Recognition and Strand Scission at Junctions between Single- and Double-Stranded DNA by a Trinuclear Copper Complex

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Transition metal complexes have been extensively studied for their nuclease-like activity.[1−3] Many of these utilize the redox properties of the metal and dioxygen to produce reactive oxygen species that oxidize DNA, yielding direct strand scission or base modification.[4,5] Copper complexes are capable of both reactions, although neither exhibit significant nucleotide sequence specificity. The best studied of these is bis(orthophenanthroline)copper, $Cu(OP)_2^{2+}$. Under standard conditions this Cu(II) complex is reduced to Cu(I), binds in the minor groove, and in the presence of hydrogen peroxide induces direct strand scission.[6,7] The proposed mechanism for this process requires 3 equiv of the Cu(II) species to produce a nondiffusible intermediate equivalent to hydroxyl radical. Because of our interest in copper/dioxygen chemistry[8−11] and its potential application in molecular biology and medicine, we initiated nucleic acid studies using copper complexes that possess pyridyl ligands and react with dioxygen. Herein we present the reactivity of the trinuclear copper complex, $[Cu_3(L)(H_2O)_3(NO_3)_2](NO_3)_4 \cdot 5H_2O$ (**1**),[12] as determined by a series of deoxyoligonucleotides that identify its surprising specificity for reaction at junctions between single- and double-stranded DNA.

Activation of dioxygen by transition metals, including copper, is strongly dependent on the coordination environment.[9−11,13] Copper(I), when coordinated by pyridyl(alkyl)amine ligands such as tris(pyridylmethyl)amine (TMPA), binds dioxygen yielding peroxo dimers stable at low temperature.[14] Dinuclear complexes based on the same motif are entropically stabilized and more rapidly form peroxo intermediates when exposed to dioxygen.[15,16] A third copper can similarly be added to model the active site of the blue copper proteins[17] that are known to effect O−O bond cleavage and reduction of O_2 to water.[13] The structure and synthesis of **1** (Figure 1)[18] as well as its reactivity with plasmid pBR322 in the presence of 3-mercaptopropionic acid (MPA) plus oxygen or just H_2O_2 has been previously established.[12] The cleavage efficiency of **1** is less than that of $Cu(OP)_2^{2+}$ with MPA as a reductant, but is comparable when H_2O_2 is used to initiate the reaction.

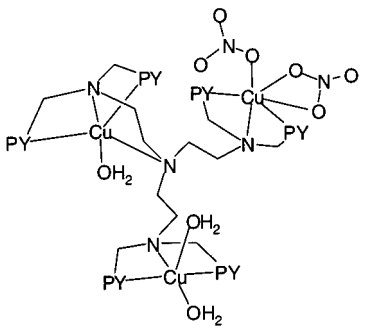

Figure 1. Structure of $[Cu_3(L)(H_2O)_3(NO_3)_2]^{4+}$ based on the X-ray structure of **1**, where PY is 2-pyridyl (see ref 12).

A hairpin forming oligonucleotide (OD1) was treated with **1** in the presence of excess MPA and then quenched with diethyl dithiocarbamic acid. PAGE analysis revealed direct and specific strand scission at position A_{22} (Figure 2, lanes 4 and 5). Quantitation of the products by phosphorimage analysis indicated that 78% of the observed cleavage occurs at A_{22} in the presence of 0.5 μM **1** and 5 mM MPA. Selectivity is reduced to 66% if the concentration of **1** is increased 10-fold to 5 μM, due in part to an increase in reactivity primarily at A_9, T_{10}, and T_{21} (Figure 2, lane 6). In the absence of either the thiol or **1**, no cleavage of OD1 is observed (Figure 2, lanes 2 and 3). When a complementary deoxyoligonucleotide (OD2) is added in 30% excess to favor duplex formation, selectivity is greatly reduced for A_{22} in OD1 (Figure 2, lane 7). In addition, there is no enhancement of reaction at A_9, T_{10}, or T_{21}. Instead, strand scission proceeds with equivalent efficiency at all sites, much like results with $Cu(OP)_2^{2+}$ and OD1+OD2. The persistent specificity for A_{22} when OD2 is present is likely due to residual hairpin formed in competition with duplex DNA. Also, the unique modification at A_{22} appears to be dominated by the recognition properties of **1** rather than the intrinsic reactivity of DNA since A_{22} is not a major target of reaction with $Cu(OP)_2^{2+}$ and either the hairpin-forming OD1 or the duplex-forming OD1+OD2.

Since a reductant is necessary for strand scission, the ultimate oxidant is likely formed by the reduction of the Cu(II) species and its subsequent reaction with dioxygen, analogous to the proposed mechanism for $Cu(OP)_2^{2+}$.[6,19] DNA fragmentation does not require piperidine treatment and suggests direct oxidation of the oligonucleotide backbone.[4,5] When the reaction products of OD1 and **1** are treated with piperidine, a 40% enhancement in

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(18) The structure of **1** reveals two different coordination environments for copper with respect to the ligand, L. Two of the coppers are tridentate with two exchangeable coordination sites each, occupied by water and nitrate in the crystal structure, while the third copper atom is tetradentate possessing one exchangeable site. In aqueous solution at basic pH, these sites are occupied by hydroxide/water.[23]
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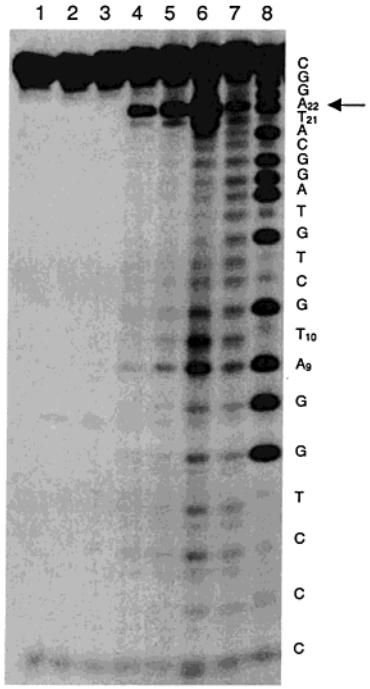

Figure 2. Autoradiogram of a 20% polyacrylamide denaturing gel (7 M urea) showing cleavage products of 100 nM 5′-^{32}P-labeled OD1 incubated with the **1** and MPA for 15 min in sodium phosphate (10 mM, pH 7.5) at ambient temperature. Lane 1: OD1 alone. Lane 2: OD1 with 5 μM **1**. Lane 3: OD1 with 5 mM MPA. Lane 4−6: 0.5, 1, and 5 μM **1** with 5 mM MPA and OD1. Lane 7: OD1+OD2 with 10 μM **1** and 5 mM MPA. Lane 8: A+G sequencing lane.

strand scission results, but the relative selectivity remains unchanged. Lack of reaction at other adenines indicates that the complex does not recognize a specific base, but instead targets a distinct structural feature of the deoxyoligonucleotide. This observation is supported by comparing the specificity of scission to the hairpin conformation of OD1, a structure that was independently confirmed via chemical modification using $CoCl_2$ and KBr with oxone and $KMnO_4$ (see Supporting Information).[20−22] Position A_{22} is directly 3′ to the stem region which contains A_9 and T_{10} (Figure 3a). The proximity of these target residues suggests a common site for association of **1**. A reaction comparable to that at A_{22} is not observed in the single-stranded loop region of the hairpin, and therefore this structure does not contain the necessary recognition elements for strand scission.

To determine the origin of specificity for the trinuclear copper complex **1**, a new target (OD3+OD4) was designed to present a central duplex region flanked by 3′ and 5′ single-stranded regions on both strands (Figure 3b). This provided two junctions between single- and double-stranded DNA in which a cytosine (C_{20}) and a guanine (G_{22}) occupy the 3′ sites for potential cleavage. Under equivalent conditions to those described above (Figure 2), strand

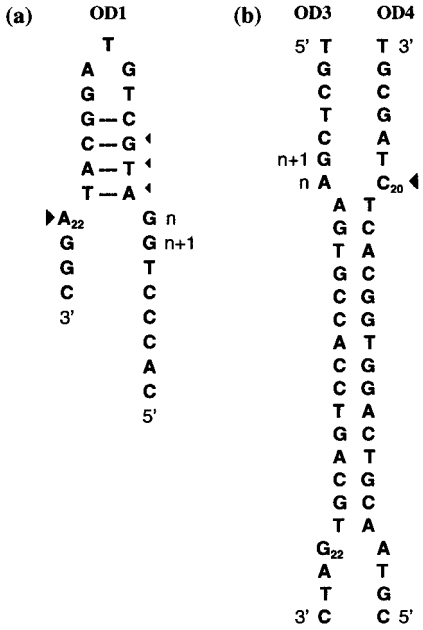

Figure 3. Secondary structure of (A) OD1 and (B) OD3+OD4. Pairing in the hairpin is designated by (- - -). Primary cleavage sites are indicated by a large arrowhead and secondary sites by a small arrowhead.

scission was observed uniquely at C_{20} in OD4 (see Supporting Information). Only low-level nonspecific reactivity was evident for OD3 similar to that for OD1+OD2 (Figure 2, lane 7). When the concentration of **1** was raised to 5 μM, nonspecific reactions also became evident for OD4. Selective reaction at A_{22} of OD1 and C_{20} of OD4 and the absence of reaction at G_{22} of OD3 indicates that recognition is not controlled by interaction with the target nucleobase. Truncation of the 5′ overhang of OD3 leaving a single nonhelical adenine led to loss of reaction at C_{20} of OD4. In contrast, a more limited truncation of OD3 leaving the nonhelical 5′-GA continued to support reaction at C_{20} of OD4. This reaction decreased to background levels when the 5′ guanine of the truncated OD3 was substituted with thymine, adenine, or cytosine. Therefore, the minimal requirement for recognition by **1** appears to be a guanine at position $n + 1$ on the 5′ overhang of OD3 (Figure 3b). This result is consistent with that observed for OD1, wherein the $n + 1$ position is also occupied by guanine (Figure 3a).

This unprecedented specificity now has the potential to aid in the recognition and characterization of nonhelical structures of nucleic acids. Elucidation of the mechanism and applicability to other substrates, both in vitro and in vivo, are currently underway.

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Supporting Information Available: Experimental procedures for the DNA cleavage experiments and PAGE analysis, and autoradiograms of 5′-^{32}P-labeled OD3 and OD4 after their chemical modification reactions (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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