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Aggregation Behavior of Amphiphiles Functionalized with Dipeptide Segments and Enantioselective Ester Hydrolysis in Their Bilayer Membranes†

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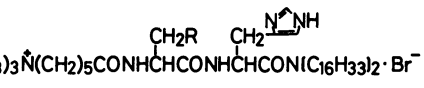
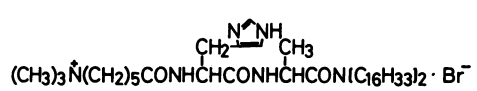
Four amphiphiles having dipeptide segments, which consist of one histidyl residue and another amino acid residue, and the dihexadecyl moiety for the double-chain segment (N^+C_5AlaHis2C_{16}, N^+C_5LeuHis2C_{16}, N^+C_5PheHis2C_{16}, and N^+C_5HisAla2C_{16}) were synthesized, and the aggregate morphology of them was characterized by electron microscopy and differential scanning calorimetry. The amphiphiles, which have the histidyl residue bound to the dihexadecylamine component (N^+C_5AlaHis2C_{16}, N^+C_5LeuHis2C_{16}, and N^+C_5PheHis2C_{16}) formed both multi- and single-walled aggregates in the aqueous dispersion state, while their sonicated solutions involved only single-walled vesicles. On the other hand, the amphiphile, which has the alanyl residue bound to the dihexadecylamine component (N^+C_5HisAla2C_{16}) afforded tubular aggregates in the aqueous dispersion. The hydrolysis of enantiomeric esters of various hydrophobic nature, which was carried out in the single-walled vesicles, showed relatively small enantioselectivity. The reasons for such catalytic performance of the vesicles were discussed from the kinetic and mechanistic viewpoints; the kinetic analysis being carried out on the basis of the organic pseudo-phase concept.

We have previously clarified that peptide amphiphiles having various amino acid residues form chemically and morphologically stable bilayer membranes in aqueous media.[1] Among them, amphiphiles bearing the histidyl residue behave not only as apoenzyme models of vitamin B_6-dependent enzymes effective in the transamination reaction of amino acids[2] but also as a functional model of hydrolytic enzymes acting on enantiomeric esters.[3]

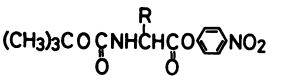
In this work, we prepared amphiphiles having dipeptide segments, which consist of one histidyl residue and another amino acid residue, and the dihexadecyl moiety for the double-chain segment. The amphiphiles having such double-chain segment are expected to show the phase transition between the gel and liquid-crystalline states in the room temperature range,[1c] so that organic reactions in the vesicular phase can be readily carried out above and below their phase transition temperatures. The aggregate morphology of these amphiphiles in aqueous media and the enantioselective ester hydrolysis in their single-walled bilayer membranes were investigated. The molecular formulae and abbreviations of the present peptide amphiphiles and of the ester substrates are shown above.



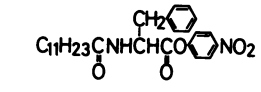
N^+C_5AlaHis2C_{16} : R =-H
N^+C_5PheHis2C_{16} : R =-C_6H_5
N^+C_5LeuHis2C_{16} : R =-$CH(CH_3)_2$



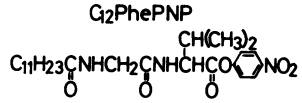
N^+C_5HisAla2C_{16}



R = -CH_3 : BocAlaPNP
R = -$CH(CH_3)_2$: BocValPNP
R = -$CH_2CH(CH_3)_2$: BocLeuPNP



C_{12}PhePNP



C_{12}GlyValPNP

Experimental

Spectroscopic data were taken on a JASCO DS-403G grating IR spectrophotometer and a Hitachi Perkin-Elmer R-20 NMR spectrometer. Melting points were measured with a Yanagimoto MP-Sl apparatus (hot-plate type). Experimental procedures for electron microscopy and differential scanning calorimetry (DSC) have been described elsewhere.[1-3] Optical rotations were measured with a Union Giken PM-71 high-sensitivity polarimeter.

N-(*t*-Butoxycarbonyl)-L-alanine, *N*-(*t*-butoxycarbonyl)-L-leucine, *N*-(*t*-butoxycarbonyl)-L-phenylalanine, *N*-(*t*-butoxycarbonyl)-N^{im}-benzyl-L-histidine, and the corresponding D-isomers were prepared from the corresponding amino acids and *O*-*t*-butyl *S*-(4,6-dimethyl-2-pyrimidinyl) thiocarbonate after the methods of Nagasawa *et al.*[4] *p*-Nitrophenyl esters of several *N*-(*t*-butoxycarbonyl)-L/D-amino acids (BocAlaPNP, BocValPNP, and BocLeuPNP) were obtained according to the procedures reported by Schnabel[5] and the products were identified by elemental analyses as well as by spectroscopic (NMR and IR) and polarimetric measurements. N^{α}-Hexadecanoyl-L-histidine (HisC$_{16}$) was prepared in a manner similar to that reported by Gitler *et al.*[6] for the preparation of N^{α}-tetradecanoylhistidine.[7] Octadecyltrimethylammonium

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chloride (STAC) of Ishizu Pharmaceutical Co. was recrystallized from aqueous ethanol.

p-Nitrophenyl N^{α}_{val}-*(*N^{α}_{gly}-*Dodecanoylglycyl)*-L-*valinate (L-C_{12}GlyValPNP).* L-BocValPNP (1.1 g, 3.3 mmol) was dissolved in dry dichloromethane (30 ml). To the solution was added trifluoroacetic acid (25 g, 0.22 mol), and the mixture was stirred for 40 min at room temperature. The solvent was removed *in vacuo*, and the solid residue was washed with diethyl ether to give a white solid (0.8 g; mp 159—162 °C (decomp)). Elimination of the *t*-butoxycarbonyl group was confirmed by NMR spectroscopy. The amine component (0.8 g, 2.3 mmol) thus obtained, *N*-dodecanoylglycine (0.6 g, 2.3 mmol), and 1-hydroxybenzotriazole[8] (0.38 g, 2.8 mmol) were dispersed in dry dichloromethane (20 ml), and the mixture was cooled to 0 °C. Triethylamine (0.24 g, 2.3 mmol) and dicyclohexylcarbodiimide (0.58 g, 2.8 mmol) were added to the mixture in this sequence. The resulting mixture was stirred for 3 h at 0 °C, and then precipitates were removed by filtration. The solvent was removed *in vacuo*, and the residue was dissolved in ethyl acetate (100 ml). The solution was washed sequentially with 10% aqueous citric acid, 4% aqueous sodium hydrogencarbonate, and saturated aqueous sodium chloride. After being dried (Na_2SO_4), the solution was evaporated *in vacuo* to give a yellow oil which was subsequently purified by gel-filtration chromatography (Sephadex LH-20, methanol as an eluant): a white solid, yield 400 mg (10%); R_f on Silica Gel IB of J. T. Baker, 0.65 (ethyl acetate) and 0.14 (benzene); mp 76—78 °C, $[\alpha]_D^{25}$ −30.3° (*c* 1.0, dioxane). Found: C, 62.46; H, 8.20; N, 8.75%. Calcd for $C_{25}H_{39}N_3O_6$: C, 62.87; H, 8.23; N, 8.80%.

p-Nitrophenyl N^{α}_{val}-*(*N^{α}_{gly}-*Dodecanoylglycyl)*-D-*valinate (D-C_{12}GlyValPNP)*: mp 74—76 °C, $[\alpha]_D^{25}$ +30.5° (*c* 1.0, dioxane). Found: C, 62.63; H, 8.54; N, 8.32%.

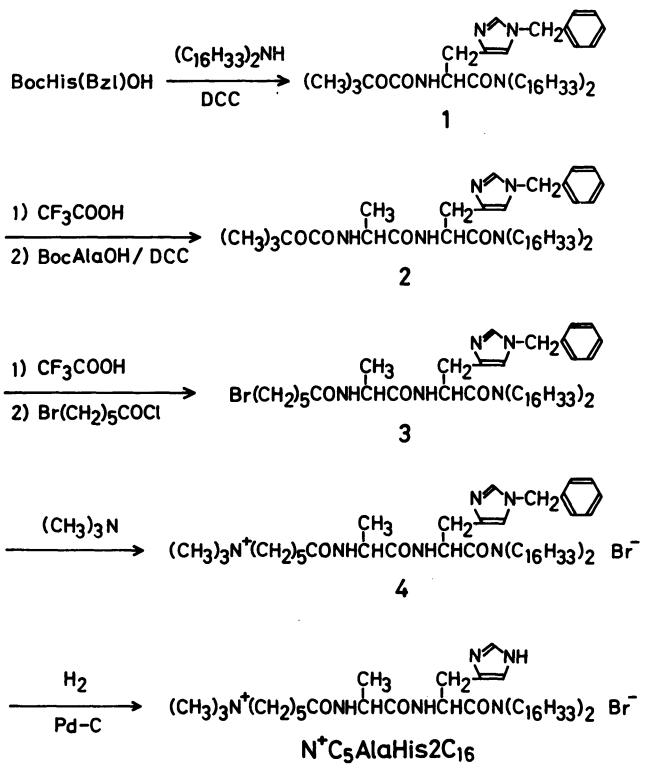
p-Nitrophenyl N^{α}-dodecanoyl-D/L-phenylalaninates were synthesized according to the procedure by Ingles.[9]

p-Nitrophenyl N^{α}-*Dodecanoyl-L-phenylalaninate (L-C_{12}-PhePNP)*: mp 110.5—111.0 °C (lit,[10] 107.0—107.5 °C), $[\alpha]_D^{25}$ −11.9° (*c* 2.0, $CHCl_3$) [lit,[10] −11.4° (*c* 2.0, $CHCl_3$)]. Found: C, 68.90; H, 7.76; N, 6.01%. Calcd for $C_{27}H_{36}N_2O_5$: C, 69.20; H, 7.74; N, 5.97%.

p-Nitrophenyl N^{α}-*Dodecanoyl-D-phenylalaninate (D-C_{12}-PhePNP)*: mp 110.5—111.0 °C (lit,[10] 107.5—108.0 °C), $[\alpha]_D^{25}$ +11.0° (*c* 2.0, $CHCl_3$) [lit,[10] +11.9° (*c* 2.0, $CHCl_3$)]. Found: C, 68.98; H, 7.76; N, 6.00%.

The synthetic procedure for *N,N*-dihexadecyl-N^{α}_{his}-[N^{α}_{ala}-(6-trimethylammoniohexanoyl)-L-alanyl]-L-histidinamide bromide (N^+C_5AlaHis2C_{16}) is outlined in Scheme 1.

N,N-Dihexadecyl-N^{α}-*(t-butoxycarbonyl)-*N^{im}-*benzyl-L-histidinamide (1).* Dicyclohexylcarbodiimide (4.7 g, 23 mmol) was added with stirring to a dry dichloromethane solution (100 ml) of *N*-(*t*-butoxycarbonyl)-N^{im}-benzyl-L-histidine (6.6 g, 19 mmol), dihexadecylamine (8.9 g, 19 mmol) and 1-hydroxybenzotriazole[8] (3.9 g, 29 mmol) at 0 °C. The mixture was stirred for 3 h at 0 °C and for further 15 h at room temperature, and precipitates (*N,N'*-dicyclohexylurea) were removed by filtration. The solvent was removed *in vacuo*, and the residual oil was dissolved in ethyl acetate (200 ml). The solution was then washed sequentially with 10% aqueous citric acid, 5% aqueous sodium hydrogencarbonate, and saturated aqueous sodium chloride. After being dried (Na_2SO_4), the mixture was evaporated *in vacuo* to give an oil which was subsequently purified by centrifugal liquid chromatography with a combination of silica gel



(Boc, t-butoxycarbonyl; Bzl, benzyl; DCC, dicyclohexylcarbodiimide)

Scheme 1.

(Wako Gel B5) and chloroform: a pale yellow solid, yield 8.1 g (54%); R_f (Silica Gel IB of J. T. Baker, ethyl acetate) 0.18, mp 63.0—65.5 °C; IR (Nujol) 3300 (NH), 1700 and 1640 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$, TMS) δ=0.88 [6H, br t, $CH_3(CH_2)_{15}N$], 1.25 [56H, s, $CH_3(CH_2)_{14}CH_2N$], 1.40 [9H, s, $(CH_3)_3COCO$], 2.85 [2H, d, $CH(CH_2ImBzl)$], 2.9—3.4 [4H, m, $CH_3(CH_2)_{14}CH_2N$], 4.95 [2H, s, benzyl CH_2], 5.20 [1H, br t, $CH(CH_2ImBzl)$], 6.63 [1H, s, Im-5H], 7.0—7.3 [5H, m, phenyl H's], and 7.33 [1H, s, Im-2H].

N,N-Dihexadecyl-N^{α}_{his}-*[*N^{α}_{ala}-*(t-butoxycarbonyl)-L-alanyl]-*N^{im}-*benzyl-L-histidinamide (2).* Trifluoroacetic acid (25 g, 220 mmol) was added to a dry dichloromethane solution (20 ml) of **1** (4.7 g, 5.9 mmol), and the mixture was stirred for 2 h at room temperature. Evaporation of an excess amount of trifluoroacetic acid *in vacuo* below 40 °C gave a pale yellow oil (**1a**). Elimination of the *t*-butoxycarbonyl group was confirmed by NMR spectroscopy. The amine (**1a**) was dissolved in dry dichloromethane (30 ml) and the solution was cooled to 0 °C. Triethylamine (1.2 g, 11 mmol), *N*-(*t*-butoxycarbonyl)-L-alanine (1.1 g, 5.9 mmol), and 1-hydroxybenzotriazole[8] (1.2 g, 8.8 mmol), and subsequently dicyclohexylcarbodiimide (1.5 g, 7.1 mmol) were added to the solution at 0 °C with stirring. The resulting mixture was stirred for 3 h at 0 °C and for further 15 h at room temperature, and precipitates (*N,N'*-dicyclohexylurea) were removed by filtration. The solvent was removed *in vacuo*, and the residual oil was dissolved in ethyl acetate (200 ml). The solution was then washed sequentially with 10% aqueous citric acid, 4% aqueous sodium hydrogencarbonate, and saturated aqueous sodium chloride. After being dried (Na_2SO_4), the mixture was evaporated *in vacuo*

to give an oil which was subsequently purified by centrifugal liquid chromatography with a combination of silica gel (Wako Gel B5) and a dual solvent system (chloroform and then methanol): a pale yellow oil, yield 1.8 g (35%); R_f (Silica Gel IB of J. T. Baker, ethyl acetate) 0.56; IR (Nujol) 3200 (NH), 1720 and 1630 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$, TMS) δ=0.85 [6H, br t, $C\underline{H}_3(CH_2)_{15}N$], 1.25 [59H, s, $CH_3(C\underline{H}_2)_{14}CH_2N$ and $CH(C\underline{H}_3)$], 1.40 [9H, s, $(CH_3)_3$-COCO], 2.85 [2H, d, $CH(C\underline{H}_2ImBzl)$], 2.7—3.8 [4H, m, $CH_3(CH_2)_{14}C\underline{H}_2N$], 3.8—4.4 [2H, m, $C\underline{H}(CH_2ImBzl)$ and $C\underline{H}(CH_3)$], 4.95 [2H, s, benzyl CH_2], 6.60 [1H, s, Im-5H], 6.9—7.4 [5H, m, phenyl H's], and 7.33 [1H, s, Im-2H].

N,N-*Dihexadecyl*-N^{α}_{his}-[N^{α}_{ala}-*(6-bromohexanoyl)*-L-*alanyl*]-N^{im}-*benzyl*-L-*histidinamide (3).* Trifluoroacetic acid (25 g, 220 mmol) was added to a dry dichloromethane solution (10 ml) of **2** (1.8 g, 2.1 mmol), and the mixture was stirred for 2 h at room temperature. Evaporation of an excess amount of trifluoroacetic acid *in vacuo* below 40 °C gave a pale yellow oil (**2a**). Elimination of the *t*-butoxycarbonyl group was confirmed by NMR spectroscopy. The amine (**2a**) was dissolved in dry dichloromethane (30 ml), and the solution was cooled to 0 °C. After triethylamine (1.7 g, 16 mmol) being added dropwise, 6-bromohexanoyl chloride (0.9 g, 4.2 mmol) dissolved in dry dichloromethane (20 ml) was added dropwise to the mixture at 0 °C with stirring. The resulting mixture was stirred for 3 h at room temperature and then washed with 4% aqueous sodium hydrogencarbonate, 10% aqueous citric acid, and saturated aqueous sodium chloride in this sequence. After being dried (Na_2SO_4), the solution was evaporated *in vacuo* to give an oil which was subsequently purified by gel-filtration chromatography on a column of silica gel (Wako Gel C-100) with ethyl acetate as an eluant: a pale yellow oil, yield 0.88 g (45%); R_f (Silica Gel IB of J. T. Baker, ethyl acetate) 0.13; IR (Nujol) 3220 (NH) and 1650 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$, TMS) δ=0.90 [6H, br t, $C\underline{H}_3(CH_2)_{15}N$], 1.25 [59H, s, $CH_3(C\underline{H}_2)_{14}CH_2N$ and $CH(C\underline{H}_3)$], *ca.* 2.0 [6H, m, $BrCH_2(C\underline{H}_2)_3CH_2CO$], 2.30 [2H, br t, $Br(CH_2)_4C\underline{H}_2CO$], 2.9—3.5 [8H, m, $CH_3(CH_2)_{14}C\underline{H}_2N$, $CH(C\underline{H}_2ImBzl)$, and $BrC\underline{H}_2(CH_2)_4CO$], 4.0—4.5 [2H, m, $C\underline{H}(CH_2ImBzl)$ and $C\underline{H}(CH_3)$], 5.40 [2H, s, benzyl CH_2], 6.95 [1H, s, Im-5H], 7.30 [5H, s, phenyl H's], and 8.95 [1H, s, Im-2H].

N,N-*Dihexadecyl*-N^{α}_{his}-[N^{α}_{ala}-*(6-trimethylammoniohexanoyl)*-L-*alanyl*]-N^{im}-*benzyl*-L-*histidinamide Bromide (4).* A benzene solution (60 ml) of **3** (880 mg, 0.94 mmol) was saturated with dry trimethylamine gas, and the solution was stirred overnight at room temperature. The solvent was removed *in vacuo* and the crude product was purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol as an eluant: a pale yellow solid, yield 610 mg (65%); R_f [Silica Gel IB of J. T. Baker, *t*-butyl alcohol–water–acetic acid (4:2:1 by volume; BWA)] 0.22; IR (Nujol) 3200 (NH) and 1630 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$, TMS) δ=0.85 [6H, br t, $C\underline{H}_3(CH_2)_{15}N$], 1.25 [59H, s, $CH_3(C\underline{H}_2)_{14}CH_2N$ and $CH(C\underline{H}_3)$], *ca.* 2.0 [6H, m, $N^+CH_2(C\underline{H}_2)_3CH_2CO$], 2.30 [2H, br t, $N^+(CH_2)_4C\underline{H}_2CO$], 2.8—3.7 [8H, m, $CH_3(CH_2)_{14}C\underline{H}_2N$, $CH(C\underline{H}_2ImBzl)$, and $N^+C\underline{H}_2(CH_2)_4CO$], 3.40 [9H, s, $(CH_3)_3N^+$], 4.50 [2H, m, $C\underline{H}(CH_2ImBzl)$ and $C\underline{H}(CH_3)$], 5.30 [2H, s, benzyl CH_2], 7.00 [1H, s, Im-5H], 7.30 [5H, s, phenyl H's], and 8.10 [1H, s, Im-2H].

N,N-*Dihexadecyl*-N^{α}_{his}-[N^{α}_{ala}-*(6-trimethylammoniohexanoyl)*-L-*alanyl*]-L-*histidinamide Bromide* ($N^+C_5AlaHis2C_{16}$). A 700-mg sample of 10% PdC was added to **4** (610 mg, 0.61 mmol) dissolved in 80% aqueous acetic acid (40 ml), and hydrogen gas was introduced into the solution at 60 °C for 30 h with stirring. The catalyst was removed by filtration on Celite (Wako No. 545) with methanol. The filtrate was evaporated off *in vacuo*, and the residue was purified by gel-filtration chromatography on a column of Toyopearl HW-40 Fine with methanol as an eluant: a glassy solid, yield 360 mg (65%); R_f (Silica Gel IB of J. T. Baker, BWA) 0.20; mp 176 °C,[11] $[\alpha]_D^{26}$ −24.5° (*c* 1.0, ethanol); IR (Nujol) 1630 cm^{-1} (C=O); ^{1}H-NMR (CD_3OD, TMS) δ=0.90 [6H, br t, $C\underline{H}_3(CH_2)_{15}N$], 1.25 [59H, s, $CH_3(C\underline{H}_2)_{14}CH_2N$ and $CH(C\underline{H}_3)$], *ca.* 2.0 [6H, m, $N^+CH_2(C\underline{H}_2)_3CH_2CO$], 2.30 [2H, br t, $N^+(CH_2)_4C\underline{H}_2CO$], 2.8—3.4 [8H, m, $CH_3(CH_2)_{14}C\underline{H}_2N$, $CH(C\underline{H}_2Im)$, and $N^+C\underline{H}_2(CH_2)_4CO$], 3.10 [9H, s, $(CH_3)_3N^+$], 6.80 [1H, s, Im-5H], 7.60 [1H, s, Im-2H], the signals of $C\underline{H}CH_3$ and $C\underline{H}(CH_2Im)$ being obscured by the signal due to CD_3OH.

Similar experimental procedures were applied to the preparation of *N,N*-dihexadecyl-N^{α}_{his}-[N^{α}_{leu}-(6-trimethylammoniohexanoyl)-L-leucyl]-L-histidinamide bromide (N^+C_5-LeuHis2C_{16}), *N,N*-dihexadecyl-N^{α}_{his}-[N^{α}_{phe}-(6-trimethylammoniohexanoyl)-L-phenylalanyl]-L-histidinamide bromide (N^+C_5PheHis2C_{16}), and *N,N*-dihexadecyl-N^{α}_{ala}-[N^{α}_{his}-(6-trimethylammoniohexanoyl)-L-histidyl]-L-alaninamide bromide (N^+C_5HisAla2C_{16}).

$N^+C_5LeuHis2C_{16}$: mp 126 °C,[11] $[\alpha]_D^{26}$ −27.8° (*c* 1.0, ethanol), R_f (Silica Gel IB of J. T. Baker, BWA) 0.14; IR (Nujol) 1630 cm^{-1} (C=O); ^{1}H-NMR (CD_3OD, TMS) δ=0.70 [12H, m, $C\underline{H}_3(CH_2)_{15}N$ and $CH_2CH(C\underline{H}_3)_2$], 1.05 [59H, br s, $CH_3(C\underline{H}_2)_{14}CH_2N$ and $C\underline{H}_2CH(CH_3)_2$], *ca.* 2.0 [6H, m, $N^+CH_2(C\underline{H}_2)_3CH_2CO$], 2.05 [2H, br t, $N^+(CH_2)_4C\underline{H}_2CO$], 2.4—3.3 [8H, m, $CH_3(CH_2)_{14}C\underline{H}_2N$, $CH(C\underline{H}_2Im)$, and $N^+C\underline{H}_2(CH_2)_3CH_2CO$], 2.90 [9H, s, $N^+(CH_3)_3$], 6.60 [1H, s, Im-5H], 7.30 [1H, s, Im-2H], the signals of $C\underline{H}CH_2CH$-$(CH_3)_2$ and $C\underline{H}(CH_2Im)$ being obscured by the signal due to CD_3OH.

$N^+C_5PheHis2C_{16}$: mp 130 °C,[11] $[\alpha]_D^{26}$ −11.4° (*c* 1.0, ethanol), R_f (Silica Gel IB of J. T. Baker, BWA) 0.09; IR (Nujol) 1640 cm^{-1} (C=O); ^{1}H-NMR (CD_3OD, TMS) δ= 0.90 [6H, br t, $C\underline{H}_3(CH_2)_{15}N$], 1.20 [56H, s, $CH_3(C\underline{H}_2)_{14}$-$CH_2N$], *ca.* 2.0 [6H, m, $N^+CH_2(C\underline{H}_2)_3CH_2CO$], 2.30 [2H, br t, $N^+(CH_2)_4C\underline{H}_2CO$], 2.7—3.5 [10H, m, $CH_3(CH_2)_{14}$-$C\underline{H}_2N$, benzyl CH_2, and $N^+C\underline{H}_2(CH_2)_4CO$], 3.20 [9H, s, $(CH_3)_3N^+$], 7.00 [1H, s, Im-5H], 7.20 [5H, s, phenyl H's], the signals of Im-2H, $C\underline{H}Bzl$, and $C\underline{H}(CH_2Im)$ being obscured by the signal due to CD_3OH.

$N^+C_5HisAla2C_{16}$: mp 105 °C,[11] $[\alpha]_D^{26}$−11.9° (*c* 1.0, ethanol), R_f (Silica Gel IB of J. T. Baker, BWA) 0.20; IR (Nujol) 1625 cm^{-1} (C=O); ^{1}H-NMR (CD_3OD, TMS) δ=0.90 [6H, br t, $C\underline{H}_3(CH_2)_{15}N$], 1.28 [59H, s, $CH_3(C\underline{H}_2)_{14}CH_2N$ and $CH(C\underline{H}_3)$], *ca.* 2.0 [6H, m, $N^+CH_2(C\underline{H}_2)_3CH_2CO$], 2.30 [2H, br t, $N^+(CH_2)_4C\underline{H}_2CO$], 2.9—3.5 [8H, m, $CH_3(CH_2)_{14}$-$C\underline{H}_2N$, $CH(C\underline{H}_2Im)$, and $N^+C\underline{H}_2(CH_2)_4CO$], 3.10 [9H, s, $(CH_3)_3N^+$], 7.05 [1H, s, Im-5H], 8.10 [1H, s, Im-2H], the signals of $C\underline{H}(CH_2Im)$ and $C\underline{H}CH_3$ being obscured by the signal due to CD_3OH.

All the elemental analysis data for the present amphiphiles are summarized in Table 1.

Kinetic Measurements. Rates of *p*-nitrophenol liberation from *p*-nitrophenyl esters were measured at 400 nm with a Union Giken SM-401 high-sensitivity spectrophotometer.

Each run was initiated by adding a dioxane solution (20 μl) of a *p*-nitrophenyl ester (*ca.* 1.0×10^{-3} mol dm^{-3}) to a reaction medium (2.0 ml) which was pre-equilibrated at a desired temperature in a thermostated cell set in the spectrophotometer. The reaction medium was prepared as follows: an ethanol solution of a dipeptide amphiphile (90 μl, *ca.* 5.0×10^{-2} mol dm^{-3}) was taken into a sample tube and the solvent was removed completely. A 4.5-ml sample of an aqueous buffer was added to the residue, and the mixture was sonicated for 3 min with a probe-type sonicator at 30 W (W-220F, Heat Systems-Ultrasonics) and allowed to stand for 1 min at 5 °C. The ionic strength of sample solutions for the measurements was maintained at 0.05 with KCl.

Results and Discussion

Phase Transition and Aggregate Morphology. The phase transition parameters (enthalpy change, ΔH; temperature at peak maximum, T_m) of the molecular aggregates formed with the present amphiphiles in the aqueous dispersion were directly measured by differential scanning calorimetry (DSC) as summarized in Table 1. The T_m values of these amphiphiles are nearly identical with those of peptide amphiphiles having the same alkyl moieties (hexadecyl) in the double-chain portion. This indicates that the T_m value of the peptide amphiphile primarily depends on the alkyl-chain length of double-chain portion.[1c] On the other hand, the ΔH values are somewhat smaller than those of amphiphiles having the same double-chain segment prepared previously in our laboratory; *e.g.*, $N^+C_5Ala2C_{16}$, 32.6 kJ mol^{-1}.[1c] Since the ΔH value for the single-walled aggregate is generally much smaller than that for the corresponding multiwalled one,[1c] the present ΔH values seem to indicate that both single- and multi-walled aggregates are present in the aqueous dispersion state. This was clearly confirmed by electron microscopy. As shown in Fig. 1A, the aqueous dispersion of $N^+C_5LeuHis2C_{16}$ apparently involves single-walled vesicles in addition to multiwalled lamellae and vesicles. Similar electron micrographs were obtained with the aqueous dispersions of $N^+C_5AlaHis2C_{16}$ and $N^+C_5PheHis2C_{16}$. Such morphological behavior is reflected on the solubility properties of these amphiphiles; these three amphiphiles are more soluble and much less turbid in the

Table 1. Physical and analytical data of amphiphiles

Amphiphile	Formula	Elemental analysis[a] /%			DSC	
		C	H	N	T_m/°C	ΔH/kJ mol^{-1}
$N^+C_5AlaHis2C_{16}$	$C_{50}H_{97}BrN_6O_3+5/2H_2O$	62.78 (62.87)	10.57 (10.76)	8.60 (8.80)	24.8	27.6
$N^+C_5LeuHis2C_{16}$	$C_{53}H_{103}BrN_6O_3$	66.54 (66.84)	11.03 (10.90)	8.67 (8.83)	24.5	22.6
$N^+C_5PheHis2C_{16}$	$C_{56}H_{101}BrN_6O_3+3/2H_2O$	66.28 (66.37)	10.45 (10.34)	7.96 (8.29)	22.5	22.6
$N^+C_5HisAla2C_{16}$	$C_{50}H_{97}BrN_6O_3+3H_2O$	62.42 (62.28)	10.43 (10.76)	8.43 (8.71)	27.0	25.9

a) Calculated values are given in parentheses.

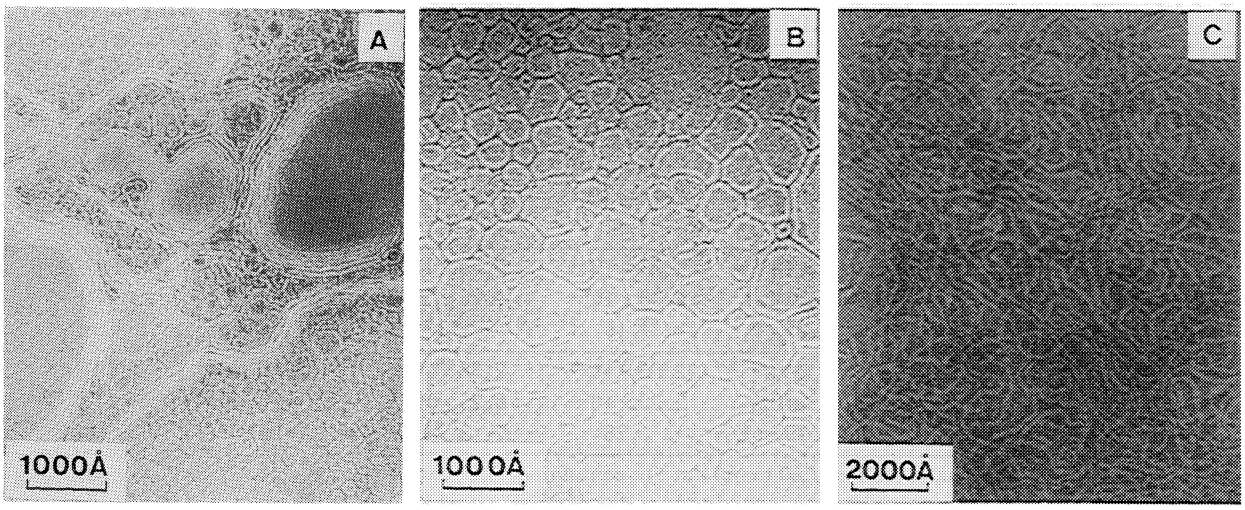


Fig. 1. Electron micrographs negatively stained with uranyl acetate: A, 5 mmol dm^{-3} aqueous dispersion of $N^+C_5LeuHis2C_{16}$; B, 5 mmol dm^{-3} aqueous solution of $N^+C_5LeuHis2C_{16}$ sonicated for 2 min at 30 W and subsequently allowed to stand for 5 min at 5 °C; C, 5 mmol dm^{-3} aqueous dispersion of $N^+C_5HisAla2C_{16}$.

dispersion state than other amphiphiles which have been synthesized previously in our laboratory.[1-3] The single-walled vesicles have never been observed with the latter amphiphiles in the dispersion state without sonication. The multiwalled aggregates were completely converted into the single-walled vesicles upon sonication of these aqueous dispersions for 2 min with a probe-type sonicator (Fig. 1B). Meanwhile, the aqueous dispersion of $N^+C_5HisAla2C_{16}$ involves exclusively tubular aggregates as clarified by electron microscopy (Fig. 1C). This indicates that the sequence of amino acid residues in the hydrogen-belt domain may largely control the aggregate morphology in the dispersion state.

Apparent Enantioselectivity in Ester Hydrolysis. The enantiomeric selectivity of the single-walled vesicles of the present amphiphiles exercised in the hydrolysis of various ester substrates was evaluated on the basis of apparent pseudo-first-order rate constants in an aqueous phosphate buffer (pH 7.47, μ 0.05 with KCl) at 15.0±0.1 °C. The first-order kinetics was found to hold at least up to 60% conversion of the substrate species under the conditions [S] ≪ [D]; S and D stand for substrate and amphiphile species, respectively (Table 2). In the micellar system of $HisC_{16}$ and STAC, the L-substrates are more favorably hydrolyzed than the corresponding D-isomers. On the other hand, the enantioselectivity demonstrated by the membrane systems depends on the hydrophobic nature of substrates employed; the L-isomers are more favorably hydrolyzed when less hydrophobic substrates are used (BocAlaPNP, BocValPNP, and BocLeuPNP), while the D-isomer is more reactive than the corresponding L-isomer in the case of C_{12}GlyValPNP that is largely hydrophobic. Above all, all the D-isomers are preferentially hydrolyzed in the single-walled vesicles of $N^+C_5LeuHis2C_{16}$ although the extent of selectivity is rather small.

Kinetic Analysis by Pseudo-Phase Concept. Various micellar reactions have been quantitatively analyzed on the basis of the Michaelis-Menten-type kinetics,[12] the ion-exchange model treatment,[13] and the pseudo-phase concept.[14] We employed in this work the pseudo-phase concept due to the following reasons. (i) The Michaelis-Menten treatment is based on the assumption that the interaction between substrate and aggregate species takes place at the 1:1 ratio; each aggregate incorporates only one substrate molecule. The aggregation number of the present single-walled vesicles is at least 4000—5000,[1c] while that of ordinary micelles is *ca.* 100.[12] In the light of our previous study, a much larger number of substrate molecules are incorporated into each vesicle and the binding site concept needs to be introduced.[15] (ii) In the present reaction systems, the histidyl residue is covalently bound to amphiphile molecules, and consequently the catalyst species are exclusively placed in the vesicular domain and not in the bulk aqueous

TABLE 2. APPARENT FIRST-ORDER RATE CONSTANTS FOR MICELLE- AND VESICLE-CATALYZED HYDROLYSIS OF ENANTIOMERIC ESTERS[a]

Catalyst[b]		Substrates				
		BocAlaPNP	BocValPNP	BocLeuPNP	C_{12}PhePNP	C_{12}GlyValPNP
$N^+C_5AlaHis2C_{16}$	k_L/s^{-1}	6.68×10^{-3}	1.81×10^{-3}	2.08×10^{-2}	3.90×10^{-2}	8.38×10^{-3}
(1.00×10^{-3})	k_D/s^{-1}	6.31×10^{-3}	1.34×10^{-3}	2.02×10^{-2}	4.09×10^{-2}	1.15×10^{-2}
	k_L/k_D	1.06	1.35	1.03	1.05[c]	1.37[c]
$N^+C_5PheHis2C_{16}$	k_L/s^{-1}	5.70×10^{-3}	2.19×10^{-3}	2.08×10^{-2}	5.05×10^{-2}	1.08×10^{-2}
(1.01×10^{-3})	k_D/s^{-1}	4.80×10^{-3}	1.56×10^{-3}	1.97×10^{-2}	5.11×10^{-2}	1.29×10^{-2}
	k_L/k_D	1.19	1.40	1.06	1.01[c]	1.20[c]
$N^+C_5LeuHis2C_{16}$	k_L/s^{-1}	7.05×10^{-3}	7.00×10^{-4}			1.06×10^{-2}
(1.05×10^{-5})	k_D/s^{-1}	7.77×10^{-3}	7.74×10^{-4}			1.26×10^{-2}
	k_D/k_L	1.10	1.11			1.19
$N^+C_5HisAla2C_{16}$	k_L/s^{-1}	2.46×10^{-3}	4.83×10^{-4}	5.55×10^{-3}	1.76×10^{-2}	3.95×10^{-3}
(1.01×10^{-3})	k_D/s^{-1}	2.35×10^{-3}	4.08×10^{-4}	4.76×10^{-3}	1.88×10^{-2}	7.60×10^{-3}
	k_L/k_D	1.05	1.18	1.17	1.07[c]	1.92[c]
$HisC_{16}$/STAC	k_L/s^{-1}	8.51×10^{-3}	2.56×10^{-3}	2.38×10^{-2}	1.10×10^{-1}	1.68×10^{-2}
(6.73×10^{-5}/	k_D/s^{-1}	7.14×10^{-3}	2.00×10^{-3}	8.55×10^{-3}	2.80×10^{-2}	8.37×10^{-3}
1.01×10^{-3})	k_L/k_D	1.19	1.28	2.78	3.93	2.01

a) In an aqueous phosphate buffer (5.0 mmol dm^{-3}) containing 1.0%(v/v) dioxane, 15.0±0.1 °C, pH 7.47, μ 0.05 (KCl). Initial concentrations in mol dm^{-3}: L-BocAlaPNP, 1.04×10^{-5}; D-BocAlaPNP, 1.01×10^{-5}; L-BocValPNP, 1.22×10^{-5}; D-BocValPNP, 1.29×10^{-5}; L-BocLeuPNP, 1.01×10^{-5}; D-BocLeuPNP, 1.07×10^{-5}; L-C_{12}PhePNP, 1.23×10^{-5}; D-C_{12}PhePNP, 1.10×10^{-5}; L-C_{12}GlyValPNP, 1.03×10^{-5} (vesicle), 5.15×10^{-6} (micelle); D-C_{12}GlyValPNP, 1.09×10^{-5} (vesicle), 5.45×10^{-6} (micelle). b) At the concentration (mol dm^{-3}) given in parentheses. c) Given as k_D/k_L.

phase. In addition, the ion-exchange treatment is not applicable to the present systems since all the substrates employed here are hydrophobic and nonionic. (iii) The bilayer domain may provide a steady and hydrophobic reaction site which is considered to be an organic pseudo-phase.

According to the Berezin's treatment based on the pseudo-phase concept, the pseudo-first-order rate constant, k_{obsd}, is given by Eq. 1 under the conditions employed for the present systems.[14,16]

$$k_{obsd} = \frac{k_m K_s D}{1 + K_s D} \quad (1)$$

This can be rearranged to give Eq. 2.

$$\frac{1}{k_{obsd}} = \frac{1}{k_m K_s D} + \frac{1}{k_m} \quad (2)$$

Here, k_m and K_s represent the intramembrane rate constant and the apparent binding constant for incorporation of a substrate into the single-walled vesicle, respectively; D designates the stoichiometric amphiphile concentration minus its critical aggregate concentration (CAC). A correlation of k_{obsd} with D is typically shown in Fig. 2a for the degradation of L-BocValPNP as catalyzed by N^+C_5AlaHis2C$_{16}$ and followed the saturation-type kinetics. The plot of $1/k_{obsd}$ *vs.* $1/D$ on the basis of Eq. 2 gave a straight line as illustrated in Fig. 2b. The k_m and K_s values for selected cases are summarized in Table 3. The K_s values for C_{12}GlyValPNP are larger than those for BocValPNP. This indicates that the substrate incorporation is largely controlled by the hydrophobic property of the substrates in addition to some orientational effect provided by the bilayer membranes.

As for the incorporation of BocValPNP, the binding constant increases in the following order with respect

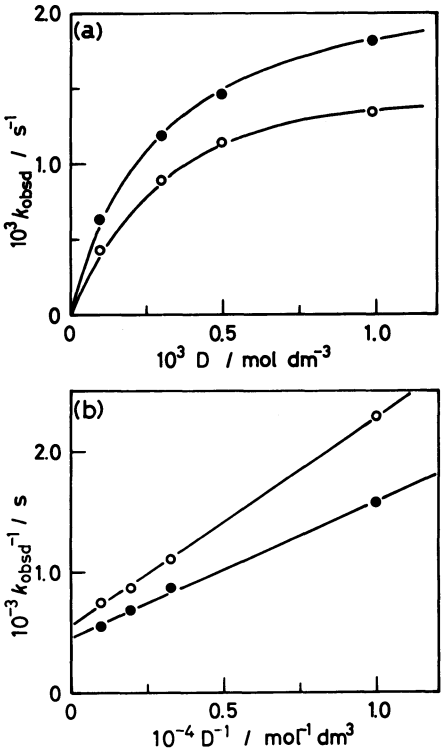


Fig. 2. Saturation-type kinetic behavior (a) and the corresponding double reciprocal plots (b) for the hydrolysis of L- (●) and D-BocValPNP (○) as catalyzed by N^+C_5AlaHis2C$_{16}$ in an aqueous phosphate buffer (5 mmol dm^{-3}) containing 1.0%(v/v) dioxane: pH 7.47, μ 0.05 (KCl), 15.5±0.1 °C. Initial concentrations: L-BocValPNP, 1.22×10^{-5}; D-BocValPNP, 1.29×10^{-5} mol dm^{-3}.

Table 3. Kinetic parameters for the hydrolysis of enantiomeric esters as catalyzed by vesicles[a]

Amphiphile[b]	Substrate		k_m/s^{-1}	K_s/mol^{-1} dm^3
N^+C_5AlaHis2C$_{16}$ (1.00×10^{-3}—1.00×10^{-4})	BocValPNP	L	2.15×10^{-3}	4.19×10^3
		D	1.65×10^{-3}	3.63×10^3
		L/D[c]	1.30	1.15
	C_{12}GlyValPNP	L	8.69×10^{-3}	2.16×10^4
		D	1.21×10^{-2}	1.13×10^4
		L/D[c]	1.39[d]	1.91
N^+C_5LeuHis2C$_{16}$ (1.05×10^{-3}—1.05×10^{-4})	BocValPNP	L	4.68×10^{-3}	1.76×10^2
		D	6.26×10^{-3}	1.41×10^2
		L/D[c]	1.34[d]	1.25
	C_{12}GlyValPNP	L	1.17×10^{-2}	9.95×10^3
		D	1.38×10^{-2}	1.06×10^4
		D/L[d]	1.18	1.07
N^+C_5PheHis2C$_{16}$ (1.01×10^{-3}—1.01×10^{-4})	BocValPNP	L	5.26×10^{-3}	6.90×10^2
		D	4.10×10^{-3}	6.00×10^2
		L/D[c]	1.28	1.15
N^+C_5HisAla2C$_{16}$ (1.01×10^{-3}—1.01×10^{-4})	C_{12}GlyValPNP	L	4.40×10^{-3}	1.93×10^4
		D	8.47×10^{-3}	7.20×10^3
		L/D[c]	1.93[d]	2.68

a) In an aqueous phosphate buffer (5.0 mmol dm^{-3}) containing 1.0%(v/v) dioxane, 15.0±0.1 °C, pH 7.47, μ 0.05 (KCl). As for initial concentrations of the substrates, see Table 2. b) At the concentration range (mol dm^{-3}) given in parantheses. c) Given as k_m^L/k_m^D and/or K_s^L/K_s^D. d) Given as k_m^D/k_m^L and/or K_s^D/K_s^L.

to the amphiphiles: N^+C_5LeuHis2C_{16}<N^+C_5PheHis2C_{16}<N^+C_5AlaHis2C_{16}. This means that the less the steric hindrance between the amino acid residue of the substrate and those of the amphiphiles is, the larger the substrate binding is. On the other hand, the k_m value does not change appreciably by the nature of the amphiphiles but increases largely by raising the hydrophobicity of the substrate: BocValPNP, $(1.6—6.3)\times10^{-3}$ s^{-1}; C_{12}GlyValPNP, $(0.4—1.4)\times10^{-2}$ s^{-1}. In addition, the apparent enantioselectivity (see the previous section) is primarily reflected on the k_m value.

pH Effect on Ester Hydrolysis. The pH-rate profiles for the hydrolysis of C_{12}GlyValPNP carried out in the micellar system composed of $HisC_{16}$ and STAC and in the vesicular phase of N^+C_5HisAla2C_{16} are shown in Fig. 3. The overall kinetic feature is in agreement with those of the previous studies which claim that the imidazolyl group is the catalytic species.[6,17] The acid dissociation from the cationic imidazolium group to the corresponding uncharged species gives the following pK_a values from the pH-rate profiles: $HisC_{16}$/STAC, 6.2; N^+C_5HisAla2C_{16}, 7.2. Thus, the present kinetic study mentioned above was carried out under the conditions that the uncharged imidazolyl group acts as the catalytic species. The imidazolyl group in the present micellar system must be placed in the cationic polar domain in the light of the previous investigations: *N*-tetradecanoylhistidine/CTAB, pK_a 6.20;[6] $HisC_{16}$/N^+C_5AlaC_{12}, pK_a 6.16.[7] On the other hand, the higher pK_a value observed in the vesicular system indicates that the imidazolyl group is placed in the significantly hydrophobic environment.

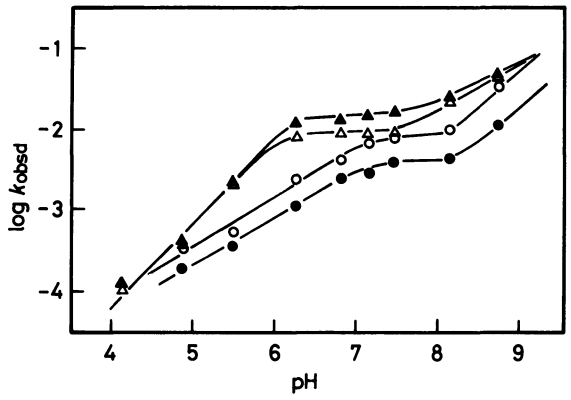


Fig. 3. Correlations of pH *vs.* rate for the hydrolysis of C_{12}GlyValPNP (L-Val, closed; D-Val, open) as catalyzed by the micellar system ($HisC_{16}$/STAC, triangle) and the vesicular system (N^+C_5HisAla2C_{16}, circle) in aqueous buffers containing 1.0% (v/v) dioxane at 15.0±0.1 °C and μ 0.05 (KCl): pH 4—6, citrate–borate buffer; pH 6—9, phosphate–borate buffer. Initial concentrations for the vesicular system: N^+C_5HisAla2C_{16}, 1.01×10^{-3}; L-C_{12}GlyValPNP, 1.03×10^{-5}; D-C_{12}GlyValPNP, 1.09×10^{-5} mol dm^{-3}. Initial concentrations for the micellar system: STAC, 1.01×10^{-3}; $HisC_{16}$, 6.73×10^{-5}; L-C_{12}GlyValPNP, 5.15×10^{-6}; D-C_{12}GlyValPNP, 5.45×10^{-6} mol dm^{-3}. Values of k_{obsd} are given in s^{-1}.

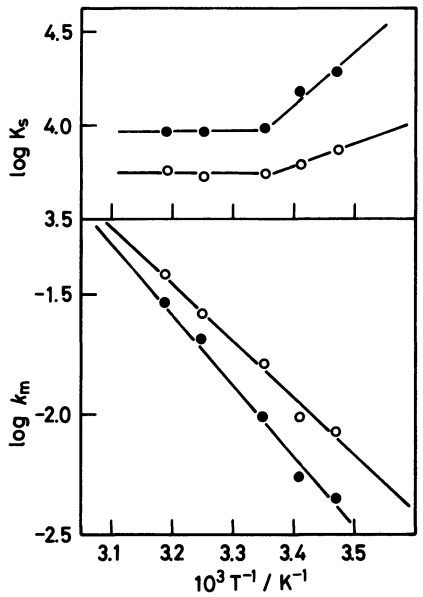


Fig. 4. Arrhenius plots for the hydrolysis of C_{12}-GlyValPNP (L-Val, ●; D-Val, ○) as catalyzed by N^+C_5HisAla2C_{16} in an aqueous phosphate buffer (5 mmol dm^{-3}) containing 1.0% (v/v) dioxane at pH 7.47 and μ 0.05 (KCl). Initial concentrations: N^+C_5HisAla2C_{16}, 1.01×10^{-3}; L-C_{12}GlyValPNP, 1.03×10^{-5}; D-C_{12}GlyValPNP, 1.09×10^{-5} mol dm^{-3}. Values of k_m and K_s are given in s^{-1} and mol^{-1} dm^3, respectively.

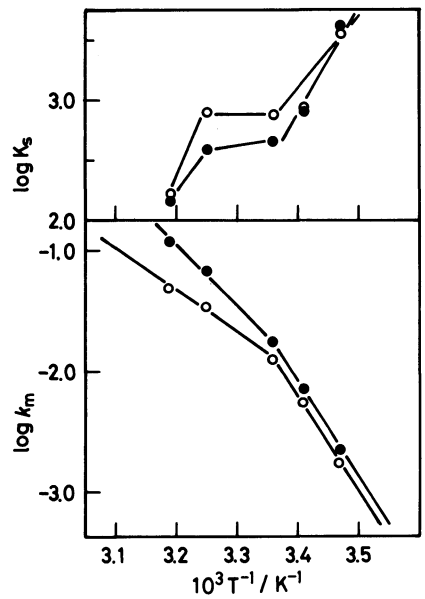


Fig. 5. Arrhenius plots for the hydrolysis of Boc-ValPNP (L-Val, ●; D-Val, ○) as catalyzed by N^+C_5AlaHis2C_{16} in an aqueous phosphate buffer (5 mmol dm^{-3}) containing 1% (v/v) dioxane at pH 7.47 and μ 0.05 (KCl). Initial concentrations: N^+C_5Ala-His2C_{16}, 1.00×10^{-3}; L-BocValPNP, 1.22×10^{-5}; D-BocValPNP, 1.29×10^{-5} mol dm^{-3}. Values of k_m and K_s are given in s^{-1} and mol^{-1} dm^3, respectively.

Table 4. Activation parameters for the hydrolysis of enantiomeric esters as catalyzed by vesicles[a)]

Amphiphile[b)]	Substrate	ΔG^{*}_{308} / kJ mol^{-1}	ΔH^{*} / kJ mol^{-1}	ΔS^{*} / J K^{-1} mol^{-1}
$N^{+}C_5HisAla2C_{16}$	L-C_{12}GlyValPNP	85.4	56.1	−95.8
(1.01×10^{-3})	D-C_{12}GlyValPNP	84.9	43.9	−133
	L-BocValPNP[c)]	82.4	77.4	−16.7
$N^{+}C_5AlaHis2C_{16}$	L-BocValPNP[d)]		144	−205
(1.00×10^{-3})	D-BocValPNP[c)]	84.1	67.8	−54.0
	D-BocValPNP[d)]		142	−197

a) In an aqueous phosphate buffer (5.0 mmol dm^{-3}) containing 1.0%(v/v) dioxane, pH 7.47, μ 0.05 (KCl). Initial concentrations in mol dm^{-3}: L-C_{12}GlyValPNP, 1.03×10^{-5}; D-C_{12}GlyValPNP, 1.09×10^{-5}; L-BocValPNP, 1.22×10^{-5}; D-BocValPNP, 1.29×10^{-5}. b) At the concentration (mol dm^{-3}) given in parentheses. c) For the range of 25—40 °C. d) For the range of 15—25 °C.

Phase-Transition Effect on Substrate-Binding and Intravesicular Rate Constants. The Arrhenius plots of the K_s and k_m values are shown in Figs. 4 and 5 for the combinations of $N^{+}C_5HisAla2C_{16}$–C_{12}GlyValPNP and $N^{+}C_5AlaHis2C_{16}$–BocValPNP, respectively. Apparently, the K_s values are much lower above the phase transition temperatures than the corresponding values below them. This seems to indicate that the fluidity of vesicular systems, which tends to be reduced as the hydrophobic and hydrogen-bonding interactions are enhanced, controls the substrate-binding process. As regards the Arrhenius plots of k_m values, there are break points in the phase transition range for the hydrolysis of both enantiomers of BocValPNP, while only a single straight line is drawn for the hydrolysis of each enantiomer of C_{12}GlyValPNP. This must reflect the fact that the BocValPNP molecules are placed in the vesicular surface domain between the charged head group and the hydrogen-belt region and consequently the molecular mobility of such substrates is much sensitive to their hydrogen-bonding interaction with the vesicular phase. On the other hand, the C_{12}GlyValPNP molecules are tightly fixed in the vesicular phase through the effective hydrophobic interaction, and the mobility of these molecules are hardly affected by the change in hydrogen-bonding mode. As for the hydrolysis of C_{12}GlyValPNP with $N^{+}C_5HisAla2C_{16}$ over the whole temperature range studied and that of BocValPNP with $N^{+}C_5AlaHis2C_{16}$ above the phase transition temperature, the unfavorable activation enthalpy is largely compensated by the favorable activation entropy. This is the reason why the enantioselectivity is not significantly large. Moreover, the absolute values of activation entropy are much smaller above T_m than those observed below it for the hydrolysis of BocValPNP. This must be due to the increased mobility of the substrate species above the phase transition temperature.

As regards the reaction mechanism, we have confirmed that the micelles formed with the peptide amphiphile having the histidyl residue enhances the ester hydrolysis partly by the general base catalysis.[7)] In the present vesicular systems, approximately half of the reaction proceeds through the general base mechanism; the hydrolysis of BocValPNP with $N^{+}C_5LeuHis2C_{16}$, 54% general base catalysis.[18)] This mechanism is operative in these molecular aggregate systems so as to reduce the direct steric hindrance between the substrate molecules and the active reaction sites within the vesicles. Thus, this mechanistic aspect provides the important reason why the enantioselectivity is not significantly large in the present systems.

In conclusion, the peptide amphiphiles having dipeptide segments yield characteristic molecular aggregates in the dispersion state, and the sequence of the amino acid residues may control the aggregate morphology. The hydrolysis reactions of enantiomeric esters of various hydrophobic nature, which were carried out in the single-walled vesicles, did not show large enantioselectivity primarily due to thermodynamic and mechanistic reasons. Since enzymes generally incorporate their specific substrates into their reaction sites most adequately for the transformation to the transition state from the stereochemical viewpoint,[19)] amphiphile molecules need to be designed so as to provide the efficient enantiomer-recognition sites through host-guest interactions.

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16) The observed reaction velocity, v, is given by the following equation.

$$v = v_o D\overline{V} + v_w(1-D\overline{V}) = k_o[A]_o[S]_o D\overline{V} + k_w[A]_w[S]_w(1-D\overline{V}) \quad (1\text{-}1)$$

Here, D, $\overline{V}$, and k stand for the amphiphile concentration minus its critical aggregate concentration (CAC), the partial molar volume of the amphiphile in the membrane, and the second-order rate constant, respectively; [A] and [S] are the concentrations of the amphiphile and the ester substrate, respectively; the subscripts, o and w, denote the quantities in the organic pseudo-phase and the bulk aqueous phase, respectively. The distribution coefficient, P_s, and the initial substrate concentration, $[S]_T$, are given as follows.

$$P_s = [S]_o/[S]_w \quad (1\text{-}2)$$
$$[S]_T = [S]_o D\overline{V} + [S]_w(1-D\overline{V}) \quad (1\text{-}3)$$

The binding constant for incorporation of the substrate into the organic pseudo-phase, K_s, is defined by Eq. 1-4, and $[S]_w$ and $[S]_o$ are given by Eqs. 1-5 and 1-6, respectively.

$$K_s = (P_s-1)\overline{V} \quad (1\text{-}4)$$
$$[S]_w = [S]_o/(1+K_sD) \quad (1\text{-}5)$$
$$[S]_o = P_s[S]_o/(1+K_sD) \quad (1\text{-}6)$$

By combining the relations, $[A]_o=1/\overline{V}$ and $[A]_w$=CAC, together with Eqs. 1-1, 1-5, and 1-6, we obtain Eq. 1-7.

$$v = k_o\frac{P_s[S]_o}{\overline{V}(1+K_sD)}D\overline{V} + k_w(\mathrm{CAC})\frac{[S]_o}{1+K_sD}(1-D\overline{V}) = \frac{k_oP_sD+k_w(\mathrm{CAC})(1-D\overline{V})}{1+K_sD}[S]_o = k_{obsd}[S]_o \quad (1\text{-}7)$$

Since the $(1-D\overline{V})$ term approximately equals 1 because of the low amphiphile concentration and k_w(CAC) can be neglected under the present conditions in addition to the fact that K_s nearly equals $P_s\overline{V}$, Eq. 1-7 is rearranged and simplified to give Eq. 1-8.

$$k_{obsd} = \frac{k_oK_sD}{\overline{V}(1+K_sD)} \quad (1\text{-}8)$$

By substitution of k_m for $k_o/\overline{V}$, Eq. 1-8 is converted into Eq. 1.

17) For example: Y. Ihara, R. Hosako, M. Nango, and N. Kuroki, *J. Chem. Soc., Chem. Commun.*, **1981**, 393.
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19) M. L. Bender, F. J. Kézdy, and C. R. Gunter, *J. Am. Chem. Soc.*, **86**, 3714 (1964).