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Artificial Metallonuclease. Cu^{II}-Promoted DNA Strand Scission as Effected by Bisresorcinol Derivatives Having a Metal-Ion Binding Site

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Bisresorcinol derivatives having an imidazolyl or an aminoalkyl group as a metal-ion binding site promotes an oxidative strand-scission of supercoiled ϕX-174 plasmid DNA in water at pH 7.4 in the presence of Cu^{II}. The essential role of the intramolecular bisresorcinol moiety is to reduce amine-coordinated Cu^{II} ion. The resulting Cu^{I} then reacts with O_2 to give reactive oxygen species capable of oxidative DNA degradation.

Metal-complex promoted strand scission of double helical DNA is a subject of much recent concern.[1] The redox-mediated oxidative degradation of the sugar moieties requires a redox-active metal complex such as $Cu^{II}(Phen)_2$[2] and $Fe^{III}(EDTA)$[3] and a reducing cofactor such as thiol; this combination leads to generation of oxy-radical species as a result of reductive activation of O_2.[4] In the present work, we have studied the Cu^{II}-promoted cleavage of plasmid DNA as effected by bisresorcinol derivatives **1a** and **1b** having a metal-ion binding site. We report here that the bisresorcinol moiety serves as an intramolecular *reductant*,[5] so that the **1**-Cu^{II} system induces oxidative DNA degradation without an external reductant.

Supercoiled ϕX 174 RF I plasmid DNA (form I, 94 µg/ml or 0.3 mM per base) in 10 mM Tris buffer (pH 7.4) underwent nicking to give a mixture of open-circular form II and linear form III derivatives (Scheme 1) upon incubation for 2 h at 30 °C with imidazolyl-bisresorcinol compound **1a**[5a] (0.1mM) in the presence of $CuCl_2$ (0.1mM), as revealed by gel electrophoresis (Fig. 1, lane 4). Such a nicking reaction did not take place in the absence of **1a** (lane 1) or $CuCl_2$ (lane 2).[6] Compound **1a** could be replaced by an aminoalkyl-substituted compound **1b** (lane 13)[7] without loss of activity but never by a phenyl-bisresorcinol derivative **2** (lane 3), 2-isopropylimidazole (**3**) (lane 5), or n-propylamine (lane 12). Furthermore, nicking was not detected in the co-presence of references **2** and **3** (lane 6). Careful removal of O_2 from the reaction mixture was also effective in inhibiting the reaction. These results indicate that the bisresorcinol moiety, an *intramolecular* imidazolyl or aminoalkyl group, Cu^{II}, and O_2 are essential for the present DNA-cleavage reaction.

The requirement of O_2, coupled with the failure of the nicked form II product to undergo ligation by T4 DNA ligase (Scheme 1), suggests that the present DNA-cleavage reaction, as in many other related systems, proceeds via a radical mechanism involving reactive oxygen species, which could be generated by reaction of O_2 with a reduced metal center (Cu^{I}). This was further supported by the inhibitory effects of catalase (60

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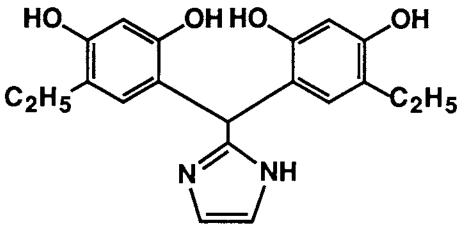
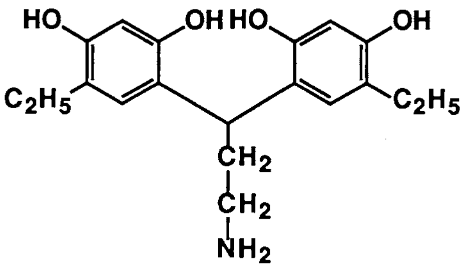
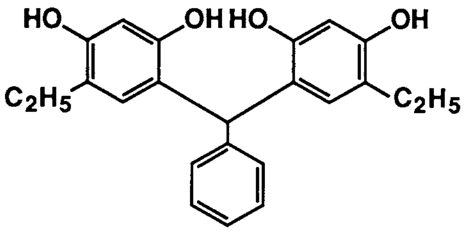


1a



1b



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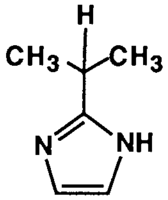

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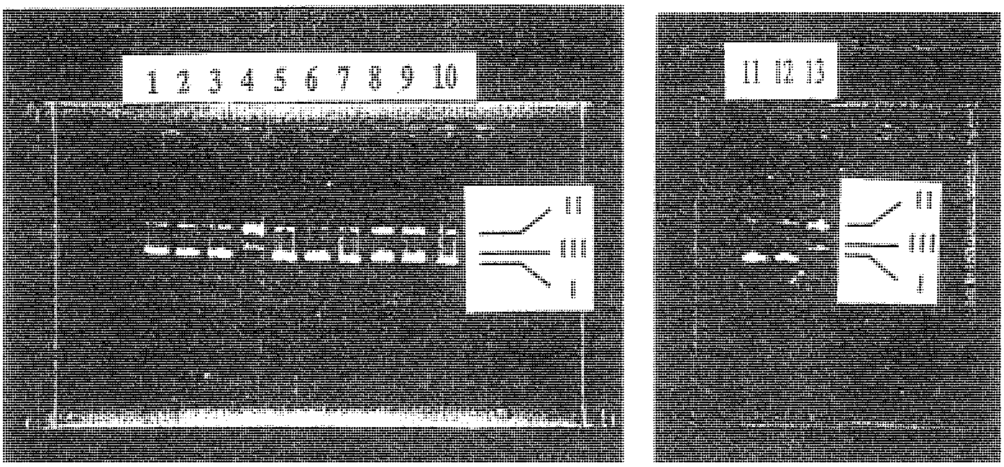
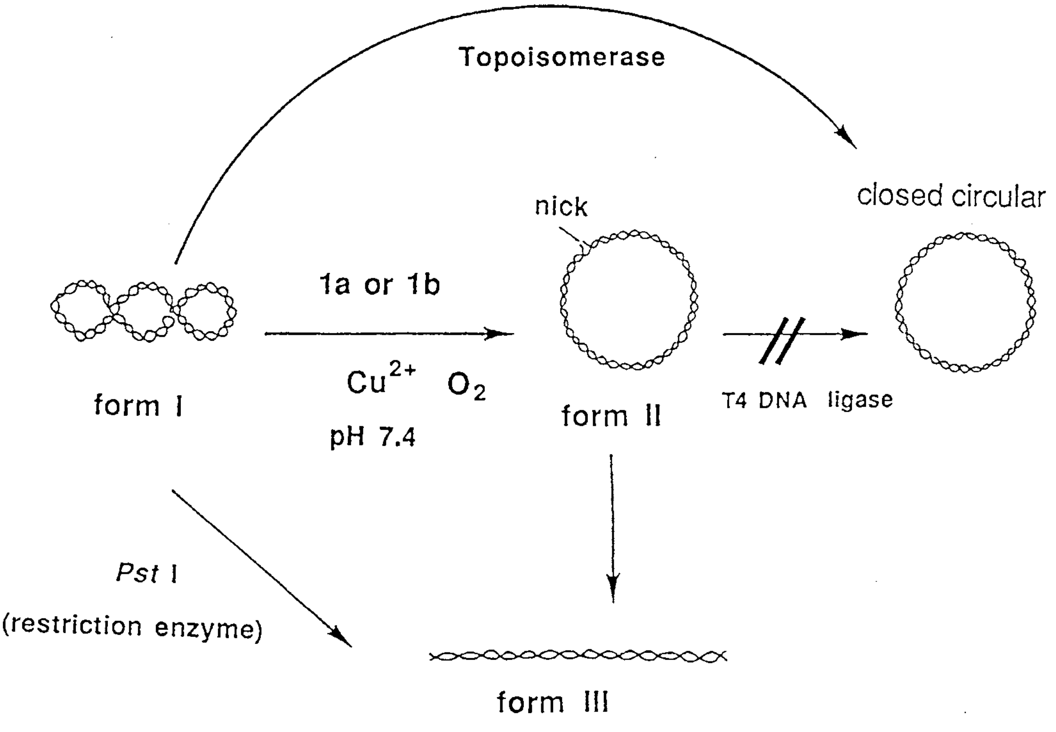


Fig. 1. The agarose gel electrophoreses for the reaction mixtures of ϕx 174 RF I plasmid DNA (94 μg/ml) in 10 mM Tris buffer (pH 7.4) at 30 °C in the presence of : lane 1, $CuCl_2$ (0.1 mM) for 2 h; lane 2, **1a** (0.1 mM) for 2 h; lane 3, **2** (0.1 mM) + $CuCl_2$ (0.1 mM) for 2 h; lane 4, **1a** (0.1 mM) + $CuCl_2$ (0.1 mM) for 2 h; lane 5, **3** (0.1 mM) + $CuCl_2$ (0.1 mM) for 2 h; lane 6, **2** (0.1 mM) + **3** (0.1 mM) + $CuCl_2$ (0.1 mM) for 2 h; lane 7, **1a** (0.1 mM) + $CuCl_2$ (0.1 mM) + catalase (60 units/μl) for 1 day; lane 8, **1a** (0.1 mM) + $CuCl_2$ (0.1 mM) + SOD (178 units/μl) for 1 day, lane 9, **1a** (0.1 mM) + $CuCl_2$ (0.1 mM) + DMSO (0.1 mM) for 1 day; lanes 10 and 11, ϕx 174 RF I plasmid DNA (94 μg/ml); lane 12, n-propylamine (0.1 mM) + $CuCl_2$ (0.1 mM) for 10 h; lane 13, **1b** (0.1 mM) + $CuCl_2$ (0.1 mM) for 10 h.

units/µl, lane 7), superoxide dismutase (SOD,178 units/µl, lane 8), or DMSO (0.10 mM, lane 9). These are well known scavengers of H_2O_2, $O_2^{\bullet -}$, and •OH , respectively.8) Reductive activation of O_2 is well documented, but what is the *reductant* in the present system?

The following observations are relevant to elucidate the mechanism. (1) Addition of $CuCl_2$ to a degassed solution of compound **1a** results in an instantaneous buildup of an absorption band at 365 nm; this is characteristic of a Cu^{II}-coordinated imidazolyl group.9) (2) DNA-cleavage reaction does not take place at pH 4.9, where the imidazolyl nitrogen is protonated to prohibit Cu^{II}-coordination. (3) The d-d transition band for $CuCl_2$ (0.62 mM) at 740 nm undergoes gradual bleaching upon addition of compound **1a** (7.2 mM). Introduction of O_2 to the resulting solution restores the intensity of this band. (4) The bisresorcinol moiety of **1a** in water (pH 7.4) at 60 °C undergoes $CuCl_2$-promoted oxidation to give a difficult-to-separate mixture of products. Items 1 and 2 indicate that coordination of Cu^{II} to the imidazolyl group is essential for the present reaction. Item 3 suggests that ligand **1a** reduces coordinated Cu^{II} to Cu^{I}.10) Item 4 shows that the reduction of Cu^{II} and the oxidation of the bisresorcinol moiety in **1a** are coupled. The precoordination of Cu^{II} to the imidazolyl group is essential for this coupling, since the corresponding oxidation of the phenyl-substituted reference **2**, if any, is very slow in the presence of $CuCl_2$.



Scheme 1.

To summarize, compound **1** has an imidazolyl or aminoalkyl group as a Cu^{II}-binding site as well as a bisresorcinol moiety which serves as a potential intramolecular reductant. It can autoreduce or autoactivate Cu^{II} for the generation of reactive oxygen species resposible for DNA-cleavage. Metal-ion mediated biomimetic reductive activation of O_2 can usually be achieved only in the presence of a large excess amount of an *external* reductant. Further work is now under way to shed more light on the structure and mechanism of the present systems.11)

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5) The bisresorcinol moiety in compound **1a** and analogous systems has previously been shown to serve as an intramolecular general acid in protic media. a) For the **1a**-catalyzed hydrolysis of ester, see: T. Motomura, K. Inoue, K. Kobayashi, and Y. Aoyama, *Tetrahedron Lett.*, **32**, 4757 (1991); b) For the two-point fixation of phosphate with a pyridyl derivative analogous to **1a**, see: T. Motomura and Y. Aoyama, *J. Org. Chem.*, **56**, 7224 (1991).

6) The form II spot seen in lanes 1-3, 5, and 6 is due to form II impurity present in the starting DNA (lane 10).

7) Compound **1b** obtained as hydrochloride salt: R_f 0.0 (silicagel/methanol); mp 203 °C (dec); ^{1}H NMR (DMSO-d_6) δ 8.89 and 8.90 (each s, each 2 H, OH), 7.89 (s, 3 H, NH_3^+), 6.32 and 6.73 (each s, each 2 H, aromatic-H), 4.31 (t, 1 H, $C\mathit{H}CH_2CH_2NH_3^+$), 2.59 (m, 2 H, $C\mathit{H}_2NH_3^+$), 2.35 (m, 4 H, $C\mathit{H}_2CH_3$), 2.12 (m, 2 H, $C\mathit{H}_2CH_2NH_3^+$), 1.02 (t, 6 H, CH_3). Anal. Found: C, 60.66; H, 7.21; N, 4.07%. Calcd for $C_{19}H_{25}O_4N{\bullet}HCl$: C, 62.03; H, 7.12; N, 3.81%.

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10) Preliminary ESR study indicates that $CuCl_2$ is rendered ESR-silent at room temperature in the presence of compound **1a**.

11) The stoichiometry of the **1a**-Cu^{II} or **1b**-Cu^{II} complexation could not be determined by a colorimetric continuous-variation analysis (Job plot) because of concurrent redox reactions between these (cf. items 1, 3, and 4). ESR characterization of the complexes is now in progress in colaboration with Dr. Nishida.

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