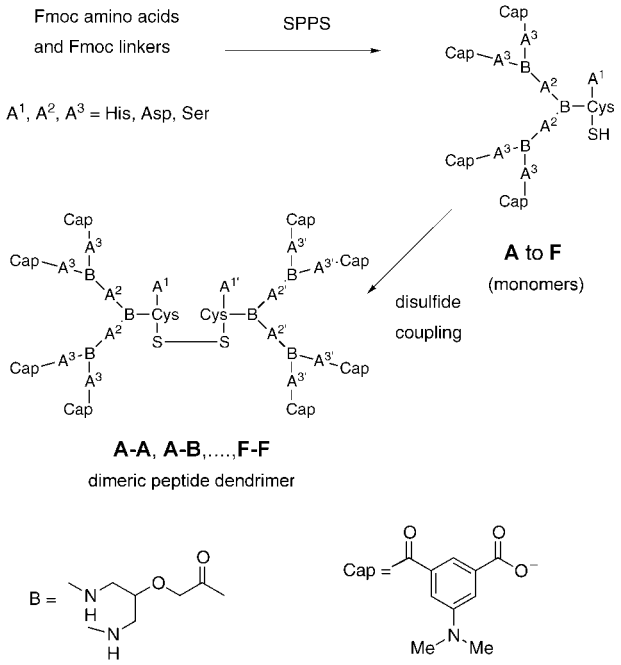
Biocatalyst Design

Catalytic Peptide Dendrimers**

*Annamaria Esposito, Estelle Delort, David Lagnoux, Françis Djojo, and Jean-Louis Reymond**

Enzyme catalysis is made possible by the folding of linear peptide chains into well-defined three-dimensional structures. The aim of de novo enzyme design is to create selective catalysts through the catalytically productive combination of amino acids.[1] A variety of α-helical peptides have been designed that are catalytically active.[2] Thus, the spatial proximity of amino acid side chains placed one turn apart in an α helix were shown to induce catalysis. Protected β-turn peptides have been found to be selective catalysts in organic solvents.[3] Such approaches have required the linear peptides to form a properly folded structure for catalysis to be observed. Alternatively one can design three-dimensional structures by chemical synthesis using a dendrimeric architecture, which enforces a globular shape by steric crowding and therefore obviates the folding requirement and greatly simplifies design.[4] Whereas catalytic dendrimers based on ether linkages and incorporating catalytically active subunits such as metal complexes and cofactors are known,[5] peptide dendrimers have to date only been reported as antigen-display units (multiple antigenic peptides)[6] or in structural studies.[7] Herein we report the preparation of the first catalytic peptide dendrimers. The peptide dendrimers exhibit enzymelike kinetic properties, including selective substrate binding and rate acceleration in aqueous media.

We prepared dendrimeric peptides with the sequence $((CapCONH\text{-}A^3)_2BA^2)_2B\text{-}Cys\text{-}A^1\text{-}NH_2$ (Scheme 1). The symmetrical, achiral diamino acid (1,3-diaminoisopropyloxy)acetic acid was chosen as the branching unit B, which provides the dendrimeric architecture. The sequences were terminated by acylation with the 3-dimethylaminoisophthaloyl group (= Cap, introduced as the mono-*tert*-butyl ester), which provided a spectroscopic signature and allowed the homogeneous solubility of the dendrimers under both the acidic conditions of HPLC purification and the neutral conditions of aqueous buffers. Permutations of the catalytic triad of the amino acids histidine (His), aspartate (Asp), and serine (Ser) were chosen as the variable positions A^1, A^2, and A^3 to generate a family of esterolytic peptide dendrimers. The solid-phase peptide synthesis was carried out on a Rink amide resin with Fmoc-



Scheme 1. Synthesis of catalytic peptide dendrimers. SPPS = solid-phase peptide synthesis.

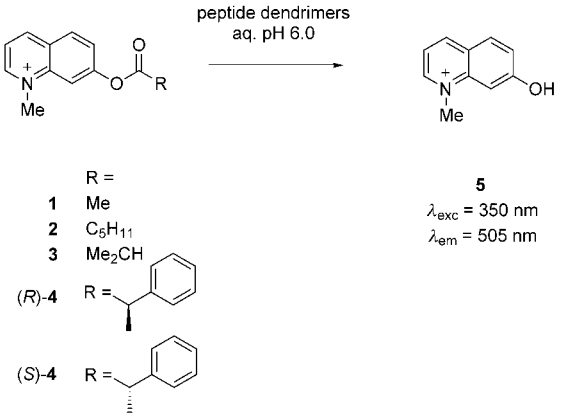
[*] Prof. Dr. J.-L. Reymond, Dr. A. Esposito, E. Delort, D. Lagnoux, Dr. F. Djojo
Department of Chemistry and Biochemistry, University of Bern
Freiestrasse 3, 3012 Bern (Switzerland)
Fax: (+41) 31-631-8057
E-mail: reymond@ioc.unibe.ch

[**] This work was supported by the Swiss National Science Foundation, the Swiss Office Fédéral de L'Education et de la Science, and the Novartis Foundation (postdoctoral grant to A.E.).

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

protected building blocks. Each of the six resulting dendrimeric peptides were then used to prepare all possible dimeric combinations by disulfide-bond formation with aldrithiol,[8] resulting in a family of 21 different peptide dendrimers. Each of the dimeric peptides was prepared on a milligram scale and purified by semipreparative reversed-phase HPLC.

The dendrimers were assayed for the catalysis of ester hydrolysis in both a neutral (pH 7.4) and a slightly acidic (pH 6.0) aqueous buffer, using chromogenic and fluorogenic acetyl esters and acetoxymethyl ethers of umbelliferone,[9] 8-acetoxypyrene-1,3,6-trisulfonate,[10] and 7-acetoxy-1-methylquinolinium sulfate,[11] with 200 μM substrate and 5 μM catalyst (2.5 mol%).[12] While there was no detectable activity with most substrates, several dendrimers catalyzed the hydrolysis of 7-acetoxy-1-methylquinolinium sulfate **1** (Scheme 2, Table 1). The activity pattern indicated that



Scheme 2. Peptide-dendrimer-catalyzed ester hydrolysis.

catalysis was correlated with the presence of histidine residues at the outermost positions A^3 of the dendrimers. The three most active dendrimers were selected for further characterization.

All three of the most active catalytic peptide dendrimers, **E-E, E-F**, and **F-F**, catalyzed the hydrolysis of **1**, the related hexanoyl ester **2**, and isobutyroyl ester **3** with enzymelike kinetics, with a Michaelis–Menten constant of $K_M \sim 200$ μM, turnover numbers of $k_{cat} \sim 0.25\ min^{-1}$, and specific rate acceleration over background of up to $k_{cat}/k_{uncat} \sim 10^3$ (Figure 1 and Table 2). A pH-profile analysis between pH 5.5 and 8.5 showed that catalysis was most efficient at pH 6.0. The specific rate acceleration over that of 4-methylimidazole (MeIm), as a model for the catalytic histidine side chain, amounted to $k_{cat}/K_M/k_2 \sim 350$. The catalytic effect observed corresponds to a 40-fold reactivity increase per histidine side chain, which might be caused by productive interactions at the surface of the dendrimer. These results are similar to those observed by Baltzer and co-workers

Table 1: Apparent hydrolysis rates measured with 200 μM ester **1** and 5 μM dendrimer at 25 °C in 20 mM aqueous Bis-Tris buffer pH 6.0.[a]

A^3, A^2, A^1		–	A	B	C	D	E	F
Ser, Asp, His	**A**	0.5	0.2					
Asp, Ser, His	**B**	0.0	0.7	0.5				
Asp, His, Ser	**C**	0.6	0.3	0.6	0.4			
Ser, His, Asp	**D**	0.4	0.2	0.5	0.7	0.4		
His, Asp, Ser	**E**	2.1	3.1	1.9	1.6	1.4	**9.2**	
His, Ser, Asp	**F**	1.7	2.1	0.7	1.8	1	**12**	**6.4**

[a] The ratio V_{net}/V_{uncat} is reported for monomeric dendrimers **A** to **F** (column 1) and all combinations of dimers. V_{uncat} is the apparent spontaneous rate of formation of **5** in buffer alone, and $V_{net} = V_{app} - V_{uncat}$ (V_{app} is the apparent rate of formation of **5** in the presence of dendrimer). The reactions were run in 96-well polystyrene half-area microtiter plates and monitored by a SpectraMAX fluorescence detector with $\lambda_{exc} = 350$ nm, $\lambda_{em} = 505$ nm. Fluorescence was converted into product concentration by using a calibration curve, which was linear in the concentration range used.

with a four-helix-bundle peptide catalyst of their design, containing six histidine residues,[2d] which suggests that histidine catalysis occurs without direct participation of the serine and aspartate residues. The reaction of α-methylphenacetyl ester **4** was also catalyzed by the dendrimers. Reaction with the pure enantiomeric substrates showed modest yet significant enantioselectivity, with the best dendrimer (**E-F**) showing an E value of 2.0 in favor of the (*S*)-**4** enantiomer (Table 2).

In summary, peptide dendrimers were assembled from a branching diamino acid building block by means of a disulfide-dimerization strategy. The synthetic strategy chosen defines a broad structural family in which structural variations are readily accessible by changing the amino acids, branching diamino acids, and capping components. Out of a total of 32 million possible dimeric peptide dendrimers with the given branching and capping units, and 20 proteinogenic amino acids ($n = 20^3$ monomers, $n(n+1)/2$ dimers), a subset of 21 dendrimers incorporating a catalytic triad of amino acids at the variable positions was prepared and tested for ester hydrolysis. Dendrimers bearing histidine residues at the outermost position in the sequence showed selective substrate recognition and enzymelike catalytic behavior in an aqueous

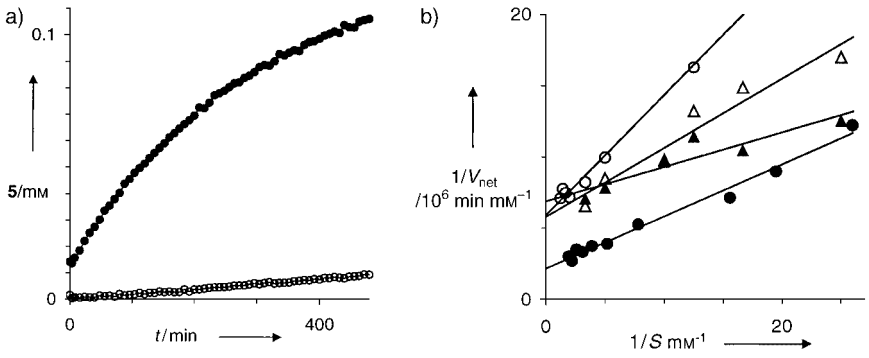


Figure 1. a) Hydrolysis of **3** to form **5** in the presence (●) or absence (○) of dendrimer **E-F**, with 200 μM substrate and 5 μM catalyst; b) double reciprocal plot for ester hydrolysis catalyzed by peptide dendrimer **E-F**. (○) acetate ester **1**; (△) α-methylphenacetyl ester (*S*)-**4**; (▲) α-methylphenacetyl ester (*R*)-**4**; (●) isobutyroyl ester **3**; *S* = substrate concentration. Conditions as in Tables 1 and 2.

  0044-8249/03/11512-1420 $ 20.00+.50/0

Table 2: Michaelis–Menten parameters for the three most active dendrimers on substrates **1**–**4**.[a]

		1	**2**	**3**	(*S*)-**4**	(*R*)-**4**	E[b]
	k_{uncat} [min^{-1}]	3.6×10^{-4}	2.4×10^{-4}	2.0×10^{-4}	3.7×10^{-4}	3.7×10^{-4}	
4-MeIm	k_2 [mM^{-1} min^{-1}]	6.7×10^{-3}	5.6×10^{-3}	7.0×10^{-3}	8.4×10^{-3}	8.0×10^{-3}	
E-E	K_M [mM]	0.20	0.21	0.11	0.10	0.13	
	k_{cat} [min^{-1}]	0.31	0.26	0.28	0.21	0.20	
	k_{cat}/k_{uncat}	860	1050	1380	570	540	
	$k_{cat}/K_M/k_2$	230	220	350	240	190	1.27
	v_{net}/v_{uncat}[c]	9.2	12.5	23.3	9.2	8.3	
F-F	K_M [mM]	0.52	0.34	0.27	0.021	0.016	
	k_{cat} [min^{-1}]	0.28	0.22	0.20	0.037	0.035	
	k_{cat}/k_{uncat}	770	890	1020	102	97	
	$k_{cat}/K_M/k_2$	80	110	110	210	270	0.77
	v_{net}/v_{uncat}[c]	6.4	8.4	12	2.3	2.2	
E-F	K_M [mM]	0.14	0.12	0.17	0.030	0.080	
	k_{cat} [min^{-1}]	0.12	0.33	0.33	0.10	0.12	
	k_{cat}/k_{uncat}	340	1380	1680	290	340	
	$k_{cat}/K_M/k_2$	130	360	280	360	180	2.00
	v_{net}/v_{uncat}[c]	12.0	18.8	31.5	6.2	6.1	

[a] Conditions and measurement method as in Table 1 with 5 μM dendrimer and 40–700 μM substrate. The kinetic constants given are derived from the linear double-reciprocal plots of $1/V_{net}$ versus $1/S$ (Figure 1b), with $r^2 > 0.95$ (r = correlation coefficient); [b] $E = (k_{cat}/K_M((S)\text{-}\mathbf{4}))/(k_{cat}/K_M((R)\text{-}\mathbf{4}))$; [c] V_{net}/V_{uncat} observed with S = 200 μM and 5 μM dendrimer (see footnote Table 1).

medium. Although the catalytic activities observed occur with a relatively labile class of ester substrates, this work opens the way for further development of peptide dendrimers as enzyme mimics. Further structural characterization and mechanistic studies and the exploration of building-block variations to improve the activity and diversity of catalytic peptide dendrimers are underway.

Received: October 9, 2002
Revised: December 11, 2002 [Z50330]

Keywords: dendrimers · enantioselectivity · enzyme catalysis · hydrolysis · peptides

[1] a) J. W. Bryson, S. F. Betz, H. S. Lu, D. J. Suich, H. X. Zhou, K. T. O'Neill, W. F. DeGrado, *Science* **1995**, *270*, 935; b) L. Baltzer, H. Nilsson, J. Nilsson, *Chem. Rev.* **2001**, *101*, 3153; c) M. Mutter, S. Vuilleumier, *Angew. Chem.* **1989**, *101*, 551; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 535; d) A. J. Kirby, *Angew. Chem.* **1996**, *108*, 770; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 707.

[2] a) B. Gutte, M. Daeumigen, E. Wittschieber, *Nature* **1979**, *281*, 650; b) K. Johnsson, R. K. Allemann, H. Widmer, S. A. Benner, *Nature* **1993**, *365*, 530; c) K. S. Broo, L. Brive, P. Ahlberg, L. Baltzer, *J. Am. Chem. Soc.* **1997**, *119*, 11362; d) S. Kerstin, H. Nilsson, J. Nilsson, L. Baltzer, *J. Am. Chem. Soc.* **1998**, *120*, 10287.

[3] a) G. T. Copeland, E. R. Jarvo, S. J. Miller *J. Org. Chem.* **1998**, *63*, 6784; b) M. M. Vasbinder, R. J. Elizabeth, S. J. Miller, *Angew. Chem.* **2001**, *113*, 2906; *Angew. Chem. Int. Ed.* **2001**, *40*, 2824; c) T. E. Horstmann, D. J. Guerin, S. J. Miller, *Angew. Chem.* **2000**, *112*, 3781; *Angew. Chem. Int. Ed.* **2000**, *39*, 3635; d) T. E. Hortstmann, D. J. Guerin, S. J. Miller, *Org. Lett.* **1999**, *1*, 1247; e) B. R. Sculimbrene, S. J. Miller, *J. Am. Chem. Soc.* **2001**, *123*, 10125.

[4] For selected references on dendrimers, see: a) S. Hecht, J. M. J. Fréchet, *Angew. Chem.* **2001**, *113*, 76, *Angew. Chem. Int. Ed.* **2001**, *40*, 74; b) S. M. Grayson, J. M. J. Fréchet, *Chem. Rev.* **2001**, *101*, 3819; c) G. R. Newkome, C. N. Moorefield, F. Vögtle, *Dendritic Molecules: Concepts, Synthesis, Perspectives*, VCH, Weinheim, **1996**; d) A. W. Bosman, H. M. Janssen, *Chem. Rev.* **1999**, *99*, 1665; e) D. A. Tomalia, *Adv. Mater.* **1994**, *6*, 529; f) H.-F. Chow, T. K.-K. Monga, M. F. Nongruma, C.-W. Wan, *Tetrahedron* **1998**, *54*, 8543.

[5] a) D. A. Tomalia, P. R. Dvornic, *Nature* **1994**, *372*, 617; b) J. W. J. Knapen, A. W. Van der Made, J. C. De Wilde, P. W. N. M. van Leeuwen, P. Wijkens, D. M. Grove, G. van Koten, *Nature* **1994**, *372*, 659; c) L. Balogh, D. A. Tomalia, *J. Am. Chem. Soc.* **1998**, *120*, 7355; d) M. Zhao, R. M. Crooks, *Angew. Chem.* **1999**, *111*, 375; *Angew. Chem. Int. Ed.* **1999**, *38*, 364; e) B. Bhyrappa, G. Vaijayanthimala, *J. Am. Chem. Soc.* **1999**, *121*, 262; f) S. Nlate, J. Ruiz, V. Sartor, R. Navarro, J. C. Blais, D. Astruc, *Chem. Eur. J.* **2000**, *6*, 2544; g) P. Weyermann, F. Diederich, *Helv. Chim. Acta* **2002**, *85*, 599; h) H. Sellner, P. B. Rheiner, D. Seebach, *Helv. Chim. Acta* **2002**, *85*, 352; i) L. J. Twyman, A. S. H. King, I. K. Martin, *Chem. Soc. Rev.* **2002**, *31*, 69.

[6] K. Sadler, J. P. Tam, *Mol. Biotechnol.* **2002**, *90*, 195.

[7] a) R. G. Denkelwalter, W. J. Lukasavage, , US Pat. 44101081 **1983**; b) T. M. Chapman, G. L. Hillyert, E. J. Mahan, K. A. Shaffer, *J. Am. Chem. Soc.* **1994**, *116*, 11195; c) Y. Kim, F. Zeng, S. C. Zimmermann, *Chem. Eur. J.* **1999**, *5*, 2133.

[8] G. Sengle, A. Jenne, P. S. Arora, B. Seelig, J. S. Nowick, A. Jäschke, M. Famulok, *Bioorg. Med. Chem.* **2000**, *8*, 1317.

[9] N. Bensel, M. T. Reymond, J.-L. Reymond, *Chem. Eur. J.* **2001**, *7*, 4604.

[10] J. E. Whitaker, R. P. Haugland, P. L. Moore, P. C. Hewitt, M. Reese, R. P. Haugland, *Anal. Biochem.* **1991**, *198*, 119.

[11] F. M. Menger, K. H. Nelson, Y. Guo, *Chem. Commun.* **1998**, *18*, 2001.

[12] These ester substrates all have similar uncatalyzed reaction rates, for example, at pH 6.0 (20 mM aqueous Bis-Tris buffer): umbelliferyl acetate: $k_{uncat} = 2\times10^{-5}\,s^{-1}$; umbelliferyl acetoxymethyl ether: $k_{uncat} = 6\times10^{-6}\,s^{-1}$; 8-acetoxypyrene-1,3,6-trisulfonate: $k_{uncat} = 2\times10^{-6}\,s^{-1}$, 7-acetoxy-1-methylquinolinium sulfate: $k_{uncat} = 6\times10^{-6}\,s^{-1}$.

  0044-8249/03/11512-1421 $ 20.00+.50/0