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Efficient and Specific Strand Scission of DNA by a Dinuclear Copper Complex: Comparative Reactivity of Complexes with Linked Tris(2-pyridylmethyl)amine Moieties

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Abstract: The compound $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ ($\mathbf{D^1}$ = dinucleating ligand with two tris(2-pyridylmethyl)-amine units covalently linked in their 5-pyridyl positions by a $-CH_2CH_2-$ bridge) selectively promotes cleavage of DNA on oligonucleotide strands that extend from the 3′ side of frayed duplex structures at a site two residues displaced from the junction. The minimal requirements for reaction include a guanine in the *n* (i.e. first unpaired) position of the 3′ overhang adjacent to the cleavage site and an adenine in the *n* position on the 5′ overhang. Recognition and strand scission are independent of the nucleobase at the cleavage site. The necessary presence of both a reductant and dioxygen indicates that the intermediate responsible for cleavage is produced by the activation of dioxygen by a copper(I) form of the dinuclear complex. The lack of sensitivity to radical quenching agents and the high level of site selectivity in scission suggest a mechanism that does not involve a diffusible radical species. The multiple metal center exhibits a synergy to promote efficient cleavage as compared to the action of a mononuclear analogue $[Cu^{II}(\mathbf{TMPA})(H_2O)](ClO_4)_2$ (**TMPA** = tris(2-pyridylmethyl)amine) and $[Cu(OP)_2]^{2+}$ (OP = 1,10-phenanthroline) at equivalent copper ion concentrations. The dinuclear complex, $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$, is even capable of mediating efficient specific strand scission at concentrations where $[Cu(OP)_2]^{2+}$ does not detectably modify DNA. The unique coordination and reactivity properties of $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ are critical for its efficiency and site selectivity since an analogue, $[Cu^{II}_2(\mathbf{DO})(Cl_2)](ClO_4)_2$, where **DO** is a dinucleating ligand very similar to $\mathbf{D^1}$, but with a $-CH_2OCH_2-$ bridge, exhibits only nonselective cleavage of DNA. The differences in the reactivity of these two complexes with DNA and their previously established interaction with dioxygen suggest that specific strand scission is a function of the orientation of a reactive intermediate.

Introduction

The previous two decades have seen a proliferation of research on the interactions of transition metal complexes with nucleic acids.[1−4] Numerous parameters including the size, shape, and chirality of complexes as well as the structure and composition of target DNA control both binding and modification.[5,6] Although many coordination compounds have been characterized extensively, the 1,10-phenanthroline−copper complex, $[Cu(OP)_2]^{2+}$, has garnered a large degree of attention due to its high nucleolytic efficiency. This complex has also been widely applied as a footprinting agent of both proteins and DNA,[7,8] a probe of the dimensions of the minor groove,[9] and an identifier of transcription start sites.[10,11] All of these investigations were conducted with the 2:1 complex of $[Cu(OP)_2]^{2+}$, in which two phenanthroline ligands chelate one copper ion and are assumed to require 3 equiv of complex for DNA cleavage. This complex is proposed to bind to DNA through intercalation of one phenanthroline into the DNA or general hydrophobic interactions between the ligand and the minor groove of DNA. Reaction has also been directed to specific nucleotide sequences by covalently linking phenanthroline to a DNA binding element (i.e. proteins or oligonucleotides) through a derivatized amido moiety.[12,13] In both cases,

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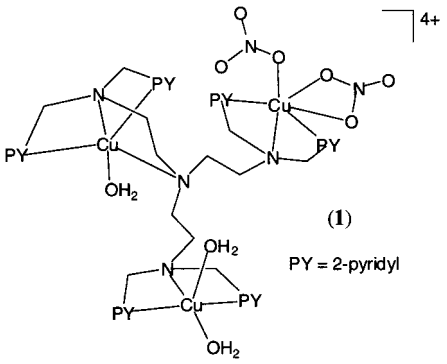
the binding and recognition of DNA are dominated by ligand interactions with the double-helical structure. The copper ion, although crucial for generating the intermediate responsible for strand cleavage, does not directly interact with DNA.



Our laboratories have begun to explore the intriguing chemistry of copper-mediated O_2 chemistry with DNA by using a complementary set of coordination compounds. Attention has initially focused on multinuclear copper complexes due to their potential for efficient intramolecular activation of bound O_2 and for binding in a selective manner to particular nucleic acid conformations. Both features were recently demonstrated by the ability of a trinuclear complex, $[Cu^{II}_3(\mathbf{L})(H_2O)_3(NO_3)_2](NO_3)_4 \cdot 5H_2O$ (**1**) (**L** = 2,2′,2″-tris(dipicolylamino)triethylamine),[14−16] to recognize a helix/coil junction of DNA and promote selective strand scission upon reduction of **1** in the presence of dioxygen. Unlike $[Cu(OP)_2]^{2+}$, the copper ions in **1** appear to perform a dual role in both recognition and reactivity. The unique reactivity of **1** poses several questions regarding how the ligand and copper ion centers control binding and how the multiple metal ions produce the oxygen-derived reactive intermediate.

The unique recognition and specificity of **1**[14,15] and the high nucleolytic efficiency of $[Cu(OP)_2]^{2+}$ encouraged us to examine other multinuclear copper complexes for related properties. This survey identified a dinuclear complex $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**) (see Chart 1 for identity of $\mathbf{D^1}$) that mediated strand scission at junctions between single-stranded and double-helical regions of DNA. The site selected by **2** is not recognized or cleaved by **1**, although both compounds mediate cleavage on the 3′ overhang strand at single target positions. Most importantly, the nucleolytic efficiency of $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**) is much greater than that of $[Cu^{II}_3(\mathbf{L})(H_2O)_3(NO_3)_2](NO_3)_4 \cdot 5H_2O$ (**1**) and $[Cu(OP)_2]^{2+}$ at low concentrations and correlates well with the established dioxygen reactivity of the reduced form of **2**, $[Cu^{I}_2(\mathbf{D^1})(MeCN)]^{2+}$.[17] Further comparison with known analogues of **2** that are both mono- and dinuclear provides insight into the origins of recognition and reactivity. The mononuclear compound, $[Cu^{II}(\mathbf{TMPA})(H_2O)](ClO_4)_2$ (**3**), and the dinuclear analogue, $[Cu^{II}_2(\mathbf{DO})(Cl)_2](ClO_4)_2$ (**4**) (Chart 2), possess similar metal coordination environments[18,19] to **2** and

Chart 1

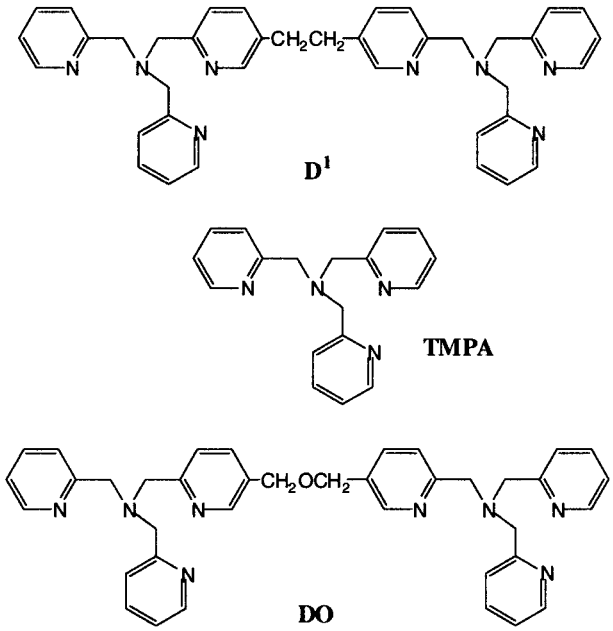
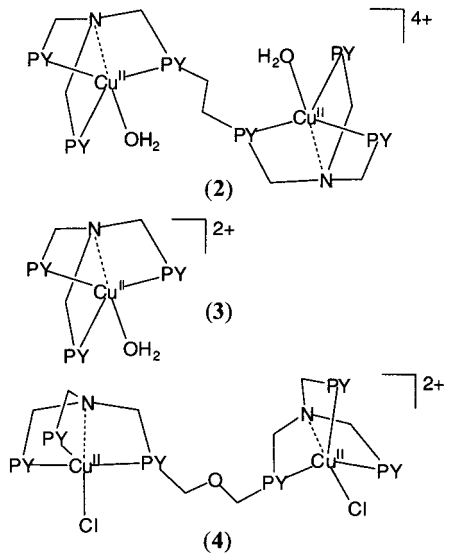


Chart 2



are also known to activate dioxygen.[20,21] The observed differences in overall reactivity and specific cleavage by the three complexes may be linked to the formation and orientation of O_2-derived intermediates.

Experimental Section

Materials. Oligodeoxynucleotides were purchased from Gibco BRL. T4 kinase and its buffer were obtained from New England Biolabs and [γ-^{32}P]ATP (3000 Ci/mmol) was obtained from Amersham. The binucleating ligand $\mathbf{D^1}$ and the mononucleating analogue **TMPA** and its coordination complex (**3**)[17,18,22] were synthesized according to published procedures. Synthesis of the ligand **DO** and its corresponding dicopper(II) complex (**4**) will be published elsewhere. Solutions of [Cu-

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$(OP)_2]^{2+}$ were prepared according to the literature[23] and examined under conditions identical with those described for reaction of **2.** Solutions of the metal complexes and other reagents for strand scission were prepared fresh daily in distilled−deionized water (18 MΩ·cm). Stock solutions of 3-mercaptopropionic acid (MPA), glutathione (GSH), and dithiothreitol (DTT) were titrated with Ellman's reagent to determine free thiol concentration.[24] All other chemicals were used as supplied by the manufacturer.

Methyl(6-bromomethyl)nicotinate. Recrystallized *N*-bromosuccinimide (13.36 g, 75 mmol), 13.4 g of methyl(6-methyl)nicotinate (89 mmol), and 174 mg of benzoyl peroxide (0.7 mmol) were added to 350 mL of CCl_4. The mixture was heated to reflux and irradiated with a 90 W bulb for 18 h. The orange solution containing a brown solid was filtered through Celite to remove the solid and washed three times with 100 mL of saturated sodium carbonate. The organic layer was dried over $MgSO_4$ for 30 min, filtered through Celite, and concentrated by rotary evaporation to give a pink solid. The solid was purified by flash column chromatography on silica (70−230 mesh), eluting with 1:4 ethyl acetate/hexanes. Fractions containing the monobrominated product ($R_f = 0.22$) were concentrated by rotary evaporation to give a crystalline white solid in 57% yield. ^{1}H NMR ($CDCl_3$): δ 3.97 (s, 3 H), 4.59 (s, 2 H), 7.53−7.55 (d, 1 H), 8.29−8.32 (d, 1 H), 9.17 (d, 1 H).

TMPAE. To 6.28 g of methyl(6-bromomethyl)nicotinate (27 mmol) was added 6.65 g of PY1 (dipicolylamine) (33 mmol), 300 mL of THF, and 8 mL of diisopropylethylamine (46 mmol). The mixture was stirred for 24 h at room temperature, filtered through Celite, and concentrated by rotary evaporation to give a dark brown oil. The oil was dissolved in diethyl ether, filtered through Celite, and placed at −20 °C to give a pale yellow solid, which was isolated by filtration. The solid was dried in vacuo resulting in a 64% yield ($R_f = 0.27$, alumina, ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 3.80 (s, 3 H), 3.87 (s, 4 H), 3.88 (s, 2 H), 7.15−7.20 (m, 2 H), 7.54−7.73 (m, 6 H), 8.18−8.22 (d, 1 H), 8.45−8.47 (d, 2 H), 9.01 (d, 1 H).

TMPAOH. To 0.85 g of lithium aluminum hydride (22 mmol) under argon and cooled to 4 °C in an ice bath was added dropwise 3.48 g of TMPAE (10 mmol) dissolved in 225 mL of dioxygen-free diethyl ether. The reaction mixture was stirred for 18 h at ambient temperature, then cooled to 4 °C in an ice bath and quenched by the dropwise addition of 1 mL of H_2O, 1 mL of 10% NaOH, and 1 mL of H_2O. The quenched reaction was allowed to stir for 2 h at room temperature, extracted with 500 mL diethyl ether, and filtered through Celite. The filtrate was dried over $MgSO_4$, filtered through Celite, and concentrated by rotary evaporation to give a pale yellow oil in 99% yield. This crude product was used without further purification ($R_f = 0.22$, alumina, 5:95 methanol/ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 3.83 (s, 6 H), 4.65 (s, 2 H), 7.13−7.15 (m, 2 H), 7.52−7.62 (m, 6 H), 8.42−8.47 (m, 3 H).

TMPACl. To a solution of 2.93 g of crude TMPA-OH (9 mmol) in 75 mL of $CHCl_3$ cooled to 4 °C in an ice bath was added dropwise 5 mL of thionyl chloride (69 mmol). The reaction was stirred for 30 min at 4 °C, then warmed to room temperature and allowed to stir for another 18 h. The dark green solution was concentrated by rotary evaporation to give a green solid. The solid was redissolved in 100 mL of CH_2Cl_2 and washed three times with 100 mL of saturated sodium carbonate. The organic layer was dried over $MgSO_4$ for 2 h, then filtered through Celite and absorbed onto alumina. Purification by column chromatography on alumina, eluting with ethyl acetate, and concentration by rotary evaporation yielded a pale yellow solid in 79% yield ($R_f = 0.33$, alumina, 5:95 methanol/ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 3.90 (s, 6 H), 4.57 (s, 2 H), 7.13−7.17 (m, 2 H), 7.54−7.69 (m, 6 H), 8.53−8.55 (m, 3 H).

D^1. To 1.45 g of $[Cu(CH_3CN)_4]PF_6$ (3.9 mmol) in a 100 mL Schlenk flask under argon was added dropwise 40 mL of freshly distilled and degassed CH_3CN containing 1.05 g of TMPA-Cl (3.11 mmol). The reaction was allowed to stir under argon for 18 h, then concentrated by rotary evaporation to give a green solid. The solid was dissolved in 100 mL of CH_2Cl_2 and extracted three times with 100 mL of concentrated NH_4OH(aq). The organic layer was washed three times with 100 mL of saturated sodium carbonate and dried over $MgSO_4$. Filtration through Celite and concentration yielded a yellow oil. The oil was purified on alumina by initial elution with ethyl acetate to remove any remaining TMPA-Cl followed by elution with 5:95 methanol/ethyl acetate to give the reductively coupled product ($R_f = 0.15$). Rotary evaporation of the product fractions yielded a white solid in 84% yield. ^{1}H NMR ($CDCl_3$): δ 2.88 (s, 4 H), 3.85 (s, 4 H), 3.87 (s, 8 H), 7.12−7.14 (m, 4 H), 7.41−7.68 (m, 12 H), 8.34 (s, 2 H), 8.53 (d, 4 H). FAB mass spectrum: m/z 607 $(M + 1)^+$.

$[Cu^{II}_2(D^1)(H_2O)_2](ClO_4)_4$ (2). A methanolic solution (5 mL) of $Cu(ClO_4)_2 \cdot 6H_2O$ (0.25 g, 0.67 mmol) was added dropwise to a test tube containing a 5 mL solution of **D^1** (0.2 g, 0.3 mmol) in MeOH. After 1 h at room temperature, blue crystals developed which were isolated by filtration, washed with Et_2O, and dried in vacuo to yield 0.35 g of crystalline solid (88%). Anal. Calcd (Found) for $C_{38}H_{42}N_8Cu_2Cl_4O_{18}$: C, 39.09 (38.46); H, 3.63 (3.63); N, 9.6 (9.33). UV−vis λ_{max} 870 nm (ϵ 420 M^{-1} cm^{-1}). IR (Nujol, cm^{-1}): 3217 (m, br, OH), 1610 (m, HOH), 1107 (vs, ClO_4^-). EPR: $g_\perp$ 2.198, $g_\parallel$ 2.0155, $A_\perp$ 85 G. The EPR spectrum is characteristic of that observed for trigonal bipyramidal Cu(II) complexes of **TMPA**.[25,26]

Purification and Labeling of DNA. Oligonucleotides were purified prior to use by denaturing (7 M urea) polyacrylamide gel electrophoresis and elution with 50 mM NaOAc and 1 mM EDTA (pH 5.2). The resulting solution was extracted with phenol/chloroform and the DNA was precipitated by the addition of ethanol. DNA was dried under reduced pressure and redissolved in water. Concentrations were determined spectrophotometrically at 260 nm using calculated extinction coefficients.[27] DNA was radiolabeled by incubation with [γ-^{32}P]ATP and T4 kinase according to the supplier. The 5′-^{32}P-labeled DNA was isolated by passage over a MicroBioSpin P-6 column (Bio-Rad). Frayed duplex structures containing a 5′-^{32}P-labeled oligonucleotide (100 nM, 90 nCi) and a complementary sequence (200 nM) were annealed in sodium phosphate (10 mM, pH 7.5) by heating to 90 °C followed by slow cooling to room temperature. Single-stranded constructs were formed by mixing 5′-^{32}P-labeled oligonucleotides (100 nM, 90 nCi) in sodium phosphate (10 mM, pH 7.5).

Copper-Dependent Strand Scission. Various concentrations of the dinuclear or mononuclear copper complexes were combined with a labeled DNA sample (100 nM, 90 nCi) in sodium phosphate buffer (10 mM, pH 6.8) and strand scission was initiated by addition of the reductant (5 mM). The reaction was quenched after a 15-min incubation at ambient temperature with 10 mM diethyl dithiocarbamic acid (5 μL).[28] DNA was then isolated by ethanol precipitation and dried under high vacuum. As indicated, certain samples were further treated with 20 μL of piperidine (0.2 M) for 30 min at 90 °C. These samples were dried under reduced pressure and twice redissolved with 20 μL of water, then subsequently dried to remove trace quantities of piperidine. The isolated DNA was resuspended in water, normalized to 45 nCi per sample, and mixed with loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 3% sucrose, and 7 M urea). The samples with loading buffer were then separated by denaturing (7 M urea) polyacrylamide (20%) gel electrophoresis and visualized by

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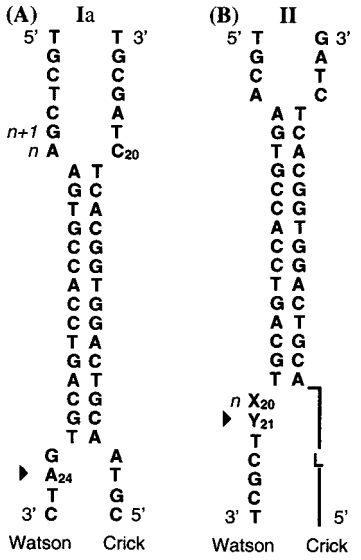

Sequence	X_{20}	Y_{21}	5′–L–3′[a]
IIa	G	A	TCGCGTA
IIb	G	T	TCGCGTA
IIc	G	G	TCGCGTA
IId	G	C	TCGCGTA
IIe	C	A	TCGCGTA
IIf	A	A	TCGCGTA
IIg	G	A	TCGCTTA
IIh	G	A	–
IIi	G	A	TA
IIj	G	A	TT
IIk	G	A	TG

[a]Sequence of the 5′ overhang on the Crick strand

Figure 1. Structures and sequences of the frayed duplex constructs **I** and **II**. The sites of specific strand scission by **2** are indicated by an arrowhead.

autoradiography and PhosphorImager (Molecular Dynamics). Quantitation of the products relied on ImageQuant software.

Dioxygen Dependence of Strand Scission. To test the dependence of the strand scission reaction on O_2, the standard reaction conditions were modified to limit the amount of dioxygen in the reaction mixture. To achieve an O_2-limited environment, a solution containing 100 nM DNA **IIa** and 1 μM **2** was degassed by bubbling the solution with prepurified nitrogen using a syringe needle for 15 min prior to initiation with 5 μL of undegassed MPA (5 mM). During the 15 min incubation, the reaction was kept under a nitrogen atmosphere. Quenching and analysis followed the same procedures as described above.

Strand Scission in the Presence of Radical Scavengers. To test for the presence of radical intermediates formed during strand scission, 100 mM ethanol, D-mannitol, and *tert*-butyl alcohol were added alternatively to a standard reaction yielding a total volume of 50 μL and final concentrations of 1 μM **2**, 100 nM **IIa**, and 10 mM radical scavenger. After initiation with 5 μL of MPA (50 mM) and incubation for 15 min at ambient temperature, quenching and analysis followed that described above.

Results and Discussion

Selective Strand Scission Induced by $[Cu^{II}_2(D^1)(H_2O)_2](ClO_4)_4$ (2). The dinuclear complex, $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**), was initially investigated for reactivity with a range of DNA structures to test the properties of multiple metal ions for selective oxidation. Specific strand scission was observed on the Watson strand at A_{24} of the frayed duplex **IA** (Figure 1a, Watson strand shown on the left) during reaction with **2** and 5 mM MPA for 15 min at ambient temperature (Figure 2A, lanes 5 and 6). The site preferentially cleaved by **2** is on the 3′ overhang and displaced from the central duplex by one intervening nucleotide, G_{23}. Reaction with the Crick strand of **Ia** (shown on the right in Figure 1A) did not result in specific cleavage. However, **2** demonstrated weak recognition of the junction by a slightly enhanced level of cleavage at relatively high concentrations of the complex (Figure 2B, lane 6).

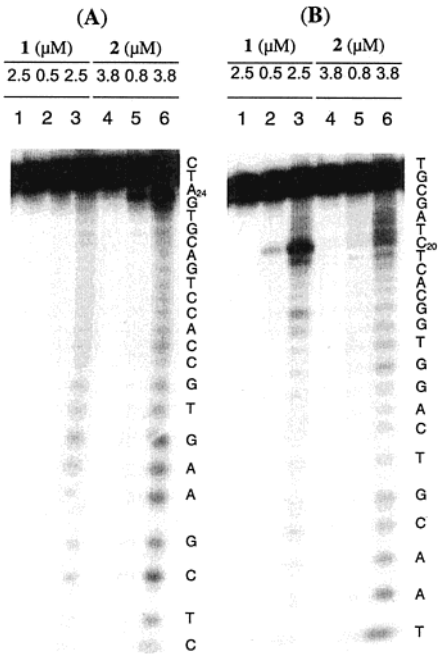

Figure 2. Autoradiograms of 20% polyacrylamide denaturing gels (7 M urea) showing cleavage products of 5′-^{32}P-labeled **Ia** (100 nM) induced by multinuclear copper complexes in the presence of MPA for 15 min in sodium phosphate (10 mM, pH 6.8) at ambient temperature. (A) Watson strand of **Ia** and (B) Crick strand of **Ia**.

The observed activity of **2** directly contrasted with that of the previously studied trinuclear complex, $[Cu^{II}_3(\mathbf{L})(H_2O)_3(NO_3)_2](NO_3)_4 \cdot 5H_2O$ (**1**),[14,15] which mediated specific cleavage at C_{20} of the Crick strand of **Ia** under the same conditions (Figure 2B, lane 3). Reaction of **1** with the Watson strand of **Ia** did not produce specific strand scission (Figure 2A, lane 3). The selectivity of **1** had previously been characterized and found to promote scission directly adjacent to a junction between single- and double-stranded DNA on the 3′ single-stranded overhang and require adjacent purine and G at the *n* (i.e. first unpaired) and $n + 1$ (i.e. second unpaired) positions.[14,15] Although both complexes mediate specific cleavage near helix/coil junctions, they do not promote these reactions at the same junctions. This may indicate similar, but not identical structural requirements for recognition and selective cleavage. The absence of a guanine in the $n + 1$ position of the 5′ overhang of the Crick strand explains the lack of specific reactivity of **1** at this junction. Reaction of **2** also appears to be more efficient than **1** at equivalent copper ion concentrations as evident from the greater level of selective and background cleavage observed (Figure 2A, lane 5 vs Figure 2B, lane 2).

To elucidate the origins of recognition, specific strand scission, and reactivity of $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**), a series of new targets was designed to identify positional and possible sequence dependences for efficient reaction at helix/coil junctions. The new constructs (**II**, Figure 1B) moved the cleavage

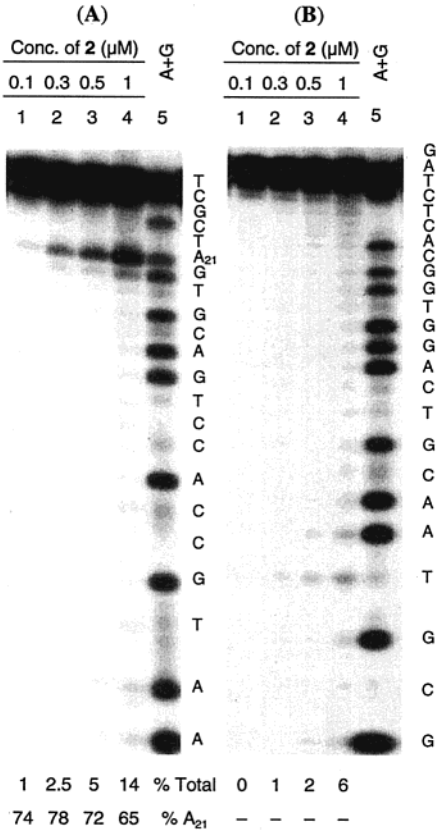

Figure 3. Autoradiogram of a 20% polyacrylamide denaturing gel (7 M urea) showing cleavage products of 5′-^{32}P-labeled **IIa** (100 nM) incubated with **2** and MPA for 15 min in sodium phosphate (10 mM, pH 6.8) at ambient temperature. (A) Lanes 1−4: **IIa** (Watson strand) and 5 mM MPA with 0.1, 0.25, 0.5, and 1 μM **2**. Lane 5: A+G sequencing lane. (B) Lanes 1−4: **IIa** (Crick strand) and 5 mM MPA with 0.1, 0.25, 0.5, and 1 μM **2**. Lane 5: A+G sequencing lane.

site further from the 3′ terminus while retaining the same central duplex region, single-stranded portions, and sequence near the helix/coil junction as **Ia** (Figure 1A). The features of the frayed duplex that were conserved between **Ia** and **IIa** were those that had a potential to play a role in selective cleavage near the junction. Extension of the overhangs near the junction was necessary to enable facile observation and analysis of specific strand scission with 5′-^{32}P-labeled oligonucleotides. The constructs were also varied at the cleavage site and key residues thought to be crucial in reactivity.

Reaction of **IIa** with **2** and MPA under the standard conditions demonstrated specific cleavage at A_{21} on the 3′ overhang (Figure 3A) identical with that observed at A_{24} of **Ia** (Figure 2B). Notably, **2** once again revealed its greater efficiency relative to **1** by mediating specific cleavage of the new target in the presence of only 0.1 μM **2** as compared to the previous standard of 0.5 μM set by **1**.[14,15] At the lower concentration of **2**, approximately 1% of the oligonucleotide **IIa** was degraded with 79% of that cleavage directed at A_{21} (Figure 3A, lane 1). This represents a 5-fold decrease in complex concentration relative to the lowest concentration of **1** required for mediation of selective cleavage. Recognition and specific cleavage by **2** proceeded not only at a lower concentration than **1**, but with significantly less copper present in the reaction mixture. As the concentration of **2** is increased 10-fold to 1 μM, the total cleavage of **IIa** approached 14% with 65% of that occurring at A_{21} (Figure 3A, lane 4). There was a steady increase in the amount of cleavage with a concomitant decrease in the percentage of that directed to A_{21} due to increasing amounts of sequence-neutral background cleavage in the presence of higher concentrations of **2**. A similar response was observed for cleavage of **Ia** by **1** at a higher concentration range of 0.5 to 5 μM.[14]

Both the selective strand cleavage at A_{21} and general background cleavage of **IIa** mediated by $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**) and MPA occurred spontaneously and did not require further treatment of the DNA with heat or alkali. The cleavage products of these reactions are therefore likely derived through oxidation of C-3′, C-4′, or C-5′, but not the C-1′ carbon of the deoxyribose moiety of the DNA backbone.[29] The degree of competing oxidation resulting in an abasic site or occurring directly at the nucleobases may be indicated by cleavage induced by piperidine (90 °C) after reaction with **2**. In the case of **IIa**, the degree of base release and base oxidation was dependent on the concentration of **2**. At 0.1 μM of the dinuclear complex, the amount of total cleavage both before and after piperidine treatment was small and approximately equivalent.[30] However, as the concentration of **2** increased from 0.25 to 1 μM, the amount of total cleavage following piperidine treatment increased to 41 ± 9%. At these higher complex concentrations, alternative modes of oxidation accounted for nearly 50% of the total reaction, demonstrating that **2** can be equally efficient at oxidizing various functional groups within DNA, and yet the general site selectivity of reaction remained the same whether spontaneous or piperidine-induced strand scission was observed.

Specific strand scission was not observed after treatment with **2** and MPA on the complementary Crick strand at either junction in contrast to the Watson strand of **IIa**. The total yield of cleavage for the Crick strand was only 6% at 1 μM **2** and no single nucleotide accounted for more than 10% of this value (Figure 3B, lane 4). At all concentrations of **2**, the total cleavage on the Crick strand of **IIa** was no more than half that observed on the Watson strand. However, when the specific cleavage at A_{21} on the Watson strand was subtracted from its cleavage yield at low concentrations of **2**, the sequence-neutral background cleavage on the two strands was nearly equal. As the concentration of **2** was increased, the difference between the levels of total cleavage on the Watson and Crick strands decreased and corresponded to a decrease in the percentage of this cleavage directed at A_{21} on the Watson strand of **IIa**. At low concentrations of **2**, strand scission is more frequently directed at A_{21} than at any other site on the Watson and Crick strands of **IIa**. In contrast, when this site is not present as in the Crick strand of **IIa** or at higher complex concentrations, sequence-neutral strand scission becomes more prevalent. This partitioning of reactivity between specific and nonspecific sites indicates a preferential interaction of **2** with the junction of **IIa** that results in specific cleavage at A_{21} of the Watson strand of this frayed duplex.

Copper-Induced Cleavage of Single-Stranded DNA. Reaction with either the Watson or Crick strand of **IIa** in the absence of its complementary strand produced very little cleavage unless the concentration of $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**) was raised to levels greater than 5 μM.[30] At concentrations less than 1 μM,

(29) Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089−1107.
(30) See Supporting Information.

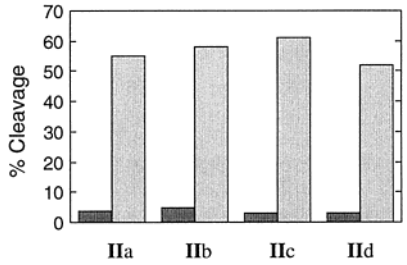

Figure 4. Effect of Y_{21} on specific and total cleavage of **IIa**, **IIb**, **IIc**, and **IId** (Watson strands) with 0.5 μM **2** and 5 mM MPA under the standard reaction conditions (see Supporting Information). Total degradation of each construct is designated by the dark shading and the percentage of specific strand scission at Y_{21} by the light shading.

degradation of the single strands was comparable to the nonspecific reaction detected for the Crick strand of **IIa** and showed little preference for any individual nucleotide either before or after piperidine treatment.[30] Interestingly, at higher complex concentrations preferential cleavage of unclear origin was observed at C_{11} of the Watson strand, but no other nucleotides. Thus, reaction of A_{21} in **IIa** duplex cannot be due to an intrinsic reactivity of that particular adenosine. Similarities between the amount of nonspecific cleavage observed with single-stranded and double-helical structures of DNA argue against association by **2** in the major or minor groove of the duplex region of **IIa**. This result suggests that specific strand scission observed for the Watson strand of **IIa** arises in part from interaction of the dinuclear complex with a unique structure such as the helix/coil junction.

Role of the Targeted Nucleotide, Y_{21}. To determine whether there is also a sequence requirement for reaction at the junction, systematic variations were made on both the 3′ and 5′ overhangs near the duplex region of the frayed duplex construct **IIa**. The first sequence modifications of **IIa** were made at the site of reaction, A_{21}, on the 3′ overhang. The nucleobase at this site was determined to have no effect on specific strand scission by replacing the target with thymine, guanine, or cytosine (**IIb**, **IIc**, and **IId**, Figure 1B). With 0.5 μM $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**), the amount of total cleavage of **IIa**, **IIb**, **IIc**, and **IId** remained relatively constant at 4 ± 1% and the percentage of the total cleavage directed at Y_{21} varied little from 56 ± 3% (Figure 4). Because specific strand scission was maintained at the same level for all four sequence variants, the nucleobase at the cleavage site could not be involved in binding or reaction of the dinuclear complex.

Role of Guanine at the *n* (First Unpaired) Position of the 3′ Overhang, X_{20}. The dinuclear complex, $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_2$ (**2**), could not have similar sequence requirements to $[Cu^{II}_3(\mathbf{L})(H_2O)_3(NO_3)_2](NO_3)_4\cdot 2.5H_2O$ (**1**) since **2** mediates strand scission at a site that is not recognized by **1**. However, the previous work with **1** suggested that interaction with a guanine near the cleavage site might be important for recognition.[14,15] In the frayed duplex construct **IIa** (Figure 1B), two guanines are in close proximity to the cleavage site. One of these, G_{20}, precedes the reactive A_{21} on the 3′ overhang and is directly adjacent to the central duplex. The other guanine is located on the 5′ overhang at a site three nucleotides removed from the duplex. The role of G_{20} was investigated first by replacing this guanine with cytosine and adenine (**IIe** and **IIf**, Figure 1B). Thymine could not be used equivalently since it would base-pair with the adjacent adenine on the 5′ overhang and shift the position of the helix/coil junction.

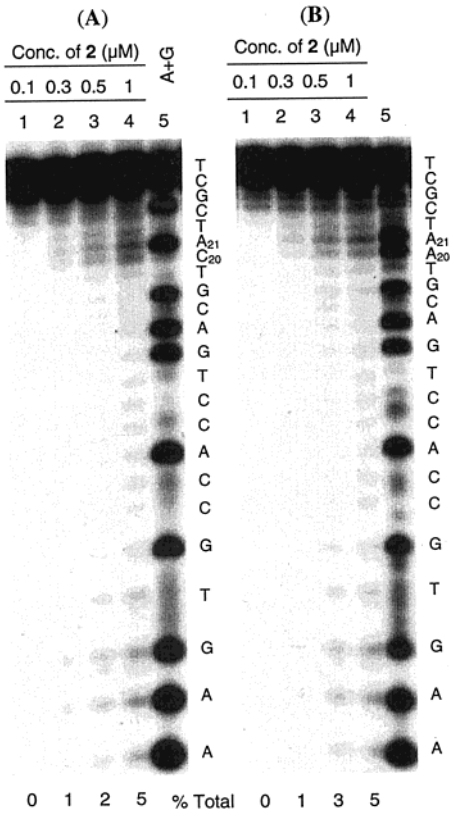

Figure 5. Autoradiogram of a 20% polyacrylamide denaturing gel (7 M urea) showing cleavage products of 5′-^{32}P-labeled **IIe** (Watson strand, 100 nM) and **IIf** (Watson strand, 100 nM) incubated with **2** and MPA for 15 min in sodium phosphate (10 mM, pH 6.8) at ambient temperature. (A) Lanes 1−4: **IIe** and 5 mM MPA with 0.1, 0.25, 0.5, and 1 μM **2**. Lane 5: A+G sequencing lane. (B) Lanes 1−4: **IIf** and 5 mM MPA with 0.1, 0.25, 0.5, and 1 μM **2**. Lane 5: A+G sequencing lane.

The total cleavage of the Watson strands of **IIe** and **IIf** by **2** (Figure 5) decreased nearly 3-fold to 5% relative to that of the Watson strand of **IIa**. The strands lacking G_{20} produced only nonspecific cleavage in quantities comparable to that detected for the Crick strand of **IIa** under the same concentration of **2**. Neither **IIe** nor **IIf** was subject to efficient oxidation of A_{21}. Instead a 5-fold decrease in the percentage of total cleavage directed at A_{21} from 53% to 10% was observed for **IIe** at 1 μM **2** (Figure 5A, lane 4), and this was accompanied by an increase in reaction at C_{20} and T_{22} flanking A_{21}. A similar cleavage pattern was detected from reaction of **IIf** (Figure 5B, lane 4). In this case, a slight enhancement in cleavage at A_{20} and T_{22} was observed, indicating that selectivity for A_{21} requires a guanine. The small increase in cleavage near the junction in **IIe** and **IIf** resembled the pattern observed for reaction of **2** with the Crick strand of **Ia** (Figure 2B). Even in the absence of the guanine at X_{20} or other possible sequence requirements, the dinuclear complex appears to have a weak intrinsic affinity for junctions of single- to double-stranded DNA. This is further illustrated by preferential oxidation of A_4, A_5, and G_6, which are part of the other helix/coil junctions in **IIa**, **IIe**, and **IIf** (Figure 5). The enhancement at both junctions is distributed over several contiguous nucleotides in contrast to the specific reaction, which targets a single nucleotide.

The ability of the guanine in the *n* position of the 3′ overhang (X_{20}, Figure 1B) to direct specific strand scission at Y_{21} by

$[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**) was considered likely due to coordination of copper to the N7 of the base and its ability to influence the site selectivity of **1**.[14,15] Coordination of Cu(II) salts to G−N7 is well precedented in duplex DNA.[31] However, in metal complexes the steric bulk of the ligand may restrict binding of the metal center to single-stranded regions as proposed for several macrocyclic nickel complexes.[32−34] Coordination through G−N7 is consistent with a lack of reactivity when the guanine is replaced with either cytosine (**IIe**) or adenine (**IIf**), which do not interact as strongly with transition metals.[31] Although coordination of copper to the N7 of adenine has also been observed,[31,35] binding to the G−N7 is preferred because of its greater basicity.[36] The redox chemistry of guanine, which determines many reactions, is likely not involved in selective strand scission of DNA by **2**. Despite a requirement for its presence, G_{20} is neither the site of specific strand scission nor subject to base oxidation[30] during reaction (Figures 2A and 3A). Since there are no other guanines on the 3′ overhang close to the junction, G_{20} is then likely to be the primary regulator of recognition at the helix/coil junction. Of course, this would also rely on the ability of copper to bind without oxidizing G_{20}. This is possible since oxidation is likely controlled by an O_2-derived radical rather than the copper itself. Orientation of a possible copper-peroxo intermediate may be key to the contrasting selectivity of **2** and **1**.[14,15] Unique recognition of a G·A mismatch could provide an alternative source of selectivity. This proposal would not necessarily suggest G−N7 binding and is currently subject to further investigation.

Role of the 5′ Overhang. Single-stranded guanines are also present in the 5′ overhang near the site of strand scission induced by $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**) and consequently their role in reactivity was next examined. Recognition by the trinuclear complex, $[Cu^{II}_3(\mathbf{L})(H_2O)_3(NO_3)_2](NO_3)_4\cdot 5H_2O$ (**1**), was entirely directed through a guanine and another purine on the 5′ overhang, even though cleavage occurred on the opposite 3′ extension.[15] The dependence of guanine on the 5′ overhang for reaction by **2** was first investigated with DNA **IIg** containing a thymine in place of the guanine nearest to the junction (Figure 1B). Reaction of this DNA and **2** proceeded equivalently to that of **IIa** and exhibited no loss in efficiency or selectivity for A_{21}.[37] The only other guanine on the 5′ overhang is five nucleotides away from the junction and was not present in the frayed duplex **Ia**. This original DNA target also exhibited selective strand cleavage at the $n + 1$ site and thus the distal guanine in **IIa**−**g** is not required for selective reaction.

To test the general dependence of reaction on the presence of a 5′ overhanging sequence, modification of a truncated structure **IIh** completely lacking in the overhang was characterized (Figure 6). If only the 3′ overhang were required for recognition, then specific strand scission on the 3′ overhang would have proceeded without change. However, no specific strand scission at A_{21} was detected for **IIh**.[30] Moreover, the absence of the 5′ overhang resulted in a decrease in the total cleavage of this DNA by 5-fold compared to that of **IIa** at 1 μM $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**). These results suggest that the 5′ overhang is required for recognition and likely helps to stabilize the frayed duplex structure and its interaction with **2**. Selective cleavage at A_{21} was recovered by restoring the first two nonhelical nucleotides of the 5′ overhang in **IIi** (Figure 7). Still, the total cleavage of the Watson strand of **IIi** at all concentrations of **2** was less than that observed under identical conditions for the Watson strand of **IIa**. The decrease in overall reactivity was also accompanied by a relative decrease in selectivity for A_{21}, which was targeted only 50% of the time (1 μM **2**) relative to the 65% for **IIa**.[30] A similar result had previously been observed for $[Cu^{II}_3(\mathbf{L})(H_2O)_3(NO_3)_2](NO_3)_4\cdot 5H_2O$ (**1**) when comparing another helix/coil junction and its derivative with a partially truncated 5′ overhang.[15]

Stimulation of selective scission at A_{21} by the presence of adenine and thymine on the 5′ overhang suggested that one or both of these nucleotides could be involved in recognition by **2**. The sequence requirements of this truncated 5′ extension were

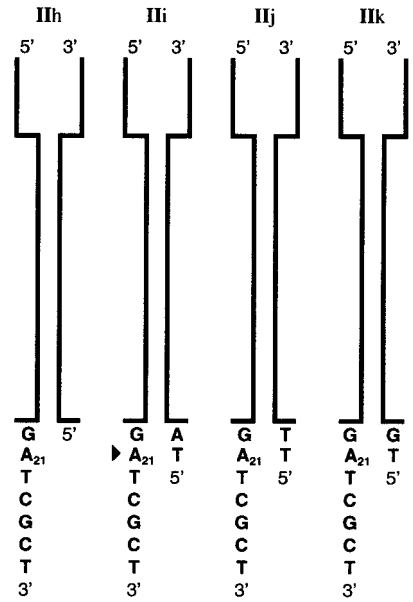

Figure 6. Structures of the frayed duplex structures **IIh**, **IIi**, **IIj**, and **IIk** with truncated 5′ overhangs. Specific strand scission is indicated by an arrowhead.

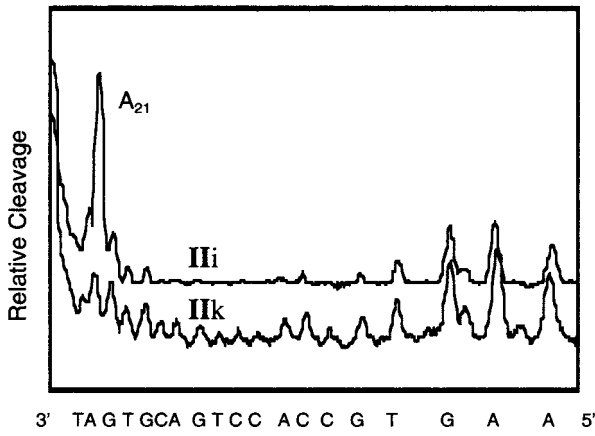

Figure 7. Densitometer trace exhibiting specific and nonspecific cleavage of **IIi** and **IIk** by 1 μM **2** under standard conditions.

(31) Sigel, H. *Chem. Soc. Rev.* **1993**, *22*, 255−267.
(32) McLachlan, G. A.; Muller, J. G.; Rokita, S. E.; Burrows, C. J. *Inorg. Chim. Acta* **1996**, *251*, 193−199.
(33) Shih, H.-C.; Kassahun, H.; Burrows, C. J.; Rokita, S. E. *Biochemistry* **1999**, *38*, 15034−15042.
(34) Shih, H.-C.; Tang, N.; Burrows, C. J.; Rokita, S. E. *J. Am. Chem. Soc.* **1998**, *120*, 3284−3288.
(35) Sigel, H. In *Metal-DNA Chemistry*; Tullius, T. D., Ed.; American Chemical Society: Washington, DC, 1989; pp 159−204.
(36) Burrows, C. J.; Rokita, S. E. *Acc. Chem. Res.* **1994**, *27*, 295−301.
(37) Data not presented.

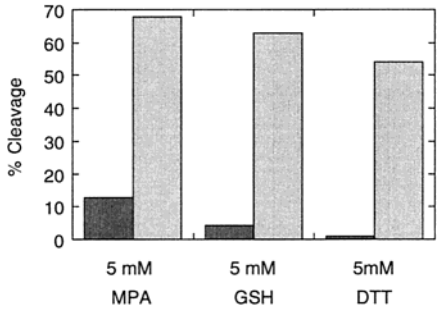

Figure 8. Effect of varying reductants on specific and total cleavage of **IIa** (Watson strand) with 1 μM **2** and 5 mM reductant under the standard reaction conditions. Total degradation of the construct is designated by the dark shading and the percentage of specific strand scission at A_{21} by the light shading.

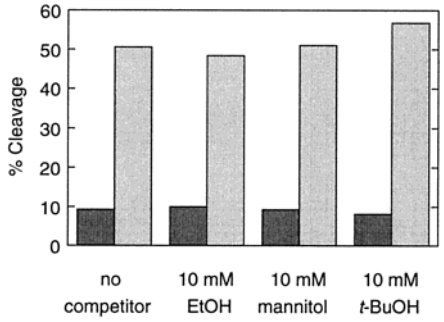

Figure 9. Effect of standard radical scavengers on cleavage of **II**a (Watson strand) by 1 μM **2** and 5 mM MPA. Total degradation of the construct is designated by the dark shading and the percentage of specific strand scission at A_{21} by the light shading.

explored next by substituting the nonhelical adenine of the truncated frayed duplex (**IIi**) with either a thymine or a guanine (**IIj** and **IIk**, Figure 6). No specific strand scission at A_{21} on the 3′ overhang was observed after reaction of either **IIj** or **IIk** and $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**) (Figure 7).[30] At most, the nonhelical thymine in **IIi**, **IIj**, and **IIk** could be relegated to a minor role in recognition of **2** since it was present in both reactive (**IIi**) and unreactive targets (**IIj** and **IIk**). The loss of specific strand scission when the nonhelical adenine is replaced on the 5′ overhang with thymine or guanine implicates adenine as an absolute requirement for recognition and specific reactivity at helix/coil junctions. Selective cleavage of the DNA also requires a guanine in a corresponding position on the 3′ overhang.

Role of the Reductant. Generation of the dicopper(I) form of $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**) appears to be crucial for formation of the reactive intermediate based on the lack of strand scission when either 3-mercaptopropionic acid (MPA) or **2** is absent from the reaction mixture. To determine if MPA played a unique role in strand scission either as a reductant or through interactions with **2**, the extent and pattern of DNA scission were assessed by testing alternative reductants, glutathione (GSH), and dithiothreitol (DTT). The efficiency of the three reductants to promote scission of the Watson strand of **IIa** varied according to MPA > GSH > DTT (Figure 8). The total cleavage of **IIa** (Watson strands) by 1 μM **2** and 5 mM GSH was approximately 3-fold less than with an equivalent concentration of MPA. Reaction in the presence of 5 mM DTT resulted in even less total cleavage with only 1% of the Watson strand degraded. However, in the presence of either GSH or DTT, the amount of cleavage directed at A_{21} was relatively consistent at 62 $\pm$ 6% and exhibited the same selectivity observed in the presence of MPA.[30] Despite the differences in the overall reactivity, the similarities in the cleavage patterns suggest that a common intermediate is formed in each case resulting from reduction of Cu(II) complex to Cu(I).[38,39] The observed differences in total cleavage are likely related to the reduction potentials of the thiols and their respective abilities to generate Cu(I) from Cu(II).

Role of Dioxygen. The expected Cu(I) species generated by the thiols has the potential to activate O_2 by forming intermediates such as superoxide anion, peroxide, and hydroxyl radical. To directly assess the importance of dioxygen in specific and background cleavage of **IIa**, a standard reaction mixture was degassed with nitrogen for 15 min prior to the addition of MPA. The reaction was further kept under a nitrogen atmosphere during its standard incubation at ambient temperature. This resulted in a 3.5-fold decrease in reactivity relative to a parallel sample that was not degassed.[30] However, little relative change was observed in the cleavage directed at A_{21} (41%). Based on these results, the reactive intermediate or intermediates responsible for specific cleavage and sequence-neutral background cleavage appear to be derived from O_2. This is also consistent with the O_2 requirement of strand scission by **1**.[14,15] The dependence on O_2 for both complexes is indicative of a mechanism akin to those proposed for $[Cu(OP)_2]^{2+}$, which also requires dioxygen to mediate cleavage of DNA.[8]

Effect of Standard Radical Scavengers. The presence of a diffusible radical species can be diagnosed by monitoring quenching of DNA cleavage in the presence of alternative H-atom donors, which would scavenge radicals (such as $^{\bullet}OH$) in solution. To this end, standard radical scavengers were added to the reaction of **IIa** and $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**) prior to initiation with MPA. The individual addition of ethanol, D-mannitol, and *tert*-butyl alcohol (10 mM) was found to have no effect on sequence-neutral cleavage, which remained constant at 9 $\pm$ 1% (Figure 9).[30] Selective cleavage at A_{21} represented 52 $\pm$ 3% of the total yield in the presence or absence of each scavenger. Thus, oxidation of the deoxyribose within the DNA backbone leading to both specific and nonspecific strand scission by **2** is not mediated by a diffusible, or at least trappable, radical species. Additional cleavage effected by subsequent treatment with piperidine also remained unaffected by these H-atom donors. The lack of diffusible intermediates is consistent with the ability of **2** to direct cleavage with single nucleotide resolution.

Target selective reaction mediated by distamycin or oligonucleotide conjugates of EDTA·Fe(II) generates a range of cleavage products 7−8 bases on either side of the binding site of their recognition element.[40,41] This is most typical of localized generation of a diffusible hydroxyl radical species. When such a complex is not linked to a DNA recognition element, then scission occurs randomly at all four nucleotides and is inhibited by addition of radical scavengers. In contrast, $[Cu(OP)_2]^{2+}$, which is believed to react through a coordinated radical species generated close to the cleavage site, cannot be quenched by

(38) John, D. C. A.; Douglas, K. T. *Biochem. J.* **1993**, *289*, 463−468.
(39) Veal, J. M.; Merchant, K.; Rill, R. L. *Nucleic Acids Res.* **1991**, *19*, 3383−3388.
(40) Taylor, J. S.; Schultz, P. G.; Dervan, P. B. *Tetrahedron* **1984**, *40*, 457−465.
(41) Dreyer, G. B.; Dervan, P. B. *Proc. Natl. Acad. Sci.* **1985**, *82*, 968−972.

mannitol or alcohols.[42] Specific and nonspecific cleavage of DNA by the trinuclear complex, $[Cu^{II}_3(\mathbf{L})(H_2O)_3(NO_3)_2](NO_3)_4 \cdot 5H_2O$ (**1**), also was not subject to inhibition by the hydrogen atom donors ethanol, D-mannitol, and *tert*-butyl alcohol.[15] In addition, specific strand scission was directed at one nucleotide suggestive of a nondiffusible intermediate. These results indicate that the reactive species that effects selective cleavage by $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**) is likely formed close to the site of reaction and not released from the complex for diffusion to the cleavage site. The reactive intermediate in strand scission mediated by **2** may be related to the known μ-peroxo dicopper(II) (Cu−O−O−Cu) intermediate formed when the dicopper(I) complex of **2** reacts with dioxygen.[17] A similar end-on μ-O−O dimer was proposed as the reactive intermediate in the nucleobase specific oxidation of a 514-bp fragment of pBR322 by a mononuclear Co(II)·Lys-Gly-His complex.[43] The formation of the peroxo species in this example was postulated from the known dioxygen chemistry of the cobalt-peptide and an observed dependence in specific oxidation on the concentration of the cobalt-peroxo dimer.

Reactivity of $[Cu^{II}(TMPA)(H_2O)](ClO_4)_2$ (3) or $[Cu^{II}_2(DO)(Cl)_2](ClO_4)_2$ (4) with IIa. The dinuclear metal complex $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**) likely controlled both its recognition and reactivity with DNA by associating with the frayed duplex and generating the intermediate responsible for site specific strand scission. This dual role is intimately linked to the influence of the ligand in defining the coordination environment about each copper, which in turn determines the orientation and type of intermediates and their rate of formation. Since at least one of the coppers in the dinuclear complex **2** may coordinate to the N7 of G_{20}, the coordination environment surrounding the metals could also be crucial for recognition of the helix/coil junction in **IIa**. Each copper(II) ion in **2** is chelated through three pyridyl nitrogens and a tertiary amine yielding the trigonal bipyramidal geometry that is characteristic for this tripodal tetradentate motif.[26,44] The fifth coordination site could alternatively be occupied by water, hydroxide ion, or substrate.[22] The mononuclear compound, $[Cu^{II}(\mathbf{TMPA})(H_2O)](ClO_4)_4$ (**3**), possesses a nearly identical coordination sphere to that of **2** and its Cu(I) and Cu(II) complexes have been characterized by X-ray crystallography, EPR, electrochemistry, and UV−vis spectroscopy.[17,19] The effects of the coordination environment and nuclearity (i.e. di- vs mononuclear) on DNA cleavage were assessed by comparing nonspecific and specific strand scission of **IIa** by **2** and **3**. When **3** was reacted with **IIa** under the standard reaction conditions, no degradation of the Watson strand was observed (Figure 10).[30] This lack of reactivity precluded any observation of specific strand scission. The Crick strand of **IIa** was also not cleaved by **3** under standard reaction conditions.[30] The absence of strand scission was not related to differences in the amount of metal ion present since equivalent copper ion concentrations of **2** and **3** were used (1 and 2 μM, respectively). Reacting $Cu(ClO_4)_2 \cdot 6H_2O$ with **IIa** under the standard reaction conditions also failed to produce either specific or nonspecific cleavage of the Watson and Crick strands.[30] The

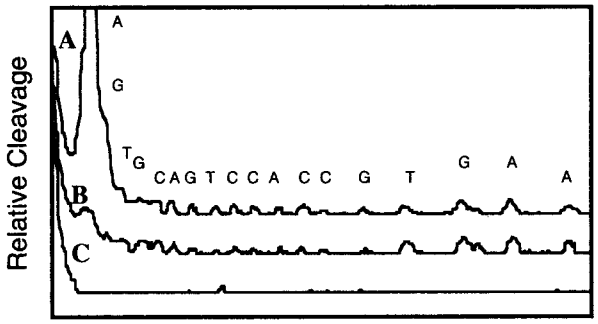

Figure 10. Densitometer traces demonstrating differential reactivity of **2**, **3**, and **4** with **IIa** and 5 mM MPA: (A) 1 μM **2**, (B) 1 μM **4**, and (C) 2 μM **3**.

results of these experiments demonstrate that multiple metal ions within the same complex are essential for efficient cleavage of DNA.

The lack of activity exhibited by $[Cu^{II}(\mathbf{TMPA})(H_2O)](ClO_4)_2$ (**3**) vs $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**) may be related to their different ability in activating dioxygen. In nitrile solvent both complexes initially form a short-lived superoxo intermediate that is observable only by stopped-flow kinetics.[45] One Cu(II)−O_2^- can then react with an additional Cu(I) ligand complex to form an end-on μ-peroxo ($Cu-O_2^{2-}-Cu$). Formation of the peroxo species by the Cu(I) form of **3** requires that two complexes come together yielding an intermolecular μ-peroxo dimer.[45−47] The tethering of two copper binding units in the dicopper(I) form of **2** gives rise to an intramolecular peroxo that is entropically stabilized.[17] Dioxygen and reductant dependent cleavage of DNA by **2** clearly indicates that the dinuclear complex is reduced to its dicopper(I) form, which subsequently reacts with O_2 to generate the reactive species responsible for DNA oxidation. The contrasting reactivity of **2** and **3** with DNA cannot by ascribed to differences in their reduction potentials since they are nearly identical.[17] The absence of nonspecific background cleavage of DNA when **3** is reacted with **IIa** in the presence of MPA and O_2 suggests difficulties in forming the appropriate O_2-derived intermediate and is not primarily related to a lack of specific DNA recognition. Instead, the rate of forming the reactive species of **3** may by too slow to mediate strand scission during the standard incubation time. Alternatively, the mononuclear complex **3** might interact with DNA (i.e. by binding to the biopolymer) in such a manner that it never comes into contact with another copper complex to form a reactive dicopper intermediate. The lack of activity by **3** implies that the dinuclearity of **2** as well as its metal coordination environment and dioxygen reactivity are crucial for efficient strand scission.

Another dinuclear complex, $[Cu^{II}_2(\mathbf{DO})(Cl)_2](ClO_4)_2$ (**4**), was tested to correlate DNA cleavage with O_2 reactivity. The copper ions in **4** share similar coordination environments to those in $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**) and $[Cu^{II}(\mathbf{TMPA})(H_2O)](ClO_4)_2$ (**3**).[48] Additionally, the dicopper(I) form of **4** yields an intramolecular μ-peroxo species in the presence of O_2 with a rate of formation faster than that formed by **2**.[21] The 2-atom linker of

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2 produces a considerable amount of strain in the peroxo complex and contributes to enthalpic destabilization of its intermediate. This effect is avoided by addition of an oxygen atom to the linker of **3**. If a dinuclear complex possessing similar properties with O_2 is needed for strand scission, then **4** should promote cleavage of **IIa**. Indeed, sequence-neutral cleavage of the DNA was detected when **4** was reacted with the Watson strand of **IIa** under the standard conditions (Figure 10).[30] The sequence neutral reaction was equally efficient for the Watson and Crick strands and similar to that formed by **2**. However, highly selective strand scission did not occur at any site on **IIa** and consequently, the amount of total cleavage on the Watson strand was about 3-fold less than that generated with **2**. The cleavage pattern generated by **4** did show a slight enhancement in cleavage at A_{21} and T_{22} near the helix/coil junction, suggesting a weak interaction between the DNA and this alternative dinuclear complex may be retained.[30] The reactivity of both **2** and **4** again demonstrates that multinuclearity is essential in mediating strand scission. However, site selectivity depends on more subtle features related to the recognition of DNA and the structure of the copper complexes, topics of continuing investigation.

Reactivity of $[Cu(OP)_2]^{2+}$ with IIa. The unique recognition and efficiency of $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**) is also highlighted by its comparison to the activity of $[Cu(OP)_2]^{2+}$. At equivalent copper ion concentrations both **2** and $[Cu(OP)_2]^{2+}$ cleaved the Watson strand of **IIa** under the standard conditions, but their dependence on concentration differed dramatically. At 0.5 μM $[Cu^{2+}]$, a total of 2% of the strand was cleaved by **2** whereas no detectable cleavage was evident for $[Cu(OP)_2]^{2+}$. When $[Cu^{2+}]$ was doubled to 1.0 μM, total degradation by **2** doubled to 4% while degradation by $[Cu(OP)_2]^{2+}$ remained below detectable levels (Figure 11A). However, after the $[Cu^{2+}]$ was increased to 2 μM, $[Cu(OP)_2]^{2+}$ became much more reactive than **2**, cleaving 50% of the oligonucleotide in contrast to 9% cleaved by the dinuclear compound (Figure 11B). However, $[Cu(OP)_2]^{2+}$ was not capable of mediating specific strand scission at A_{21} under any of the concentrations tested. The phenanthroline−copper complex demonstrated a preference for oxidation of double-helical 5′ GT sequences consistent with prior reports.[49,50] Cleavage of the Crick strand of **IIa** by 2 μM $[Cu(OP)_2]^{2+}$ was similar to that of the Watson strand of **IIa** and again a threshold $[Cu^{2+}]$ of 2 μM was necessary for reaction.[30] There is no cleavage on either strand by $[Cu(OP)_2]^{2+}$ at concentrations below 2 μM. Earlier studies had shown that strand scission by $[Cu(OP)_2]^{2+}$ appeared to require 2 equiv of the complex, one bound to the minor groove and one free of DNA.[51] Consequently, if all of the 1,10-phenanthroline−copper complex is interacting with the biopolymer, then none is left to form the dimeric intermediate and reaction is prevented. However, as the complex concentration increases, some complex will remain unbound and free to interact with the DNA-bound

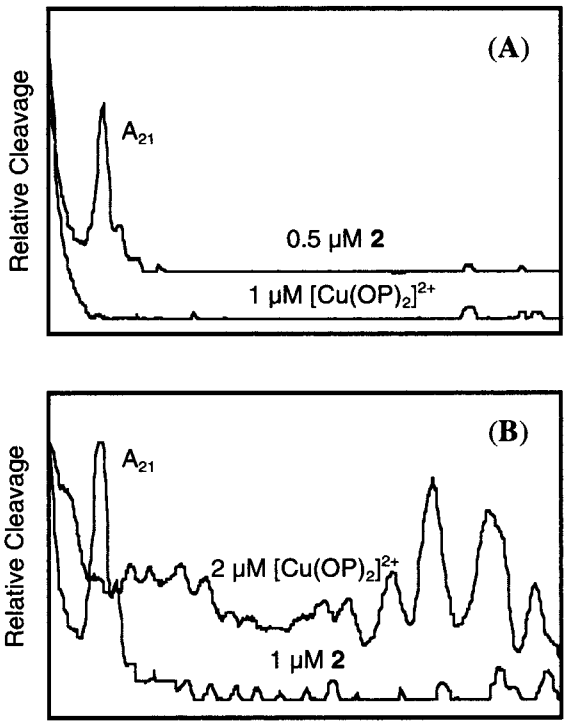

Figure 11. Densitometer traces demonstrating differential reactivity of **2** and $[Cu(OP)_2]^{2+}$ with **II**a and 5 mM MPA: (A) 1 μM $[Cu^{2+}]$ and (B) 2 μM $[Cu^{2+}]$.

complex. If a related dicopper complex must form around the site of strand scission induced by **2**, then no intermolecular assembly of copper is required. The ligand maintains the two coppers intramolecularly to promote efficient oxidation of DNA at low copper concentrations.

Proposed Model of Reactivity and Recognition by $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (2). The key to understanding the differences between $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**) and $[Cu^{II}_2(\mathbf{DO})(Cl)_2](ClO_4)_2$ (**4**) in cleavage of DNA may lie in understanding the details of their interactions with dioxygen. Formation of a μ-peroxo intermediate $[Cu{-}O_2^{2-}{-}Cu]$ from the dicopper(I) form of **2** was shown to be destabilized enthalpically relative to the same intermediate formed between two monomeric copper complexes, i.e., in the reaction of $[Cu^{II}(\mathbf{TMPA})(H_2O)](ClO_4)_2$ (**3**) with dioxygen. This destabilization is caused by strain in the peroxo intermediate manifested in the short two-atom bridge connecting the copper binding moieties.[17,52] In contrast, the three-atom ether bridge of **4** participates in an intramolecular μ-peroxo species that is both entropically and enthalpically stabilized when the dicopper(I) complex is reacted with dioxygen.[17,21,52] Once formed, the intermediates of **2** and **4** likely initiate H-atom abstraction from the deoxyribose moiety of DNA and lead to direct strand scission. The slight enhancement of cleavage at the junction of **IIa** produced by **4** suggests that this analogue possessed similar recognition properties to **2**.

Although the μ-peroxo intermediate of **4** is thermodynamically more stable than that of **2**,[21] their similar abilities to oxidize DNA nonspecifically suggest that neither the rate of formation nor the lifetime of the intermediate are responsible for selective cleavage. Molecular mechanics modeling demonstrates that one of the primary differences between the μ-peroxo intermediates

(48) Spectroscopic studies revealed similar EPR spectra for **2**, **3**, and **4** typical of reverse tetragonal coordination. Chloride coordination in **4** produced a distinct UV−vis spectrum that compared favorably with that of a chloride coordinated form of **3**. Since both of these relate to the electronics surrounding the metal as affected by the ligand environment, the compounds are then presumed to have closely related coordination spheres. This is also supported by the similar bond distances and angles from X-ray structures of each dicopper(II) complex. Data to be published elsewhere.
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of **2** and **4** is the tilt of the two equatorial planes encompassing the metal ion and the three pyridyl nitrogens in relation to one another.[52] All other constraints, including the calculated Cu···Cu distances, Cu−O−O−Cu bond angles, and torsion angles around the peroxide bond, are nearly identical for both **2** and **4**. Therefore, the difference in the ability of the two μ-peroxo complexes to promote selective cleavage may be the orientation of the O−O moiety. Further investigations will be needed to ascertain the identity of and the exact effect of copper−dioxygen intermediates in mediating specific cleavage.

Conclusion

A dicopper(II) complex, $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**), has been shown to exhibit efficient and specific strand scission at junctions between single- and double-stranded DNA. Although the general requirements of a helix/coil junction are similar to those of previously studied trinuclear complex $[Cu^{II}_3(\mathbf{L})(H_2O)_3(NO_3)_2](NO_3)_4 \cdot 5\ H_2O$ (**1**)[14,15] studied previously, the exact cleavage sites and nucleotide sequence requirements of these complexes are quite different. Specific strand scission by **2** occurs at a site that is two nucleotides displaced from the junction on the 3′ overhang of a frayed duplex structure. Cleavage is not dependent on the identity of the base at the cleavage site, but does require a guanine on the 3′ overhang directly adjacent to the central duplex and an adenine on the 5′ overhang in the same position. This is in contrast to **1**, for which both sequence requirements were found on the 5′ overhang opposite the cleavage site on the 3′ overhang. Again, these differing recognition elements may reflect the available geometries of the dicopper(II)-peroxo intermediate and suggest that orientation of the intermediate may be more important than its thermodynamic stability.

Generation of the reactive intermediate responsible for specific and sequence-neutral strand scission requires both a reductant and dioxygen, implying the formation of an intermediate derived from the activation of O_2 by Cu(I). Addition of radical scavengers to the standard reaction failed to inhibit cleavage by $[Cu^{II}_2(\mathbf{D^1})(H_2O)_2](ClO_4)_4$ (**2**), demonstrating that the intermediate responsible for selective and nonspecific strand scission is not a freely diffusible radical. At equivalent copper ion concentrations, **2** is more efficient than its mononuclear analogue, $[Cu^{II}(\mathbf{TMPA})(H_2O)](ClO_4)_2$ (**3**), and $[Cu(OP)_2]^{2+}$. The dinuclear analogue, $[Cu^{II}_2(\mathbf{DO})(Cl)_2](ClO_4)_2$ (**4**), exhibits a similar yield of sequence-neutral DNA cleavage, but only a very a weak preference for reaction at a helix/coil junction. Site-specific strand scission, best illustrated by **2**, may derive from the formation and orientation of its μ-peroxo derivative. Studies have now been initiated to identify the subtle requirements of target recognition and dioxygen activation that may reveal fundamental information about copper and related complexes as well as provide tools for nucleic acid analysis in vitro and in vivo.

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Supporting Information Available: Autoradiograms of sequence variants of **IIa** in reactions with complex **2**, piperidine treated samples, strand scission in the presence of different reductants and standard radical scavengers, and cleavage mediated by complexes **3**, **4**, and $[Cu(OP)_2]^{2+}$ (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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