Electronic Supplementary Material for Chemical Communications

Supporting Information for

A Combinatorial Approach to Minimal Peptide Models of Metalloprotein Active Sites

by

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General materials and instrumentation. The Fmoc-protected amino acids, O-benzotriazole-*N*,*N*,*N'*,*N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU), *N*-*N'*-diisopropylethylamine (DIPEA) and the solvent *N*-methyl-2-pyrrolidinone (NMP) were purchased from Advanced ChemTech. TentaGel-S-NH_2 and Fmoc-Phe-Wang resins were obtained from Peptides International. Benzotriazol-1-yloxy-tripyrrolidinophosphonium hexafluorophosphate (PyBop), methyl sulfide (DMS), ethane thiol (EtSH), 5-bromo-4-chloro-3-indolyl phosphate (BCIP), 4-nitrophenyl acetate (4-NA), $CoCl_2$•$6H_2O$ and the solvents DMF and TFA were purchased from Acros and Fischer and used without further purification. Piperidine, tris(2-carboxyethyl)phosphine (TCEP), dithiothreitol (DTT), $Zn(OAc)_2$•$2H_2O$ and HEPES (*N*-(1-hydroxyethyl)piperazine-*N*-2-ethanesulfonic acid) were obtained from Sigma Aldrich. Phenol was obtained from J. T Baker. A LeicaMZ 7.5 stereo zoom optical microscope equipped with a digital camera was used for library screening. Edman sequencing of single peptide-resin beads was performed on a Perkin-Elmer/Applied Biosystems Procise protein sequencing system at the Synthesis and Sequencing Facility of Johns Hopkins University. ESI mass spectra were acquired with a Finnigan LCQDeca ion-trap mass spectrometer equipped with an electrospray ionization source (Thermo Finnigan, San Jose, CA). Kinetic studies and cobalt optical spectra were

collected on an Agilent 8453 photodiode-array spectrophotometer equipped with a thermostable cell holder and the Agilent biochemical analysis software package.

Peptide library synthesis. The peptide library (resin-Phe-His-X-X-Glu-His-X-Gly-Leu-Cys-X-NH_2) was manually synthesized by standard Fmoc procedures and split/pool methodology using Tentagel-S-NH_2 (90 μm, 0.24 mmol/g) resin on a 0.2 g scale. Couplings were performed by using 2 equiv of Fmoc-L-amino acid, 2 equiv of PyBOP, and 3% N-methyl morpholine in DMF for 1 h. Deprotections were performed by using 20% piperidine in DMF. For variable amino acids at the X positions, the resin was split into 5 equivolume portions and each of the variable amino acids (Arg, Ser, Ala, Gly or Asp) was added to a different portion. After coupling was complete, the 5 portions of resin were pooled together and deprotected as usual. Coupling completion was checked by both the bromophenol blue and the kaiser tests. After the last cycle, side chain protecting groups were cleaved using a mixture of TFA: EtSH:DMS:phenol; 90:5:2.5:2.5. The peptide library was stored in DMF at 4 °C.

Screening of the peptide library. An amount of the peptide library (5 mg, ~ 2500 beads) was washed with H_2O and then HEPES buffer (20 mM) and transferred to a Petri dish for easy viewing under the microscope. The peptide library was then incubated with $Zn(OAc)_2$ (1 μmol) in 2 mL of HEPES buffer (20 mM) at pH 6 for 15 min. An amount of BCIP (1 μmol) dissolved in HEPES buffer (20 mM, pH 6) was then added, and the peptide library was monitored every 6 – 12 h by viewing under the microscope. After 48 h, 1 – 3 dark blue beads were seen, and one dark blue bead was selected and isolated by microsyringe. A control experiment in the absence of $Zn(OAc)_2$ was run under identical conditions, and only a faint blue color due to background

hydrolysis was observed in the solution. The selected dark blue beads were analyzed by Edman sequencing.

Synthesis of isolated peptides. The peptides **A**, **B**, and **C** (Figure 3) were prepared on a Symphony Quartet Multiple automated synthesizer on Fmoc-Phe-Wang resin by using standard Fmoc procedures. Efficient coupling for 100 mg of resin (loading capacity of 0.62 mmol/g) involved double couplings at each step. Each single coupling step was done using 4 equiv of Fmoc-protected amino acids, 4 equiv of HBTU, and excess DIPEA in NMP for 30 min. After the last step, side chain deprotection and cleavage from the resin was performed by suspending the resin in a cleavage cocktail containing TFA:EtSH:DMS:Phenol/ 90:5:2.5:2.5 for 3 h. Crude peptides were precipitated and triturated with cold diethyl ether, dissolved in water (0.1% TFA) and lyophilized to give a white fluffy solid that was analyzed by ESI-MS to confirm the presence of the desired peptide in the crude product. Crude peptides were dissolved in water and incubated with excess DTT or TCEP for 30 min at 55 °C to reduce any disulfides prior to purification by reversed-phase C_{18} HPLC on a Waters 626 gradient HPLC system fitted with nonmetallic (PEEK) tubing. An acetontirile/water gradient containing 0.1% (v/v) TFA was used. The single major HPLC peak for each peptide was collected and dried in an anaerobic chamber under a 10% hydrogen/90% nitrogen atmosphere. The identity of the purified peptides was confirmed by ESI-MS obsd (calcd) (*m/e*): **A** 1397.8 (1397.6) $[M]^+$; **B** 1312.8 (1312.5) $[M]^+$ **C** 1255.7 (1255.4) $[M]^+$.

Hydrolysis of BCIP in homogeneous solution. An amount of peptide **A**, **B**, or **C** (1 mL of a 1 mM solution) was incubated with $Zn(OAc)_2$ (1 mL of a 1 mM solution) in HEPES buffer (20

mM, pH 6) for 1 h under anaerobic conditions in a closed glass vial. BCIP (1 mL of a 1 mM solution) was then added, and the reaction mixture was exposed to air. Parallel control reactions involving BCIP combined with a) apopeptide b) $Zn(OAc)_2$ alone and c) neat buffer were set up in the same manner (see Figure 4). For peptides **A** and **B**, a dark blue color formed within 72 h. Peptide **C** and all control experiments showed only a faint blue color due to background hydrolysis after this time.

Hydrolysis of 4-nitrophenyl acetate (4-NA). The rate of hydrolysis of 4-NA was monitored by UV-spectroscopy, following the production of the 4-nitrophenolate anion at 405 nm. A typical experiment was run as follows: Under anaerobic conditions, a 1.5 mL quartz UV-vis cuvette was loaded with peptide and $Zn(OAc)_2$ in 900 μL of HEPES buffer (20 mM) at pH 7.5, and allowed to incubate for 1 h. An amount of 4-NA in dry CH_3CN (100 μL) was then added to the cuvette to initiate the reaction. Concentrations of peptide, Zn^{2+}, and 4-NA in the cuvette were each 1 mM. The initial rate of hydrolysis was monitored up to 3% completion. Linear initial rates were observed for peptides **A**, **B** and background hydrolysis (buffer alone).