Novel stereoselective incorporation and hydrolysis of long-chain amino-acid substrates by vesicular membrane systems which include tri- or tetra-peptide catalysts

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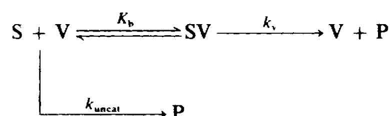
In the vesicular membrane systems comprising *N,N*-didodecyl-*N,N*-dimethylammonium bromide and tri- or tetra-peptide catalysts (pH 7.68; ionic strength μ = 0.15; 298 K), Z-L-Leu-L-His-L-Leu (or Z-L-Leu-L-His-L-Leu-L-Leu), having a hydrophobic terminal L-leucine (or L-leucyl-L-leucine) unit, contributed to the stereoselective binding of long-chain *p*-nitrophenyl *N*-hexadecanoyl-L (and D)-phenyalanates with the remarkably high binding ratio K_b(L/D) = 11 (or 7.3) and overall stereoselectivity [k_{cat}(L/D) = 35 (or 8.1)].

Although there has been much interest in the stereoselective hydrolysis of enantiomeric amino acid esters with peptide catalysts in bilayer membranes,[1–5] there have been no reports of enzyme-resemblant membrane systems containing peptide catalysts which are capable of exhibiting stereospecificity during the substrate binding process; previously reported peptide catalysts (mainly dipeptides) were ineffective for the stereoselective incorporation of enantiomeric substrates (*e.g.* stereospecific substrate–catalyst complex formation). This report first focuses on the enhancement of the stereoselective hydrolysis of amino acid esters through the highly stereoselective substrate-incorporation by vesicular membrane systems containing L-histidyl group-containing, tri- or tetra-peptide catalysts.

The hydrolysis of *p*-nitrophenyl *N*-decanoyl (or hexadecanoyl)-L (and D)-phenyalanates **1a–b** (10.0 μmol dm^{-3}) with the peptide catalysts **2–5** (indicated in Table 1; 0.10 mmol dm^{-3}) were carried out in the vesicular membrane of *N,N*-didodecyl-*N,N*-dimethylammonium bromide **6** (1.0 mmol dm^{-3}) in 3% (v/v) MeCN/Tris buffer ([Tris] = 0.083 mol dm^{-3}; pH 7.68; μ = 0.15) involving 0.083 mol dm^{-3} KCl at 298 K. The apparent catalytic second-order rate constants k_{cat} were evaluated in the usual way,[1] together with the binding constants K_b/N (aggregation number N = 2162[6]) for the catalyst-substrate binding process and rate constants k_v for the reaction step of the catalyst–substrate complex (Table 1).†

Although Z-L-His **2** and Z-L-Ala-L-His **3a** hydrolysed the enantiomeric substrates **1a,b** without notable stereoselectivity, the dipeptide Z-L-Leu-L-His **3b**, which has earlier been accepted as being a most efficient catalyst,[1,2,5] hydrolysed **1a,b** stereoselectively [k_{cat}(L/D = 14 (**1a**) – 20 (**1b**)] with the high stereoselectivity in the reaction step [k_v(L/D) = 6.8 (**1a**) – 8.2 (**1b**)] despite the very small selectivity in the binding constant [K_b(L/D) = 1.1 (**1a**) – 1.6 (**1b**)]; the high stereoselectivity in the reaction (k_v) step results from membrane-promoted interactions between the dipeptide catalysts and the substrates (see Fig. 1).[7] In contrast, the tripeptide catalyst of Z-L-Leu-L-His-L-Leu **4a** having a terminal and hydrophobic L-leucine unit shows quite high stereoselectivity in the binding process [K_b(L/D) = 11], a relatively high ratio of k_v(L/D) = 4.2, and the highest overall selectivity [k_{cat}(L/D) = 35]. Compound **4a** and other peptide catalysts failed to show stereoselective substrate-binding ability in the hydrolyses of **1b** and other short-chain *p*-nitrophenyl *N*-acetyl-L (and D)-phenylalanates. In the hydrolysis of **1a**, a change of the amino acid sequence from that of **4a** to Z-L-Ala-L-His-L-Leu **4b** considerably decreased the overall selectivity [from k_{cat}(L/D) = 35 to k_{cat}(L/D) = 3.0], although continuing to demonstrate the predominance of the substrate selectivity in the binding process [K_b(L/D) = 3.4] over that of the reaction step [k_v(L/D) = 1.1]. Z-L-Leu-L-His-L-Ala **4c**, however, which possesses a less hydrophobic terminal L-alanine unit showed loss of stereoselective substrate-binding ability in the hydrolysis of **1a** [K_b(L/D) = 1.3]; the Z-L-Leu-L-His portion in **4c** still contributed, however, to give approximately the same degree of stereoselectivity in the reaction step [k_v(L/D) = 3.8] as compared with that [k_v(L/D) = 4.2] of **4a**. It is also notable that Z-L-Leu-L-His-D-Leu bound D-**1a** in preference to L-**1a** [k_{cat}(L/D) = 0.04] with a high rate ratio of k_v(L/D) = 15 and low overall selectivity of k_{cat}(L/D) = 2.2. Therefore, the hydrophobicity and chirality of the terminal amino acid in tripeptide catalysts play an essential role in the enhancement of stereoselectivity in the substrate binding process. In this regard, the increase of the hydrophobicity of the terminal amino acid unit for L-Leu-L-Leu in Z-L-Leu-L-His-L-Leu-L-Leu **5** also resulted in the relatively high K_b(L/D) value of 7.3, but a low k_v(L/D) value of 1.6. In conclusion, the amino acid sequence Z-L-Leu-L-His-L-Leu is very important to the enhancement of the stereoselective hydrolysis of the long-chain compound **1a** through substrate stereoselection in both the binding and reaction steps; the interaction mode of the stereoselective substrate-binding was assumed to be as indicated in Fig. 1.

† The values of K_b/N and k_v were obtained in the following simplifed reaction process:



where S = substrate, V = vesicular membrane system, and P = products.

Experimental

Typical experimental procedure

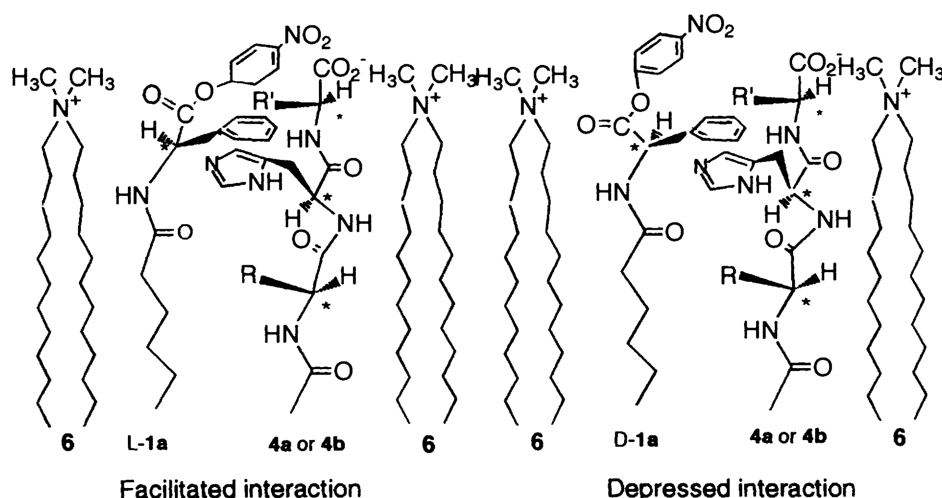
Hydrolysis of the ester substrates (10.0 μmol dm^{-3}) by the vesicular system of the peptide catalyst (0.10 mmol dm^{-3}) and

Table 1 Kinetic parameters for the hydrolysis of *p*-nitrophenyl *N*-hexadecanoyl (or decanoyl)-L (and D)-phenyalanates (**1a** or **1b**) by the peptide catalysts **2–5** in the membrane of *N,N*-didodecyl-*N,N*-dimethylammonium bromide **6**[a]

		k_{cat}/mol^{-1} dm^3 s^{-1}			(K_b/N)/mol^{-1} dm^3			10^2 k_v/s^{-1}		
Catalyst[b]	Substrate	L	D	L/D	L	D	L/D	L	D	L/D
Z-L-His **2**	**1b**	14	20	0.7	430	490	0.88	3.9	3.5	1.1
Z-L-AlaL-His **3a**	**1b**	41	11	3.7	725	719	1.0	3.0	2.3	1.3
Z-L-Leu-L-His **3b**	**1a**	677	49	14	1280	1120	1.1	17	2.5	6.8
	1b	2590	129	20	1140	730	1.6	42	5.1	8.2
		(310	19	16	130	110	1.2	28	2.5	11)[c]
Z-L-His-L-Leu **3c**	**1a**	69	27	2.6	2250	1160	2.2	2.0	1.8	1.1
	1b	87	66	1.3	640	690	0.93	4.4	3.8	1.2
Z-L-Leu-L-His-L-Leu **4a**	**1a**	623	18	35	2560	230	11	10	2.4	4.2
	1b	958	65	15	610	630	0.97	36	5.7	6.3
		(149	34	4.4	360	400	0.90	6.1	1.5	4.1)[c]
Z-L-Ala-L-His-L-Leu **4b**	**1a**	23	7.6	3.0	2300	710	3.4	1.6	1.5	1.1
	1b	97	38	2.6	460	470	0.98	4.4	2.7	1.6
Z-L-Leu-L-His-L-Ala **4c**	**1a**	330	35	9.3	700	540	1.3	7.9	2.1	3.8
	1b	860	70	12	290	170	1.7	40	6.5	6.2
Z-L-Leu-L-His-D-Leu **4d**	**1a**	155	69	2.2	51	1234	0.04	69	2.6	15
Z-L-Leu-L-His-L-Leu-L-Leu **5**	**1a**	314	39	8.1	1275	174	7.3	7.2	4.4	1.6

[a] [Substrate] = 10.0 μmol dm^{-3}, [Catalyst] = 0.10 mmol dm^{-3} and [**6**] = 1.0 mmol dm^{-3} in 3% (v/v) MeCN/Tris buffer ([Tris] = 0.083 mol dm^{-3}; pH 7.68; μ = 0.15) including 0.083 mol dm^{-3} KCl at 298 K. The K_b/N and k_v values were obtained with [Substrate] = 10.0 μmol dm^{-3} and [**6**] = 1.0–0.67 mmol dm^{-3} under [**6**]/[Catalyst] = 10. Experimental errors are within 5%. [b] For instance, Z-L-Leu-L-His-L-Leu denotes N^{α}-(*N*-benzyloxycarbonyl-L-leucyl-L-histidyl)-L-leucine. [c] Obtained for *p*-nitrophenyl *N*-acetyl-L (and D)-phenyalanates.

Interaction mode A



Interaction mode B

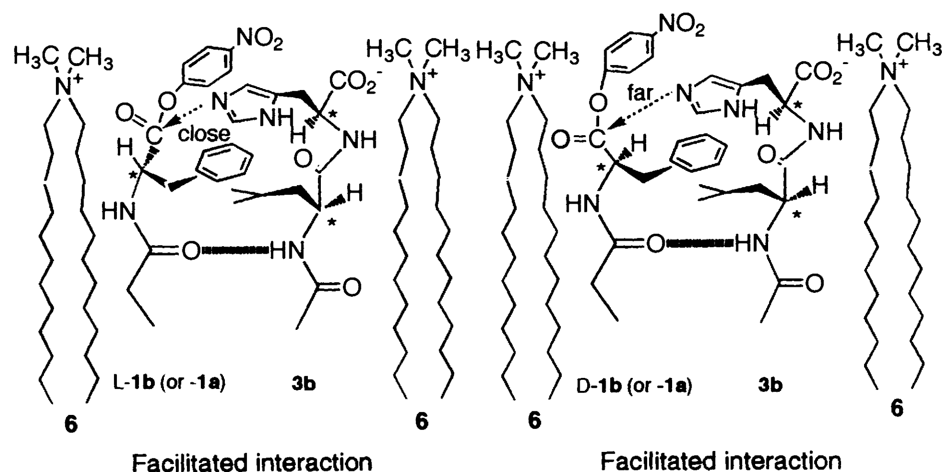


Fig. 1 Modes of interactions between peptide catalysts and enantiomeric substrates for (A) stereoselective substrate-binding and (B) stereoselective reaction

the surfactant (1.0 mmol dm^{-3}) was carried out in 3% (v/v) MeCN–Tris buffer (0.083 mol dm^{-3}; pH 7.68; μ = 0.15) involving 0.083 mol dm^{-3} KCl at 298 K, where the vesicular system was sonicated at 318 K for 1 h before the hydrolysis. The hydrolysis rate which obeyed well a pseudo-first-order rate law (up to *ca.* 80% conversion) was followed by spectroscopic determination of *p*-nitrophenolate (400 nm); the second-order catalytic rate constant (k_{cat}) was evaluated in the usual way.[1]

References

1 K. Ohkubo and S. Miyake, *J. Chem. Soc., Perkin Trans. 2*, 1987, 995.
2 K. Ohkubo, M. Kawata, T. Orito and H. Ishida, *J. Chem. Soc., Perkin Trans. 1*, 1989, 666.
3 K. Ohkubo, H. Ishida, K. Yamaki and M. Kawata, *Chem. Lett.*, 1991, 1723.
4 R. Ueoka, Y. Matsumoto, R. A. Moss, S. Swarup, A. Sugii, K. Harada, J. Kikuti and Y. Murakami, *J. Am. Chem. Soc.*, 1988, **110**, 1588.
5 Y. Ihara, S. Akasaka, K. Igata, Y. Matsumoto and R. Ueoka, *J. Chem. Soc., Perkin Trans. 2*, 1991, 543 and references cited therein.
6 T. Kunitake, Y. Okahata, R. Ando, S. Shinkai and S. Hirakawa, *J. Am. Chem. Soc.*, 1980, **102**, 7877.
7 K. Ohkubo, K. Urabe, J. Yamamoto, H. Ishida, S. Usui and T. Sagawa, *J. Chem. Soc., Chem. Commun.*, in the press.

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