CHEMCOMM Communication
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Highly specific oxidative damage of double-strand DNA by copper aminoglycosides†

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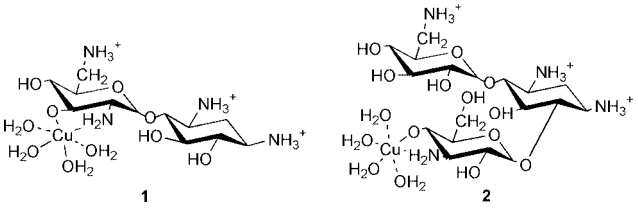
Received (in Cambridge, UK) 26th April 2001, Accepted 22nd June 2001
First published as an Advance Article on the web 23rd July 2001

Oxidative cleavage of double-strand DNA, mediated by either Cu^{2+}-neamine 1 or Cu^{2+}-kanamycin A 2, is shown to follow a highly specific C-4′ H mediated pathway and suggests a mechanism for efficient double-strand scission of duplex DNA.

Oxidative degradation of DNA has been demonstrated to follow a number of pathways, depending on the cleavage agent.[1] Proton abstraction from one of the C-1′, C-2′, C-3′, C-4′ or C-5′ ribose carbons is followed by a series of elimination reactions that afford a variety of small molecule products characteristic of the specific cleavage pathway. Fe–bleomycin mediated cleavage shows evidence of both a major C-4′H cleavage path and for C-1′H abstraction.[2,3] While copper phenanthroline and copper desferal had previously been assigned a major C-1′H cleavage path,[4–8] more recent work has indicated that C-4′H and C-5′H abstractions are the only routes that lead to backbone scission.[9] Enediynes, oxochromium reagents and manganese porphyrin derivatives have also been shown to mediate C-1′H, C-4′H, and C-5′H abstraction.[10,11] An example of C-2′H abstraction has been reported for 5-iodouracil.[12] With the exception of Barton's rhodium complexes, which mediate photocleavage *via* C-3′H abstraction,[13] most cleavage agents typically yield a mixture of products, indicative of relatively non-specific cleavage pathways.

Previously we have synthesized and characterized copper derivatives of aminoglycosides (**1** and **2**) and have demonstrated these compounds to be highly efficient catalysts for cleavage of both RNA and DNA under physiological conditions.[14,15] Here we characterize the mechanism for oxidative cleavage of DNA mediated by **1** or **2**, and demonstrate a highly specific C-4′H mediated pathway (Fig. 1). This observation provides an explanation for the efficient conversion of closed circular to linear plasmid DNA, following the double-strand cleavage path identified in our earlier work.[14]



1 **2**

The HPLC profile for Cu^{2+}–kan cleaved plasmid DNA (Fig. 2A) shows the release of cytosine (C), guanine (G), thymine (T), and adenine (A) following residual DNA and larger product fragments. An additional peak corresponding to 5-methylene furanone (5-MF) (Fig. 2) appears when the reaction mixture is heated at 85 °C for 20 min following incubation. C-1′H abstraction generates a C-1′ radical that is further oxidized to a carbocation and forms either a 2′-deoxyribonolactone[6] or a 1′,2′-dehydronucleotide intermediate after subsequent attack by H_2O.[8] Both intermediates are stable at pH 7.4 and 37 °C, the conditions of our cleavage reaction, and the HPLC elution profile corroborates heat treatment to be a prerequisite for the release of 5-MF (Fig. 2). Thus C-1′H abstraction leads to the formation of abasic sites with no apparent strand cleavage, confirming the recent observation by Sugiyama *et al.*[12]

C-4′H abstraction yields distinct reaction products, depending on the attacking species following formation of the radical intermediate. In the presence of dioxygen, base propenals (detected by the TBA assay, ESI†) and 3′-phosphoglycolate terminae are generated (Fig. 1; products C and D, respectively). When water is the attacking species the pathway again does not give rise to direct strand cleavage, but base release is observed. Under anaerobic conditions the TBA assay was negative,

† Electronic supplementary information (ESI) available: experimental section and complete HPLC trace for the truncated version shown in Fig. 2B. See http://www.rsc.org/suppdata/cc/b1/b103789g/

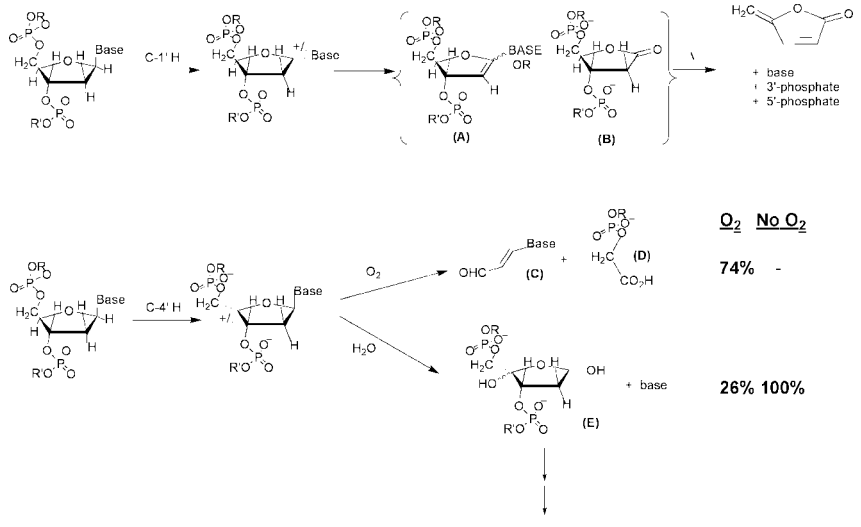


Fig. 1 Porposed radical mediated cleavage mechanisms for oxidative degradation of DNA. (A) 1′,2′-dehydronucleotide; (B) 2′-deoxyribonolactone; (C) base propenal; (D) 3′-phosphoglycolate; (E) hydroxyabasic site.

 DOI: 10.1039/b103789g

indicating the absence of a C-4′H (O_2) path. None of the expected products for C-2′H, C-3′H and C-5′H degradation paths were observed,[10–13] consistent with C-4′H abstraction paths as the only route leading to plasmid cleavage.

A C-1′H path was observed only in the case of supercoiled plasmid with peroxide as coreactant and the fraction of C-1′H abstraction was never found to be more than 12% of the C-4′H path, based on quantitation of the 5-MF released (ESI†). Reaction with ascorbate/O_2 appears to proceed *via* an intermediate copper species that does not mediate C-1′H abstraction. Neither linear plasmid, nor general polynucleotide duplex DNA (discussed below) show evidence for C-1′H loss, whereas C-4′H chemistry is observed with both substrates, and also using either H_2O_2 or ascorbate/O_2 as coreactants. This difference most likely reflects structural perturbations of the supercoiled strands that make C-1′H accessible for abstraction (Fig. 3 illustrates the close proximity of the C-1′ and C-4′ H's).

The reactivity of Cu^{2+}–kan A and Cu^{2+}–neamine toward two DNA duplexes, poly(dA)–poly(dT) and poly(dG)–poly(dC), was also examined in the presence of either hydrogen peroxide or ascorbate, under reaction conditions that were similar to those used for plasmid DNA. Base release was observed and quantitated by HPLC analysis (ESI†), and the occurrence of base propenals was confirmed with the thiobarbituric acid assay. Neither 5-MF (following heat treatment), nor products for other decay paths were observed, and so again the duplexes exhibit exclusive C-4′H abstraction chemistry in the presence of copper neamine or copper kanamycin in the presence of either H_2O_2 or ascorbate/O_2. C-4′H abstraction is normally associated with minor groove binding, and such a binding mode is consistent with our studies conducted in the presence of netropsin or Hoechst 33258 (minor groove binding drugs) which inhibit the cleavage of DNA duplexes (ESI†). Quantitation of the ratio of C-4′H (O_2) to C-4′H (H_2O) paths (ESI†) revealed a preference for the C-4′H (O_2) path, both for the polynucleotide duplexes (74 *vs.* 26%), and for a plasmid DNA (59 *vs.* 41%). These two pathways have previously been observed for $Cu(phen)_2^+$ cleavage of plasmid and duplex sequences,[6] although the factors that favor one path over another are not clear. Base release following peroxide mediated cleavage of either plasmid or duplex DNA by 1 or 2 also shows no obvious trend, other than a variable tendency for release of pyrimidine over purine.

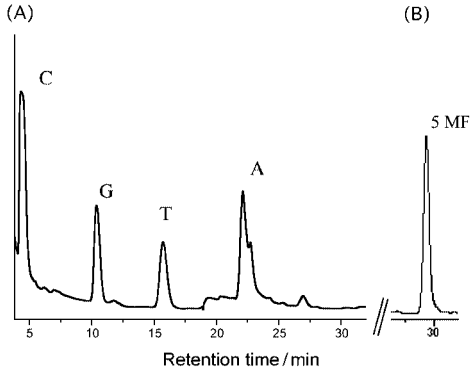


Fig. 2 HPLC profile for the reaction, 100 μg plasmid + 0.5 mM Cu–kan + 5 mM H_2O_2, incubated at 37 °C for 1 h (A) and 100 μg plasmid + 0.5 mM Cu–kan + 5 mM H_2O_2, incubated at 37 °C for 1 h followed by 20 min at 85 °C (B). Excess complex was used to promote rapid reaction, but multiturnover cleavage with sub-stoichiometric complex has been documented.[16]

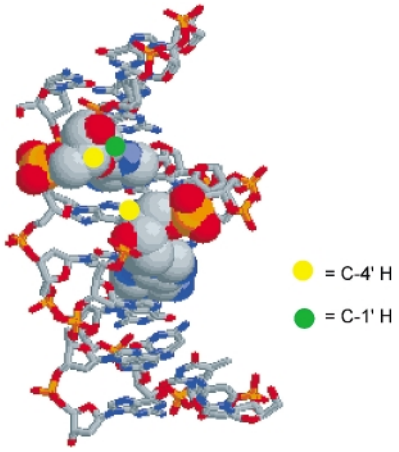


Fig. 3 The proximity of the ribose C-4′H's on opposing strands (*x*, *x* + 3 base positions) in the minor groove of B-conformer DNA suggests a pathway for double-strand scission of duplex DNA by formation of reactive oxygen intermediates in the vicinity of proximal ribose hydrogens. CPK coloring has been used and the two nucleotides on opposing strands are highlighted.

Under hydrolytic conditions,[16] in the absence of added H_2O_2 or ascorbate, treatment of DNA with either 0.5 mM $CuSO_4$ or 0.5 mM aminglycoside, under otherwise similar reaction conditions, produced neither free base, nor 5-MF, nor other products normally observed for the oxidative degradation of duplex or plasmid DNA. Reaction of $CuSO_4$ and a ten-fold excess of either H_2O_2 or ascorbate, again all other conditions being maintained, showed minimal (< 10%) base release, as compared to treatment with either **1** or **2**, but without the production of base propenals. The latter observation suggests an alternative path for production of nucleotide base. With increasing amounts of free Cu^{2+} (maintaining ten times excess of ascorbate or H_2O_2) the concentration of released bases increased. However, base release was found to be random with free Cu^{2+} and coreactant, and did not show any preference for either pyrimidines or purines, consistent with a distinct reaction path. No reaction was observed with H_2O_2 or ascorbate (with or without added neamine or kanamycin A) in the absence of Cu^{2+} (aq), **1** or **2**.

In contrast to other DNA cleavage agents that follow an oxidative path (cited earlier), copper aminoglycosides (**1** and **2**) follow a highly specific route mediated by abstraction of the C-4′H. Close proximity of C-4′H's on opposing strands separated by two additional base-paired nucleotides (Fig. 3) also provides a rational explanation for the observed linearization of plasmid (or double-strand cleavage of duplex DNA). A reactive copper moiety placed in the minor groove would be well placed to execute a double-strand scission with minor structural rearrangement between cleavage reactions. Taken with the unique enzyme-like character of these reagents,[16] these results demonstrate copper aminoglycosides to possess several valuable traits for a metallonuclease mimetic.

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