Recl. Trav. Chim. Pays-Bas **112**, 001–006 (1993) 0165–0513/93/01001–06$06.00

Full Papers

Enantioselective cleavage of esters by histidine-containing tripeptides in micellar solutions of various hexadecyltrialkylammonium bromide surfactants

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(Received February 17th, 1992)

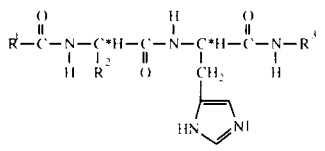
Abstract. Cleavage of chiral *p*-nitrophenyl esters derived from the amino acid phenylalanine by histidine-containing tripeptides has been studied in micellar solutions of four quaternary ammonium surfactants. Enzyme-like enantioselectivities up to $k_L/k_D = 131$ (at 0°C) are observed. The enantioselectivity can be rationalized by assuming a hydrophobically driven stabilizing hydrogen bond between the L enantiomer of the ester and the tripeptide in the transition state of the reaction. This hydrogen bond is absent in the reaction with the D enantiomer of the ester. The transition state has an amphipolar character and is stabilized by the micellar environment. The hydrophilic–hydrophobic balance of the reactants, which affects the transition state, was optimized by varying the composition of the tripeptide and the length of the N-protecting groups in the tripeptide and the substrate. The activities and enantioselectivities depend on the structure of the quaternary ammonium surfactant headgroup. Increasing the size of this headgroup leads to an increase in rate of hydrolysis of the L ester and hence to an increase in enantioselectivity. This effect is attributed to a change in the degree of ion-pair formation with a carboxylate group that is present in the peptides. Compared to previous studies the results indicate that a chiral surfactant is not required for obtaining high enantioselectivities.

Introduction

Reactions in micellar solutions have received a great deal of interest in the last decades[1–3]. The reason is that such reactions are believed to mimic certain aspects of enzymatic catalysis. Micelles, just like enzymes form microdomains in water. These microdomains may bind apolar or semipolar molecules and may promote the conversion of these molecules into products. Under certain conditions, *e.g.* when a properly functionalized chiral catalyst is bound to the micellar domain, the conversion takes place at high rate and with high enantioselectivity. A number of micellar reactions reported to display high enantioselectivities, deal with the cleavage of chiral amino acid *p*-nitrophenyl esters by histidine-containing oligopeptides[3]. Until recently, the origin of the observed enantioselectivities was not well understood. In our previous papers we presented a detailed model which explains these phenomena[1,2].

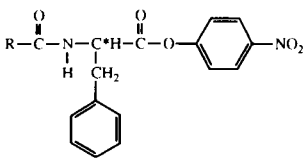
In aqueous solution the imidazolyl group is an active catalyst in the hydrolysis of *p*-nitrophenyl esters. In the first step of this hydrolysis the imidazolyl group is acylated. In the second, relatively slow, step the acylated intermediate is hydrolysed and the free imidazolyl group is regenerated. On increasing the substrate concentration typical Michaelis–Menten kinetics are observed. At high substrate concentrations the imidazolyl group is completely acylated and the rate is independent of substrate concentration.

This paper describes the esterolytic activity and enantioselectivity of a number of imidazolyl-containing tripeptide derivatives dissolved in an aqueous micellar solution. These peptides, including their abbreviations, are listed in Chart 1. The imidazolyl moiety is part of the chiral amino acid residue L-histidine at the 2-position of the tripeptide. The amino acid residues adjacent to this His residue are varied and the effect on the esterolytic activity is investigated. The C-terminal end is unprotected which implies that under the employed pH condition (pH 7.3) the carboxyl moiety is deprotonated and in its anionic form. The N-protecting group of the peptide chain is of the alkoxycarbonyl type which proved to be the most effective protecting group[1]. The chiral substrates, which are shown in Chart 2, are N-acyl-*p*-nitrophenyl (ONp) esters of the amino acid L- or D-phenylalanine (C_n-Phe-ONp). The micelles are formed by the achiral cationic surfactants hexadecyltrimethylammonium bromide (CTMABr) (C = cetyl = hexadecyl), hexadecyltriethylammonium bromide (CTEABr), hexadecyltripropylammonium bromide (CTPrABr) and tributylhexadecylammonium bromide (CTBABr) which are shown in Chart 3. Attention is focussed on the first step in the catalytic cycle, *i.e.* the acylation of the imidazolyl moiety for the same reason as in the previous papers[1,2]. The enantioselectivity is expressed as the ratio of the rate constants obtained for this acylation step employing L and D substrate: k_L/k_D. Special attention will be given to the effect of the surfactant headgroup structure on the observed reaction rates and enantioselectivities.



R^1	R^2	R^3	Abbreviation
$CH_3(CH_2)_{11}O$-	CH_3	$CH[CH_2CH(CH_3)_2]$-COOH	S_{12}-L-Ala-L-His-L-Leu
..	CH_2CH_3	..	S_{12}-L-Abu-L-His-L-Leu
..	$CH_2CH_2CH_3$	..	S_{12}-L-Nva-L-His-L-Leu
..	$CH_2CH(CH_3)_2$	..	S_{12}-L-Leu-L-His-L-Leu
..	$CH_2C_6H_5$	..	S_{12}-L-Phe-L-His-L-Leu
..	..	CH_2-COOH	S_{12}-L-Phe-L-His-Gly
..	..	$CH(CH_3)$-COOH	S_{12}-L-Phe-L-His-L-Ala
..	..	$CH(CH_2CH_3)$-COOH	S_{12}-L-Phe-L-His-L-Abu
..	..	$CH(CH_2CH_2CH_3)$-COOH	S_{12}-L-Phe-L-His-L-Nva
..	..	$CH(CH_2C_6H_5)$-COOH	S_{12}-L-Phe-L-His-L-Phe
..	..	$CH(CH_2C_8H_6N)$-COOH	S_{12}-L-Phe-L-His-L-Trp
CH_3CH_2O-	..	$CH[(CH_2CH(CH_3)_2]$-COOH	S_2-L-Phe-L-His-L-Leu
$CH_3(CH_2)_3O$-	..	$CH[(CH_2CH(CH_3)_2]$-COOH	S_4-L-Phe-L-His-L-Leu
$CH_3(CH_2)_5O$-	..	$CH[(CH_2CH(CH_3)_2]$-COOH	S_6-L-Phe-L-His-L-Leu
$CH_3(CH_2)_{11}O$-	..	CH_3	S_{12}-L-Phe-L-His-NMA

Chart 1.

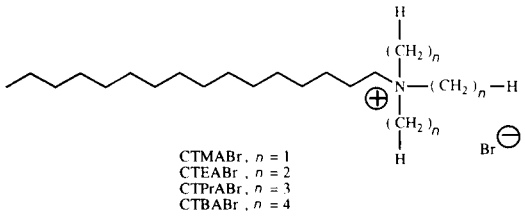


C_n-L (or D)-Phe-ONp: $R = CH_3(CH_2)_{n-2}$-; $n = 2,4,7,12,16$

Chart 2.

Results

The reaction rates and enantioselectivities (k_L/k_D) for the cleavage of the chiral ester C_{12}-Phe-ONp (L and D) by the catalysts S_{12}-L-X-L-His-L-Leu (X = Ala, Abu, Nva, Leu, Phe, Trp) in the presence of CTBABr micelles are presented in Table I. The reaction conditions (pH and surfactant concentration) are such that the imidazolyl moiety (pK_a 6.3) is completely in its unprotonated form. Both ester and catalyst are completely bound to the micelles as we have shown in our previous papers[1,2]. Increasing the hydrophobicity of the amino acid residue X from X = Ala to X = Phe results in a 10-fold rate enhancement for the L enantiomer of the ester. The reaction rate for the D enantiomer increases less than 2-fold. Consequently, the enantioselectivity increases from $k_L/k_D = 7$ up to $k_L/k_D = 40$. This clearly shows that increasing the hydrophobicity of X gives rise to a selective stabilization of the transition state leading to the tetrahedral intermediate of the L ester. However, if X = Trp, the activity toward the L-ester is strongly reduced and a much lower enantioselectivity is found ($k_L/k_D = 4$). We followed the binding of the S_{12}-L-Trp-L-His-L-Leu peptide to the micelles formed by CTBABr by means of fluorescence spectroscopy[1,2,4-7]. In Figure 1, curve a, the position of the fluorescence emission maximum of the indolyl moiety of the Trp residue is plotted as a function of the surfactant concentration. The position of the emission maximum in the absence of surfactant is λ_{em} 360 nm. The addition of surfactant causes a gradual shift of the emission maximum to a plateau value of λ_{em} 350 nm at a surfactant concentration of 2 mmol/dm^3. The shift of the emission maximum to a shorter wavelength indicates that the environment of the Trp residue becomes less polar when the tripeptide binds to the micelles. At a surfactant concentration of 2 mmol/dm^3 the peptide is completely bound. An emission maximum of λ_{em} 350 nm is also observed when this peptide is dissolved in a 1,4-dioxane/water mixture of 55:45(v/v). This indicates that the indolyl moiety remains exposed to the aqueous phase when the tripeptide is bound to the micellar interface.



CTMABr, $n = 1$
CTEABr, $n = 2$
CTPrABr, $n = 3$
CTBABr, $n = 4$

Chart 3.

Table I Rate constants [$k_{a,obs}/(M^{-1}s^{-1})$] and enantioselectivities (k_L/k_D) in the cleavage of C_{12}-Phe-ONp (L and D) by S_{12}-L-X-L-His-L-Leu in the presence of CTBABr at 25°C.

X	k_L	k_D	k_L/k_D	$\Delta\Delta G^{\ddagger}$/(kcal/mol)
Ala	249	34	7	1.15
Abu	333	37	9	1.30
Nva	799	40	20	1.77
Leu	1077	39	28	1.97
Phe	2441	61	40	2.18
Trp	150	37	4	0.83

[a] Conditions: pH 7.3 (0.08 M Tris/HCl and 0.40 M KCl); acetonitrile/water 3/97 (v/v); $c_{catalyst}$ $5\cdot10^{-5}$ M; c_{ester} $1\cdot10^{-5}$ M; c_{CTBABr} $2.5\cdot10^{-3}$ M.

Table II gives the rate constants and enantioselectivities (k_L/k_D) for the cleavage of the chiral ester C_{12}-Phe-ONp (L and D) by the nucleophiles S_{12}-L-Phe-L-His-L-Y in the presence of CTBABr micelles. The observed effects upon changing the hydrophobicity of residue Y are completely different from the effects observed for residue X in Table I. Increasing the hydrophobicity of Y from Y = Gly to Y = Leu slightly reduces the reaction rates toward both enantiomers of the ester. The enantioselectivity remains fairly constant around $k_L/k_D = 35$. For Y = Leu, the enantioselectivity is somewhat higher and amounts to $k_L/k_D = 40$. Remarkably, for Y = Phe, a considerable drop in activity toward the L ester is observed, whereas the activity toward the D ester is only slightly affected. Consequently, the enantioselectivity is reduced to $k_L/k_D = 20$. For Y = Trp, the activity and enantioselectivity are very similar to those of the other nucleophiles in Table II (except Y = Phe). For the S_{12}-L-Phe-L-His-L-Trp tripeptide the binding to the micelles was also followed by fluorescence spectroscopy (Figure 1, curve b). In the absence of surfactant the position of the emission maximum appeared at λ_{em} 361 nm. Just as in curve a the addition of surfactant caused a gradual shift of the position of the emission maximum to a plateau value of λ_{em} 351 nm at a surfactant concentration of 2 mmol/dm^3. An emission maximum of λ_{em} 351 is also observed when this tripeptide is dissolved in a 1,4-dioxane/water mixture of 60:40 (v/v). This indicates that the Trp residue remains exposed to the aqueous phase.

In Table III the rate constants and enantioselectivities are listed which were determined for the cleavage of C_{12}-Phe-ONp (L and D) by S_n-L-Phe-L-His-L-Leu (n = 2, 4, 6 and 12) in the presence of CTBABr micelles in the temperature range between 0 and 25°C. The values of $\Delta\Delta G^{\ddagger}$ are

Table II Rate constants $[k_{a,o}b_x/(M^{-1}s^{-1})^{-1}]$ *and enantioselectivity* (k_L/k_D) *for the cleavage of* C_{12}*-Phe-ONp (L and D) by* S_{12}*-L-Phe-L-His-L-Y in the presence of CTBABr at 25°C* [a].

Y	k_L	k_D	k_L/k_D	$\Delta\Delta G^{\neq}$/(kcal/mol)
Gly	2795	82	34	2.09
Ala	2686	77	35	2.10
Abu	2591	77	34	2.08
Nva	2574	74	35	2.10
Leu	2441	61	40	2.18
Phe	1111	55	20	1.78
Trp	2378	66	36	2.12

[a] Conditions as described in Table I.

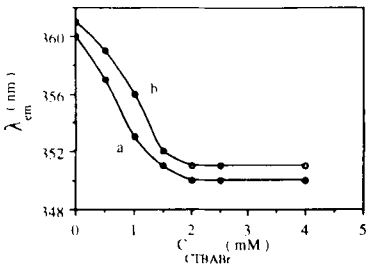


Figure 1. Position of the fluorescence emission maximum of S_{12}*-L-Trp-L-His-L-Leu (curve a) and* S_{12}*-L-Phe-L-His-L-Trp (curve b) in the presence of varying concentrations of CTBABr.* λ_{exc} *286 nm; 0.08 mol/dm³ 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) HCl; 0.40 mol/dm³ KCl; pH 7.3;* $c_{peptide} = 2 \cdot 10^{-5}$ mol/dm³.

also included in this Table. Increasing the hydrophobicity of the S_n moiety results in a large rate enhancement toward both enantiomers of the ester. This effect is caused by the fact that an increasing fraction of the catalyst becomes bound to the micelles where the very hydrophobic ester resides: for the S_2 compound this fraction is only 0.02, whereas for the S_{12} compound it approaches 1. The enantioselectivity peaks at S_4 and amounts to $k_L/k_D = 62$ at 25°C. Reducing the temperature causes a considerable increase in enantioselectivity. The highest value, $k_L/k_D = 131$, is observed for S_4-L-Phe-L-His-L-Leu at 0°C. This ratio corresponds to a $\Delta\Delta G^{\neq}$ value of 11 kJ/mol (2.6 kcal/mol). The activation parameters $\Delta H^{\neq}$, $\Delta S^{\neq}$ and $\Delta G^{\neq}$ are listed in Table IV. As shown in this Table the activation enthalpy for the L enantiomer of the ester is 17–21 kJ/mol (4–5 kcal/mol) smaller than that for the D enantiomer. On the other hand the activation entropy for the L ester is more negative than for the D ester. This indicates that the reaction of the former compound proceeds through a more ordered transition state than the reaction of the latter compound.

Table IV Thermodynamic parameters for the cleavage of C_{12}*-Phe-ONp (L and D) by* S_n*-L-Phe-L-His-L-Leu in the presence of CTBABr* [a]

S_n	Ester	$\Delta H^{\neq}$ [b]	$\Delta S^{\neq}$ [c]	$\Delta G^{\neq}$ [b,d]
S_4	L	6.8	−21.7	13.2
S_6	L	6.3	−22.7	13.0
S_{12}	L	6.9	−19.9	12.8
S_4	D	11.7	−13.2	15.7
S_6	D	10.8	−15.2	15.4
S_{12}	D	10.8	−14.1	15.0

[a] Conditions as described in Table I. [b] In kcal/mol, estimated error ±0.4. [c] In cal/mol·K, estimated error ±2. [d] At 25°C.

Table V Rate constants $[k_{a,obs}/(M^{-1}s^{-1})]$ *and enantioselectivities* (k_L/k_D) *in the cleavage of* C_n*-Phe-ONp (L and D) by* S_4*-L-Phe-L-His-L-Leu in the presence of CTBABr micelles* [a]

Ester	k_L	k_D	k_L/k_D
C_2-Phe-ONp	230	11	21
C_4-Phe-ONp	389	15	26
C_7-PHe-ONp	875	18	49
C_{12}-Phe-ONp	1241	20	62
C_{16}-Phe-ONp	1258	21	60

[a] Conditions as described in Table I.

Table V presents the reaction rates and enantioselectivities for the cleavage of a series of C_n-Phe-ONp esters by S_4-L-Phe-L-His-L-Leu in the presence of CTBABr micelles. Increasing the acyl chain length in the ester from $n = 2$ to 16 causes a large rate enhancement, in particular the L enantiomer, and the enantioselectivity increases from $k_L/k_D = 21$ up to 62. Both the activity and enantioselectivity reach a limiting value at approximately $n = 12$. A further increase to $n = 16$ has no effect.

The effect of the size of the surfactant headgroup was investigated for the cleavage of C_{12}-Phe-ONp (L and D) by S_{12}-L-Phe-L-His-L-Leu (Table VI). The conditions were chosen such that both ester and nucleophile are completely bound to the micelles. Increasing the size of the quaternary ammonium headgroup from a trimethylammonium up to a tributylammonium moiety surprisingly results in a 4-fold rate enhancement of the L ester. The rate of the D ester is hardly affected. Consequently, the enantioselectivity increases almost 4-fold from $k_L/k_D = 11$ in the presence of CTMABr up to 40 in the presence of CTBABr. This corresponds to an increase in $\Delta\Delta G^{\neq}$ of 3 kJ/mol (0.8 kcal/mol).

Table III Rate constants $[k_{a,obs}/(M^{-1}s^{-1})]$ *and enantioselectivities* (k_L/k_D) *in the cleavage of* C_{12}*-Phe-ONp (L and D) by* S_n*-L-Phe-L-His-L-Leu in the presence of CTBABr at various temperatures* [a]

S_n	T/(°C)	k_L	k_D	k_L/k_D	$\Delta\Delta G^{\neq}$/(kcal/mol)
S_2 [b]	25	46	1.9	24	1.89
S_4	0	366	2.8	**131**	2.64
	5	471	4.1	115	2.62
	10	609	6.2	98	2.58
	15	743	8.6	86	2.55
	20	922	13.2	70	2.47
	25	1241	20.1	62	2.44
S_6	0	593	5.3	112	2.56
	10	852	10.2	84	2.49
	25	1709	33	52	2.33
S_{12}	0	844	8.8	96	2.47
	5	1090	12.9	85	2.45
	10	1365	17.9	76	2.44
	15	1740	28.2	62	2.36
	20	2165	42.2	51	2.29
	25	2441	61	40	2.18

[a] Conditions as described in Table I. [b] $c_{catalyst}$ $1.5 \cdot 10^{-4}$ M.

Table VI Rate constants $[k_{a,obs}/(M^{-1}s^{-1})]$ *and enantioselectivity* (k_L/k_D) *for the cleavage of* C_{12}*-Phe-ONp (L and D) by* S_{12}*-L-Phe-L-His-L-Leu in the presence of various surfactants at 25°C* [a]

Surfactant [b]	k_L	k_D	k_L/k_D	$\Delta\Delta G^{\neq}$/(kcal/mol)
CTMABr	623	58	11	1.40
CTEABr	1324	58	23	1.85
CTPrABr	2110	60	35	2.10
CTBABr	2441	61	40	2.18

[a] Conditions as described in Table I. [b] $c_{surfactant}$ $2.5 \cdot 10^{-3}$ M.

Table VII Rate constants $(k_{a,obs}/(M^{-1}s^{-1})]$ *and enantioselectivity* (k_L/k_D) *for the cleavage of* C_{12}*-Phe-ONp (L and D) by* S_{12}*-L-Phe-L-His-NMa in the presence of various surfactants at 25°C* [a]

Surfactant	k_L	k_D	k_L/k_D	$\Delta\Delta G^{\neq}$/(kcal/mol)
CTMABr	289	50	5.8	1.04
CTEABr	235	41	5.7	1.03
CTPrABr	183	31	5.9	1.05
CTBABr	146	26	5.6	1.02

[a] Conditions as described in Table I. [b] $c_{surfactant}$ $2.5 \cdot 10^{-3}$ M.

Table VII shows the effect of the size of the surfactant headgroup on the cleavage of C_{12}-Phe-ONp (L and D) by S_{12}-L-Phe-L-His-NMA. This catalyst lacks a carboxylate moiety. A number of interesting differences are observed when the data in Table VII are compared with those in Table VI. The activity of the catalyst toward the L ester is very low, resulting in a 2- to 7-fold decrease in enantioselectivity. Increasing the size of the surfactant headgroup has no significant effect on the enantioselectivity which remains constant between $k_L/k_D = 5.6$ and 5.9. However, the reaction rates decrease 2-fold. This result is probably due to a dilution effect. For all surfactants a concentration of 2.5 mmol/dm^3 is used: the CTBABr surfactant, however, is much bulkier than the CTMABr surfactant. Consequently, the volume of the micellar pseudo-phase also increases when the size of the quaternary ammonium group increases. This dilution effect will also occur in Table VI but is more than compensated for by other phenomena.

Under the applied conditions our *p*-nitrophenyl esters will also undergo spontaneous hydrolysis, in particular by hydroxide ion. Table VIII gives the rate constants for the spontaneous hydrolysis of C_{12}-L-Phe-ONp in the presence of the various surfactants. Increasing the size of the quaternary ammonium headgroup from trimethyl to tributylammonium causes a 1.7-fold increase in the rate of spontaneous ester hydrolysis. A similar but larger effect was observed for the reaction rates in the presence of S_{12}-L-Phe-L-His-L-Leu (see Table VI). The ester is completely micellar bound in all cases. Consequently, the observed effect is not caused by a change in the distribution of the ester between the aqueous phase and the micellar pseudo-phase.

Table VIII First-order rate constants of spontaneous hydrolysis (k_{sp}/s^{-1}) *of* C_{12}*-L-Phe-ONp in the presence of various surfactants at pH 7.3* [a]

Surfactant	$k_{sp} \cdot 10^{-3}$ (s^{-1})
CTMABr	0.90
CTEABr	1.05
CTPrABr	1.35
CTBABr	1.55

[a] Conditions as described in Table I, c_{ester} $1 \cdot 10^{-5}$ M, $c_{surfactant}$ $2.5 \cdot 10^{-3}$ M.

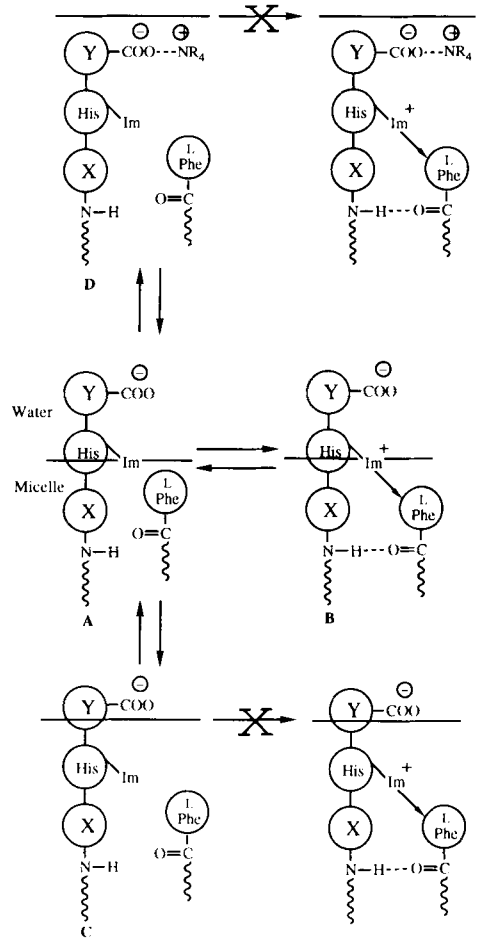


Figure 2. Schematic representation of the tripeptide and L ester bound to the micelles (A). The formation of the transition state at the micellar interface (B). Complete transfer of the imidazolyl moiety to the micellar hydrocarbon phase which prevents the formation of the transition state (C). Ion-pair formation between the carboxylate moiety of the tripeptide and the surfactant headgroup resulting in the complete transfer of the tripeptide to the micellar hydrocarbon phase which prevents the formation of the amphipolar transition state (D).

Discussion

Our micellar solutions are basically two-phase systems consisting of an aqueous phase and a micellar hydrocarbon-like phase. A solute that is added to such a solution may dissolve in either of the two phases or may be adsorbed at the micelle–water interface. Our *p*-nitrophenyl esters are very hydrophobic species, particularly the most frequently employed C_{12}-Phe-ONp ester. The latter compound is highly soluble in apolar organic solvents, whereas the solubility in water is very low ($< 1 \cdot 10^{-6}$ mol/dm^3). If this ester is added to the micellar solutions, it can be expected to dissolve completely into the micellar hydrocarbon phase as is schematically shown in Figure 2A. The hydrophilic acyl CO moiety which is located between the hydrophobic part of the C_{12} group and the hydrophobic Phe side chain will also dissolve into the micellar hydrocarbon phase. In this apolar environment it can form a hydrogen bond with the catalyst (*vide infra*). Our tripeptides are amphiphilic compounds consisting of a distinct very hydrophobic part and a very hydrophilic part (COO^-). They will adsorb to the micellar interface with their hydrophobic part dissolved into the micellar hydrocarbon phase and their hydrophilic part dissolved into the aqueous phase (Figure 2A). Just as in the previous papers[1,2], we propose that the peptide chain

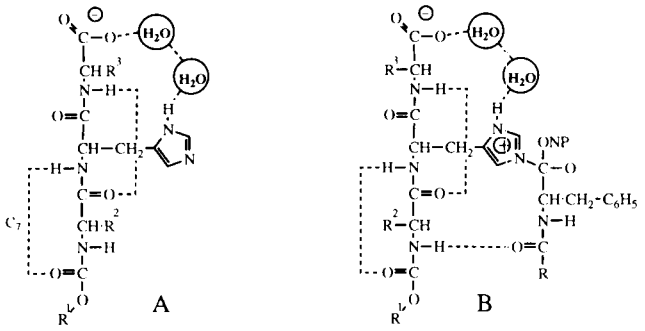


Figure 3. The S_n-L-X-L-His-L-Y tripeptide in the proposed internally hydrogen-bonded (dashed lines) C_7 conformation. Two water molecules that form hydrogen bonds with the carboxylate moiety and the imidazolyl group are also shown (A). The tetrahedral intermediate with a hydrogen bond between the L ester and the tripeptide backbone (dashed line) and the positive charge on the imidazolyl group (B).

adopts an internally hydrogen-bonded conformation, the so-called C_7 conformation. This allows the transfer of this part of the peptide chain to the micellar hydrocarbon phase. These internal C_7 hydrogen bonds are shown by the dashed lines in Figure 3A. The hydrophilic NH moiety of the X residue is not involved in internal hydrogen bonding and can form a hydrogen bond with the acyl CO moiety of the ester provided that is located in the micellar hydrocarbon phase. The proposed tetrahedral intermediate of the reaction is shown in Figures 2B and 3B. Only the L-enantiomer of the ester can form the hydrogen bond in the transition state as was explained in our previous papers[1,2]. Consequently, the transition state with the L ester will be selectively stabilized. The reactivity of the imidazolyl moiety depends on the polarity of its environment. In the transition state the imidazolyl moiety becomes positively charged (Figs. 2B and 3B), *i.e.* in the transition state the imidazolyl group is more polar than in the initial state.

Consequently, a polar environment strongly enhances the reactivity of the imidazolyl moiety. In our two-phase system, the polar environment is formed by the aqueous phase. Here, the NH moiety of the imidazolyl group is hydrogen bonded to water molecules (Figure 3B). To prevent the Im group from dissolving into the micellar hydrocarbon phase where it loses its reactivity, it must be located in the hydration mantle of the very hydrophilic COO^- moiety. A more detailed study of this proximity effect of the carboxylate moiety was given in a previous paper[2]. The resulting transition state is amphiphilic: the imidazolyl moiety requires a polar environment, whereas the formation of the hydrogen bond between the L ester and the tripeptide backbone requires an apolar environment. This is why we need micelles, to provide the proper environment for such a transition state. Increasing the hydrophobicity of the amino acid side chain of X in S_{12}-L-X-L-His-L-Leu will promote the transfer of the NH group of X to the micellar hydrocarbon phase and favour hydrogen bond formation with the L ester (Table 1). Consequently, the reaction rate toward the L ester will increase as will the enantioselectivity. If X = Trp, the hydrophilic indolyl NH group in the amino acid side chain will prevent the transfer of the Trp residue to the micellar phase. Consequently, the hydrogen bond with the L ester cannot be formed efficiently and the activity and the enantioselectivity remain low (Table I). The fact that the Trp residue remains exposed to the aqueous phase can be concluded from the fluorescence measurements.

Table III shows that increasing the hydrophobicity of S_n in S_n-L-Phe-L-His-L-Leu from S_2 to S_4 causes an increase in enantioselectivity. This increase may also be the result of a more facilitated transfer of the Phe NH group to the micellar hydrocarbon phase. A further increase in hydrophobicity of S_n causes a reduction in enantioselectivity. If S_n becomes highly hydrophobic, it may not only assist the transfer of the Phe NH group to the micellar hydrocarbon phase but also the unfavorable transfer of the imidazolyl moiety to this apolar phase (Figure 2C). An optimum in hydrophobicity of the peptide is to be expected for an amphiphilic transition state, since a too low or a too high hydrophobicity causes the peptide to dissolve completely in one of the two phases.

If residue Y in S_{12}-L-Phe-L-His-L-Y becomes highly hydrophobic, the peptide chain, including the His residue, will also tend to dissolve completely into the micellar hydrocarbon phase (Figure 2C) where the imidazolyl moiety loses most of its nucleophilicity. This occurs for *e.g.* Y = Phe (Table II) resulting in a decrease of the activity particularly toward the L ester and consequently of a reduction in the enantioselectivity.

Increasing the hydrophobicity of the C_n moiety of the ester leads to the complete transfer of the ester to the micellar hydrocarbon phase which will facilitate the formation of the hydrogen bond with the tripeptide. The enantioselectivity increases up to a ceiling value when the transfer to the micellar hydrocarbon phase is complete. This effect is shown in Table V.

In our previous studies we used micelles of the chiral quaternary ammonium surfactant (*R* or *S*)-N-hexadecyl-*N*,*N*-dimethyl(α-methylbenzyl)ammonium bromide as the reaction medium for the hydrolysis reactions. Enantioselectivities up to $k_L/k_D = 40$ were observed. The results presented in this paper indicate that a chiral matrix is not required for obtaining a high enantioselectivity. In fact the k_L/k_D values in Table III are much higher than those reported in our previous papers. Table VI suggests that the enantioselectivity depends on the bulkiness of the surfactant headgroup. This result may be explained in the following way. The quaternary ammonium headgroups of the surfactant molecules can form ion pairs with the carboxylate groups of the tripeptide catalysts. Ion-pair formation decreases the hydrophilicity and the tripeptide including the imidazolyl group can dissolve completely into the micellar hydrocarbon phase (Figure 2D). This will cause a reduction in activity, particularly toward the L ester, and a decrease in enantioselectivity. The bulkier the surfactant headgroups the lower the tendency for ion-pair formation, while the rate toward the L ester and the enantioselectivity increase (Table VI). This effect is not observed for S_{12}-L-Phe-L-His-NMA since this peptide lacks a carboxylate moiety (Table VII). As a result of this deficiency the latter peptide will tend to dissolve completely into the micellar hydrocarbon phase, including its imidazolyl group. (This peptide also dissolves well in apolar organic solvents.) This explains the very low activity and enantioselectivity that is observed for this compound.

Ion-pair formation also explains the effect of the surfactant headgroup size on the hydroxide ion promoted spontaneous hydrolysis of the *p*-nitrophenyl esters (Table VIII). The tendency of the quaternary ammonium headgroups to form ion pairs with OH^- ions will decrease when the size of the headgroup increases. This results in a reduction in OH^- concentration in the micellar pseudophase where the ester resides. This reduced concentration would result in a reduction of the reaction rate. However, this effect is more than compensated for by an increase in reactivity of the hydroxide ions caused by the weak interaction with the bulky quaternary ammonium

headgroups. The overall result is that the reaction rate increases when the surfactant headgroup size increases. This effect of reduced binding of counterions (Cl^-, Br^-) but increased reactivity of these ions in micellar solutions has been reported before in the literature[8,9].

Experimental

General remarks

Thin-layer chromatography (TLC) was performed on silica (Merck DC-Plastikrolle, Kieselgel 60 F_{254}) and detection was effected by ultraviolet light or iodine. Column chromatography was performed with silica (Merck Kieselgel 60, 230–400 mesh). Solvents and reagents were of analytical grade. The preparation of the surfactant solutions with and without catalyst is described elsewhere[1]. The kinetic and fluorescence measurements were carried out as described in Refs. 1 and 2.

Synthesis

N-(Dodecyloxycarbonyl)-L-X-L-histidyl-L-leucine-OMe (S_{12}-L-X-L-His-L-Leu-OMe). The synthesis of S_{12}-L-X-L-His-OH (X = Ala, Abu, Nva, Leu, Phe and Trp) has been described in a previous paper[1]. H-L-Leu-OMe·HCl was coupled to N-(dodecyloxycarbonyl)-L-X-L-histidine in DMF with the aid of 1,3-dicyclohexyl carbodiimide according to a method described in literature; *Cf.* our previous papers[1,2]. Although this method is not completely free of racemization, the desired products can be easily obtained by column chromatography (Silica 60, eluant chloroform/methanol, 15:1 (v/v)) and subsequent recrystallization from acetone. The final yield amounted to approximately 60%. TLC: R_f 0.3 [Silica: chloroform/methanol, 10:1 (v/v)] one spot for all compounds. IR (KBr): 1745 (CO ester), 1690 (CO carbamate), 1650 (CO amide) cm^{-1}. For individual compounds ^1H NMR [$CDCl_3/CD_3OD$, 1/1 (v/v)]; $[\alpha]_D^{20}$/(*c* 1.0, methanol): m.p./°C; yield/%:

S_{12}-L-Ala-L-His-L-Leu-OMe. δ 0.9 (m, 9H, 3 CH_3); 1.3 [m, $((CH_2)_{10}$ and CH_2CH (Leu)]; 1.45 [d, CH_3 (Ala)]; 3.1 [d (dist), CH_2 (His)]; 3.65 (s, OCH_3); 4.0 (t, CH_2O); 4.2 [m, CH (Ala) and CH (Leu)]; 4.8 [t, CH (His)]; 6.8 and 7.5 [2 s, 2 CH (Im)]; 7.3 (s, ArH) ppm; −18.1°; 87.4; 62.

S_{12}-L-But-L-His-L-Leu-OMe. δ 0.9 (m, 4 CH_3); 1.3 [m, $CH_2)_{10}$, $CHCH_2$ (Leu) and CH_2 (But)]; 3.1 [d (dist). CH_2 (His)]; 3.7 (s, OCH_3); 4.0 (t, CH_2O); 4.2–4.8 (m, 3·CH); 6.7 and 7.4 (2 s, 2 CH (Im)) ppm; −16.8°; 81.9; 63.

S_{12}-L-Norval-L-His-L-Leu-OMe. δ 0.9 (m, 4 CH_3); 1.3 [m, $(CH_2)_{10}$, $CHCH_2$ (Leu) and $(CH_2)_2$ (Norval)]; 3.1 [d (dist), CH_2 (His)]; 3.7 (s, OCH_3); 4.0 (t, CH_2O); 4.2–4.8 (m, 3 CH); 6.7 and 7.4 [2 s, 2 CH (Im)] ppm; −16.5°; 79.8; 59.

S_{12}-L-Leu-L-His-L-Leu-OMe. δ 0.9 (m, 5 CH_3); 1.3 [m, $(CH_2)_{10}$ and 2 CH_2CH (Leu)]; 3.1 [d (dist); CH_2 (His)]; 3.65 (s, OCH_3); 4.0 (t, CH_2O); 4.2–4.9 (m, 3 CH); 6.7 and 7.4 [2 s, 2 CH (Im)] ppm; −14.5°; 74.0; 64.

S_{12}-L-Phe-L-His-L-Leu-OMe. δ 0.9 (m, 3 CH_3); 1.3 [m, $(CH_2)_{10}$ and $CHCH_2$ (Leu)]; 3.1 [2 d (dist), CH_2 (PHe) and CH_2 (His)]; 3.7 (s, OCH_3); 4.0 (t, OCH_2); 4.2–4.9 (m, 3 CH); 7.2 (s, ArH); 6.7 and 7.4 [2 s, 2 CH (Im)]; ppm; −9.4°; 118.3; 61.

S_{12}-L-Trp-L-His-L-Leu-OMe. δ 0.9 (m, 3 CH_3); 1.3 [m, $(CH_2)_{10}$ and $CHCH_2$ (Leu)]; 3.0 [m, CH_2 (Trp) and CH_2 (His)]; 3.65 (s, OCH_3); 3.9 (t, CH_2O); 4.2–4.9 (m, 3 CH); 6.7 and 7.3 [2 s, 2 CH (Im)]; 6.8–7.7 [m, 5 CH (indolyl)] ppm; −10.5°; 109.7; 58.

N-(Dodecyloxycarbonyl)-L-X-L-histidyl-L-leucine (S_{12}-L-X-L-His-L-Leu-OH). Deprotection of the corresponding methyl esters was performed in aqueous ethanol using NaOH. The crude product was recrystallized from acetone. The final yields amounted to approximately 75%. IR (KBr): 1685 cm^{-1} (CO carbamate); 1640 cm^{-1} (broad; CO mide). ^1H NMR (methanol-d_4); $[\alpha]_D^{20}$/(*c* 1.0, methanol); m.p./°C:

S_{12}-L-Ala-L-His-L-Leu-OH. δ 0.9 (m, 3 CH_3), 1.3 [m, $(CH_2)_{10}$, CH_3 (Ala) and $CHCH_2$ (Leu)]; 3.1 [d, CH_2 (His)]; 3.9 (t, CH_2O); 4.2–4.8 (m, 3 CH); 7.1 and 8.1 [2 s, 2 CH (Im)] ppm; +9.6°; 176.1. Anal. calcd. for $C_{28}H_{49}N_5O_6$: C 60.96, H 8.95, N 12.69, O, 17.40; found: C 60.71, H 9.05, N 12 56%.

S_{12}-L-Abu-L-His-L-Leu-OH. δ 0.9 (m, 4 CH_3), 1.3 [m, $(CH_2)_{10}$, CH_2 (But) and $CHCH_2$ (Leu)]; 3.1 [d, CH_2 (His)]; 3.9 (t, CH_2O); 4.2–4.8 (m, 3 CH); 7.1 and 8.1 [2 s, 2 CH (Im)] ppm; +9.8°; 171.7. Anal. calcd. for $C_{29}H_{51}N_5O_6$: C 61.57, H 9.09, N 12.38, O 16.97; found: C 61.41, H 9.16, N 12.25%.

S_{12}-L-Nva-L-His-L-Leu-OH. 0.9 (m, 4 CH_3), 1.3 [m, $(CH_2)_{10}$, $(CH_2)_2$ (Norval) and $CHCH_2$ (Leu)]; 3.1 [d, CH_2 (His)]; 3.9 [t, CH_2O]; 4.2–4.8 (m, 3 CH); 7.2 and 8.2 [2 s, 2 CH (Im)] ppm; +8.8°; 174.2. Anal. calcd. for $C_{30}H_{53}N_5O_6$: C 62.15, H 9.21, N 12.08, O 16.56; found: C 61.99, H 9.31, N 12.00%.

S_{12}-L-Leu-L-His-L-Leu-OH. δ 0.9 (m, 5 CH_3); 1.3 [m, $(CH_2)_{10}$ and 2 CH_2CH (Leu)]; 3.1 [d, CH_2 (His)]; 3.9 (t, CH_2O); 4.2–4.8 (m, 3 CH); 7.1 and 8.1 [2 s, 2 CH (Im)] ppm; +9.1°; 168.5. Anal. calcd. for $C_{31}H_{55}N_5O_6$: C 62.70, H 9.34, N 11.79, O 16.17; found: C 62.51, H 9.46, N 11.66%.

S_{12}-L-Phe-L-His-L-Leu-OH. δ 0.9 (m, 3 CH_3); 1.3 [m, $(CH_2)_{10}$ and $CHCH_2$ (Leu)]; 3.0 [2 d (dist), CH_2 (Phe) and CH_2 (His)]; 3.9 (t, CH_2O); 4.2–4.9 (m, 3 CH); 7.1 and 8.1 [2 s, 2 CH (Im)]; 7.1 (s, ArH) ppm; −0.2°, 185.5. Anal. calcd. for $C_{34}H_{53}N_5O_6$: C 65.05, H 8.51, N 11.15, O 15.29; found: C 64.94, H 8.60, N 11.04%.

S_{12}-L-Trp-L-His-L-Leu-OH. δ 0.9 (m, 3 CH_3); 1.3 [m, 23H, $(CH_2)_{10}$ and $CHCH_2$ (Leu)]; 3.1 [2 d (dist), CH_2 (Trp) and CH_2 (His)]; 4.0 (t, CH_2O); 4.2–4.8 (m, 3 CH); 7.0 and 8.0 [2 s, 2 CH (Im)]; 7.2 (s, ArH); 6.8–7.7 [m, 5 CH (indolyl)] ppm; +10.4°; 186.4. Anal. calcd. for $C_{36}H_{54}N_6O_6$: C 64.84, H 8.16, N 12.60, O 14.40; found: C 64.69, H 8.26, N 12.48%.

Hexadecyltrimethylammonium bromide (CTMABr)

This compound was a commercial product purchased from Aldrich. It was purified by standard methods[10]; m.p. 248°C [lit.[10], m.p. 227–235°C (dec.)].

Hexadecyltriethylammonium bromide (CTEABr); hexadecyltripropylammonium bromide (CTPrABr); hexadecyltributylammonium bromide (CTBABr)

These compounds were prepared by quaternization of 0.1 mol of the symmetrical trialkylamine with hexadecyl bromide (0.15 mol) in 25 cm^3 of absolute ethanol[9]. After refluxing the mixture for 14 h, the ethanol was removed *in vacuo*. The oily or solid residue was stirred with diethyl ether. The solid material was collected by filtration and thoroughly washed with ether. The crude product was recrystallized three times from acetone. The $(C_4)_3$-surf 16 compound was recrystallized from acetone/diethyl-ether 3:1 (v/v); after two recrystallizations the melting point remained constant. The final yields amounted to 50–60%.

CTEABr. m.p. 178.6°C. ^1H NMR ($CDCl_3$): δ 0.9 (t, CH_3), 1.3 [m, $(CH_2)_{14}$]; 1.35 (t, 3 CH_3); 3.4 (t, CH_2N); 3.55 (q, 3 CH_2N) ppm. Anal. calcd. for $C_{22}H_{48}NBr$: C 65.00, H 11.90, N 3.45, Br 19.66; found: C 64.59, H 11.96, N 3.51, Br 19.41%.

CTPrABr. m.p. 119.9°C. ^1H NMR ($CDCl_3$): δ 0.9 (t, 4 CH_3); 1.3 [m, $(CH_2)_{14}$ and 3 $CH_3CH_2CH_2N$]; 3.4 (m, 4 CH_2N) ppm. Anal. calcd. for $C_{25}H_{54}NBr$: C 66.93, H 12.13, N 3.12, Br 17.81; found: C 66.73, H 12.33, N 3.22, Br 17.61%.

CTBABr. m.p. 78.4°C. ^1H NMR ($CDCl_3$): δ 0.9 (t, 4 CH_3); 1.3 [m, $(CH_2)_{14}$ and 3 $(CH_2)_2$]; 3.4 (m, 4 CH_2N) ppm. Anal. calcd. for $C_{28}H_{60}NBr$: C 68.54, H 12.32, N 2.85, Br 16.28; found: C 68.99, H 12.66, N 2.75, Br 16.58%.

The synthesis of the *p*-nitrophenyl esters was described in our previous papers[1,2].

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