Efficient Multi-point Interaction between Peptide Catalysts and Amino Acid Esters in a Bilayer Vesicular Membrane for Highly Stereoselective Hydrolysis Reactions

Katsutoshi Ohkubo,*[a] Kenji Urabe,[b] Junji Yamamoto,[b] Hitoshi Ishida,[b] Satoshi Usui[b] and Takashi Sagawa[b]

[a] *Institute for Fundamental Research of Organic Chemistry, Kyushu University, Fukuoka 812, Japan*
[b] *Department of Applied Chemistry, Faculty of Engineering, Kumamoto University, Kumamoto 860, Japan*

The ^{1}H NMR NOESY spectra of the multi-point interaction between *N*-(*N*-benzyloxycarbonyl-L-leucyl)-L-histidine-[(*Z*)-L-Leu-L-His] and methyl *N*-hexanoyl-L-phenylalanate in a *N,N*-bisdodecyl-*N,N*-dimethylammonium bromide membrane confirms that the membrane-assisted, hydrophobic interaction between the peptide catalyst and enantiomeric substrates enhances the stereoselective hydrolysis of *p*-nitrophenyl *N*-acetyl (or decanoyl)-L (or D)-phenylalanate with (*Z*)-L-Leu-L-His (most effective in di-, tri- or tetra-peptide catalysts with an introduced L-histidyl group).

Although the membrane-assisted stereoselective hydrolysis of amino acid esters has already been performed, mainly with L-histidyl group-introduced dipeptide catalysts and dialkyl-chain surfactants as a simplified reaction enzymes model,[1,2] the investigation of the membrane-promoted interaction between an efficient peptide catalyst and an amino-acid substrate in connection with the stereoselective hydrolysis of amino-acid substrates has not been reported. Here we report the determination of the most efficient amino acid sequence in a series of di-, tri- and tetra-peptide catalysts which include nucleophilic L-histidine and hydrophobic L- (or D)-leucine. We also report on the multi-point interaction between the most efficient peptide catalyst and an amino acid substrate in a bilayer vesicular membrane by means of a 600 MHz ^{1}H NMR NOESY analysis.

The peptide catalysts **1–5** (see Table 1), the substrates of *p*-nitrophenyl *N*-acetyl (or decanoyl)-L- (or D-)-phenylalanate **6** and the surfactant of *N,N*-bisdodecyl-*N,N*-dimethylammonium bromide **7** were prepared by previously reported methods.[1] The hydrolyses of **6a–c** (10 μmol dm^{-3}) by **1–5** (0.1 mmol dm^{-3}) with **7** (1.0 mmol dm^{-3}) were carried out in a 3% *v/v* MeCN–Tris buffer (pH 7.68, ionic strength μ = 0.15) at 298 K after sonication of the vesicular membrane (of **7**) containing one of **1–5**, and the pseduo-first-order hydrolysis rate was followed spectrophotometrically by monitoring the *p*-nitrophenolate anion formation at 400 nm. The catalytic rate constants k_{cat} summarized in Table 1 were obtained by the usual methods.

Among the peptide catalysts tested, the stereoselectivity of the peptide catalysts possessing the L-Leu-L-His unit were appreciably higher than ones without the L-His-L-Leu unit (**1**, **2** and **4a**) and followed the order **3b** ≫ **4b** > **5a** > **5b**. The stereoselectivity of (*Z*)-L-Leu-L-His **3b** was considerably lowered by changing the L-Leu unit into D-Leu (or L-Ala) or by removing the membrane from the reaction system. The catalytic efficiency of **3b** was also reduced by changing the substrate from **6b** to C_{10}-L (or D)-Ala-PNP **6c** having a less hydrophobic side-chain. These facts suggest the importance of the membrane-assisted hydrophobic interaction between the side-chains of L-Leu (or L-His) in the peptide catalyst and the enantiomeric substrates for making the reacting positions of the active imidazolyl group of L-His in the catalyst and the susceptible carbonyl group of L (or D)-substrate closer (or further apart), respectively, since the stereoselectivity occured in the catalyst–substrate complex reaction step rather than in the catalyst–substrate binding process.† The 600 MHz ^{1}H NMR NOESY spectrum (measured by GE NMR OMEGA, mixing time 300 ms) of a D_2O solution (pH 7.0; μ = 0.02) including **3b** (10 mmol dm^{-3}), a substrate analogue of C_6-L-Phe-OMe (methyl *N*-hexanoyl-L-phenylalanate; 10 mmol dm^{-3}), **7** (20 mmol dm^{-3}), and KCl (20 mmol dm^{-3}) indicated the multi-point hydrophobic interaction between the L-Leu or L-His side-chain of **3b** and the Ph ring in the side-chain of the substrate. An expected inter-amide hydrogen bonding between the reactants was not observed, probably because of proton–deuterium exchange (Fig. 1).‡ The same mode of the interaction between **3b** and the D-substrate probably makes the reacting groups further apart resulting in the efficient stereoselectivity of **3b**. Such a favourable membrane-promoted multi-point interaction

Table 1 Stereoselective hydrolyses of *p*-nitrophenyl *N*-acetyl (or decanoyl)-L(or D)-phenylalanate [**6a** (or **6b**)] by peptide catalysts in the membrane of *N,N*-bisdodecyl-*N,N*-dimethylammonium bromide **7**[a]

	k_{cat}/mol^{-1} dm^3 s^{-1}					
	6a			**6b**		
Catalyst	L	D	L/D	L	D	L/D
(*Z*)-L-His **1**	26	15	1.6	53	45	1.2
(*Z*-L-His-L-Leu **2**	17	33	0.52	87	66	1.3
(*Z*)-L-Ala-L-His **3a**	7.7	1.8	4.3	41	11	3.7
(*Z*)-L-Leu-L-His **3b**	310	19	16	2590	129	20
	(0.99	0.8	1.2)[b]			
	(0.007	0.007	1.0)[c]			
(*Z*)-D-Leu-L-His **3c**	40	37	1.1	254	108	2.4
(*Z*)-L-His-L-Leu-L-Leu **4a**	5.2	5.7	0.91	27	28	0.9
(*Z*)-L-Leu-L-His-L-Leu **4b**	149	34	4.4	958	65	15
(*Z*)-L-Leu-L-Leu-L-His **4c**	96	1.8	4.3	268	430	0.62
(*Z*)-L-Leu-L-His-L-Leu-L-Leu **5a**	30	6.8	4.4	386	35	11
(*Z*)-L-Leu-L-Leu-L-His-L-Leu **5b**	28	16	1.8	291	263	1.1

[a] [Catalyst] = 0.10 mmol dm^{-3}, [**6a–b**] = 10.0 μmol dm^{-3}, and [**7**] = 1.0 mmol dm^{-3} in 3% *v/v* MeCN–Tris buffer (pH 7.68, μ = 0.15) at 298 K. Experimental errors were within 5%. [b] Obtained with 3 vol% MeOH instead of **7**. [c] k_{uncat} values obtained without **3b**.

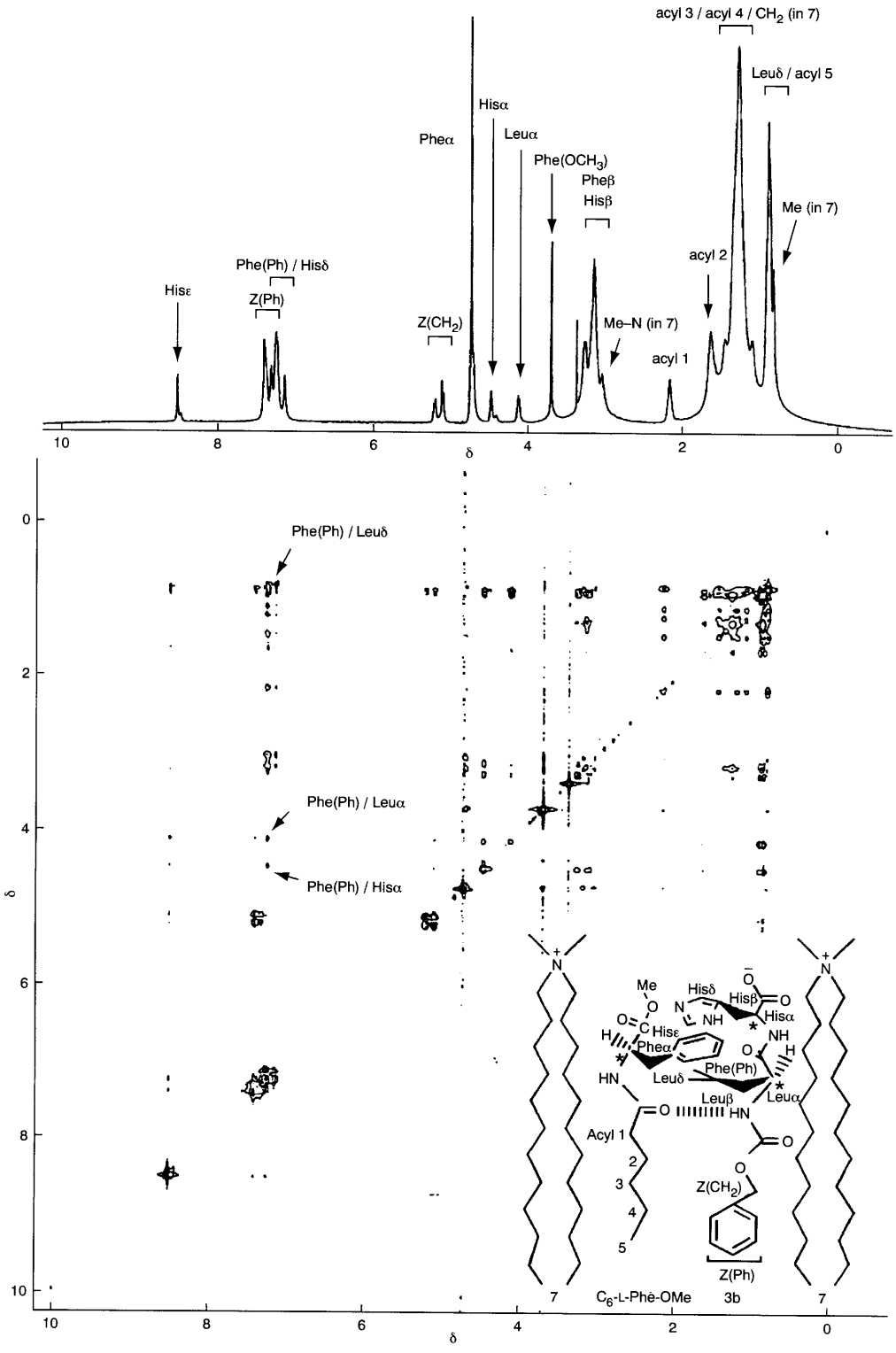

Fig. 1 600 MHz ^{1}H NMR NOESY spectrum of the interaction between **3b** (10 mmol dm^{-3}) and C_6-L-Phe-OMe (10 mmol dm^{-3}) in the membrane of **7** in D_2O (pH 7.0) including 20 mmol dm^{-3} KCl (μ = 0.02)

cannot be established by using peptide frameworks other than **3b**.

Received, 6th July 1995; Com. 5/04407C

Footnotes

† The enantiomeric ratio (L/D) of the catalyst–substrate (**3b–6b**) binding constant and its reaction rate were calculated to be 1.6 and 8.2, respectively.

‡ Resonance of the surfactant **7** which was not deuterated was not observed in the mixing time of 300 ms, presumably because the lateral motion of **7**. MeOH (single peak at δ 3.35) which originated not from C_6-L-Phe-OMe but from stock MeOH solutions.

References

1 K. Ohkubo and S. Miyake, *J. Chem. Soc., Perkin Trans. 2*, 1987, 995.
2 Y. Ihara, S. Akasaka, K. Igata, Y. Matsumoto and R. Ueoka, *J. Chem. Soc., Perkin Trans. 2*, 1991, 543 and references cited therein.