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Letter

The role of membrane-promoted multi-point hydrophobic interactions between peptide catalysts and enantiomeric substrates in highly stereoselective hydrolyses of amino acid esters

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Abstract

The 500 MHz ^{1}H NMR NOESY spectra of membrane-promoted multi-point hydrophobic interactions between peptide catalysts and enantiomeric amino acid esters were detected for clarifying the role of muti-point interactions between the reactants in the highly stereoselective hydrolysis reactions in a vesicular membrane.

Keywords: NMR NOESY; Hydrophobic interaction; Supramolecular catalysis; Bilayer membrane; Stereoselectivity; Hydrolysis

The membrane-assisted stereoselective hydrolyses of *N*-acylated amino acid esters with the L-histidyl group-bearing di- or tri-peptide catalysts and dialkyl-chain surfactants have hitherto been investigated as a reaction model of enzymes, [1–5] and the hydrophobic interaction between the reactants in the membrane has been considered to give the substrate stereoselectivity. However, there is no direct investigation of the hydrophobic interaction between the peptide catalysts and the enantiomeric substrates in the stereoselective hydrolysis in the membrane. This paper reports the role of membrane-promoted multi-point interactions (detected by means of ^{1}H NMR NOESY spectra) between a peptide catalyst and enantiomeric substrates in the stereoselective hydrolysis of amino-acid substrates by di- or tripeptide catalysts in a vesicular membrane (Scheme 1).

For descriptions of the synthetic methods of the materials used in the present investigation see [1].

The stereoselective hydrolyses of 10.0 μmol dm^{-3} *p*-nitrophenyl *N*-acetyl (or decanoyl)-(L or D)-phenylalanate (**1a** or **1b**, respectively) by 0.1 mmol dm^{-3} peptide catalyst of *Z*-L-His (**2**), *Z*-L-Ala–L-His (**3**), *Z*-L-Leu–L-His (**4a**), *Z*-D-Leu–L-His (**4b**), *Z*-L-His–L-Leu (**4c**), *Z*-L-Leu–L-His–L-Leu (**5a**), *Z*-D-Leu–L-His–L-Leu (**5b**), or *Z*-L-Leu–L-Leu–L-His (**5c**) in the vesicular membrane of 1.0 mmol dm^{-3} *N*,*N*-bisdodecyl-*N*,*N*-dimethylammonium bromide (**6**) were car-

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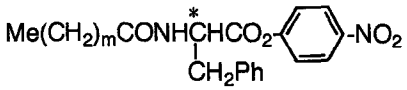
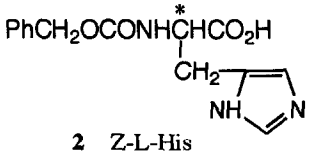
Substrate

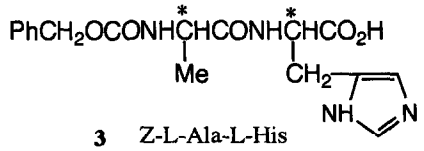


1a (m = 0)
1b (m = 8)

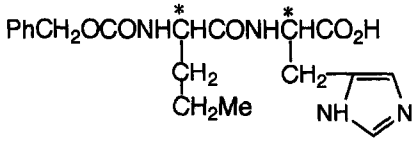
Peptide catalyst



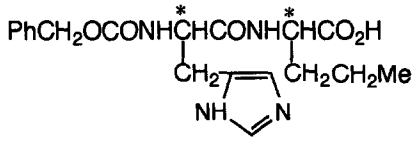
2 Z-L-His



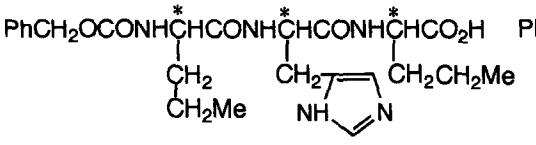
3 Z-L-Ala-L-His



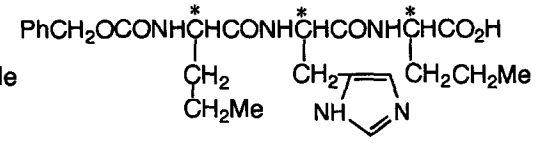
4a Z-L-Leu-L-His
4b Z-D-Leu-L-His



4c Z-L-His-L-Leu

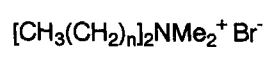


5a Z-L-Leu-L-His-L-Leu
5b Z-D-Leu-L-His-L-Leu



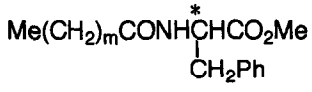
5c Z-L-Leu-L-Leu-L-His

Surfactant



6 (n = 11)

Substrate analogue



7 (m = 8)

Scheme 1.

ried out in 3% v/v CH_3CN/Tris buffer (pH 7.68, ionic strength $\mu = 0.15$) at 298 K after sonication of the membrane including the catalyst for 1 h. The catalytic rate constants k_{cat} were evaluated by the usual way [1], together with binding constants K_b/N (N is an aggregation number of 2162 [6] for the membrane of **6**) and rate constants k_v in the simplified reaction

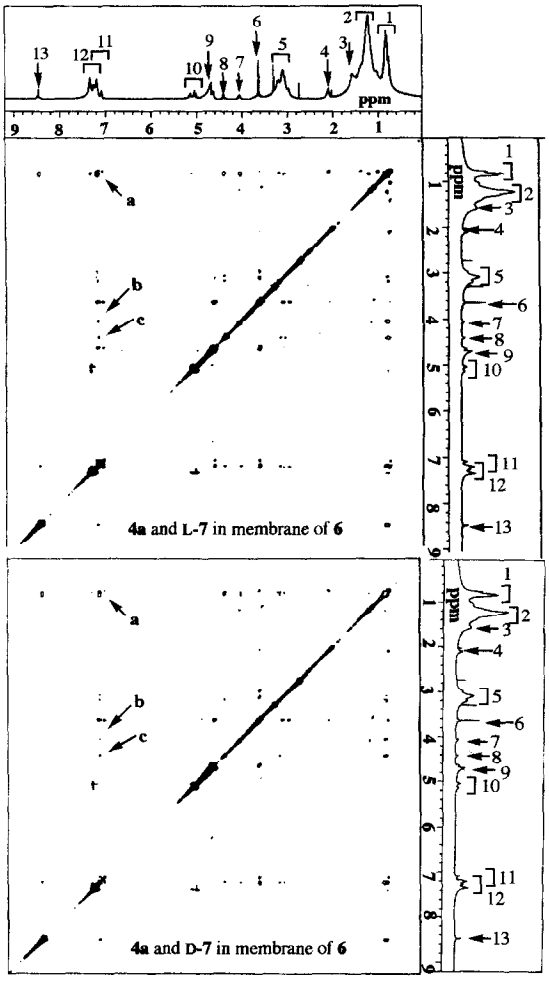


Fig. 1. NOESY spectra of the interaction between **4a** and L (or D)-**7** in the membrane of **6**. 1: Leuδ/acyl 5–8/CH_3. 2: acyl 3/acyl 4/CH_2. 3: acyl 2. 4: acyl 1. 5: Pheβ/Hisβ/CH_3–N. 6: Phe(OMe). 7: Leuα. 8. Hisα. 9: Pheα. 10: Z(CH_2). 11: Phe(Ph)/Hisδ. 12: Z(Ph). 13: Hisε. **a**: Phe(Ph)/Leuδ. **b**: Phe(Ph)/Leuα. **c**: Phe(Ph)/Hisα.

process [1]. It can be seen from the kinetic parameters listed in Table 1 that the dipeptide **4a** or tripeptide **5a** catalyst exhibited efficient stereoselectivity through the reaction (k_v) step rather than through the substrate-binding (K_b/N) process, while the efficiencies of **4b** and **5b** catalysts possessing D-Leu instead of L-Leu as an adjacent amino acid to the active L-His unit were very low. Neither the catalyst **2** possessing no adjacent hydrophobic amino acids nor the catalyst **3** having a less hydrophobic L-alanine instead of L-leucine were effective. Z-L-His–L-Leu (**4c**) and Z-L-Leu–L-Leu–L-His (**5c**), both of which do not have the Z-L-Leu–L-His unit in their frameworks, were also ineffective. Therefore, the Z-L-Leu–L-His unit was found to be very important in the efficient peptide catalysts for the membrane-promoted stereoselective interaction with the enantiomeric substrates.

The 500 MHz ^{1}H NMR NOESY measurements of the sonicated membrane (of undeuterated **6**; 20 mmol dm^{-3}) including the catalyst **4a** (10 mmol dm^{-3}) and the stable methyl *N*-decanoyl-(L or D)-phenylalanate substrate (**7**; 10 mmol dm^{-3}) in D_2O (pH 7.0 and $\mu = 0.02$) involving KCl (20 mmol dm^{-3}) resulted in the two identical NOESY spectra for the enantiomeric L- and D-substrates at mixing time = 500 ms (Fig. 1). As Fig. 1 shows, the NOESY cross-peaks of the hydrophobic side-chain interaction between the phenyl (Ph) ring in the side-chain ($PhCH_2$) of **7** and the Leuδ (or Leuα) proton or Hisα proton in **4a** are identical for L-**7** and D-**7**. Such a mode of multi-point hydrophobic interaction (Fig. 2) makes the distance between the reacting positions of **4a** and L (or D)-**7** closer (or farther), respectively, so as to bring about the substrate stereospecificity of **4a** through the difference in the hydrolysis rates between the enantiomeric substrates. The interamide hydrogen bond depicted in Fig. 2 might be present in the hydrophobic region of the membrane, but it was not clearly detected. In the case of less efficient catalyst **4b**, the NOESY cross-peak of Phe(Ph)/Hisα disappeared without appreciable changes of other NOESY peaks; namely, the high substrate stereoselectivity of **4b** is not expected without approximation of the His imidazolyl group toward the reacting position of the L- (or D)-substrate. The inefficient catalyst **2** also did not show the NOESY cross-peak of Phe(Ph)/Hisα under the same conditions of the NOESY measurement.

[1] The simplified reaction process is as follows:

Table 1
Stereoselective hydrolyses of *p*-nitrophenyl *N*-acetyl (or decanoyl)-(L or D)-phenylalanate (**1a** or **1b**, respectively) with peptide catalysts (**2–5**) in the membrane of $2C_{12}N2C_1$ (**6**) [a]

Catalyst	Substrate	k_{cat}/mol^{-1} dm^3 s^{-1}			K_b/N/mol^{-1} dm^3			$10^2 k_v$/s^{-1}		
		L	D	L/D	L	D	L/D	L	D	L/D
Z-L-His (**2**)	**1a**	26	15	1.6	114	447	0.3	1.5	1.1	1.3
	1b	53	45	1.2	430	490	0.9	2.7	2.5	1.1
Z-L-Ala-L-His (**3**)	**1a**	7.7	1.8	4.3	46	39	1.2	2.4	1.2	2.0
	1b	41	11	3.7	725	719	1.0	3.0	2.3	1.3
Z-L-Leu-L-His (**4a**)	**1a**	310	19	16	130	110	1.2	28	2.5	11
	1b	2590	129	20	1140	730	1.6	42	5.1	8.2
Z-D-Leu-L-His (**4b**)	**1b**	254	108	2.4	180	700	0.3	17	4.4	3.9
Z-L-His-L-Leu (**4c**)	**1b**	87	66	1.3	640	690	0.9	4.4	3.8	1.2
Z-L-Leu-L-His-L-Leu (**5a**)	**1a**	149	34	4.4	360	400	0.9	6.1	1.5	4.1
	1b	958	65	15	610	630	1.0	36	5.7	6.3
Z-D-Leu-L-His-L-Leu (**5b**)	**1b**	285	107	2.5	350	360	1.0	7.9	3.8	2.3
Z-L-Leu-L-Leu-L-His (**5c**)	**1b**	268	430	0.6	98	270	0.4	20	15	1.5

[a] [Catalyst] = 0.1 mmol dm^{-3}, [substrate] = 10.0 μmol dm^{-3}, and [**6**] = 1.0 mmol dm^{-3} in 3% v/v CH_3CN/Tris buffer (pH 7.68, ionic strength μ = 0.15) at 298 K. The experimental errors were within 5%.

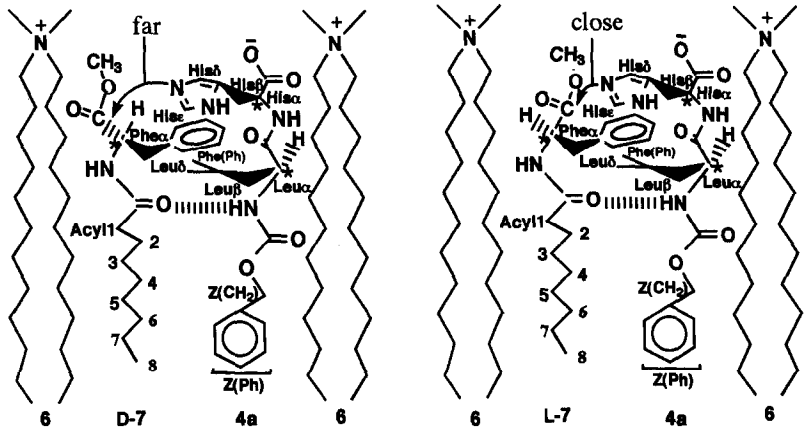


Fig. 2. Membrane-promoted multi-point interactions between the catalyst **4a** and the substrate analogue **7**.

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