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Screening of a Combinatorial Library Reveals Peptide-Based Catalysts for Phosphorester Cleavage in Water

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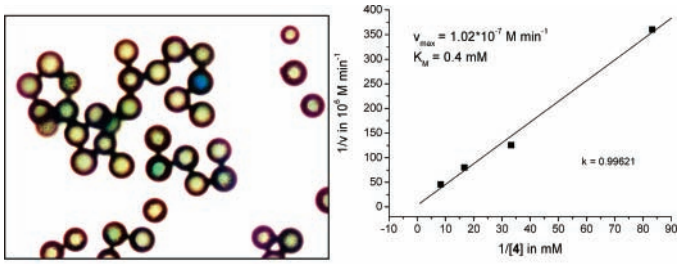
ABSTRACT



1c = Cbz-His-Gly-Gua-D-Pro-Aib-Gua-Gly-Val-NH_2

A combinatorial library of 625 octapeptides was screened for their efficiency to catalyze phosphorester cleavage in water. Octapeptides such as 1c, which contains a catalytically active histidine and two cationic residues (Gua) as potential anion-binding sites, show substrate hydrolysis in water. Metal-free catalysis with a rate enhancement of up to a factor of 175 over the uncatalyzed background reaction is observed.

In Nature, about 50% of all enzymes are metal-free and hence solely rely on simple organic functionalities in the amino acid side chains to achieve both substrate binding and then its chemical transformation. Therefore, there has been a long-standing interest in the development of small peptide-based catalysts. However, early attempts to model the active sites of enzymes such as chymotrypsin using small peptides were futile.[1] Only in the past few years has metal-free catalysis with small peptides been succesfully achieved for reactions such as hydrocyanation, acylations, and aldol or Michael reactions.[2,3] In most cases, such peptide-based catalysts are limited to nonpolar organic solvents, as substrate binding is often depending on H-bonds which are not strong enough under more competitive conditions.[4] For example, catalytically active oligopeptides with a modified *N*-methylhistidine developed by Miller mainly function in chloroform or toluene.[5] Proline-rich peptides as for example developped by Wennemers for asymmetric aldol reactions[6] or by Tsogoeva[7] for Michael additions are limited to aprotic solvents such as acetone and DMSO and low temperatures.

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(3) Amino acids and peptide-based catalysts have also been used for other reactions such as oxidations, phosphorylations, or sulfinylations. For some examples, see: (a) Licini, G.; Bonchio, M.; Broxterman, Q. B.; Kaptein, B.; Moretto, A.; Toniolo, C.; Scrimin, P. *Biopolymers* **2006**, *84*, 97−104. (b) Berkessel, A.; Koch, B.; Toniolo, C.; Rainaldi, M.; Broxterman, Q. B.; Kaptein, B. *Biopolymers* **2006**, *84*, 90−96. (c) Peris, G.; Jakobsche, C. E.; Miller, S. J. *J. Am. Chem. Soc.* **2007**, *129*, 8710−8711. (d) Morgan, A. J.; Komiya, S.; Xu, Y.; Miller, S. J. *J. Org. Chem.* **2006**, *71*, 6923−6931.;

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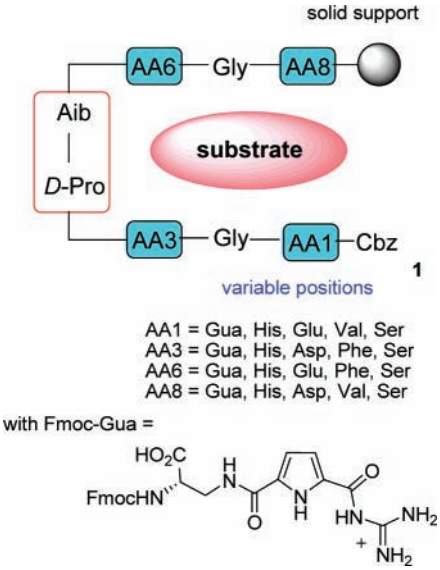
Only a few examples for peptide-based catalysis in aqueous solutions have been reported. For example, catalytically active peptide fragments such as His-Ser incorporated into a dendrimer show esterase-like activity in water as was recently reported by Reymond.[8] Also hydrophobic interactions can be used to achieve substrate binding and hence catalysis in aqueous solvents.[9] In this context, we present here new peptide based catalysts such as **1c** identified from the screening of a combinatorial library[10] **1** that are capable to hydrolyze phosphoresters in aqueous buffer solution even without the shielding environment of a dendrimer or the need for extensive hydrophobic interactions.[11]

The design of octapeptide library **1** is shown in Scheme 1. A D-Pro-Aib-turn element[4b,12,13] was incorporated at the

Scheme 1. Design of the Oligopeptide Library **1**



central positions 4 and 5 of the octapeptide to hopefully help induce a more folded conformation in solution. Positions 1 and 3 as well as 6 and 8 were chosen as the variable positions with a glycine in between to reduce the steric bulk within the oligopeptide. For each of the four variable positions five different amino acids were used in the split-mix-protocol giving rise to a total library size of 625 members. For these five amino acids different combinations of six proteinogenic amino acids (His, Ser, Glu, Asp, Phe, Val) as well as an artificial arginine analogue Gua[14,15] were used (Scheme 1). This choice of amino acids was based on the idea that the polar amino acids (His, Glu, Asp, Ser) could function as catalytically active residues or help in structuring the oligopeptide (e.g., via ion-pair formation between the arginine analogue and Glu or Asp). The two nonpolar amino acids Phe and Val could provide a less polar microenvironment further favoring substrate binding or catalysis.

The library was synthesized on amino-TentaGel using a standard Fmoc-protocol with PyBOP as the coupling reagent and the IRORI radio frequency tagging technology[16] for the decoding of the split-mix-combinatorial variation. This tagging technique provides spatially separated, macroscopic quantities of each library member and hence allows further studies on bead with individual library members later on. The amino acids (3 equiv in each coupling step) were used as their Fmoc derivatives with free carboxylic acid groups and acid-sensitive protection groups (tBoc, tBu) in the side chains where needed. After each coupling step, the Fmoc group was removed with a solution of 20% piperidine in DMF before the next amino acid was attached. The last amino acid (AA1 in Scheme 1) was coupled in form of the *N*-Cbz-protected derivative. After library synthesis, the side chain protecting groups were cleaved off using 50% TFA in CH_2Cl_2 to give the unprotected library members still attached to the solid support.

After the synthesis the catalytic activity of the individual octapeptides within the library was probed using an on-bead color assay by incubation of the beads with the indole phosphate **2**. Efficient hydrolysis of the phosphorester in **2** leads—after oxidation—to the formation of an indigo dye **3** and hence a blue staining of active beads.[17] We first probed a pooled mixture of the library members to determine suitable conditions for the assay which led to a staining of <5 % of

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(16) (a) Nicolaou, K. C.; Pfefferkorn, J. A.; Mitchell, H. J.; Roecker, A. J.; Barluenga, S.; Cao, G.-Q.; Affleck, R. L.; Lillig, J. E. *J. Am. Chem. Soc.* **2000**, *122*, 9954−9967. (b) Czarnik, A. W. *Curr. Opin. Chem. Biol.* **1997**, *1*, 60−66.

(17) Berkessel, A.; Herault, D. A. *Angew. Chem., Int. Ed.* **1999**, *38*, 102−105.

the beads. These conditions were then used to screen the individual library members in microtiter plates ([**2**] = 1 mM in 10 mM bis-tris buffer at pH = 6.0, incubation at 30 °C). This screening assay showed that indeed some—but only some—library members were capable to efficiently hydrolyze the phosphor monoester in **2** as indicated by the different degree of staining of the beads (Figure 1).

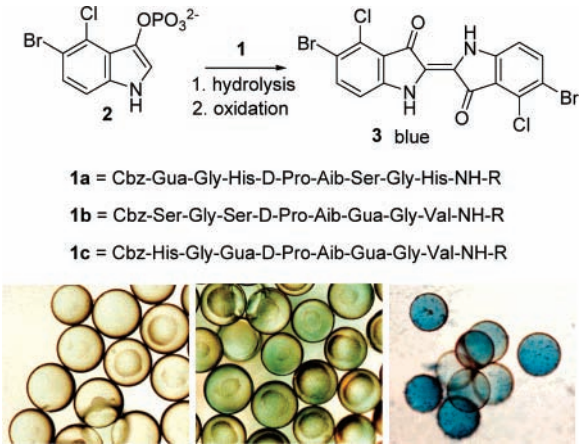

Figure 1. Activity screening of solid-phase bound library **1** (R = resin) in buffered water (bis-tris buffer, pH = 6.0) based on the formation of an indigo dye **3** upon hydrolysis of **2**. After 14 h, octapeptide **1a** shows no (left), **1b** medium (middle), and **1c** high catalytic activity (right).

Interestingly, the most active sequences in this screening all contained the artificial arginine analogue Gua in combination with either serine or histidine. For example, both the medium active sequence **1b** as well as the most active sequence **1c** in this screening contain one or two of our arginine analogues Gua as well as either Ser (**1b**) or His (**1c**), respectively. Hence, a cationic binding site[18] seems to be crucial for catalysis in aqueous solvents most likely by allowing substrate binding.[19] Serine or histidine seem to be the catalytically active residues, as replacement of Ser or His in **1b** and **1c** by either valine or phenylalanine leads to completely inactive sequences. The catalytic activity is furthermore depending on the actual sequence of the octapeptide suggesting that catalysis indeed occurs after substrate binding within a well-defined host−guest complex and not by some unspecific acid/base mechanism. For example, sequence **1a** also contains the binding site Gua as well as His and Ser similar to **1b** and **1c**, but no catalytic activity is observed as the groups are arranged in a different way.

(18) A catalytic role for the guanidiniocarbonyl pyrrole residue (e.g. as a nucleophile) is not likely, as a similar arginine-containing peptide (**1c** with two arginines instead of Gua) is also catalytically active. But arginine due to its larger basicity is unlikely to act as a nucleophile. Therefore, the cation most likely is involved in electrostatic stabilization of the TS and substrate binding. We are currently exploring the differences between Gua and Arg in more detail.

(19) Of course, it can not be completely ruled out at this point, that substrate binding is also due to some hydrophobic interactions with the peptide. However, due to the charged nature of both peptide and substrates and the necessity of a cationic group in the peptide (ref 18), this seems to be less likely.

The most active octapeptide **1c** (as well as the inactive sequence **1a**) was resynthesized in larger amounts (using standard Fmoc chemistry on Rink amide resin) and was then—after cleavage from the resin—also studied in solution using initial rates obtained from the concentration−time profiles (Figure 2). The hydrolysis of *p*-nitrophenyl

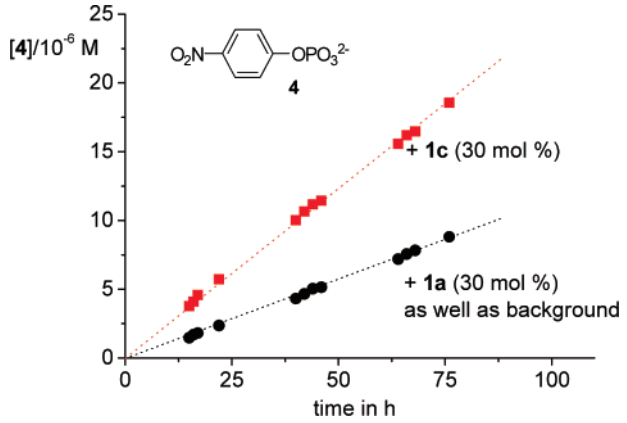

Figure 2. Concentration−time profile of the hydrolysis of **4** (0.1 mM) with or without octapeptide **1c** (R = H, 30 mol %) followed by the increase in UV absorbance at 400 nm (1 mM aqueous bis-tris buffer, pH = 6.0, temperature T = 50 °C).

phosphate **4** (0.1 mM) is efficiently catalyzed by 30 mol % **1c** (but not **1a**) in buffered water (1 mM bis-tris buffer, pH = 6.0). The addition of EDTA did not have any effect on the rates, indicating that this is indeed a metal-free catalysis. A saturation kinetic in the presence of **1c** (R = H, [**1c**] = 0.03 mM) under the same conditions (1 mM aqueous bis-tris buffer, pH = 6.0, T = 50 °C) provided the following kinetic parameters based on an initial-rate analysis from a Lineweaver−Burk plot (Figure 3): k_{cat} = 0.0032 min^{-1}, K_m = 0.4 mM, and k_{cat}/K_m = 8 M^{-1}·min^{-1}.

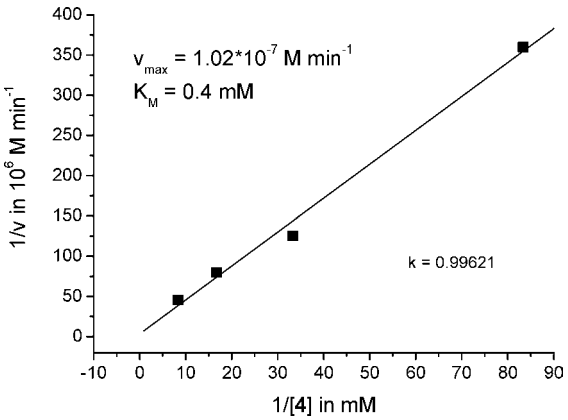

Figure 3. Lineweaver−Burk plot for the hydrolysis of **4** in the presence of octapeptide **1c** (R = H,[1c] = 0.03 mM, in 1 mM aqueous bis-tris buffer, T = 50 °C).

Hence, **1c** shows a significant rate enhancement for the hydrolysis of phosphate **4** compared to the uncatalyzed background reaction ($k_{uncat} = 1.8 \times 10^{-5}$ min^{-1}) with $k_{cat}/k_{uncat} = 175$.

In conclusion, we have shown here that oligopeptides such as **1c** can be efficient metal-free catalysts for the hydrolysis of phosphoresters also in aqueous solution. We are currently further exploring the scope and mechanism of these new peptide catalysts.

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Supporting Information Available: NMR, ESI-MS, and IR spectra of petide **1c** and selected peptide sequences from the library screening and their activity. This material is available free of charge via the Internet at http://pubs.acs.org.

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