Design and Synthesis of a Versatile DNA-Cleaving Metallopeptide Structural Domain

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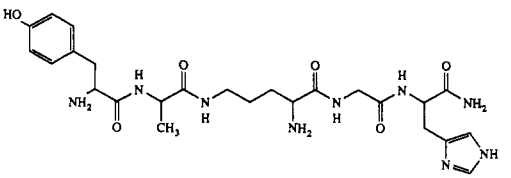
The design of metal-binding sites and their sequence-specific incorporation into proteins and peptides has become a topic of considerable current interest.[1] Previous studies have demonstrated that sites of metal complexation can be utilized to effect the conformational control and assembly of peptide[2] or protein[3] structures, alterations in enzymatic activity,[4] and the evaluation of novel biophysical properties.[5] Here we report the design and synthesis of a high-affinity metal-binding tripeptide sequence that can be activated to efficiently mediate DNA strand scission and may be easily incorporated *at any position* within an oligopeptide chain.

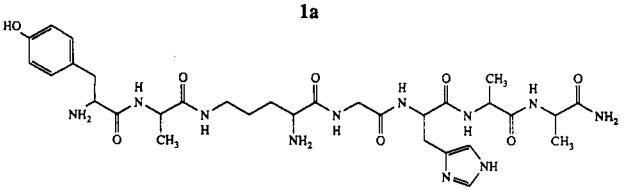
Our strategy involves the synthetic redesign of the tripeptide NH_2-Gly-Gly-His (GGH) which mimics the amino-terminal, square planar Cu(II)-chelating domain of serum albumins.[6] At physiological pH values GGH binds Cu(II) in a 1:1 complex through the histidine imidazole nitrogen, two deprotonated amide nitrogens, and the terminal α-amine with a dissociation constant on the order of 10^{-16}–10^{-17}.[6] Upon metal ion complexation and subsequent chemical activation, this simple ligand system is competent to produce oxidizing equivalents that are capable of effecting DNA[7,8] or protein cleavage.[9] In the case of the Cu(II) complex of GGH, diffusible oxidants are thought to be produced[7,8] while the Ni(II) complex[10] is believed to create a metal-bound oxidizing equivalent.[8b,c,11]

Unfortunately, however, due to the central role played by the terminal amine functionality of this tripeptide unit in the coordination of metal ions, the incorporation of GGH is limited to the amino-terminus of polypeptide structures. Accordingly, we sought to design a peptide-based system *which preserves the metal-binding, electronic, and catalytic properties of the unique GGH tripeptide while also permitting its incorporation at any site along a polypeptide chain.* Our design requirements led to the synthesis of the model peptides **1a** and **1b**, which contain a *carboxy-terminal* and *interior* Cu(II)-binding domain, respectively. As the primary structure indicates, these peptides contain a tripeptide sequence of (δ)-Orn-Gly-His through which the growing peptide chain[12] is attached via the *δ-amino* group of ornithine. In theory, this design leaves the α-amino group of ornithine free to participate in Cu(II) complexation (Scheme I) in a fashion analogous to that of the amino-terminal glycine in Gly-Gly-His.[13]



NH_2-Tyr-Ala-(δ)-Orn-Gly-His-$CONH_2$

1a



NH_2-Tyr-Ala-(δ)-Orn-Gly-His-Ala-Ala-$CONH_2$

1b

The synthesis of peptides **1a** and **1b** was accomplished by conventional solid-phase peptide synthesis (SPPS) techniques[14] using N^{δ}-Boc-N^{α}-Fmoc-ornithine as illustrated in Scheme I. After purification of the peptides by reversed-phase HPLC and verification of structure,[14] the ability of **1a** and **1b** to bind Cu(II) in the intended fashion was examined by UV–vis spectroscopy; these complexes yielded a characteristic absorbance at λ_{max} = 520–530 nm exactly analogous to that observed with Cu(II)·GGH and similar metallopeptides which utilize an α-amino functionality in metal chelation.[6,13,15] In addition, an ESR spectrum identical to those of previously characterized metallopeptides[13,16] (g_0 = 2.090, superhyperfine splitting constant = 12.8 G) provided clear evidence for the ability of **1a** or **1b** to bind Cu(II) in the manner depicted in Scheme I. Subsequently, we also sought to determine whether the Cu(II) and Ni(II) complexes of (δ)-Orn-Gly-His possessed redox properties capable of effecting DNA cleavage like that of Gly-Gly-His metal complexes. As illustrated in Figure 1, preincubation of the Cu(II)·**1a** or Ni(II)·**1a** complexes with ΦX174 RF plasmid followed by activation with ascorbate/H_2O_2 or oxone,[11] respectively, resulted in facile conversion of the intact form I DNA to forms II and III; essentially identical results were obtained for the Cu(II) and Ni(II) complexes of **1b** and the control tripeptide NH_2-Gly-Gly-His.[17]

The preceding observations demonstrate that the incorporation of the simple (δ)-Orn-Gly-His sequence into polypeptide structures

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(9) Cuenoud, B.; Tarasow, T. M.; Schepartz, A. *Tetrahedron Lett.* **1992**, *33*, 895.

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(11) Chen, X.; Rokita, S. E.; Burrows, C. J. *J. Am. Chem. Soc.* **1991**, *113*, 5884.

(12) The tyrosine residue was specifically included in order to provide a convenient means of accurately quantitating the peptide by UV–vis spectroscopy.

(13) (a) Lau, S.-J.; Laussac, J.-P.; Sarkar, B. *Biochem. J.* **1989**, *257*, 745. (b) Iyer, K. S.; Lau, S.-J.; Laurie, S. H.; Sarkar, B. *Biochem. J.* **1978**, *169*, 61. Amino acid substitutions with side chains bulkier than glycine within the parent NH_2-Gly-Gly-His sequence have little effect on metal complexation at or above physiological pH values, indicating that the histidine imidazole and α-amine are the primary determinants of metal ion complexation.

(14) Stewart, J. M.; Young, J. D. *Solid-Phase Peptide Synthesis*; Pierce Chemical Co.: Rockford, IL, 1984. (FAB-MS: 560.3 and 702.4 for **1a** and **1b**, respectively. UV–vis: λ_{max} = 275 nm, ϵ = 1500 (tyrosine), pH 7.5.)

(15) Shullenberger, D. F.; Long, E. C. *BioMed. Chem. Lett.* **1993**, *3*, 333. We have also carried out control reactions in parallel with the analogous GGH-containing peptide NH_2-GGHAY-$CONH_2$. Under our experimental conditions (pH 7.5, 25 mM sodium cacodylate buffer) we calculate identical molar extinction coefficients (ϵ = 108 cm^{-1} M^{-1}; λ_{max} = 525–527 nm; broad, flattened absorbance) for **1a**, **1b**, and GGHAY. Additionally, titration studies of each peptide with Cu(II) have indicated the formation of 1:1 peptide:Cu(II) complexes with affinities for Cu(II) similar to those previously described (K_D = 10^{-16}).[6,13]

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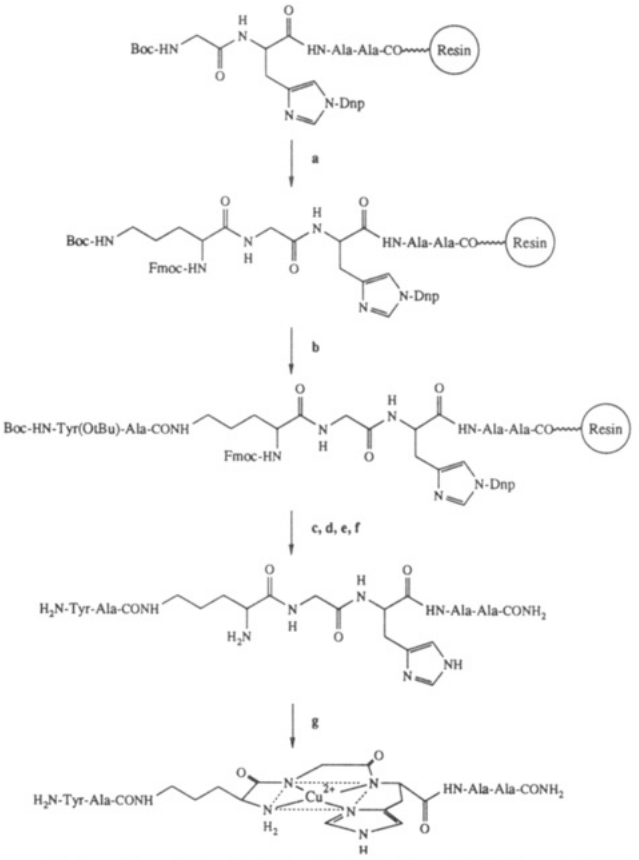
0002-7863/93/1515-11038$04.00/0

Scheme I. Stepwise Solid-Phase Peptide Synthesis (SPPS) of Ornithine-Based Metal-Binding Oligopeptides.[a]



[a] (a) Coupling of Boc-Orn(Fmoc) via Boc-benzyl SPPS method; (b) Boc-benzyl SPPS (two cycles); (c) thiophenol/DMF; (d) piperidine/DMF; (e) TFA/CH_2Cl_2; (f) NH_3/CH_3OH; (g) $Cu(OAc)_2$/10 mM sodium cacodylate.

provides an environment for metal ion binding which is similar electronically, structurally, and catalytically to that presented by NH_2-Gly-Gly-His. This modification significantly enhances the versatility of the latter metal-binding motif by allowing its synthetic or semisynthetic[18] incorporation *within* a polypeptide chain at sterically-permissible locations and, theoretically, several positions simultaneously. Importantly, this design also makes use of commercially-available amino acids and chemistries that can be incorporated into automated or manual synthesis protocols. While we have concentrated our initial efforts on the use of Boc-Orn(Fmoc) as the provider of the α-amino group used in metal ion complexation, a similar incorporation of analogously protected amino acids with side chain substitutions that are shorter or longer than ornithine (e.g., diaminopropionic acid or lysine, respectively) may permit the "optimization" of the linker chain connecting the metal-binding domain with the continuing amino-terminal peptide chain. Ongoing research will seek to explore the utility of this design and its incorporation as a structural and catalytic center in oligopeptides.

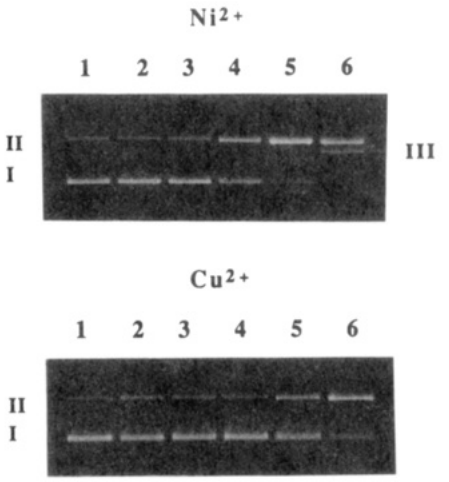


Figure 1. Cleavage of ΦX174 RF DNA (11 μM in base pairs) by Ni(II)·**1a** and Cu(II)·**1a**. Reactions with Ni(II) were initiated with oxone and quenched after 1.0 min with EDTA-containing (120 mM) loading buffer. Reactions with Cu(II) were initiated by the simultaneous addition of sodium ascorbate and H_2O_2 to preformed Cu(II) complexes and quenched after 1.5 min with EDTA buffer. All reactions were performed in 10 mM sodium cacodylate buffer, pH 7.5. Ni(II)·**1a** (top): lane 1, reaction control, DNA alone; lane 2, reaction control [100 μM **1a**, 80 μM Ni(II)]; lane 3, reaction control [80 μM Ni(II), 100 μM oxone]; lane 4, cleavage reaction [1 μM **1a**, 80 μM Ni(II), 100 μM oxone]; lane 5, cleavage reaction [5 μM **1a**, 80 μM Ni(II), 100 μM oxone]; lane 6, cleavage reaction [25 μM **1a**, 80 μM Ni(II), 100 μM oxone]. Cu(II)·**1a** (bottom): lane 1, reaction control, DNA alone; lane 2, reaction control [150 μM **1a**, 120 μM Cu(II), 150 μM sodium ascorbate]; lane 3, reaction control [150 μM **1a**, 120 μM Cu(II), 150 μM H_2O_2]; lane 4, reaction control [150 μM **1a**, 120 μM Cu(II)]; lane 5, cleavage reaction [25 μM **1a**, 20 μM Cu(II), 25 μM sodium ascorbate, 25 μM H_2O_2]; lane 6, cleavage reaction [150 μM **1a**, 120 μM Cu(II), 150 μM sodium ascorbate, 150 μM H_2O_2].

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Supplementary Material Available: UV–vis absorption by Cu(II)·**1a** and room temperature ESR analysis of Cu(II)·**1a** (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(17) In the case of Cu(II)·**1a**,**b** and Cu(II)·GGH, preformed complexes were required due to the DNA cleavage ability of Cu(II) + ascorbate alone as noted by Dervan[8a] and others.[7] Our activation conditions therefore employed a protocol similar to that used previously[8a] and incorporated the reaction Cu·peptide + ascorbate (no H_2O_2) as a control to verify complete coordination of the metal ion by **1a**,**b** or GGH. With Ni(II) + oxone activation, direct plasmid cleavage was observed in the presence of **1a**,**b** and GGH; while others have noted[8b,c] alkali-labile lesions in cleavage mediated by Ni(II)·GGH-derivatized proteins, the differences observed here may reflect (1) adventitious nicking of alkali-labile sites or (2) a random DNA strand scission event stemming from a bimolecular collision rather than an intimately-bound complex.

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