# DNA Strand Scission by Dioxygen + Light-Activated Cobalt Metallopeptides

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## Introduction

Metallopeptides constitute an emerging class of nucleic acid modification reagents[1] that, in light of other metal-based species,[2] can closely mimic the chemical recognition functionalities of natural products and proteins or act as affinity cleavage appendages to prestructured nucleic acid binding domains.[3] Currently, our laboratory is exploring Cu(II) and Ni(II) complexes of $H_2N$-Xaa-Xaa-His-$CONH_2$ (where Xaa is any amino acid) in the development of site-selective DNA strand scission agents;[4] these studies are based on the metal chelation[5] and substrate oxidation[6] capabilities of metallo-Gly-Gly-His and its derivatives.

Unfortunately, while current activation systems involving Cu(II)/Ni(II)·Xaa-Xaa-His peptides have facilitated an understanding of the selectivity of metallo-tripeptides alone[4] and their application as affinity cleavage reagents,[3] the necessity of an exogenous reductant or oxidant limits investigation to systems tolerant of these chemicals and can alter the observed products of the reaction.[7] Thus, we have sought to develop alternative metallopeptide activation protocols that require a minimum number of coreactants. Reported herein is an efficient means of inducing DNA cleavage by Co(II)·metallopeptides that requires only ambient dioxygen and light. Interestingly, although of minimal complexity, $O_2 + h\nu$ activation of Co(II) metallopeptides is reminiscent of that observed with air-oxidized Co(II) derivatives of the glycopeptide antibiotic bleomycin.[8] As a precedent for the complexes examined herein, previous studies have indicated that Co(III)·Gly-Gly-His complexes involving coordination of the peptide terminal amine, two deprotonated amide residues, and the imidazole nitrogen of His can be formed at alkaline pH values.[9]

## Experimental Section

**Peptide and Metallopeptide Syntheses.** All peptides used in this study were prepared, purified, and characterized by manual solid-phase synthesis protocols[4a] using commercially available amino acids and resins. The metallopeptide $(NH_3)_2$Co(III)·Gly-Gly-His was prepared and purified as described[9] [$\lambda_{max}$ 434 and 520 (sh) nm; $^1$H NMR ($D_2O$) $\delta$ 2.74 (m, 1), 3.48 (d, 1), 3.72 (s, 2), 4.07 (s, 2), 4.66 (s, 1), 7.27 (s, 1), 8.05 (s, 1); $NH_2$ 5.01, 5.15; $NH_3$ 2.48, 2.97; plasma desorption mass spectrum $m/z$ 325.7 $(M + H)^+$]. All other metallopeptides employed in DNA cleavage reactions were generated *in situ* through the admixture of equimolar amounts of peptide and $CoCl_2$ in aqueous buffered solutions at alkaline pH (as described below).

**DNA Cleavage Reactions.** Cleavage of ΦX174 RF DNA (11 $\mu$M base pair concentration) by Co(II)·Gly-Gly-His and Co(II)·Lys-Gly-His was initiated through the admixture of equimolar amounts of peptide and $CoCl_2$ (see Figure 1 for concentrations) in 5 mM sodium borate buffer, pH 8.0, with a final volume of 20 $\mu$L. The metallopeptide formed *in situ* was allowed to air oxidize for 1 min followed by irradiation of the entire sample with a Pyrex-filtered 1000 W Xenon lamp at a distance of 13 cm (or incubation in the dark). All reactions were quenched after 30 min by the addition of EDTA-containing (30 mM) loading buffer. Reactions containing[9] $(NH_3)_2$Co(III)·Gly-Gly-His and DNA were initiated by irradiation with a Pyrex-filtered 1000 W Xenon lamp in 5 mM sodium borate buffer, pH 8.0, with a final volume of 20 $\mu$L. The reactions described above may also be prepared in the absence of DNA, lyophilized, reconstituted with $H_2O$ (in the presence of DNA), and irradiated leading to similar extents of DNA cleavage. All reactions were analyzed on 0.9% agarose gels containing ethidium bromide that were electrophoresed at 60 V for 2 h followed by visualization on a UV transilluminator.

## Results and Discussion

As shown in Figure 1, irradiated admixtures of Co(II) + $H_2N$-Gly-Gly-His-$CONH_2$ (Co(II)·Gly-Gly-His), under ambient dioxygen tension, were found to mediate substantial DNA damage. Initially, three metallopeptides were assayed for their ability to convert supercoiled DNA (form I) to nicked-circular (form II) DNAs including the following: (i) $(NH_3)_2$Co(III)·Gly-Gly-His[9] + $h\nu$; (ii) Co(II)·Gly-Gly-His + ambient $O_2$; and (iii) Co(II)·Gly-Gly-His + ambient $O_2$ + $h\nu$. Of the metallopeptides examined, it was found that Co(II)·Gly-Gly-His + ambient $O_2$ + $h\nu$ (Figure 1, panel A, lane 6) was able to totally convert form I DNA to form II under the conditions employed.[10] In comparison, the Co(II)·Gly-Gly-His + $O_2$ with no irradiation (Figure 1, panel A, lane 5) and $(NH_3)_2$Co(III)·Gly-Gly-His + $h\nu$ (Figure 1, panel A, lane 7) activation systems converted <50% of the form I DNA to form II.[11]

In addition to the examination of Co(II)·Gly-Gly-His + ambient $O_2$ + $h\nu$, the generality of this activation was explored using $H_2N$-Lys-Gly-His-$CONH_2$ (Co(II)·Lys-Gly-His). As shown in Figure 1 (panel B) appreciable DNA cleavage by this metallopeptide was observed in the 10−20 $\mu$M range; the increased cleavage efficiency of Co(II)·Lys-Gly-His (which contains a positively-charged Lys residue that facilitates

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(7) We have found that Ni(II)·Gly-Gly-His peptides activated with Oxone ($KHSO_5$) are capable of degrading free purine nucleobases released during the course of DNA cleavage reactions making the direct quantitation and chemical analysis of reaction products problematic.

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(9) Hawkins, C. J.; Martin, J. *Inorg. Chem.* **1983**, *22*, 3879. We have found that identical complexes can also be obtained through the air oxidation of Co(II)·Gly-Gly-His in aqueous ammonia (unpublished results).

(10) Metallopeptide concentrations of 300 $\mu$M were required in this experiment due to the lower DNA affinity of the Gly-Gly-His complex in comparison to the Lys-Gly-His complex.[4a]

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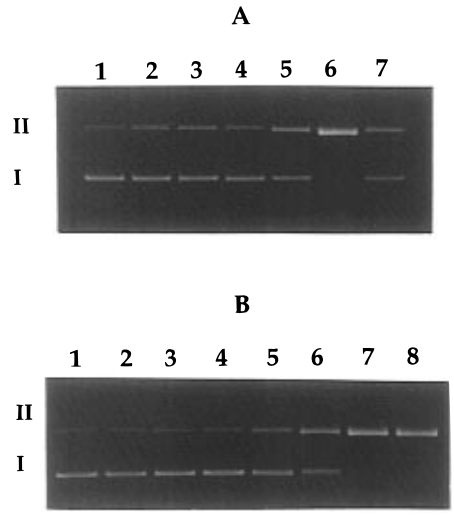


**Figure 1.** Cleavage of ΦX174 RF DNA by Co·Gly-Gly-His complexes (A) and Co(II)·Lys-Gly-His (B): (A) (lane 1), reaction control, DNA alone, (lane 2), reaction control (DNA, 30 min *hν*), (lane 3), reaction control (300 $\mu$M Gly-Gly-His, 30 min *hν*), (lane 4), reaction control (300 $\mu$M $Co^{2+}$, 30 min *hν*), (lane 5), cleavage reaction (300 $\mu$M Gly-Gly-His, 300 $\mu$M $Co^{2+}$, no *hν*), (lane 6), cleavage reaction (300 $\mu$M Gly-Gly-His, 300 $\mu$M $Co^{2+}$, 30 min *hν*), (lane 7), cleavage reaction (300 $\mu$M $Co(NH_3)_2$(Gly-Gly-His), 30 min *hν*); (B) (lane 1), reaction control, DNA alone, (lane 2), reaction control (DNA, 30 min *hν*), (lane 3), reaction control (50 $\mu$M Lys-Gly-His, 30 min *hν*), (lane 4), reaction control (50 $\mu$M $Co^{2+}$, 30 min *hν*), (lane 5), cleavage reaction (10 $\mu$M Lys-Gly-His, 10 $\mu$M $Co^{2+}$, 30 min *hν*), (lane 6), cleavage reaction (20 $\mu$M Lys-Gly-His, 20 $\mu$M $Co^{2+}$, 30 min *hν*), (lane 7), cleavage reaction (40 $\mu$M Lys-Gly-His, 40 $\mu$M $Co^{2+}$, 30 min *hν*), (lane 8), cleavage reaction (50 $\mu$M Lys-Gly-His, 50 $\mu$M $Co^{2+}$, 30 min *hν*).

electrostatic DNA binding[4a]) *vs* Co(II)·Gly-Gly-His suggests that a metallopeptide-bound oxidizing equivalent that pre-associates with the nucleic acid strand is responsible for DNA damage.

Given the decreased concentrations required for strand scission, Co(II)·Lys-Gly-His was employed for an examination of the mechanism of this activation system; DNA cleavage by Co(II)·Lys-Gly-His + ambient $O_2$ + *hν* was found to be slightly affected (~10% diminution in cleavage) by hydroxyl radical scavengers (mannitol and DMSO), unenhanced in $D_2O$ (suggesting the absence of singlet oxygen photosensitization), and unaffected by superoxide dismutase. These experiments suggest that DNA strand scission does not involve the bulk generation of freely diffusible oxygen-based radicals. Rather, given the wealth of literature precedent,[12] an oxygenated Co(III)−peptide complex likely is formed that, upon photoirradiation, is capable of inducing DNA strand scission through means similar to those reported[8e] involving hydroxyl radical generation supported by His−Co coordination[13,14] in close proximity to DNA.

In support of the above, UV−vis spectroscopy was performed to investigate the effects of oxygen on preformed Co(II)·Lys-Gly-His complexes. As shown in Figure 2, introduction of ambient $O_2$ to an Ar-purged sample of Co(II)·Lys-Gly-His resulted in the immediate formation of absorbance bands centered at 322 and 432 nm.[12] This absorbance decayed (in the dark or with ambient light) appreciably within 10 min, followed by a slower decay for up to 1 h (at 25 °C); this observation suggests the rapid formation of a $\mu$-peroxo dimer of two Co−metallopeptides[12] that decomposes in a fashion *which closely parallels the oxygenation of Co(II)·bleomycin*[8,15] resulting in the formation of an oxygenated Co(III) peptide complex. These data, in total, suggest a mechanism of Co(III)·Xaa-Gly-His DNA strand scission that mimics the activity of the Co(III)·bleomycin "brown" complex.[8]

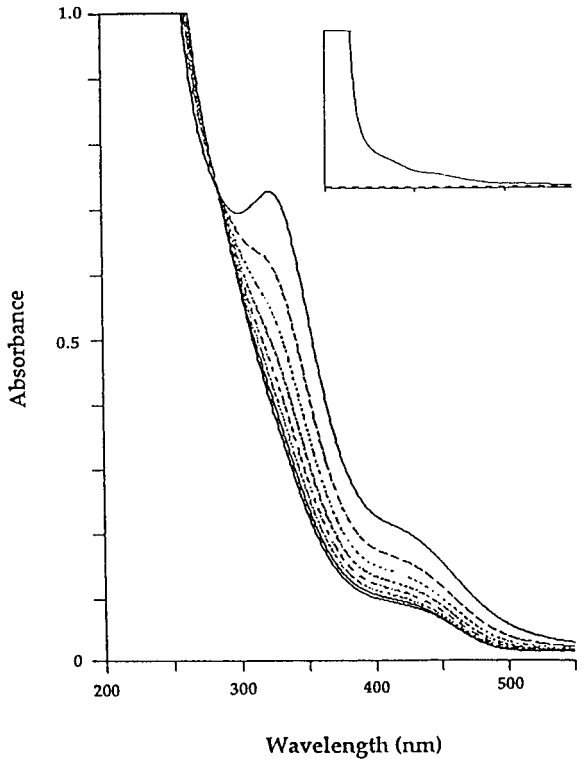


**Figure 2.** UV−vis spectra of Co(II)·Lys-Gly-His in the absence and presence of ambient dioxygen. Co(II)·Lys-Gly-His (300 $\mu$M) in pH 8.0 sodium borate buffer was prepared anaerobically and upon exposure to ambient dioxygen scanned at 1.5 min intervals (top scan, $t = 0$; bottom scan, $t = 10$ min); after 10 min of exposure, decay occurs steadily for up to ~1 h. Inset: UV−vis spectrum (200−600 nm range) of Co(II)·Lys-Gly-His prepared anaerobically (prior to $t = 0$).

The preceding observations suggest that admixtures of Co(II) + Lys-Gly-His/Gly-Gly-His under ambient dioxygen conditions result in a discrete oxygenated metallopeptide (most likely[12,15] an aquated Co(III)·peptide). The species formed, upon irradiation, is capable of DNA strand scission without exogenous chemical activating agents beyond ambient dioxygen. Further, the ability to lyophilize and reconstitute the metallopeptide responsible for DNA strand scission separates the metalation and substrate modification steps and couples them

(11) A comparison of these three cobalt-containing systems suggests that DNA cleavage by Co(II)·Gly-Gly-His + ambient $O_2$ + *hν* (Figure 1, panel A, lane 6) occurs via a mechanism that is not due solely to either (i) a Fenton-like release of reduced oxygen species as might be expected from a simple oxidation of the Co(II) metallopeptide (as in Co(II)·Gly-Gly-His + ambient $O_2$ without irradiation; Figure 1, panel A, lane 5) or (ii) excitation of the ligand field bands alone of these complexes (as in $(NH_3)_2$Co(III)·Gly-Gly-His + *hν*; Figure 1, panel A, lane 7. See: Fleisher, M. B.; Waterman, K. C.; Turro, N. J.; Barton, J. K. *Inorg. Chem.* **1986**, *25*, 3551). Further, experiments performed under anaerobic conditions indicate that the Co(II) complexes were unable to cleave DNA while cleavage by the Co(III) complex was unaffected.

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(13) Control reactions involving the use of $H_2N$-GGG-$CONH_2$ and $H_2N$-GGGG-$CONH_2$ tri- and tetrapeptides were also conducted with Co(II) to support the notion of selective activity within Xaa-Xaa-His peptides; reactions carried out in a fashion identical to those described for Gly-Gly-His/Lys-Gly-His failed to induce detectable DNA strand scission (see Supporting Information).

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to the convenience of photoactivation; these features could augment currently available affinity cleavage protocols and facilitate further studies of metallopeptide−nucleic acid interactions.

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**Supporting Information Available:** A figure containing the results of gel electrophoresis control experiments that included the peptides $H_2N$-GGG-$CONH_2$ and $H_2N$-GGGG-$CONH_2$ with $Co^{2+}$ ± light activation (1 page). Ordering information is given on any current masthead page.

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