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Changing Selectivity of DNA Oxidation from Deoxyribose to Guanine by Ligand Design and a New Binuclear Copper Complex

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Transition-metal complexes that cleave DNA have received considerable attention in the past few years.[1] One of the most thoroughly studied is the copper bis(1,10-phenanthroline) complex, which associates with the minor groove and cleaves duplex DNA by direct abstraction of the deoxyribose H1′ hydrogen.[1a,2] Recent advances on this topic have provided important new insights into the mechanism of DNA cleavage[3] and inspired investigations on many other copper complexes possessing DNA-cleaving reactivity.[4] We previously reported that binuclear and trinuclear (but not mononuclear) copper complexes utilizing pyridylalkylamine ligands efficiently promote cleavage of DNA by oxidation of selected deoxyribose moieties on single-stranded regions adjacent to junctions of single- and double-stranded (ss/ds) DNA.[5] A new dicopper(II) complex $[Cu^{II}_2(PD'O)(H_2O)_2](ClO_4)_3 \cdot 2H_2O$ (**1**) (Chart 1) reported here acts in a contrasting manner by primarily promoting nucleobase (vs deoxyribose) oxidation at unpaired guanine residues, particularly at such junctions.

Previous studies show that low temperature (organic solvent) oxygenation of $[Cu^{I}_2(D^1)]^{2+}$, a dicopper(I) analogue of **2** (Chart 1), leads to the formation a peroxo-dicopper(II) species.[6] This intermediate may be directly involved in oxidation of DNA, since reduction of **2** and the presence of O_2 are required for DNA strand cleavage.[5] The structure and reactivity of copper−dioxygen adducts (such as peroxo dicopper(II), superoxo copper(II), bis-μ-oxo dicopper(III)) strongly depend on the ligands and the resulting coordination environment.[7] Thus, we sought to manipulate the possible intermediate responsible for DNA oxidation by employing a binucleating phenolate containing chelate such as in **1** (Chart 1). Related complexes form peroxo and/or hydroperoxo dicopper(II) species, the latter of which may oxidize substrates via electrophilic reactions.[8] The binucleating ligand PD′OH forming a bis(aquo) dicopper(II) complex **1** was consequently synthesized.[9]

The reactivity of **1** was first examined with the ss/ds-forming oligodeoxynucleotides (OD1 + OD2) in the presence of excess reductant 3-mercaptopropionic acid (MPA) under ambient conditions (aerobic) and quenched by diethyl dithiocarbamic acid after 15 min.[5b] Subsequent treatment with piperidine revealed significant and selective cleavage at G_{20} and G_{21} of OD1 at the junction, while little reaction was apparent in the absence of this secondary treatment (Figure 1, lane 8 vs 4). Cleavage at these G's accounted for ca. 70% of the total reaction, and no other G's in ss or ds regions reacted above a low background level (e.g., G_{23} and G_{15}). Equivalent specificity was also observed for G_6 and G_7 in OD2.[9] This target structure had been chosen initially based on the selectivity of the previous multinuclear copper complexes for ss/ds junctions.[5] However, when OD1 itself was treated under similar conditions, all G's reacted although their efficiency was lower than that for G's in a junction.[9]

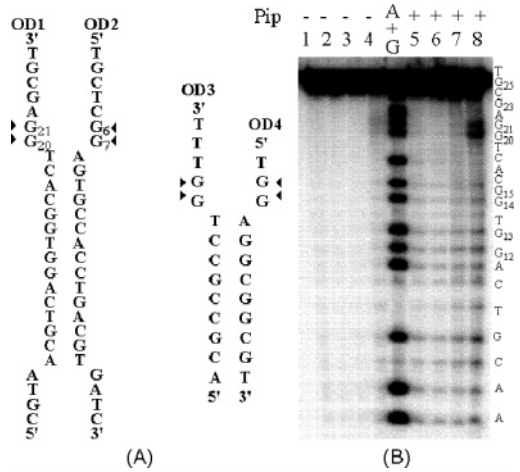
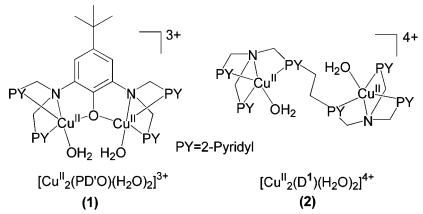


Figure 1. (A) Secondary structure of OD1/OD2 and OD3/OD4. (B) Phosphoimage of a 20% polyacrylamide denaturing gel (7 M urea) of 100 nM 5′-^{32}P-OD1+OD2 incubated in the presence and absence of **1** (100 μM) and MPA (5 mM) for 15 min in sodium phosphate (10 mM, pH 6.8) at ambient temperature. Lanes 1, 5: OD1 alone. Lanes 2, 6: OD1 + OD2 and MPA. Lanes 3, 7: OD1+OD2 with **1**. Lanes 4, 8: OD1 + OD2 with **1** and MPA. Lanes 5−8: treated with 0.2 M piperidine at 90 °C for 30 min.

Chart 1



The lack of direct strand cleavage of DNA suggested that the reactive derivative of **1** selectively oxidized the nucleobase instead of deoxyribose, the most common target of copper complexes. Hydrogen atom abstraction from all positions of the deoxyribose except for C1′ and C2′ yields at least some direct strand scission.[3d,10] The C1′ product is labile under relatively mild conditions (NaOH, 0.1 M, 37 °C, 20 min).[10] These conditions were not sufficient for inducing scission in DNA after its reaction with **1**. The nearly complete lack of spontaneous strand scission is highly unusual, but known in a few cases, for simple copper salts or complexes.[11]

8-Oxo-7,8-dihydro-2′-guanine (8-oxoG) is perhaps the most frequent product of nucleobase oxidation in DNA.[11] Although 8-oxoG is not piperidine labile, its low redox potential makes it prone to further aerobic oxidation that ultimately leads to cleavage under piperidine treatment.[12] Such oxidation may be stimulated by $IrCl_6^{2-}$,[13] but this did not enhance the cleavage of DNA products formed here by reaction of **1**.[9] Addition of 2-mercaptoethanol to a

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piperidine treatment is known to act in a reciprocal manner by preventing oxidation of 8-oxoG and strand scission.[12a] This modified treatment also had no detectable effect on the yield of DNA cleavage after reaction with **1**, MPA, and O_2.[9] Thus, 8-oxoG is not likely the product of oxidation under these conditions.

To provide further insight into the nucleobase (G) oxidation chemistry, an oligodeoxynucleotide system (OD3 + OD4; Figure 1) of lower molecular weight than the original target was chosen for analysis by mass spectrometry. Again, each strand contained unpaired G's for reaction with **1**, and each demonstrated reactivity similar to OD1 + OD2.[9] Modification at ss G's remained dominant even after extended incubations (90 min, 65% yield) used to maximize products for isolation and detection.[9] Parent strands and their derivatives were isolated from reverse-phase HPLC and analyzed by nanospray ionization mass spectrometry (NSI/MS). For OD3, major products of +18 amu and +34 amu were detected,[9] and equivalent derivatives were detected for OD4. The species with a +18 amu is consistent with formation of a 2,6-diamino-5-formamidino-4-hydroxypyrimidine (FAPy-G) residue. Such a derivative would explain the piperidine lability (see above). FAPy-G is typically generated by hydroxyl radical addition followed by one-electron reduction.[11,14] Excess MPA likely facilitates the final reduction step under the conditions used here.[15] This product is not consistent with generation of singlet oxygen ($^1O_2$) as proposed for reaction of Cu(II) and H_2O_2.[11d] We only found one proposal in the literature to explain the gain of + 34 amu.[12b] The suggested product, 5,8-dihydroxy-7,8-dihydroguanine, is likely to be hydrolytically unstable and may rearrange to a more stable isomer.

The selectivity demonstrated by the copper complex **1** is atypical at two levels. First, its preference for nucleobase rather than deoxyribose oxidation is unlike most copper complexes known to react with DNA. Although the copper-bound hydroxy radical commonly proposed for reaction has the potential to add to guanine, hydrogen abstraction of the deoxyribose is far more usual.[10c,11a] Proximity and accessibility alone cannot explain the reaction specificity because both **1** and **2** act on single-stranded regions that should not restrict nucleotide access, and yet **1** primarily oxidizes the nucleobase and **2** oxidizes the deoxyribose. Thorp has suggested that systematic variation of ligand environment and accessibility to a metal-oxo species may yield a series of oxidants selective for either sugar or base.[16] As mentioned above, $[Cu^I_2(D^1)]^{2+}$ reacts with O_2 to produce a peroxo dicopper(II) complex,[6] while the dicopper(I) analogue of **1** yields a hydroperoxo dicopper(II) complex.[17] Theoretically, this latter species is a stronger (electrophilic) oxidant.[8] Similar intermediates are expected to form during reaction with DNA through initial MPA reduction of Cu(II) to Cu(I) followed by reaction with ambient O_2.[5] Thus, the structural makeup or specific nature of the dicopper−dioxygen intermediate instead of its oxidizing power likely determines the type of DNA damage produced.

The DNA sequence targeted by **1** represents the second level of specificity that is distinct from other multinuclear copper complexes. A ss/ds junction of DNA is not an absolute requirement for **1** as it is for **2** or a related trinuclear copper complex.[5] In all cases, oxidation is excluded from duplex regions and products form in a highly localized manner. Together, these observations are consistent with the formation of copper-based intermediates that deliver their oxidizing power directly and alternatively to guanine or deoxyribose. Diffusible oxidants such as reactive oxygen species are avoided under these conditions. Direct coordination between the copper complex and DNA may be inferred but would be destroyed before electrophoretic analysis by the quenching and denaturing conditions.

In summary, we have discovered a dicopper complex that predominantly effects DNA base (rather than ribose) oxidation on G residues in single-stranded regions of DNA. FAPy-G is one of the major oxidation products derived from G. We hypothesize that (i) the nature of the ligand-induced copper−dioxygen species and (ii) copper−guanine binding[18] are important elements in the specificity of base recognition and oxidation. Further investigation into the origins of these features are in progress.

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Supporting Information Available: Synthesis of the copper complex (**1**) and PAGE analysis of DNA oxidation (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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