Efficient catalytic cleavage of DNA mediated by metalloaminoglycosides†

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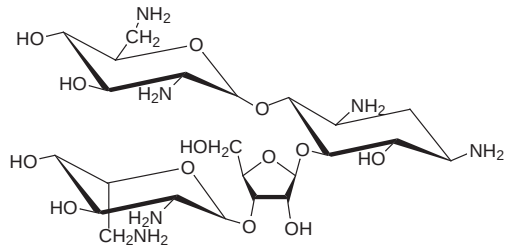
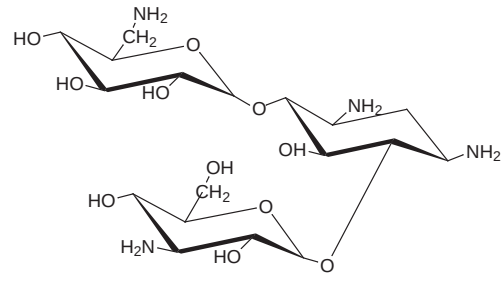
Metalloaminoglycosides have been synthesized and demonstrated to mediate catalytic cleavage of DNA at physiological pH and temperature.

Antitumor antibiotics such as neocarzinostatin,[1] bleomycin,[2] and elsamicin[3] create single or double strand nicks on duplex DNA. The latter are biologically more significant since they appear to be less readily repaired by natural DNA repair mechanisms.[4] Molecules that demonstrate DNA cleavage activity must possess either a hydrolytic, photoactivatable, or a redox active center, as well as recognition elements that target ds DNA.[5,6] We have explored the chemistry of Cu^{2+} derivatives of the aminoglycosides neomycin B (neo) and kanamycin A (kan) (Scheme 1),§ and demonstrate their ability to linearize DNA by non-random two-strand cleavage under catalytic conditions, and ultimately to effect complete degradation of plasmid DNA. Both hydrolytic and oxidative pathways have been identified.

A variety of first row transition metal ion complexes of kanamycin A were tested to assess their DNA cleavage efficiency; however, only a mixture of Cu^{2+} and kanamycin A was found capable of effecting rapid (< 1 h) degradation of plasmid DNA at 1 μM concentration. A systematic reactivity profile of the isolated and purified Cu(kan), **1**,¶ with plasmid DNA allowed observation of nicked DNA after brief (5 min) exposure to 0.5 μM complex (Fig. 1, lane 3). Both $Cu(kan)_2$, **2**, and Cu(neo), **3**, were also found capable of mediating DNA cleavage at catalytic concentrations as low as 100 nM; however, as a result of the hydrolytic instability of **2** and **3**, further DNA scission studies were focused on complex **1**.¶,‖



neomycin B



kanamycin A

Scheme 1

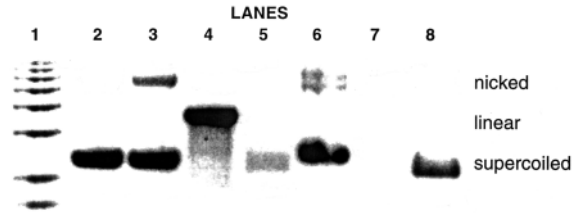


Fig. 1 Agarose gel electrophoresis of the reaction products following T4 ligase treatment of the nicked or linearized plasmid pT7-7†† obtained by reaction with Cu(kan) and BamH1, respectively. The reaction mixture (10 μl) containing plasmid DNA and metal–kanamycin complex was incubated for 1 h at 37 °C, mixed with 2 μl loading buffer (bromophenol blue, xylene cyanole and 50% glycerol) and loaded onto a 0.8% agarose gel containing ethidium bromide. Control experiments were run with plasmid DNA in the presence and absence of 5 μM kanamycin A. The gel was run at a constant voltage of 120 mV for 60–90 min in TAE buffer, washed in distilled water, visualized under a UV transilluminator and photographed on polaroid films. DNA concentrations are 50 μM: lane 1, 1 kb markers; lane 2, DNA + 0.5 μM Cu(kan), followed by treatment of reaction products with 2 units T4 ligase; lane 3, DNA + 0.5 μM Cu(kan); lane 4, DNA + 1 μl BamH1; lane 5, DNA + 0.5 μM Cu(kan) + 0.1 μM H_2O_2; lane 6, DNA + 1 μl BamH1, followed by treatment of reaction products with 2 units T4 ligase; lane 7, DNA + 0.5 μM Cu(kan) + 0.1 μM H_2O_2, followed by treatment of reaction products with 2 units T4 ligase; lane 8, DNA.

A hydrolytic degradative pathway is supported by several experimental criteria. In particular, neither standard inhibitors of reactive oxygen species (azide, superoxide dismutase, DMSO, EtOH), nor anaerobic conditions were found to inhibit DNA cleavage.[8] Further, it was found that plasmid DNA nicked by Cu(kan) (Fig. 1, lane 3) can be circularized by T4 DNA ligase (Fig. 1, lane 2),[9] while the resulting circularized plasmid demonstrates significantly higher transformation efficiencies than the nicked form.** The re-ligation and transformation efficiency indicate that DNA cleavage by Cu(kan) in the absence of external reducing agents is indeed hydrolytic.

A distinct oxidative pathway has also been identified. Addition of a reducing agent such as ascorbic acid or dithiothreitol (DTT) was found to enhance the DNA cleaving ability of **1**, and nicking was observed at nanomolar concentrations (Fig. 2). Interestingly, complete conversion of supercoiled

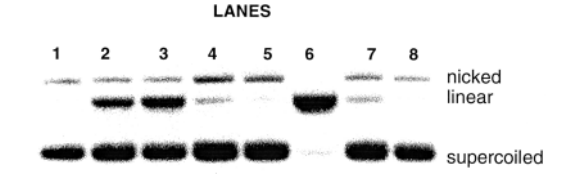


Fig. 2 Agarose gel electrophoresis of the oxidative cleavage reaction of plasmid pT7-7 by Cu(kan) **1** in the presence of ascorbate. Reactions were carried out for 1 h as follows: lane 1, 51 μM DNA; lane 2, + 1 nM **1**/10 μM asc; lane 3, + 1 nM **1**/10 μM asc; lane 4, + 1 nM **1**/1 μM asc; lane 5, + 1 nM **1**/0.1 μM asc; lane 6, + 5 nM **1**/10 μM asc; lane 7, + 5 nM **1**/1 μM asc; lane 8, + 5 nM **1**/0.1 μM asc. For lane 2, Cu^{2+}(kan) and ascorbate were mixed and preincubated at 37 °C for 30 min, to form Cu^{+}(kan), before addition of substrate DNA. The results were similar to those obtained by simultaneous mixing (lane 3). Experimental conditions for gel eletrophoresis are described in the legend to Fig. 1.

DNA (51 μM base pair) to a linear form was observed with a catalytic concentration of **1** (5 nM) in the presence of 10 μM ascorbate (Fig. 2, lane 6), while Cu^{2+}(aq) alone or with added ascorbate did not result in DNA cleavage under similar reaction conditions. This indicates non-random scission of the complementary strand in close proximity to the initial nick site, leading to production of linear DNA, since random cutting should produce a smear of fragment sizes. Complexes **1** to **3** degrade plasmid DNA completely in the presence of 10 μM H_2O_2. Fig. 1 (lane 5) shows extensive degradation of the plasmid over the time of this reaction, relative to control DNA shown in lane 8, with the range of smaller product fragments essentially undetectable. Furthermore, DNA treated with Cu(kan) and H_2O_2 is cleaved into random small and undetectable fragments that could not be ligated using T4 ligase (Fig. 1, lane 7).

In the presence of oxygen donor ligands, copper has been shown to produce a variety of oxidatively active intermediates, including a non-diffusable copper-oxene species,[10] and diffusable hydroxy radicals through Fenton-type chemistry.[11] The presence of DMSO and EtOH, which are hydroxy radical quenchers, do not have inhibitory effects on oxidative DNA cleavage by **1**. However, superoxide dismutase (SOD) and NaN_3 exhibit considerable inhibition of the oxidative DNA cleavage mediated by **1**, indicating that activated oxygen species (such as O_2^- or $^1O_2$) are most likely involved in plasmid cleavage. Also, superoxide generated by ascorbate-reduced copper can dismute to H_2O_2, which then reacts with another equivalent of cuprous ion through a Fenton mechanism to produce hydroxy radical like species, which might be metal bound. These species are analogous to metal-oxo species and are responsible for initiating the oxidative DNA strand scission chemistry. Both metal-oxo and metal-hydroxo species are expected to cleave the DNA backbone, but are not expected to be quenched by hydroxy radical quenchers,[12,13] as was observed in the case of **1**.

To address the issue of hydroxy radical involvement, production of this species was quantified using rhodamine B as a reporter molecule in the presence of **1** and ascorbic acid.[11,12] Substantial quantities of hydroxy radical were generated by Cu(kan) in the presence of ascorbate. However, in the presence of substrate plasmid no hydroxy radicals were trapped by rhodamine B. Furthermore, neither DMSO nor EtOH were able to quench the oxidative DNA scission reactions, indicating that any hydroxy radicals generated are not diffusible. A requirement of dioxygen for the oxidative scission reaction is substantiated by the fact that no hydroxy radical (determined by the rhodamine B test) and no cleavage was observed under anaerobic conditions. A mechanistic model that is consistent with these observations has a diffusible Cu^I-generated superoxide species interacting with another Cu^I center with production of the reactive metal-oxo or metal-hydroxo species.

In summary, we report the first example of catalytic cleavage and linearization of double-strand DNA by a synthetic complex at physiological pH and temperature. Both hydrolytic and oxidative pathways have been identified. Although other workers have described oxidative DNA cleavage by copper complexes,[8a,11,14–19] these are non-catalytic, requiring a large excess of reagent (from 0.1 to 1 mM copper complex with 25 nM to 10 μM DNA) and often extended reaction times of several hours to days. The cleavage efficiency and ease of synthesis of metalloaminoglycosides make them attractive molecules for therapeutic applications.

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Notes and References

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§ Aminoglycosides carry a large positive charge at physiological pH (from +3 to +6) and bind with high affinity to anions and nucleic acids *via* electrostatic and hydrogen bonding contacts.[7] Interestingly, the antitumor molecules described earlier[1–3] also contain aminated sugars in their framework.
¶ Complex **1** was synthesized by addition of $CuSO_4$ (0.0624 g, 0.25 mmol) to kanamycin A sulfate (0.1455 g, 0.25 mmol), in 5 ml water. After stirring at room temperature for 24 h, ethanol (5 ml) was added to precipitate a blue solid. This was filtered, washed twice by stirring in EtOH for 6 h, dissolved in water, and EtOH precipitated to yield **1**. Complexes **2** and **3** were formed similarly, using 2 equiv. of kan to form **2**. All products have been purified and give satisfactory elemental analysis, EPR, and UV–VIS data.
‖ Also excess metal-free aminoglycoside was found to inhibit cleavage, supporting a requirement for direct DNA binding to effect cleavage. At extreme concentrations, neomycin B alone has also been shown to mediate hydrolysis of a ribodinucleotide substrate (0.1 mM adenylyl-3,5-adenosine, ApA, and 0.3 M neomycin B).[20]
** Ligase treated Cu(kan) nicked plasmid demonstrated more than two-fold higher transformation efficiences than the nicked product itself.
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