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DESIGN AND SYNTHESIS OF A DNA-CLEAVING METALLOPEPTIDE

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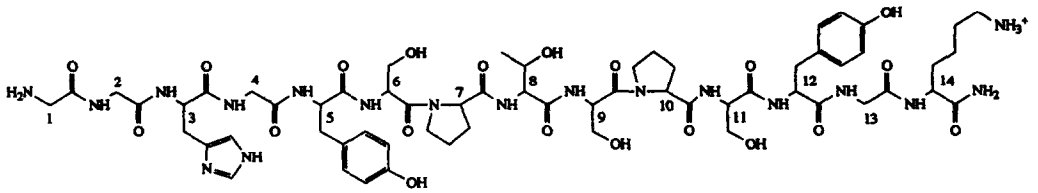
Abstract: A structured 14-residue peptide containing (1) a proposed intercalative DNA-binding domain consisting of tyrosine residues connected by tandem β-turns and (2) a redox-active Cu(II)-binding domain was synthesized and shown to readily cleave ΦX174 plasmid DNA under appropriate conditions of activation.

The design and synthesis of chemical agents that bind DNA and induce strand scission is of great current interest.[1] Research in this area has both elevated our understanding of the factors involved in the molecular recognition of DNA and produced chemical and photochemical probes of DNA structure in solution. Although it is not an absolute requirement,[2] many DNA-cleaving agents contain a complexed metal ion such that upon binding to DNA, phosphodiester bond breakage is effected via redox chemistry associated with the metal center.[1] However, despite widespread efforts devoted to the development of metal-complexing compounds that interact specifically with the DNA helix, the use of simple oligopeptides consisting of naturally-occurring L-α-amino acids is an approach that has received relatively little attention. To date, the majority of research along these lines has



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been limited to the use of large polypeptides and protein super-secondary structures of relatively high molecular weight which have been modified for the purpose of converting them into sequence-specific chemical nucleases.[3] As a consequence, the design and preparation of *low* molecular weight metallopeptides of *defined structure* that can associate with and subsequently mediate oxidative cleavage of DNA represents an essentially uncharted area of

considerable potential. In particular, given the broad access to synthetic peptides provided by current solid-phase methodologies, this approach offers an opportunity to take advantage of the growing body of information that deals with the structural features utilized by proteins for such purposes as DNA binding and metal chelation.[4]

As a starting point from which to design metallopeptides, we have integrated two unique protein structural elements: (1) the proposed DNA *bis*-intercalating octapeptide repeat of eucaryotic RNA polymerase II,[5] and (2) the redox-active[3b,c] Cu(II)-binding domain Gly-Gly-His from the serum albumin protein family.[6] These features are intended to endow our peptides with the capacity to effect the oxidative scission of DNA by allowing efficient delivery of the catalytic Cu domain to the DNA helix through the binding action of a tyrosine intercalation domain. Interestingly, this arrangement of functional "domains" is reminiscent of that observed in the widely-studied glycopeptide antitumor agent bleomycin.[1b,7]

Implementation of this strategy led to the synthesis of peptide **1**; as the primary sequence indicates, this compound contains a positively-charged Lys residue at the carboxy terminus and a metal-binding Gly-Gly-His unit at the amino terminus, each separated from the central octapeptide unit Tyr-Ser-Pro-Thr-Ser-Pro-Ser-Tyr by a Gly spacer. The presence of the Lys residue was intended both to promote water solubility of the peptide and to facilitate its initial interaction with the negatively-charged DNA through simple electrostatic attraction. Molecular modeling studies of **1** indicate that the peptide is able to assume a conformation consistent with (1) that proposed[5] for the octapeptide Tyr-Ser-Pro-Thr-Ser-Pro-Ser-Tyr in which two consecutive β-turns[8] impart a closed structure such that the Tyr side chains assume a parallel orientation poised for *bis*-intercalation into DNA, and (2) Cu(II) binding by the Gly-Gly-His tripeptide tail in a square planar complex identical to that which has previously been characterized with the model peptide Gly-Gly-His-NHMe by X-ray crystallography.[9] Peptide **1** was synthesized manually on MBHA resin by the solid-phase method of Merrifield using a Boc-Benzyl protection scheme followed by side chain deprotection and resin cleavage with 1 M TFMSA in TFA.[10]

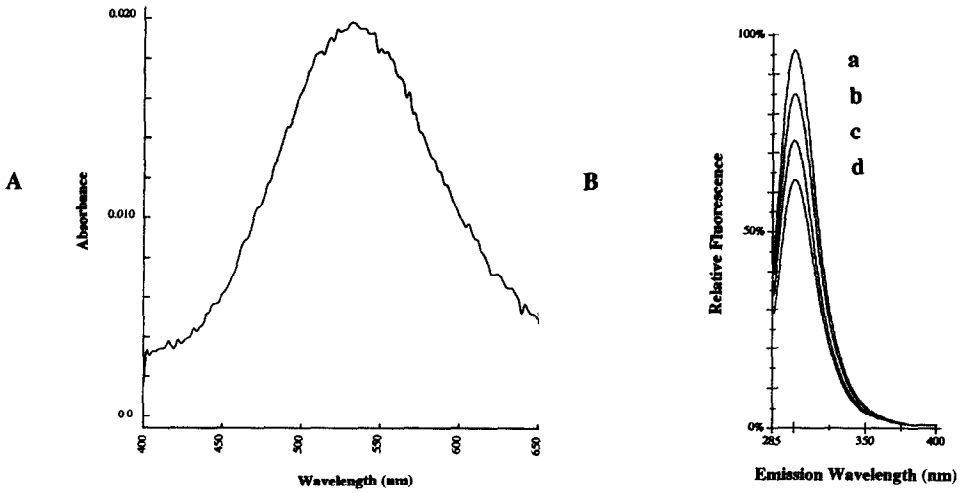

Figure 1. A: UV-vis absorption by the Cu(II) complex of peptide **1** [250 μM **1**, 200 μM $Cu(OAc)_2$] in 10 mM Na-cacodylate buffer (pH 7.5). B: Fluorescence emission (λ_{ex} 273 nm) by peptide **1** (40 μM in 1 mM Na-cacodylate, pH 7.5) upon titration with calf thymus DNA. Curve a, 0 μM DNA; curve b, 10 μM (bp) DNA; curve c, 20 μM (bp) DNA; curve d, 30 μM (bp) DNA.

The ability of **1** to bind Cu(II) in the intended fashion was demonstrated by UV-vis spectral analysis (**Figure 1A**) which showed an absorption band analogous to that observed with the Cu(II) complex of the model peptide Gly-Gly-His-NHMe.[12] In turn, binding to DNA was examined using fluorescence quenching techniques; as represented in **Figure 1B**, a decrease in tyrosine fluorescence from peptide **1** is apparent upon the addition of calf thymus DNA. After correction for the inner-filter effect[13] of DNA by the use of free tyrosine as a control, these data provided clear evidence of DNA binding by **1**, a result consistent with observations suggesting DNA intercalation of the tyrosine rings.[5,14]

DNA cleavage studies with the Cu(II) complex of peptide **1** have demonstrated that this compound can readily effect scission of ΦX174 RF plasmid DNA through redox chemistry.[3b,c] As illustrated by agarose gel electrophoresis (**Figure 2**) preincubation of the Cu(II)·**1** complex with ΦX174 followed by a 30 second activation period with sodium ascorbate and hydrogen peroxide results in facile conversion (>90% in lane 6) of the intact Form I plasmid to the nicked Form II. At lower concentrations of Cu(II)·**1** a proportionately reduced level of plasmid nicking is observed (~ 50% in lane 4, ~ 70% in lane 5) in accordance with expectations.[15]

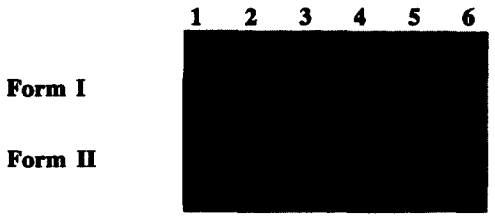

Figure 2. Cleavage of ΦX174 RF DNA (40 μM in bp) by the Cu(II) complex of peptide **1** in 10 mM Na-cacodylate (pH 7.5). Reactions were initiated by the addition of Na-ascorbate and/or H_2O_2 and quenched after 30 sec.[16] Lane 1: DNA alone; lane 2: reaction control [350 μM **1**, 200 μM Cu(II), 350 μM H_2O_2]; lane 3: reaction control [350 μM **1**, 200 μM Cu(II), 350 μM ascorbate]; lane 4: cleavage reaction [250 μM **1**, 100 μM Cu(II), 250 μM ascorbate, 250 μM H_2O_2]; lane 5: cleavage reaction [250 μM **1**, 200 μM Cu(II), 250 μM ascorbate, 250 μM H_2O_2]; lane 6: cleavage reaction [350 μM **1**, 200 μM Cu(II), 350 μM ascorbate, 350 μM H_2O_2].

In conclusion, it is clear from the experimental data presented herein that peptide **1** is able to perform each of the discrete functions for which it was designed: (1) complexation of Cu(II), (2) association with DNA, and (3) facile oxidative cleavage of DNA. These results support the notion that it is possible to rationally construct low molecular weight peptides for specific purposes through the adaptation of structural features that have been successfully employed by larger and more complex polypeptides and proteins. Of particular relevance in this regard are simple *structured* peptide motifs which can be generated from a short stretch of consecutive amino acid residues; the β-turn and Cu(II)-binding elements that have been incorporated into **1** represent good examples of protein secondary structural features that fit into this category. Since there is evidence to indicate that in addition to RNA polymerase II a number of proteins (e.g., histone H1,[17] HMG-I[18]) employ β-turns in DNA recognition through a minor groove interaction, additional studies which address the interaction of β-turn metallopeptides with DNA are in progress in our laboratory.

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15. Control studies utilizing the Cu(II) complex of Gly-Gly-His (Sigma) which lacks the DNA-binding domain of **1**, show no significant cleavage (~ 5 %) under identical conditions. This result is as expected given the lack of a defined DNA binding domain in the simple tripeptide sequence alone.
16. The 20 μl reaction mixtures (lanes 1-6) were quenched by the addition of 6 μl of 2:1 (v/v) gel loading buffer III - 0.2 M aqueous EDTA (pH 7.0) (see: Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning; A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N.Y., 1989; p. 6-12.)
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