Recognition of guanines at a double helix–coil junction in DNA by a trinuclear copper complex†

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Coordination between guanine N7 and a trinuclear copper complex appears critical for selective and efficient strand scission of DNA at a helix–coil junction as indicated by the lack of reactivity of comparable DNA containing 7-deazaguanine in place of guanine; both the base pair at the junction and coil flexibility also modulate the specificity of DNA oxidation.

Transition metal complexes stand out as exceptional candidates for artificial nucleases of DNA and RNA due to their diverse ability to recognize and react selectively with individual target sites.[1–6] Discovering new complexes with unique specificity occurs relatively frequently, but establishing the structural and chemical basis of these activities is much less common. A number of copper systems have been well described and none more than $[Cu(OP)_2]^{2+}$ (OP = 1,10-phenanthroline).[7,8] *In situ* reduction generates the Cu^{I} species $[Cu(OP)_2]^{+}$ that subsequently binds to the minor groove of DNA, combines with molecular oxygen, generates a non-diffusible oxidant and finally induces strand scission by oxidation of the ribose backbone.[1,9] Direct metal–nucleobase coordination is another mechanism by which specific modification can be achieved. For example, a series of Ni^{II} square-planar complexes can be oxidized *in situ* to their Ni^{III} octahedral derivatives that subsequently bind to the most solvent accessible G N7 positions and selectively deliver the oxidizing power of a peracid for nucleobase oxidation.[10,11]

Copper-based reactions of DNA are particularly appealing since they have the potential for application *in vivo*. Only a thiol and molecular oxygen are necessary to induce their ability to oxidize nucleic acids.[7] Our laboratories have recently applied a variety of multi-nuclear copper complexes as probes for DNA that were initially developed as models for copper-dependent enzymes responsible for molecular oxygen transport and activation.[12] Both target selection and reaction can be modulated by the copper nuclearity and ligand structure.[13–17] Two features common to their specificity are proximal G residues and a helix–coil junction. The role of each has now been clarified as described below.

Simple copper salts coordinate to the N7 position of guanine,[18,19] and similar coordination may explain the site selectivity of a trinuclear copper complex, $[Cu_3^{II}(\boldsymbol{L})(NO_3)_2(H_2O)_3](NO_3)_4{\cdot}5H_2O$ (complex **1**) (Fig. 1, $\boldsymbol{L}$ = 2,2′,2″-tris(dipicolylamino)triethylamine) as well.[14,20] Direct strand scission induced by complex **1** had previously been shown to require a purine (A or G) as the first residue extending from the 5′-terminus of a duplex and a G as the second and neighboring residue.[14] Strand scission then occurred on the opposite strand at the helix–coil junction as illustrated by the Crick strand of DS **1** (Fig. 2).

We now find that replacing either G in the proposed recognition sequence of DS **1** with 7-deazaguanine (dzG) (DS **2**, DS **3**, Fig. 2) dramatically suppressed strand scission at the target sites (T19 and T20, Fig. 3, lane 4 *vs.* lanes 8 and 12). The composite yield of scission at these sites dropped from 21% to only 2% relative to the initial starting material, just above the threshold of detection as measured by densitometry. Substitution of T with dzG on the Crick strand adjacent to the sites of strand scission also suppressed reaction somewhat although the effect was only evident for the residue immediately preceding dzG (T20 of DS **4**, Fig. 3, lane 16). Hence, loss of the strong coordination site provided by the extrahelical purine N7 residues of the Watson strand appears to limit recognition with the target site at least as evident from the lack of copper-dependent strand scission of DNA. Both residues are therefore required for productive association between complex **1** and the helix–coil junction.

The N7 of G could alternatively contribute to the selective recognition by stabilizing a specific oligonucleotide conformation that might be unusually susceptible to oxidation. However, we previously showed that this explanation is unlikely since scission is independent of the nucleobase at the reactive position 20 on the Crick strand opposite from the 5′-G-purine, and certainly this site would also affect the conformation of the helix–coil junction.[14] Finally, inclusion of dzG has the added potential to divert

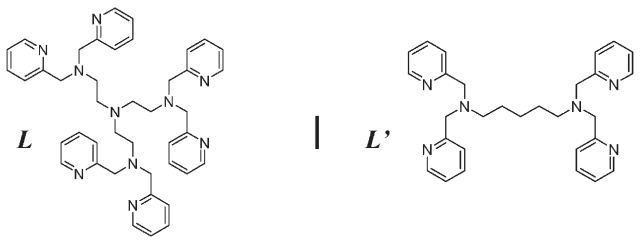

Fig. 1 Ligands $\boldsymbol{L}$ and $\boldsymbol{L}'$ used to form the trinuclear and dinuclear copper complexes **1** and **2**, respectively.

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† Electronic supplementary information (ESI) available: Synthesis and characterization of $\boldsymbol{L}'$ and its $Cu_2\boldsymbol{L}'$ derivative; protocols for preparation, oxidation and characterization of oligonucleotides; phosphoimagery of gel electrophoresis used to locate and quantify strand scission. See http://dx.doi.org/10.1039/b509690a

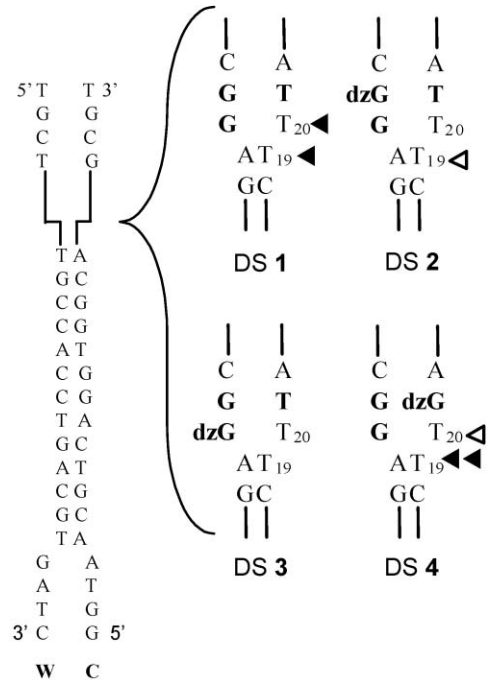

Fig. 2 Nucleotide sequences and secondary structures of DS **1–4** and indication of the Watson (W) and Crick (C) strands. Location of strand scission induced by complex **1** is indicated by (▲) for higher and (Δ) for lower efficiency of reaction.

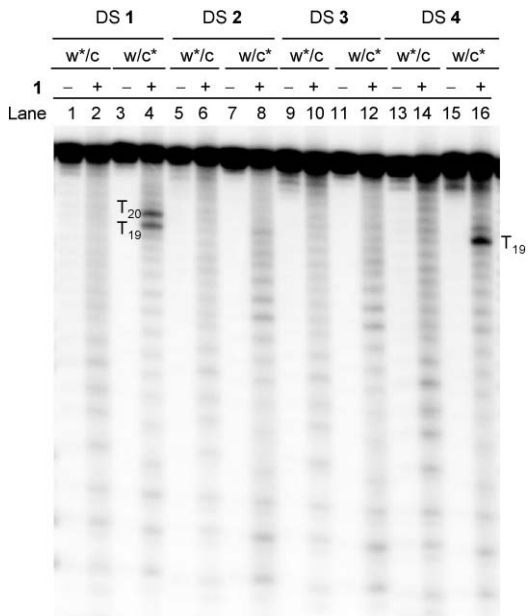

Fig. 3 Phosphoimage of a denaturing 20% polyacrylamide gel showing strand scission products of DS **1–4** (0.1 μM). 5′-Labeled Watson (W) or Crick (C) strand was annealed with the complementary strand, and incubated with complex **1** (1.5 μM) and MPA (10 mM) for 15 min in sodium phosphate (10 mM, pH 7) at ambient temperature.

oxidation from strand scission to base oxidation since this modified nucleobase is considerably more sensitive to oxidation than the already labile guanine.[4,21] No such alternative reaction was evident, and thus the results with dzG substitution are most consistent with a selective reaction that is dependent on coordination between complex **1** and the -G(A/G)- sequence extending 5′ from the helix on the Watson strand.

Strand scission was not promoted by complex **1** in the unpaired region of the Watson strand or helical region of either strand in DS **1–4** (for example, see Fig. 3, lanes 2 and 4). Similarly, oxidation had not been observed for a single oligonucleotide strand that does not fold into a stable secondary structure.[14] The helix–coil junction consequently seems to support a unique environment for the 5′-extension of a 5′-G(A/G) sequence to guide selective oxidation by complex **1**. We expect that this junction provides a favorable electrostatic potential and maintains the necessary proximity of participating groups without adding the conformational constraints or limited solvent accessibility common to duplex DNA (Fig. 4).

The sensitivity of reaction to such conformational constraints around the recognition site was examined by comparing reaction of DS **5** with DS **6** and SS **1** (Fig. 5 and S1†). First, the scission pattern of DS **5** confirmed the ability of a 5′-GA (as well as a 5′-GG extension, see DS **1**) to support reaction of complex **1**. C20 of the Crick strand was the primary site of scission in DS **5**, and the yield (15%) was comparable to those of DS **1** and DS **4**. The helix–coil junction remained the target of scission for DS **6**, but the yield (3%) diminished greatly (Fig. 5 and S2†). Continuation of a duplex structure after only one residue beyond the 5-GA recognition sequence was consequently sufficient to inhibit productive association between the unpaired DNA and complex **1**. Joining the single-stranded extensions to form the hairpin loop of SS **1** also suppressed reaction to a similar extent (Fig. 5 and S3†). Thus, neither a bulge nor hairpin site within duplex DNA has yet to mimic the reactivity of a helix–coil junction with the multi-nuclear complex.

The terminal base pair at the junction and proximal to the recognition site also had the potential to contribute to the observed selectivity since the structure and dynamics of base pairing and strand extension vary with each participating residue.[22,23] Previously, studies had been limited to terminal A–T pairs illustrated in both junctions of DS **1–6**. Interconversion of these termini to T–A did not inhibit strand scission by complex **1** at the reactive junction but rather distributed it over four residues in the Watson (A7 and T8) and Crick (C20 and T21) strands of DS **7** (Fig. 5 and S4†). In contrast, reaction of DS **5** was directed exclusively to its Crick strand and primarily to just C20 (Fig. 5). Subsequent removal of the A/T base pair resulted in a helix–coil junction with terminal G–C pairs (DS **8**). This enhanced and focused scission specifically to C18 of the Crick strand, a position equivalent to C20 in DS **5** and DS **7** (Fig. 5 and S5†). The influence of the terminal base was then similar to that of the unpaired 5′-G(A/G) extension. In both cases, reaction was directed to the oligonucleotide strand opposite to a purine-rich sequence. In addition, no scission was evident at the second and unreactive helix–coil junctions containing a 3′-extension, rather than

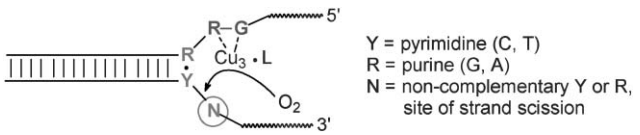

Fig. 4 Recognition elements and reaction site of complex **1**.

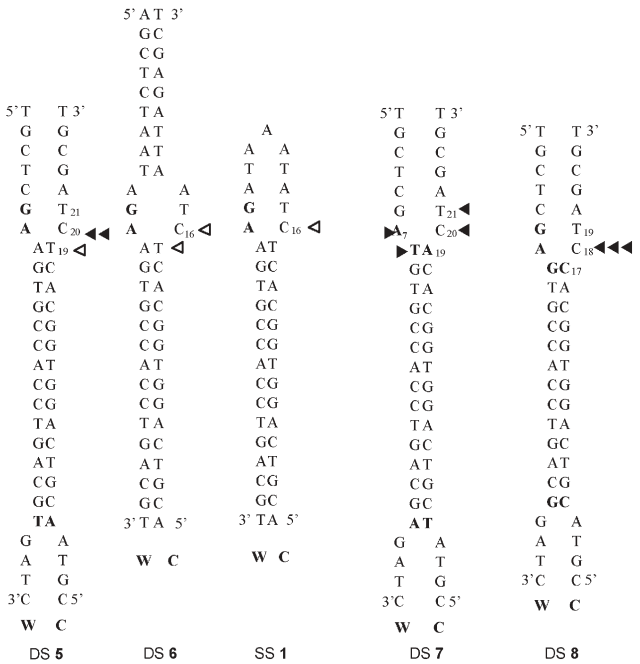

Fig. 5 Nucleotide sequences and secondary structures of DS **5–8** and SS **1**. The Watson and Crick strands are indicated by W and C, respectively. Location of strand scission induced by complex **1** is indicated by (▲) for higher and (Δ) for lower efficiency of reaction.

5′-extension, of 5′-GA regardless of the terminal base pair. Thus, strand polarity is also critical for productive interaction with complex **1**.

Our initial interest in the trinuclear copper complex was based on an assumption that one copper would be available for nucleobase recognition, and the other two coppers could activate molecular oxygen cooperatively.[12] However, DNA oxidation using a single copper is still possible as demonstrated by $[Cu(OP)_2]^{2+}$.[7] A binuclear analog, $[Cu_2^{II}(\boldsymbol{L}')(H_2O)_2](ClO_4)_4$ (complex **2**, $\boldsymbol{L}'$ = *N,N,N′,N′*-tetrakis(2-pyridylmethyl)-1,5-pentanediamine) (Fig. 1) was consequently investigated as a control for the activity of a binuclear analog of the trinuclear complex **1**. The efficiency of strand scission for DS **1** induced by complex **2** (12% scission over C18–G23) was a little lower than that for complex **1** (21% distributed over only T19–T20) (Fig. S6†). More significantly, reaction of complex **2** was dispersed over almost all of the 3′-unpaired residues of the Crick strand. This suggests that loss of one copper affected DNA recognition more than molecular oxygen activation. Perhaps the third copper in complex **1** is primarily involved in target recognition, but further investigations will be necessary to confirm and better define the function of each copper in promoting selective DNA recognition and strand scission.

All of the multi-nuclear copper complexes investigated to date express unique selectivities for particular nucleotides within a helix–coil junction.[13–16] At least for the trinuclear copper complex **1**, coordination to the first two unpaired purines extending from the 5′-strand of a duplex is critical for controlling strand scission on the 3′-strand extension. This activity can also be modulated by the terminal base-pair at the junction and the flexibility of the coil region.

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