Efficient Stereoselective Hydrolysis of Enantiomeric Amino Acid Esters by Bilayer Vesicular Systems which include Di- or Tri-peptide Histidine Catalysts

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When the stereoselective hydrolysis of enantiomeric amino acid esters, $Me[CH_2]_nCONHCH(R)CO_2C_6H_4NO_2$-*p* [R = $PhCH_2$ (**6a**), Me_2CHCH_2 (**6b**), or Me (**6c**); *n* = 0—12], by the bilayer vesicular systems of histidine catalysts (L-histidine or di- or tri-peptide L-histidine derivatives) and a double-chain surfactant, $(Me[CH_2]_{11})_2NMe_2Br$ (**5**), was carried out at 4—25 °C in Tris buffer (pH 7.68) in 3% (v/v) MeCN–H_2O, the efficient stereoselective reaction was realized with dipeptide histidine catalysts such as Z-L-Phe-L-His (**2a**) and Z-L-Leu-L-His (**2c**) and the surfactant (**5**) through the two stereoselective processes (*viz.*, binding and hydrolysis of the L-substrate by the catalytic vesicular system). In the hydrolysis of the substrates (**6a**; *n* = 8) possessing an appropriately long *N*-acyl chain and a hydrophobic substituent R (R = $PhCH_2$) by the gel-phase vesicular catalyst composed of (**2c**) and (**5**) at 4 °C, the stereoselection of the enantiomeric substrate was considerably enhanced by the close proximity of the catalyst and the substrate, giving an enantiomer rate ratio (L/D) of 148.2. The mode of interaction between the catalyst and the substrate is discussed in relation to the preferential attack of the histidine catalyst on the L-substrate in the vesicle–substrate complex.

The stereoselective hydrolysis of enantiomeric amino acid esters by chiral model biological membranes has been examined previously in the deacylation of *N*-acylamino acid *p*-nitrophenyl esters by synthetic functionalized membranes which include an L-histidine moiety [1a] or a dipeptide segment,[1b] and also by bilayer vesicular systems of *N*-acyl-L-histidine catalysts (involving di- or tri-peptide derivatives) and cationic double-chain surfactants.[2] Although the highly stereoselective deacylation of long-chain *p*-nitrophenyl phenylalanates [maximum enantiomer rate ratio (L/D) = 83.6 at 10 °C] has been performed with a dipeptide histidine catalyst (Z-L-Leu-L-His) and *N*,*N*-bisdodecyl-*N*,*N*-dimethylammonium bromide in our previous work,[2c] this extent of stereoselectivity has recently been improved in the deacylation of similar long-chain *p*-nitrophenyl phenylalanates with a tripeptide histidine catalyst (Z-L-Phe-L-His-L-Leu) and coagregates of single-chain and double-chain surfactants (*N*-hexadecyl-*N*,*N*,*N*-trimethylammonium bromide and *N*,*N*-bis-tetradecyl-*N*,*N*-dimethylammonium bromide).[3]

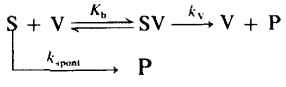
However, the catalytic efficiency of bilayer vesicular systems comprising a histidine catalyst and a double-chain surfactant in the stereoselective hydrolysis of enantiomeric amino acid esters is essentially dependent on a number of factors (the structures of histidine catalysts and substrates, the reaction conditions such as the molar ratio of a surfactant to a histidine catalyst, and the reaction temperature) which have not yet been investigated in detail. The present report describes the stereoselective esterase activity of chiral membrane models composed of the histidine catalysts (**1**)—(**4**) and a double-chain surfactant (**5**) in the hydrolysis of short-chain or long-chain esters (**6**) at 4—25 °C (pH 7.68).

Experimental

Materials.—The histidine catalysts (**1**)—(**3**) and (**4b**) were supplied as in previous papers: [2e,f] Z-L-His (**1**) was commercially available, and the dipeptide or tripeptide histidine catalysts (**2**) and (**4b**) were obtained respectively by the reactions of L-histidine with *N*-benzyloxycarbonyl-L (or D)-leucine (or phenylalanine) succinimido ester or *N*-(*N*-benzyloxycarbonyl-L-leucyl)-L-leucine succinimido esters.[2f] The long-chain dipeptide histidine catalysts (**3**) were prepared by the acylation of Z-L-Phe-L-His, Z-L-Leu-L-His, or Z-L-His-L-Leu with dodecanoic anhydride *via* the hydrobromination of the benzyloxy (Z) group in the dipeptide histidine catalysts.[2e] The tripeptide histidine catalyst (**4a**) was prepared from *N*-(L-histidyl)-L-leucine and *N*-benzyloxycarbonyl-L-leucine succinimido ester (Found: C, 55.3; H, 6.8; N, 12.5. Calc. for $C_{25}H_{36}N_5O_6$·HCl: C, 55.7; H, 6.9; N, 13.0%). The double-chain surfactant (**5**) was obtained by the reaction of *N*,*N*-dimethyldodecylamine with dodecyl bromide[4] (Found: C, 67.0; H, 12.3; N, 3.05. Calc. for $C_{26}H_{56}BrN$: C, 67.5; H, 12.2; N, 3.0%).

Hydrolysis and Kinetic Measurements.—Hydrolysis of the ester substrates (**6**) (1.0×10^{-5}M) by the vesicular system of the histidine catalysts (**1**)—(**4**) (5.0×10^{-5}M) and the surfactant (**5**) (2.5×10^{-4} to 1.5×10^{-3}M; critical micelle concentration 5×10^{-5}M) was carried out at 4—25 °C, pH 7.68, in Tris buffer (0.083M) containing KCl (0.08M) in 3% (v/v) MeCN–H_2O. The substrate was added to the vesicular catalyst after the latter had been sonicated at 45 °C. The rate constants for the hydrolysis with or without the histidine catalyst (k_{total} and k_{spont}, respectively) were obtained from good pseudo-first-order rate constants, from spectrophotometric determination of *p*-nitrophenolate concentration (400 nm), and the second-order catalytic rate constant, $k_{cat} = (k_{total} - k_{spont})/[catalyst]$, was taken as the average value from more than three reactions repeated under identical conditions.

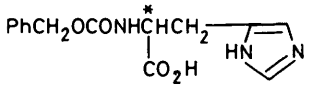
The binding constant K_b/N (*N* is the aggregation number) and the rate constant k_V were obtained in the usual way on the basis of simplifed reaction scheme shown, where S = substrate, V = vesicle, SV = vesicle–substrate complex, and P = *p*-nitrophenolate anion.



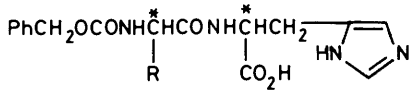
Scheme.

Results and Discussion

Effects of Surfactant Concentration on the Stereoselective Hydrolysis.—The effects of the molar ratio of the surfactant (**5**) to the histidine catalyst on the hydrolysis rate and the stereoselectivity were first examined in the deacylation of a long-chain



(1) Z-L-His



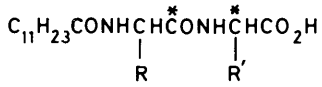
(2)

a; R = $PhCH_2$ Z-L-Phe-L-His

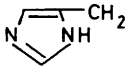
b; R = $PhCH_2$ Z-D-Phe-L-His

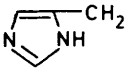
c; R = Me_2CHCH_2 Z-L-Leu-L-His

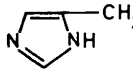
d; R = Me_2CHCH_2 Z-D-Leu-L-His

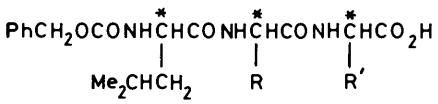


(3)

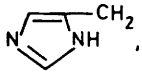
a; R = $PhCH_2$, R′ =  C_{12}-L-Phe-L-His

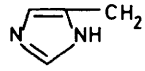
b; R = Me_2CHCH_2, R′ =  C_{12}-L-Leu-L-His

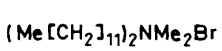
c; R =  , R′ = Me_2CHCH_2 C_{12}-L-His-L-Leu



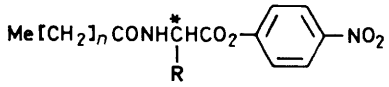
(4)

a; R =  , R′ = Me_2CHCH_2 Z-L-Leu-L-His-L-Leu

b; R = Me_2CHCH_2, R′ =  Z-L-Leu-L-Leu-L-His



(5)



(6)

a; R = $PhCH_2$ C_{n+2}-L(or D)-Phe-ONp (n = 0, 8, 12)

b; R = Me_2CHCH_2 C_{n+2}-L(or D)-Leu-ONp (n = 8)

c; R = Me C_{n+2}-L(or D)-Ala-ONp (n = 8)

substrate, C_{10}-L (or D)-Phe-ONp (**6a**), by the vesicular system of a dipeptide histidine catalyst (**2c**) and (**5**) at 25 °C, with [surfactant (**5**)]/[catalyst (**2c**)] = 5—30 {[(**2c**)] = 5.0×10^{-5}M}. As indicated in Figure 1, the increase of [(**5**)]/[(**2c**)] in the range 5—10 raised both the deacylation rate and the stereoselectivity, but decrease in the amount of the catalyst (**2c**) per vesicle when [(**5**)]/[(**2c**)] was more than 10 lowered the reaction rate, with the maximum stereoselectivity {enantiomer rate ratio (L/D) = 28.6 at [(**5**)]/[(**2c**)] = 20}. Since increase in the concentration of the surfactant with respect to that of the catalyst promotes the formation of vesicles simultaneously with the decrease in the amount of catalyst per vesicle, increase of [surfactant (**5**)]/[catalyst (**2c**)] in the range 10—30 raised the binding constant K_b/N for the formation of the vesicle–substrate complex, concomitant with the decrease of the rate constant k_V for the catalyst–substrate reaction in the vesicle–substrate complex (Figure 2). It is notable from Figure 2 that both K_b/N and k_V differ between the enantiomeric substrates under identical reaction conditions; that is, stereoselection of the substrates was realized not only through stereoselective reaction of the histidine catalyst and the substrates *via* the formation of the vesicle–substrate complex but also through stereoselective binding of the substrates by the chiral vesicular system. In terms of the effective stereoselection of the substrate, the former reaction process, in which the histidine catalyst reacts predominantly with one of the enantiomers (*viz.*, the L-substrate) *via* the formation of the vesicle–substrate complex, seems important, because the enantiomer parameter ratio (L/D) of the k_V value (L/D = 5.6—12.7) was considerably larger than that of the K_b/N value (L/D = 1.3—1.9) in the range of [(**5**)]/[(**2c**)] = 10—30. However, the highest stereoselectivity [enantiomer rate ratio (L/D) = 28.6] was obtained at [(**5**)]/[(**2c**)] = 20, even though the enantiomer k_V ratio (L/D) decreased monotonically with increasing [(**5**)]/[(**2c**)] ratio in

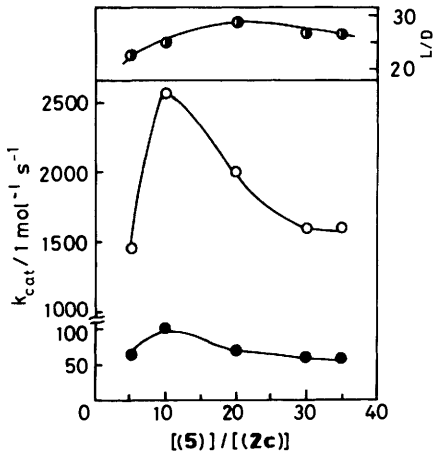


Figure 1. Concentration effects of the surfactant (**5**) on the stereoselective hydrolysis of enantiomeric esters (**6a**; n = 8) with Z-L-Leu-L-His (**2c**) at 25 °C; [(**6a**; n = 8)] = 1 × 10^{-5}M, [(**2c**)] = 5 × 10^{-5}M, and $[(5)]_{cmc}$ = 5 × 10^{-5}M; ○ L-substrate, ● D-substrate

Structural Effects of the Catalysts and Substrates on the Stereoselective Hydrolysis.—The present stereoselection of the enantiomeric amino acid esters by the catalytic vesicular system was also influenced by the structural effects of the histidine catalysts and the substrates. As can be seen from Table 1, both the hydrolysis rate and the stereoselectivity were considerably influenced by the difference in the structural frameworks of the reactants. The structural difference in the substrates might cause the difference in the extent of vesicle–substrate complex formation and of the hydrophobic interaction (and/or interamide hydrogen-bonding) between the histidine catalyst and the substrate in the vesicle–substrate complex. Such an interaction might occur in the weakly hydrophobic region (not the hydrophobic core) of the vesicular system; possible representative interaction modes are given in Figure 3. When the reacting positions of the susceptible ester group in the L-substrate and the imidazolyl group in the histidine catalyst come closer *via* the hydrophobic interaction (and/or the interamide bonding) shown in Figure 3, those of the D-substrate and the histidine catalyst are more remote; this increases the difference in hydrolysis rate between the enantiomers.

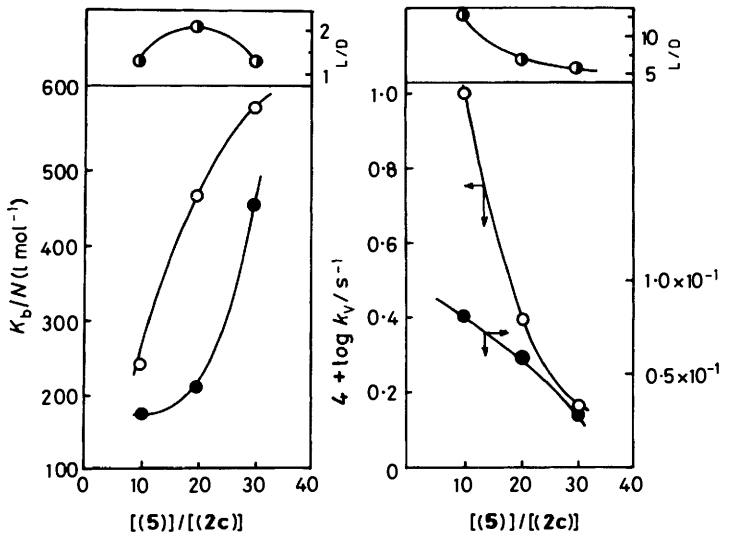


Figure 2. Concentration effects of the surfactant (**5**) on the binding and rate constants for the hydrolysis of (**6a**; n = 8) with Z-L-Leu-L-His (**2c**) at 25 °C under the conditions [(**6a**; n = 8)] = 1 × 10^{-5}M, [(**2c**)] = (3.5—5.0) × 10^{-5}M, and [(**5**)]/[(**2c**)] = 10—30; ○ L-substrate, ● D-substrate

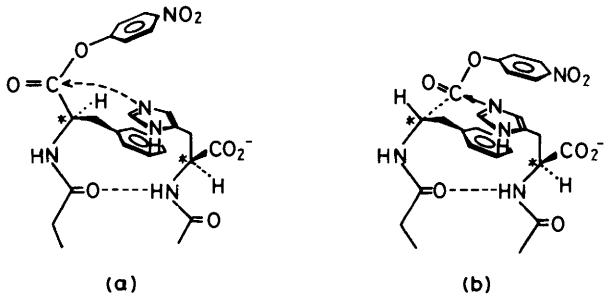


Figure 3. Schematic representation of the interaction between a histidine catalyst (Z-L-His) and enantiomeric substrates (**6a**); (a) Z-L-His and D-enantiomer, (b) Z-L-His and L-enantiomer

the range 10—30. Since the enantiomer K_b/N ratio (L/D) was largest (L/D = 1.9) at [(**5**)]/[(**2c**)] = 20, the highest stereoselectivity at [(**5**)]/[(**2c**)] = 20 was realized through co-operative stereoselective processes (*viz.*, stereoselective substrate-binding and hydrolysis reactions).

Therefore, enhancement of vesicle–substrate complex formation through condensation or incorporation of the substrate by the vesicular system might result in a raising of the hydrolysis rate, and the enhanced proximity of the substrate and the histidine catalyst in the vesicle–substrate complex might increase stereoselectivity (and/or the hydrolysis rate). As indicated in Table 1, the long-chain (n = 8—12) substrates (especially the L-enantiomers), as compared with the short-chain system (n = 0), showed a high reaction rate due to facilitation of vesicle–substrate complex formation by hydrophobic forces. Furthermore, in the hydrolysis of the substrates C_{n+2}-L (or D)-Phe-ONp (n = 0—12) (**6a**), use of all the histidine catalysts resulted in the highest stereoselectivity (and/or the highest reaction rate) for the C_{10}-L (or D)-Phe-ONp substrate possessing an appropriately long chain (n = 8). Therefore, the proximity of the substrate and the histidine catalyst was promoted through vesicle–substrate complex formation *via* incorporation of the long-chain (n = 8) substrates into the vesicular system of the double-chain ($C_{12}H_{25}$) surfactant (**5**) and the histidine catalyst. It is also notable here that, in the

Table 1. Rate constants and stereoselectivity in the hydrolysis of the substrates (**6**; n = 0, 8, or 12)[a]

		k_{cat}/l mol^{-1} s^{-1}													
		(**6a**; n = 0)			(**6a**; n = 8)			(**6a**; n = 12)			(**6b**; n = 8)			(**6c**; n = 8)	
Catalyst	T/°C	L	D	L/D	L	D	L/D	L	D	L/D	L	D	L/D	L	D	L/D
Z-L-His (**1**)	25	20	34	0.6	44	62	0.7	68	40	1.5	38	28	1.4			
	4	0.82	0.61	1.3	1.6	0.24	6.7									
Z-L-Phe-L-His (**2a**)	25	640	50	12.8	4 506	152	29.6	2 094	160	13.8	2 772	126	22.0	316	93	3.4
	23	546	42	13.0	3 940	134	29.4	1 752	118	14.8	2 274	116	19.6			
	20	506	38	13.3	3 574	122	29.3	1 690	106	15.9	2 038	100	23.4			
	15	426	26	16.4	2 922	76	38.4	1 246	54	23.1	1 770	68	26.0			
	10	340	18	18.9	2 596	62	41.9	978	42	23.3	1 592	48	33.2	130	28	4.6
	4	193	8.0	24.1	1 107	16	69.2	538	15	35.9	510	14	36.4			
Z-D-Phe-L-His (**2b**)	25	88	72	1.2	434	222	2.0	388	180	2.3	280	124	2.3			
	20	50	37	1.3	415	148	2.8	245	101	2.4	196	91	2.2			
	15				334	114	2.9	196	78	2.6						
	10	34	14	2.4	286	94	3.0	122	58	2.1	170	64	2.7			
	4	10	4.3	2.3	62	18	3.4	81	51	1.6	147	53	2.8			
Z-L-Leu-L-His (**2c**)	25	380	38	10.0	2 282	104	22.5	1 016	92	11.0	2 002	80	25.0	266	44	6.0
	20	298	20	14.9	1 554	46	33.8				1 664	60	27.4			
	15	184	10	18.4	1 016	18	56.4	476	10	47.6	970	32	30.3			
	10	154	4.0	38.0	836	10	83.6	320	4.1	78.0	798	18	44.3			
	4	72	1.8	40.0	326	2.2	148.2	130	1.4	92.9	339	7.2	47.1	46	3.0	15.4
Z-D-Leu-L-His (**2d**)	25	23	12	1.9	133	47	2.8	54	24	2.3						
C_{12}-L-Phe-L-His (**3a**)	25	764	118	6.5	3 156	340	9.3	1 664	352	4.7	2 040	360	5.7			
	15	556	54	10.3	2 254	226	10.0	802	154	5.2	1 556	226	6.9			
	10	436	38	11.5	2 030	172	11.8	546	106	5.2	1 172	158	7.4			
	4	128	5.4	23.7	1 520	62	24.5	228	21	10.9	471	37	12.7			
C_{12}-L-Leu-L-His (**3b**)	25	1 084	156	6.9	4 318	654	6.6	1 654	538	3.0	4 118	620	6.6			
	15	644	74	8.7	3 030	386	7.8	1 044	254	4.1	2 704	330	8.1			
	10	542	56	9.8	2 148	270	8.0	764	190	4.0	2 126	260	8.2			
	4	224	13	17.2	1 439	128	11.2	264	52	5.1	620	53	11.7			
C_{12}-L-His-L-Leu (**3c**)	25	122	120	1.0	844	136	6.4	576	90	6.4	722	112	6.4			
	10	58	56	1.0	456	56	8.1	210	32	6.6	420	60	7.0			
	4	17	6.0	2.8	229	20	11.5	56	2.4	23.3	37	2.8	13.2			
Z-L-Leu-L-His-L-Leu (**4a**)	25	155	38	4.1	1 099	119	9.2	566	23	24.6	680	51	13.3	122	20	6.1
	4	22	2.8	8.0	235	8.1	29.0	131	1.8	72.8	131	6.9	19.0	24	3.4	7.1
Z-L-Leu-L-Leu-L-His (**4b**)	25	99	86	1.2	245	266	0.92	95	150	0.63	114	101	1.1	37	30	1.2
	4	7.0	0.6	11.7	25	35	0.71									

[a] Tris buffer (0.08M) containing 0.08M-KCl at 4—25 °C (pH 7.68) in 3% (v/v) MeCN–H_2O; [catalyst] = 5 × 10^{-5}M, [surfactant (**5**)] = 1 × 10^{-3}M, [substrate (**6**)] = 1 × 10^{-5}M.

hydrolysis of the substrates $C_9H_{19}CONHCH(R)CO_2C_6H_4$-$NO_2$-$p$ [R = $PhCH_2$ (**6a**), Me_2CHCH_2 (**6b**), or Me (**6c**)] possessing the same long chain (n = 8) with the histidine catalysts (**2a**—**c**) and (**4a**) and the surfactant (**5**), both the hydrolysis rate and the stereoselectivity become higher in the order R = $PhCH_2$ (**6a**; n = 8) ≳ R = Me_2CHCH_2 (**6b**; n = 8) ≫ R = Me (**6c**; n = 8); that is, the hydrophobic substituent R in the substrate plays an important role in intensifying the proximity of substrate and histidine catalyst through hydrophobic interaction with the imidazolyl group in the catalyst (see Figure 3).

The extent of the contact between catalyst and substrate was also influenced by the structural difference in the histidine catalysts. The use of the L-L-type dipeptide catalysts having the L-histidine moiety in their terminal position [Z-L-Phe-L-His (**2a**), Z-L-Leu-L-His (**2c**), C_{12}-L-Phe-L-His (**3a**), and C_{12}-L-Leu-L-His (**3b**)] instead of Z-L-His (**1**) considerably enhanced both the deacylation rate and the stereoselectivity in the hydrolysis of the short-chain or long-chain substrates (Table 1). The L-phenylalanine or L-leucine part of the dipeptide catalysts (**2a** and **c**, and **3a** and **b**), which is adjacent to the L-histidine moiety, probably encourages the imidazolyl group (in the catalyst) and the susceptible ester group (in the substrate) to come closer together through hydrophobic interaction with the substituent R in the substrate (see Figure 4). In this respect, the D-phenylalanine or D-leucine part in the D-L-type dipeptide catalysts [Z-D-Phe-L-His (**2b**) and Z-D-Leu-L-His (**2d**)] was unable to participate efficiently in the hydrophobic interaction with the substituent R in the substrate, as reflected in the relatively low deacylation rate and low stereoselectivity in the hydrolysis of (**6**) by (**2b**) or (**2d**) (see Figure 4 and Table 1). Therefore, the chirality of the neighbouring amino acid part in the dipeptide histidine catalysts (*viz.*, L-amino acid part with respect to the L-histidine moiety) should be taken into

consideration for improving the contact between catalyst and substrate. On the other hand, the neighbouring amino acid part in the terminal position of such a dipeptide histidine catalyst as C_{12}-L-His-L-Leu (**3c**) was unable to enhance the deacylation rate and the stereoselectivity in the hydrolysis of the short-chain substrate (**6a**; $n = 0$), probably because of steric hindrance of the terminal L-Leu part in (**3c**) against the approach of the substrate (**6a**; $n = 0$) to the catalyst.

It is also noteworthy with respect to the efficiency of the dipeptide histidine catalysts that Z-L-Phe-L-His (**2a**) or Z-L-Leu-L-His (**2c**) showed higher stereoselective ability as compared with the corresponding C_{12}-L-Phe-L-His (**3a**) or C_{12}-L-Leu-L-His (**3b**); thus the double-chain surfactant (**5**) constituted the stereoselectively efficient vesicular system with the dipeptide catalyst (**2a**) or (**2c**) including the group Z as its hydrophobic *N*-acyl part rather than with that (**3a**) or (**3b**) possessing the long-chain (C_{12}) *N*-acyl part. This is probably attributable to the fact that the weakly hydrophobic group Z in (**2a**) or (**2c**), as compared with the hydrophobic long-chain C_{12} group in (**3a**) or (**3b**), made the catalyst–substrate interaction favourable in the vesicle–substrate complex because of the different location of the catalysts in the vesicles; that is, (**3a** and **b**) would be expected to sit neatly in the bilayer array with its hydrocarbon tail deep in the bilayer core. In this respect, (**2a**—**d**), as well as (**1**) and (**4a** and **b**), would be expected to be located in the outer shell of the bilayer, and indeed to exist significantly within an essentially aqueous environment.

The further introduction of the L-leucine into the efficient dipeptide catalyst Z-L-Leu-L-His (**2c**) as neighbouring amino acid part bound to the L-histidine moiety did not result in a highly active tripeptide catalyst, Z-L-Leu-L-His-L-Leu (**4a**) or Z-L-Leu-L-Leu-L-His (**4b**), for the stereoselective hydrolysis of the amino acid esters. As can be seen from Table 1, the relatively low catalytic activity of (**4a**) for the stereoselective hydrolysis of (**6a**; $n = 0$—8) might be ascribed to the steric hindrance of the terminal L-Leu part in (**4a**), and the markedly low activity of (**4b**) might be due to the fact that the bending of the terminal L-His part in (**4b**) to the vesicle core through the tripeptide framework retarded the approach between the reacting positions of catalyst and substrate.

The kinetic parameters (K_b/N and k_V) obtained for the hydrolysis of (**6a**; $n = 8$) at 25 °C with the vesicular system of (**5**) and a histidine catalyst [Z-L-His (**1**), Z-L-Leu-L-His (**2c**), or Z-L-Leu-L-His-L-Leu (**4a**)] also characterized the structural effects of the histidine catalysts (Table 2). The order of the k_V values or the enantiomer k_V ratios (L/D), (**2c**) > (**4a**) ≫ (**1**), which reflects the extent of contact between the catalyst and the substrate *via* the hydrophobic interaction, was in agreement with the behaviour of the k_{cat} value of the enantiomer k_{cat} ratio (L/D), and the highly stereoselective activity of the dipeptide (**2c**) catalyst was also derived from its efficient substrate-stereoselection during the binding process, as shown in the order of the enantiomer K_b/N ratios (L/D), (**2c**) > (**1**) > (**4a**).

Table 2. Binding and rate constants for the hydrolysis of (**6a**; $n = 8$) with the histidine catalyst (**1**), (**2c**), or (**4a**) and (**5**)[a]

Catalyst	K_b/N(1 mol^{-1})			$10^2 k_V$/s^{-1}		
	L	D	L/D	L	D	L/D
Z-L-His (**1**)	1 706	1 508	1.1	1.9	1.6	1.2
Z-L-Leu-L-His (**2c**)	466	211	2.2	40	6.0	6.7
Z-L-Leu-L-His-L-Leu (**4a**)	362	496	0.7	18	3.0	6.0

[a] Tris buffer (0.08M) containing KCl (0.08M) at 25 °C (pH 7.68) in MeCN–H_2O; [(**6a**; $n = 8$)] = 1.0×10^{-5}M, [catalyst] = (3.5—5.0)-$\times 10^{-5}$M, [(**5**)] = (1—7) $\times 10^{-4}$M; [(**5**)]/[catalyst] = 20.

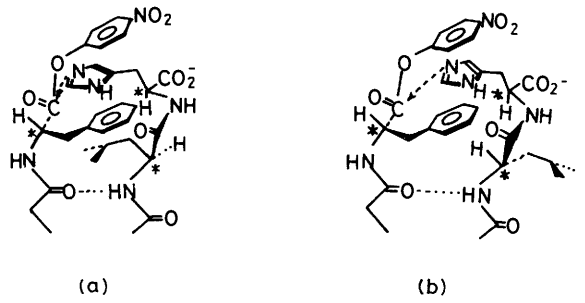


Figure 4. Schematic representation of the interaction between a dipeptide catalyst [Z-L-Leu-L-His (**2c**) or Z-D-Leu-L-His (**2d**)] and an enantiomeric L-substrate (**6a**); (a) Z-L-Leu-L-His and L-(**6a**), (b) Z-D-Leu-L-His and L-(**6a**)

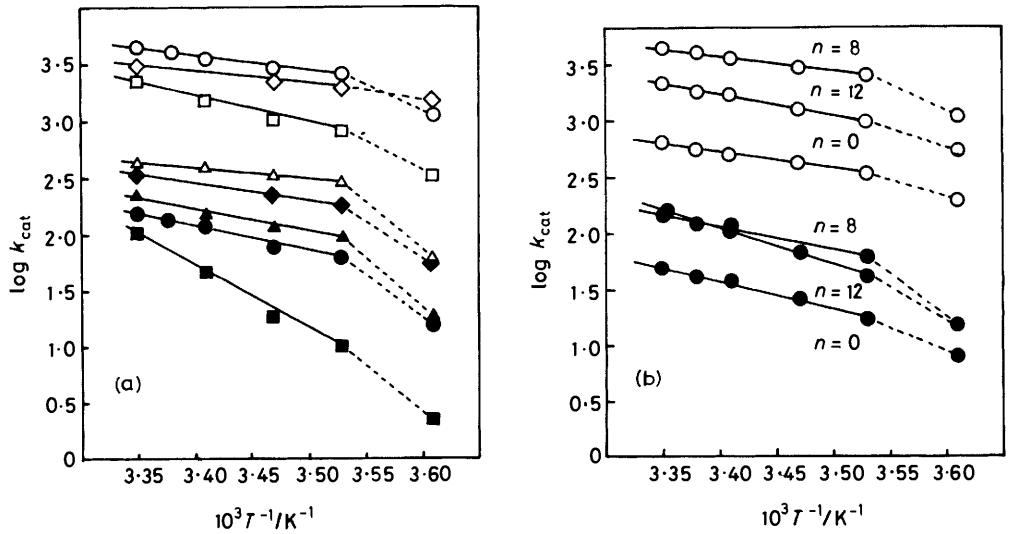


Figure 5. Typical examples of Arrhenius plots of k_{cat} *vs.* $1/T$, (a) in the hydrolysis of (**6a**; $n = 8$) with the dipeptide histidine catalysts and (**5**), and (b) in the hydrolysis of (**6a**; $n = 0$, 8, or 12) with a dipeptide catalyst (**2a**) and (**5**); (a) hydrolysis of L-substrate: (**2a**) (○), (**2b**) (△), (**2c**) (□), (**3a**) (◇), hydrolysis of D-substrate: (**2a**) (●), (**2b**) (▲), (**2c**) (■), and (**3a**) (◆); (b) L-substrate (○), D-substrate (●)

Table 3. Activation parameters for the hydrolysis of enantiomeric esters (**6a**; n = 0, 8, or 12) and (**6b**; n = 8) with histidine catalysts (**2a**—**c** and **3a**—**b**) and (**5**)[a]

Catalyst	Activation parameter[b]	(**6a**; n = 0) L	(**6a**; n = 0) D	(**6a**; n = 8) L	(**6a**; n = 8) D	(**6a**; n = 12) L	(**6a**; n = 12) D	(**6b**; n = 8) L	(**6b**; n = 8) D
(**2a**)	$\Delta H^{\ddagger}$	6.1	11.2	5.5	10.0	7.6	14.7	5.2	10.5
	$\Delta S^{\ddagger}$	−25.1	−13.3	−23.4	−15.0	−17.8	+0.7	−25.5	−13.8
(**2b**)	$\Delta H^{\ddagger}$	9.3	17.2	4.2	8.9	11.6	12.3	4.6	6.3
	$\Delta S^{\ddagger}$	−18.5	+7.5	−32.3	−17.8	−7.8	−6.9	−32.0	−27.7
(**2c**)	$\Delta H^{\ddagger}$	9.9	24.1	10.8	25.8	12.1	34.0	10.3	16.1
	$\Delta S^{\ddagger}$	−13.4	+29.6	−6.8	+37.2	−4.2	+64.7	−8.8	+4.2
(**3a**)	$\Delta H^{\ddagger}$	5.3	11.9	4.4	6.7	11.6	12.7	5.3	8.4
	$\Delta S^{\ddagger}$	−27.4	−9.0	−27.9	−24.5	−4.9	−4.3	−25.6	−18.7
(**3b**)	$\Delta H^{\ddagger}$	7.4	10.7	7.0	9.2	7.9	11.0	6.5	9.2
	$\Delta S^{\ddagger}$	−19.7	−12.5	−18.3	−14.9	−17.3	−9.1	−20.2	−15.0

[a] Obtained for the reaction carried out under the conditions in Table 1 at 10—25 °C. [b] In kcal mol^{-1} for $\Delta H^{\ddagger}$ and cal mol^{-1} K^{-1} for $\Delta S^{\ddagger}$ (1 cal = 4.184 J).

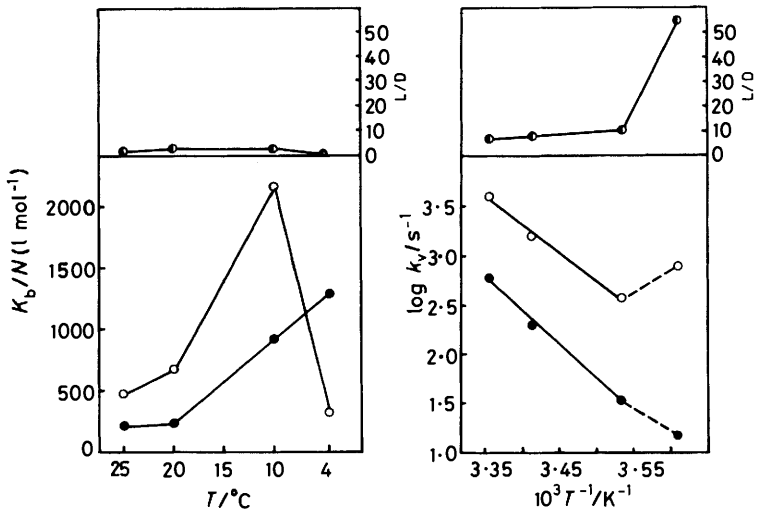


Figure 6. Temperature effects on the binding and rate constants for the hydrolysis of (**6a**; n = 8) with (**2c**) and (**5**) under the conditions [(**6a**; n = 8)] = 1 × 10^{-5}M, [(**2c**)] = (3.5—5.0) × 10^{-5}M, and [(**5**)]/[(**2c**)] = 20; ○ L-substrate, ● D-substrate

Temperature Effects on the Stereoselective Hydrolysis.—The extent of the substrate-binding by the vesicular system and of the contact between the catalyst and the substrate might be influenced by the reaction temperature. As indicated in Table 1, the lowering of the reaction temperature decreased the hydrolysis rate with increase in stereoselectivity in all cases. Since the temperature dependence of the reaction rates obtained at 10—25 °C for the present hydrolyses of the enantiomeric substrates by the different kinds of the histidine catalyses exhibits a linear Arrhenius relationship (Figure 5), the reduction in hydrolysis rate brought about by a drop in temperature with enhancement of stereoselectivity is attributable to the suppression of the mobility of the reactants; no drastic change in the microenvironment of the catalytic vesicular system occurred in the temperature range of 10—25 °C. However, all the rate constants obtained at 4 °C were found to be small as compared with those expected from the linear Arrhenius relationship in Figure 5. The deviation of the plots at 4 °C from the Arrhenius relation is related to the liquid crystal–gel phase transformation of the vesicular system, since the present kinetic results are in agreement with the two reported values[6] of the phase-transformation temperature (T_C) of the surfactant (**5**): T_C = 5—10 °C (estimated by means of a DSC method)[6a] and 10 °C (by a fusion experiment).[6b] The temperature decrease from 25 to 4 °C raised the stereoselectivity remarkably, and the change of the

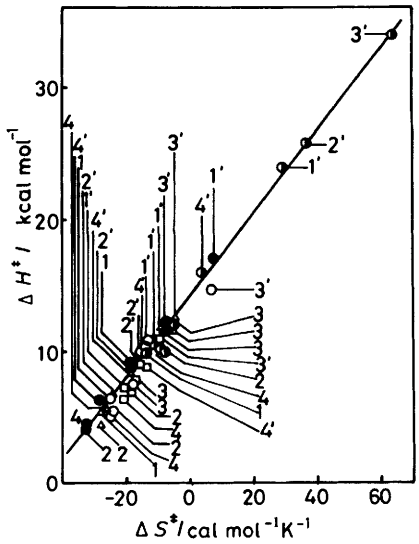


Figure 7. An isokinetic relation of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values obtained in the hydrolysis of (**6a**; n = 0, 8, or 12) and (**6b**; n = 8) with the dipeptide catalysts and (**5**); (**2a**) (○), (**2b**) (●), (**2c**) (◐), (**3a**) (△), (**3b**) (□); (**6a**) L (n = 0) (1), D (n = 0) (1′), L (n = 8) (2), D (n = 8) (2′), L (n = 12) (3), D (n = 12) (3′); (**6b**) L (n = 8) (4), D (n = 8) (4′)

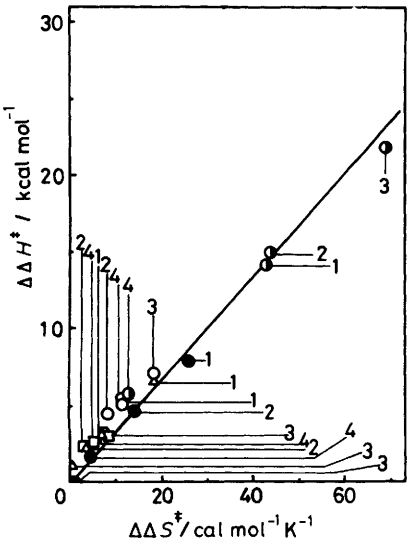


Figure 8 A closely linear correlation between $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$ values: (**2a**) (○), (**2b**) (●), (**2c**) (◑), (**3a**) (△), (**3b**) (□); (**6a**) $n = 0$ (1), $n = 8$ (2), $n = 12$ (3); (**6b**) $n = 8$ (4)

enantiomer rate ratio (L/D) from 22.5 (at 25 °C) to 148.2 (at 4 °C) in the hydrolysis of (**6a**; $n = 8$) with the efficient histidine catalyst (**2c**) and the surfactant (**5**) seems noteworthy. In the hydrolysis of (**6a**; $n = 8$) with (**2c**) and (**5**), the enhancement of substrate stereoselection by a temperature decrease is not associated with an enhanced binding process but with an increase in the extent of stereoselective reaction between the catalyst and the enantiomeric substrates in the vesicle–substrate complex (Figure 6). In this respect, it is notable that the phase transition of the vesicular system from the liquid crystal to the gel phase in the temperature range of 10—4 °C considerably encouraged reaction between the catalyst and the L-substrate in the vesicle–substrate complex, so as to result in the high stereoselectivity of the gel-phase vesicular system.

The characteristic aspects of the present stereoselective hydrolysis reaction were also reflected in the activation parameters ($\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$) for the reaction at 10—25 °C (Table 3); that is, (*a*) the smaller $\Delta H^{\ddagger}$ or larger negative $\Delta S^{\ddagger}$ values of the L-substrates, as compared with those of the D-substrates, indicate predominant attack of the present histidine catalysts on the L-substrate, even though the order of the $\Delta H^{\ddagger}$ (or $\Delta S^{\ddagger}$) values does not parallel that of the overall hydrolysis rates from both the reactions of substrate-binding and substrate-hydrolysis by the catalytic system; (*b*) the present hydrolysis reaction promoted by the catalytic vesicular system is reflected in a linear isokinetic relation (correlation coefficient 0.993; slope β = 318 K) of the activation parameters (Figure 7); and (*c*) the extent of stereoselective deacylation in the vesicle–substrate complex, which might be reflected in the difference of the activation parameters between the enantiomers ($\Delta\Delta H^{\ddagger} = \Delta H^{\ddagger}_{D} - \Delta H^{\ddagger}_{L}$ or $\Delta\Delta S^{\ddagger} = \Delta S^{\ddagger}_{D} - \Delta S^{\ddagger}_{L}$), can be expressed by a closely linear relation (correlation coefficient 0.995) between the $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$ (Figure 8).

In summary, the present stereoselective hydrolysis of the enantiomeric substrates by the vesicular system of the histidine catalyst and the double-chain surfactant required two efficient co-operative processes in the substrate-stereoselection (*viz.*, binding and deacylation). Even so, the proximity between the reacting positions of the histidine catalyst and the substrate was most important for raising the stereoselectivity during the process of substrate-deacylation in the vesicle–substrate complex. Therefore, the high catalytic activity of vesicular systems for the stereoselective hydrolysis of weakly hydrophobic substrates such as the short-chain substrates (**6a**; $n = 0$) in the present reaction might be realizable in the reaction with catalytic vesicular systems which are able to exhibit an efficient chiral microenvironment in the vesicular surface.

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