≫D-S_2 in the deacylation of L- and D-enantiomers, respectively, over the temperature range of 10—35 °C in both the bilayer systems. These orders agree fairly well with the hydrophobicity of substrates. The catalytic efficiency of MyrHis is superior to that of MyrHisLeu. The difference in catalytic efficiency between MyrHis and MyrHisLeu would be attributable to the framework of both nucleophiles. Namely, it is deduced that the bulky leucine part in MyrHisLeu might reduce the catalytic efficiency due to the steric hindrance against the substrate-incorporation by the mixed bilayer membrane.

Table 2 shows the temperature dependence of $k_{a,obsd}$ for the deacylation of D(L)-S_n (n=2 and 12) catalyzed by LauHis and LauHisLeu in the CTAB micellar systems. The $k_{a,obsd}$ values increase linearly as temperature is raised for the deacylation of all the substrates and were used for calculating activation parameters. Nucleophile LauHis is superior in catalytic efficiency to the dipeptide-type nucleophile (LauHisLeu). The difference in catalytic efficiency between LauHis and LauHisLeu in the micellar system is similar to that between MyrHis and MyrHisLeu in the bilayer system. It is suggested that the steric hindrance of the leucine part in the nucleophile cannot be neglected with respect to the substrate-incorporation into the micellar matrix. The hydrophobicity of the substrate is almost reflected in the order of its reactivity. Especially, substrate L-S_{12} having a long acyl chain is fairly superior to L-S_2 having a short acyl chain as shown in Table 2. These results for both the bilayer and micellar systems imply that the hydrophobic interaction between substrate and nucleophile and the easiness of substrate-incorporation into the aggregates are of great importance to the enhancement of catalytic efficiency.

It is known that the extent of incorporation of a substrate into a micellar phase agrees reasonably well with its binding properties.[13] The binding constants of the long-chain substrates are fairly larger than those of the short-chain substrates in both the bilayer and micellar systems.[4b] This result suggests that the hydrophobic interaction between the former and the aggregates might be stronger than that between the latter and the aggregates and is reflected in the larger rate enhancement for the deacylation of the long-chain substrates (L-S_{12} and D-S_{12}) than that of the short-chain ones (L-S_2 and D-S_2) in almost all the catalytic systems in the present study as shown in Tables 1 and 2.

Stereoselectivity. Enantioselective parameters ($k^L_{a,obsd}/k^D_{a,obsd}$) as a function of temperature for the deacylations of S_n (n=2 and 12) and ZS in the bilayer systems of MyrHis+$2C_{12}N2C_1Br$ and MyrHisLeu+$2C_{12}N2C_1Br$ are shown in Figs. 1 and 2, respectively.

As Fig. 1 indicates, the temperature dependence of enantioselectivity for the deacylation of S_n (n=2 and 12) and ZS with MyrHis and $2C_{12}N2C_1Br$ is divided into the following three trends over the temperature range of 10—35 °C: (a) the enantioselectivity in the deacylation of S_{12} having a long acyl chain tends to decrease as temperature is raised with a maximum

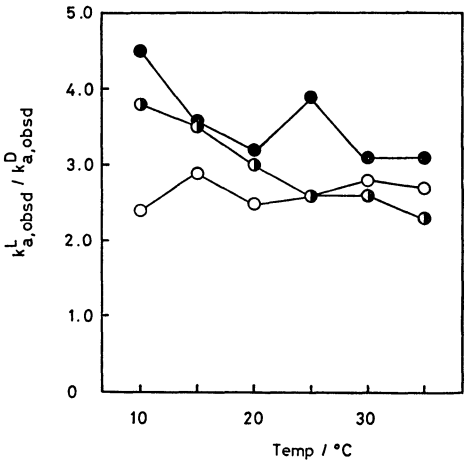


Fig. 1. Temperature dependence of enantioselectivity in the deacylation of S_n catalyzed by MyrHis with $2C_{12}N2C_1Br$.
pH 7.6, 3%(v/v) CH_3CN-H_2O, 0.083 M Tris-KCl buffer, [MyrHis]=5×10^{-5} M, [Sub]=1×10^{-5} M, [$2C_{12}N2C_1Br$]=1×10^{-3} M. ○: S_2, ●: S_{12}, ◑: ZS. The $k^L_{a,obsd}/k^D_{a,obsd}$ values include the experimental error within ±4%.

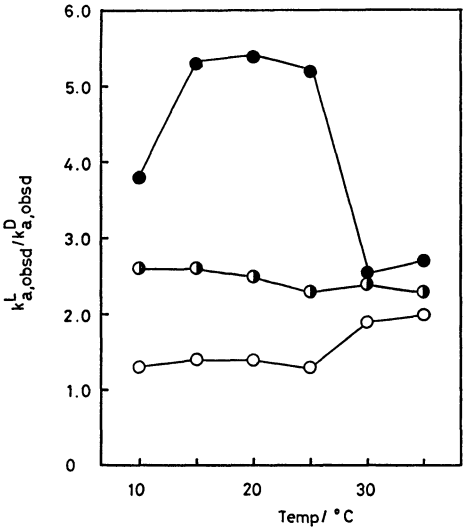


Fig. 2. Temperature dependence of enantioselectivity in the deacylation of S_n catalyzed by MyrHisLeu with $2C_{12}N2C_1Br$.
pH 7.6, 3%(v/v) CH_3CN-H_2O, 0.083 M Tris-KCl buffer, [MyrHisLeu]=3×10^{-5} M, [Sub]=1×10^{-5} M, [$2C_{12}N2C_1Br$]=1×10^{-3} M. ○: S_2, ●: S_{12}, ◑: ZS. The $k^L_{a,obsd}/k^D_{a,obsd}$ values include the experimental error within ±6%.

($k^L_{a,obsd}/k^D_{a,obsd}$=3.9) at 25 °C; (b) the $k^L_{a,obsd}/k^D_{a,obsd}$ value in the deacylation of ZS having a bulky side part decreases with rise in temperature; (c) the enantioselectivity in the deacylation of S_2 having a short acyl chain is almost constant ($k^L_{a,obsd}/k^D_{a,obsd}$=2.3—2.9) over the experimental temperature range. The difference in the temperature dependence of enantioselectivity among the deacylation of the three substrates (S_{12}, ZS, and S_2) would be attributed to the difference in the substrate-framework.

On the other hand, the temperature dependence for

the deacylation of S_n and ZS in the system of MyrHis-Leu+$2C_{12}N2C_1Br$ is different from that in the system of MyrHis+$2C_{12}N2C_1Br$ as shown in Fig. 2. It is especially worthy to note that the temperature dependence of enantioselectivity for the S_{12} deacylation is bell-shaped with a maximum ($k^L_{a,obsd}/k^D_{a,obsd}$=5.2—5.4) at 15—25 °C. The $k^L_{a,obsd}/k^D_{a,obsd}$ value for the deacylation of ZS is almost constant (2.3—2.6) over the whole temperature range examined, whereas that for the deacylation of S_2 is constant (1.3—1.4) in the temperature range of 10—25 °C and increases (1.9—2.0) at temperatures above 30 °C. Interestingly, the enantioselectivity for the deacylation of all the substrates (S_2, S_{12}, and ZS) employed converges at 30—35 °C.

These results for the bilayer and micellar systems suggest that the hydrophobic microenvironment would be changed delicately with temperature in the range of 10—35 °C and that the long-chain substrate (S_{12}) might be influenced by the change in microenvironment. At any rate, it is unique that the bilayer membrane system including the dipeptide-type nucleophile (MyrHisLeu+$2C_{12}N2C_1Br$) has displayed the high enantioselectivity in an adequate temperature range (15—25 °C) probably through a favorable hydrophobic interaction between the nucleophile (MyrHisLeu) and the substrate (D(L)-S_{12}). And, it is obvious that the leucine part of MyrHisLeu plays an important role in producing the high stereoselectivity.

Figure 3 and Table 2 show the temperature dependence of enantioselectivity for the deacylation of S_n (n=2, 12, and 16) in the micellar systems of PalHis+OTAC, LauHis+CTAB, and LauHisLeu+CTAB. Two interesting results were obtained: firstly, the temperature dependence of enantioselectivity for the S_{16} deacylation catalyzed by PalHis+OTAC is clearly bell-shaped with a maximum ($k^L_{a,obsd}/k^D_{a,obsd}$=3.6) at 30 °C and, secondly, the enantioselectivity for the S_{12} deacylation catalyzed by LauHisLeu+CTAB increases sharply as temperature is lowered. It is notable that the highest enantiomer rate ratio ($k^L_{a,obsd}/k^D_{a,obsd}$=12) is attained at 20 °C for the S_{12} deacylation in the micellar system of LauHisLeu+CTAB. This implies that the peculiar environments of the hydrophobic dipeptide (LauHisLeu) and the CTAB micelles, which are effective for the highly enantioselective deacylation of the hydrophobic substrate (S_{12}) at the lower temperature (20 °C), would be varied delicately as temperature is raised. The leucine part of LauHisLeu in the micellar system (CTAB) might play an important role in enhancing the enantioselectivity in common with that in the bilayer system (MyrHis-Leu+$2C_{12}N2C_1Br$). On the other hand, in the micellar systems of PalHis+OTAC and LauHis+CTAB, the stereoselectivity for the deacylation of S_n (n=2 and 12) remains almost constant over the temperature range of 20—35 (40) °C, though the magnitude of enantioselectivity for the long-chain substrate (S_{12}) is fairly larger than that for the short-chain substrate (S_2).

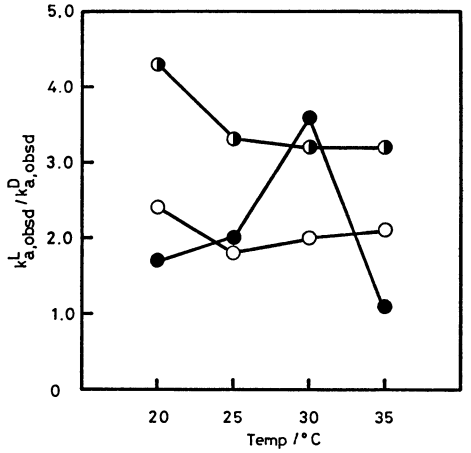


Fig. 3. Temperature dependence of enantioselectivity in the deacylation of S_n catalyzed by PalHis with OTAC.
pH 7.6, 3%(v/v) CH_3CN-H_2O, 0.083 M Tris-KCl buffer, [PalHis]=4×10^{-5} M, [S_n]=1×10^{-5} M, [OTAC]=8×10^{-4} M. ○: S_2, ◑: S_{12}, ●: S_{16}.
The $k^L_{a,obsd}/k^D_{a,obsd}$ values include the experimental error within ±3%.

Isokinetic Relationship. The isokinetic relationship appears to hold for the deacylation of D(L)-S_n (n=2 and 12) and D(L)-ZS in the bilayer and micellar systems from the relation between the entropy and enthalpy of activation on the basis of rate constant ($k_{a,obsd}$). The activation parameters (ΔG^*, ΔH^*, and ΔS^*) were evaluated by

$$\Delta G^* = 2.303RT \log (kT/hk_{a,obsd}) = \Delta H^* - T\Delta S^*, \quad (2)$$

where k and h stand for the Boltzmann and Planck constants, respectively. The linear relationship between the free energy of activation (ΔG^*) and the absolute temperature (T) is shown in Figs. 4 and 5. The ΔH^* and ΔS^* values obtained for the bilayer and micellar systems are summarized in Tables 3 and 4, respectively. Furthermore, the isokinetic temperatures (β) have been determined by plotting ΔH^* against ΔS^* according to Eq. (3)[5] and are summarized in Tables 3 and 4.

$$\Delta H^* = \Delta H^*_0 + \beta\Delta S^*, \quad (3)$$

where ΔH^*_0 is simply the intercept of ΔH^* correspond-

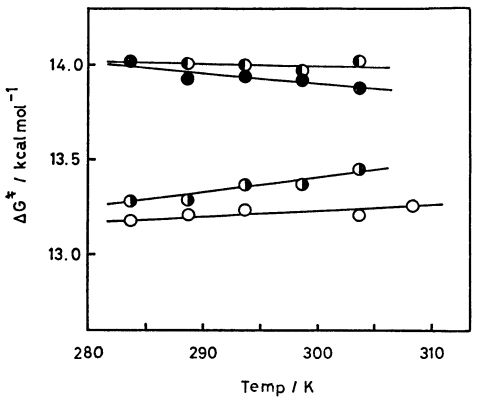


Fig. 4. Linear correlations between ΔG^* and absolute temperature (T) in the deacylation of S_n catalyzed by MyrHis with $2C_{12}N2C_1Br$.
pH 7.6, 3%(v/v) CH_3CN-H_2O, 0.083 M Tris-KCl buffer, [MyrHis]=5×10^{-5} M, [Sub]=1×10^{-5} M, [$2C_{12}N2C_1Br$]=1×10^{-3} M. ○: L-S_{12}, ●: D-S_{12}, ◑: L-ZS, ◐: D-ZS.

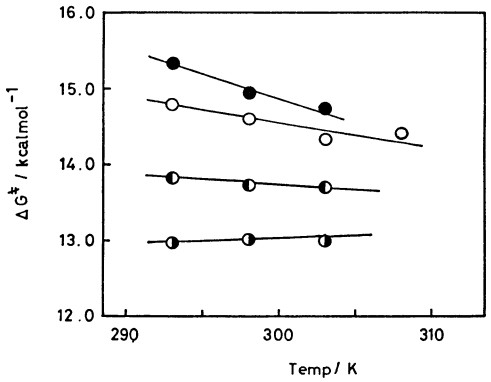


Fig. 5. Linear correlations between ΔG^* and absolute temperature (T) in the deacylation of S_n catalyzed by PalHis with OTAC.
pH 7.6, 3%(v/v) CH_3CN-H_2O, 0.083 M Tris-KCl buffer, [PalHis]$=4\times10^{-5}$ M, [S_n]$=1\times10^{-5}$ M, [OTAC]$=8\times10^{-4}$ M. ○: L-S_2, ●: D-S_2, ◑: L-S_{12}, ◐: D-S_{12}.

ing to ΔS^*.

It is known that hydrophobic interactions are mainly entropy driven, whereas lyophobic ones are mainly enthalpy driven.[14,15] On the other hand, it has been found that the β value based on apparent second-order rate constants ($k_{a,obsd}$) agrees fairly well with that based on k_m values for the simplified reaction ($M+S_n \overset{K_b}{\rightleftharpoons} MS_n \overset{k_m}{\longrightarrow} P$; M, MS_n, and P stand for an aggregate composed of nucleophile and surfactant, an aggregate-substrate complex, and *p*-nitrophenol, respectively) in the deacylation of S_n (n=2, 12, and 16) with PalHis incorporated into OTAC micelles.[16] Then, we intend to discuss on the hydrophobic microenvironment for the stereoselective deacylation in the bilayer and micellar systems in terms of the β value obtained from the $k_{a,obsd}$ values in harmony with the k_m ones.

On the basis of the β value in connection with $\overline{T}$ (the average experimental temperature), the deacylation reaction in the bilayer systems may be governed by the entropy of activation in the temperature range of 10—40 °C, that is, $\overline{T}$ (296—306 K) exceeds β (245—269 K) in the bilayer systems of PalHis+$2C_{12}N2C_1Br$, MyrHis+$2C_{12}N2C_1Br$, MyrHisLeu+$2C_{12}N2C_1Br$, and PalHis+$2C_{14}N2C_1Br$, though β (302 K) is nearly equal to $\overline{T}$ (306 K) in the system of PalHis+$2C_{16}N2C_1Br$. This difference in β value between the systems of $2C_mN2C_1Br$ (m=12 and 14) and the $2C_{16}N2C_1Br$ system would be attributed to the difference in the state of bilayer phase. Namely, the bilayer systems of $2C_{12}N2C_1Br$ and $2C_{14}N2C_1Br$ and the system of $2C_{16}N2C_1Br$ are considered to be liquid crystals and a mixed state of liquid crystal and crystal over the experimental temperature range (see Table 3), respectively, in view of the phase transition temperature ($2C_{12}N2C_1Br$, 8 °C; $2C_{14}N2C_1Br$, 18 °C; $2C_{16}N2C_1Br$, 27 °C).[17] On the other hand, interestingly, the deacylation in the micellar systems may be governed by the enthalpy of activation in the temperature range of 20—40 (35) °C, that is, β (313—330 K) exceeds $\overline{T}$ (301—303 K) in the micellar systems of LauHis+

Table 3. Activation parameters (ΔH^* and ΔS^*) and isokinetic temperature (β) for the deacylation of S_n in the bilayer systems at pH 7.6[a,b]

Nucleophilic system	Substrate	ΔH^* / kcal mol^{-1} [d]	ΔS^* / cal K^{-1} mol^{-1}	β / K	$\overline{T}$[c] / K
PalHis+$2C_{12}N2C_1Br$	L-S_2	10.9	−10.6	267	306 (25—40 °C)
	D-S_2	9.2	−17.0		
	L-S_{12}	8.5	−15.6		
	D-S_{12}	9.6	−13.9		
MyrHis+$2C_{12}N2C_1Br$	L-S_2	10.4	−12.4	269	296 (10—35 °C)
	D-S_2	9.8	−16.8		
	L-S_{12}	12.6	−2.2		
	D-S_{12}	16.1	7.4		
	L-ZS	10.9	−8.4		
	D-ZS	14.4	1.4		
MyrHisLeu+$2C_{12}N2C_1Br$	L-S_2	10.3	−14.7	252	296 (10—35 °C)
	D-S_2	10.1	−16.0		
	L-S_{12}	11.8	−5.4		
	D-S_{12}	6.7	−26.2		
	L-ZS	10.9	−10.5		
	D-ZS	11.6	−9.7		
PalHis+$2C_{14}N2C_1Br$	L-S_2	7.4	−21.7	245	301 (20—35 °C)
	D-S_2	6.7	−25.0		
	L-S_{12}	8.3	−15.0		
	D-S_{12}	9.1	15.1		
PalHis+$2C_{16}N2C_1Br$	L-S_2	25.4	36.9	302	306 (25—40 °C)
	D-S_2	7.4	−22.9		
	L-S_{12}	19.3	21.0		
	D-S_{12}	14.6	3.0		

a) 3% (v/v) CH_3CN-H_2O, 0.083 M Tris buffer (0.083 M KCl), [PalHis]=[MyrHis]$=5\times10^{-5}$ M, [MyrHisLeu]$=3\times10^{-5}$ M, [Sub]$=1\times10^{-5}$ M, and [$2C_mN2C_1Br$]$=1\times10^{-3}$ M. b) Values in the parentheses are the temperature range. c) Average value of experimental temperatures. d) 1 cal=4.184 J.

Table 4. Activation parameters ($\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$) and isokinetic temperature (β) for the deacylation of S_n in the micellar systems at pH 7.6[a,b]

Nucleophilic system	Substrate	$\Delta H^{\ddagger}$ / kcal mol^{-1}	$\Delta S^{\ddagger}$ / cal K^{-1} mol^{-1}	β / K	$\overline{T}$[c] / K
LauHis+CTAB	L-S_2	14.4	−0.4	319	303 (20—40 °C)
	D-S_2	11.3	−11.7		
	L-S_{12}	7.2	−21.8		
	D-S_{12}	7.6	−23.2		
PalHis+(*R*)-(+)-SUR_{16}	L-S_2	9.9	−13.5	313	303 (20—40 °C)
	D-S_2	10.1	−13.8		
	L-S_{12}	8.2	−16.1		
	D-S_{12}	12.1	−5.1		
PalHis[d]+OTAC	L-S_2	23.3	29.0	330	301 (20—35 °C)
	D-S_2	32.6	59.0		
	L-S_{12}	9.9	−10.1		
	D-S_{12}	17.3	12.0		

a) 3%(v/v) CH_3CN-H_2O, 0.083 M Tris buffer (0.083 M KCl), [LauHis]=[PalHis]=5×10^{-5} M, [CTAB]=3×10^{-3} M, [(*R*)-(+)-SUR_{16}]=1×10^{-3} M, and [S_n]=1×10^{-5} M. b) Values in the parentheses are the temperature range. c) Average value of experimental temperature. d) [PalHis]=4×10^{-5} M, [OTAC]=8×10^{-4} M.

CTAB, PalHis+(*R*)-(+)-SUR_{16}, and PalHis+OTAC.

The difference in the driving parameter of activation ($\Delta S^{\ddagger}$ or $\Delta H^{\ddagger}$) for the deacylation reaction between the liquid-crystalline bilayer systems and the micellar ones would be attributable to the difference in the hydrophobic microenvironment of the aggregates. After all, it is concluded that the stereoselective deacylation in the liquid-crystalline bilayer systems would proceed through a stronger hydrophobic interaction between the reactants. So far, however, we have not found the relation between the β value and the catalytic efficiency and enantioselectivity.

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