Highly Selective DNA Modification by Ambient O_2-Activated Co(II)·Lys-Gly-His Metallopeptides

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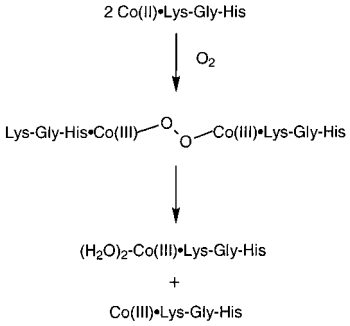
The quest for highly selective DNA modification agents and reactions continues to challenge our understanding of nucleic acid recognition phenomena and impacts the development of pharmaceuticals and reagents for biotechnology.[1] In this area, naturally occurring[2] and synthetic[3] metal complexes that activate O_2 to induce nucleic acid modification are of particular interest, given the physiological availability of molecular oxygen; systems designed with this capability could lead to agents that function in vivo.

Building on our studies of Ni(II)-based metallopeptides,[4] we have demonstrated[5] that aerobic admixtures of Co(II) + H_2N-Xaa-Xaa-His-$CONH_2$ peptides (where Xaa is an α-amino acid) form unstable μ-peroxo dimers (Scheme 1) which decompose to aquated Co(III)·Xaa-Xaa-His metallopeptides consistent with structures reported previously.[6] These metallopeptides have the wherewithal to induce DNA strand scission upon photoirradiation. In addition to *hν* activation, it was also shown[5] that aerobic admixtures of Co(II) + Xaa-Xaa-His peptides in the *absence* of light were able to cleave DNA, albeit with less overall activity than irradiated reactions. As reported presently, further analysis of the *hν*-independent reaction has revealed that aerobic admixtures of Co(II) + H_2N-Lys-Gly-His-$CONH_2$ mediate the highly selective modification of DNA, most likely via the intermediacy of a μ-peroxo dimer.

Scheme 1. Oxygenation of Co(II)·Lys-Gly-His to Form a μ-Peroxo Dimer Followed by Decomposition to Monomeric Products



As shown in Figure 1, Co(II) + Lys-Gly-His leads to the O_2-dependent modification of a single thymine residue within 5′-AGG**T**GG sites; the initial DNA lesion formed was found to be labile to a postreaction workup with piperidine (1 M piperidine/ 90 °C/30 min).[7] Analysis of additional restriction fragment substrates (representing a total of ∼1500 base pairs) and other Xaa-Xaa-His peptide-ligands[8] indicated that the high level of Co(II)/O_2 + Lys-Gly-His selectivity for this sequence is maintained (e.g., identical 5′-AGG**T**GG selective cleavage was also observed in the *Bsm*I → *Nru*I fragment of pBR322). While a lower level of Co(II)/O_2 + Lys-Gly-His-dependent DNA modification occurs at 5′-A**T**GAGA sites (observed as a slower migrating band in lane 3 of Figure 1) and some background DNA damage due to

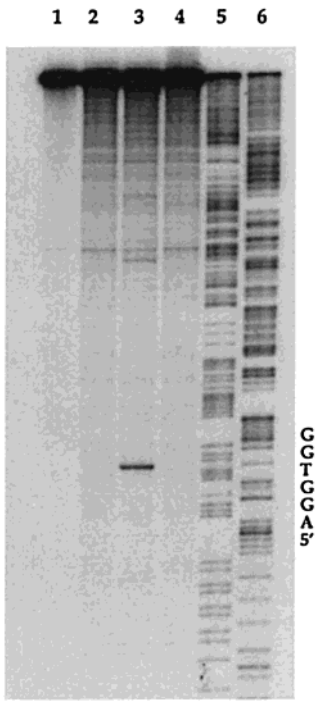


Figure 1. Autoradiogram of a 6% denaturing polyacrylamide gel demonstrating the O_2-dependent modification of a 5′-^{32}P end labeled duplex DNA substrate (514 base pair *Eco*RI → *Rsa*I fragment of pBR322) by Co(II)·Lys-Gly-His. All lanes were treated with piperidine: lane 1, reaction control, intact DNA; lane 2, reaction control, 100 μM Co(II); lane 3, 100 μM Co(II) + 100 μM Lys-Gly-His + ambient O_2; lane 4, 100 μM Co(II) + 100 μM Lys-Gly-His, Ar-purged; lanes 5 and 6, Maxam−Gilbert G + A and T + C reactions, respectively.

Co^{2+} + piperidine is evident (lanes 2−4, top, Figure 1), cleavage was not observed within (1) 5′-TGG, 5′-GGT, 5′-GTG, or other sites containing portions of the preferred 5′-AGG**T**GG sequence nor (2) the very similar sequence 5′-CGGTGG which differs from the targeted sequence by only one base pair. In addition, labeled DNA substrates containing sites complementary to the 5′-AGG**T**GG sequence (i.e., 5′-CCACCT) were not modified indicating that only the T residue within the duplex recognition sequence is targeted and suggesting the involvement of a non-diffusible modifying species. Indeed, hydroxyl radical scavengers (100 mM DMSO, mannitol, ethanol, *tert*-butyl alcohol) have no effect on the reaction (Supporting Information).

As mentioned, the DNA lesion formed by Co(II)/O_2 + Lys-Gly-His is labile to a postreaction workup with piperidine. To probe further the chemistry of this DNA lesion, its lability to treatment with NaOH was also tested (0.1 M NaOH/ 60 °C/5 min, Supporting Information); strand scission did not occur upon this mild alkali treatment suggesting that DNA modification by Co(II)/O_2 + Lys-Gly-His proceeds through a mechanism involving T nucleobase oxidation[9] and not through deoxyribose oxidation (e.g., C4′-hydroxylation).[1,2] Given that T nucleobase modification is likely, the relative accessibility and reactivity of the T residue within 5′-AGGTGG sites was evaluated through treatment of identical end-labeled DNA substrates with $KMnO_4$; accessible T residues are readily modified by this reagent, while T residues that are efficiently stacked within the double helix are less reactive.[10] It was found that the T residue within 5′-AGGTGG sequences exhibited low reactivity with this reagent (in comparison to other T residues within the same substrate), suggesting their efficient stacking within the double helix, likely due to the flanking purines of this sequence.[10a] It is possible, however, that

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upon DNA−metal complex association[11] a structural perturbation of this sequence could occur altering the accessibility of this residue.

What is the active DNA-modifying species formed in the above reaction? To test the involvement of the μ-peroxo dimer formed upon aerobic oxidation of Co(II) + Lys-Gly-His, 5′-AGG**T**GG cleavage was monitored as a function of dimer concentration (Figure 2 and Supporting Information). It was found that the intensity of T residue modification within this sequence steadily diminished in parallel with the spectroscopically monitored decomposition of the dimer. This observation strongly supports the notion that the μ-peroxo species is involved directly in DNA modification.

In addition to the above, further support for the involvement of the μ-peroxo dimer derives from molecular modeling and knowledge of the size and symmetry of the 5′-AGGTGG DNA recognition site. Structural models indicate that it is possible for the μ-peroxo dimer to recognize and bind to the six-base pair recognition sequence, whereas a monomeric complex can only interact with two to three base pairs and is thus unlikely to recognize a site of this size. Indeed, reactions that employed monomeric complexes[6] of Co(III)·Lys-Gly-His do not mediate DNA damage at these sites.[5] The above thus suggests that each of the metallopeptide halves of the dimer may interact with the two 5′-XpGpG sequences of 5′-AGG**T**GG via the DNA major groove, as similarly found with other Co complexes.[11] In such a complex with the DNA, the μ-peroxo dimer could effectively deliver its active oxidizing moiety to the proximal 5−6 double bond of the T residue residing approximately in the middle of the recognition sequence. Oxidation of this region of thymine would result in a piperidine-labile lesion.[9]

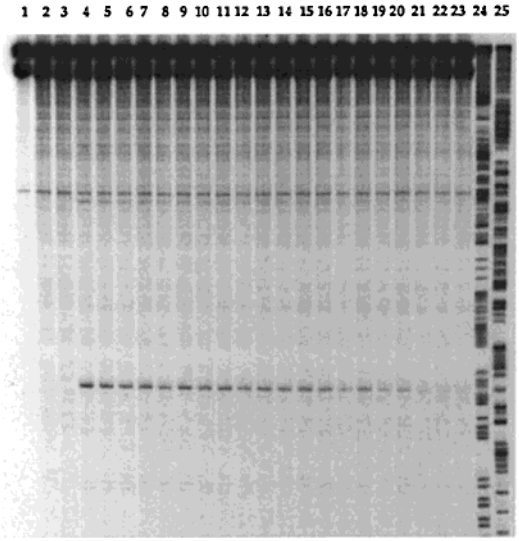

Figure 2. Autoradiogram illustrating the selective cleavage of a 5′-^{32}P end-labeled 514 base pair DNA restriction fragment (the *Eco*RI → *Rsa*I fragment of pBR322) by Co(II)·Lys-Gly-His + O_2 as a function of time and μ-peroxo dimer decomposition. Lanes 2−23 were treated with piperidine: lane 1, reaction control, DNA alone; lane 2, reaction control, DNA alone; lane 3, reaction control, 100 μM Co(II); lanes 4−23, 100 μM Co(II) + 100 μM Lys-Gly-His + ambient O_2 (preformed and added to substrate DNA for 30 min after the following elapsed time intervals: 0, 1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12, 13.5, 15, 16.5, 18, 19.5, 25, 30, 40, 50, 60, and 70 min, respectively); lanes 24 and 25, Maxam−Gilbert G + A and T + C reactions, respectively.

In summary, the above findings indicate that μ-peroxo dimers of Co(III)·Lys-Gly-His lead to a novel means of mediating the highly selective recognition and modification of DNA.[12] While this reaction, at present, does not yield a high level of modified DNA, it does represents a new strategy for the efficient activation and delivery of a reactive oxidant *derived from ambient* O_2 to the DNA double helix. Further investigation of this reaction in conjunction with alternative Xaa-Xaa-His tripeptide-ligands may result in a generalized means of selectively modifying alternative DNA sequences.

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Supporting Information Available: Autoradiograms illustrating the results of hydroxyl radical scavenger experiments, reactions involving alternative Xaa-Xaa-His peptide-ligands, a comparison of postreaction alkali treatment with piperidine vs NaOH, and DNA modification as a function of time and μ-peroxo dimer decomposition are available. Also available is a plot comparing the % μ-peroxo dimer decomposition (as monitored by UV−vis) and the quantitated decrease in 5′-AGG**T**GG modification as a function of time (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) DNA cleavage reactions were carried out in 20 μL total volumes containing 3×10^4 cpm of ^{32}P end-labeled restriction fragment substrate and 150 μM (base pair concentration) calf thymus carrier DNA. Reactions were initiated through the admixture of equimolar amounts of peptide and $CoCl_2$ in 5 mM Na-cacodylate buffer (pH 8.0). Reactions performed in the presence of ambient O_2 were allowed to react for 30 min before quenching with 3 μL of a 0.2 M EDTA solution; anaerobic reactions were carried out similarly through the reconstitution of lyophilized solutions and their subsequent admixture in an Ar-purged glovebag. After quenching, all reactions were EtOH precipitated, dried, and redissolved in freshly diluted 1.0 M piperidine, heated to 90 °C for 30 min, and then lyophilized. Prior to gel loading, each sample was resuspended in 3 μL of 80% formamide loading buffer, heat denatured (90 °C/5 min) and quick-chilled on ice.

(8) Admixtures of Co(II) + Gly-Gly-His, Arg-Gly-His, Gly-Asn-His, and Pro-Lys-His, although capable of forming μ-peroxo dimers (as determined through UV−vis analyses) and, in some instances, mediating the relaxation of supercoiled plasmid DNA, did not selectively modify DNA within the restriction fragments employed as substrates for the current study (see Supporting Information).

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