Relaxation of pBR322 Form I DNA by Copper(II) Complex and Hydrogen Peroxide

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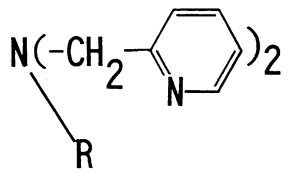
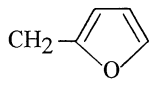
Relaxation of pBR322 form I DNA by copper(II) complex and hydrogen peroxide systems was found to be highly dependent on the chelate structure of copper(II) complex used. This is suggesting that an active species for nicking DNA may be a copper(II)-hydroperoxide adduct.

Chemical methods for nicking DNA have several uses that include structural variations in nucleic acids,[1,2] identifying binding sites of DNA ligands,[1,2] designing artificial nuclease and restriction enzyme,[3-5] and serving as chemotherapeutic agents, such as bleomycin.[6] The use of copper(II) compounds to promote such DNA cleavage has also been subject of recent research.[7,8] In this study we have investigated relaxation of supercoiled plasmid DNA by the mononuclear copper(II) complex and hydrogen peroxide systems, and found that the nicking of the DNA is highly dependent on the chelate structure of the complex used. These seem to be quite important to determine an intrinsic active species for DNA damage, because free copper(II) ion cannot exist in the human plasma.

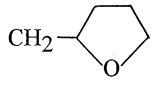
The copper(II) complexes used in this study are of a general formula, $Cu(L)Cl^+$, where (L) denotes the tetradentate ligands illustrated below, and these are newly prepared in this work,[9] except for the (tpa) complex((tpa)=tris(2-pyridylmethyl)amine).



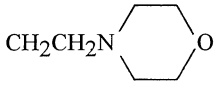
R=CH_2COOH H(dpa)



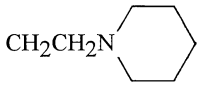
(fpy)



(tfpy)



(mopy)



(pipy)

The crystal structures of all the new complexes used in this study have been determined in our laboratory; ORTEP drawing of $[Cu(mopy)Cl]^+$,[10] and $[Cu(pipy)Cl]^+$,[11] are illustrated in Figure 1. The geometrical features around the copper(II) ion of the complexes used in this study are quite similar to each other; the copper(II) ion is of a square pyramidal structure, and in the planar coordination sites, a copper(II) ion is surrounded by two pyridine and one amine nitrogen atoms, and one chloride ion.

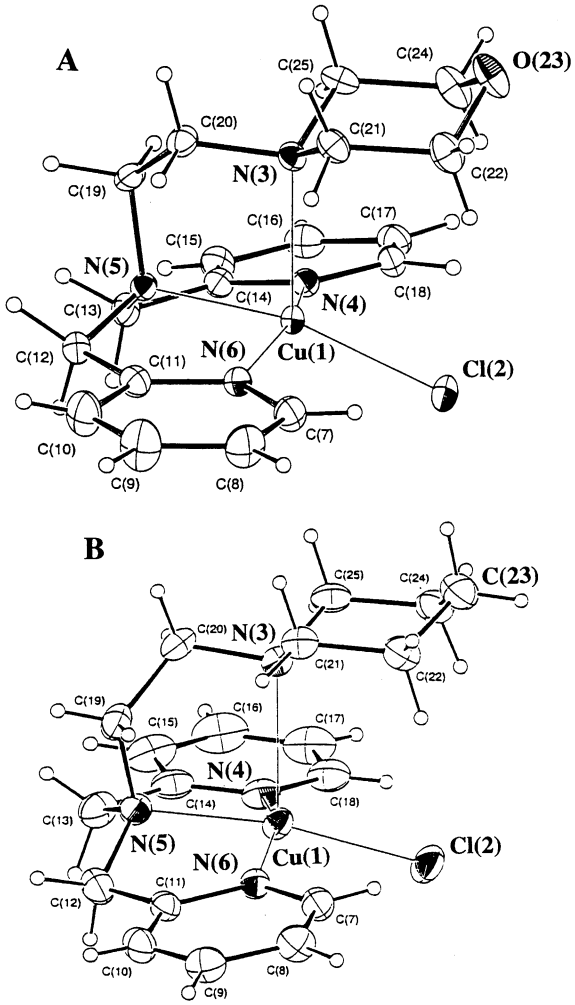


Figure 1. ORTEP drawing of copper(II) compounds.
A. $Cu(mopy)Cl^+$: Cu(1)-N(3), 2.408(5); Cu(1)-N(4), 2.002(5); Cu(1)-N(5), 2.062(5); Cu(1)-N(6), 1.998(5), Cu(1)-Cl(2), 2.243(2) Å.
B. $Cu(pipy)Cl^+$: Cu(1)-N(3), 2.430(5); Cu(1)-N(4), 2.002(5); Cu(1)-N(5), 2.056(8); Cu(1)-N(6), 1.998(5); Cu(1)-Cl(2), 2.241(2) Å.

The patterns of the DNA degradation by several copper(II) complexes in the presence of hydrogen peroxide are illustrated in Figure 2.[12] Under the present experimental conditions, free copper(II) ion does not give the effect on the relaxation of the DNA(see lanes 2 and 3), which is similar to that observed for the

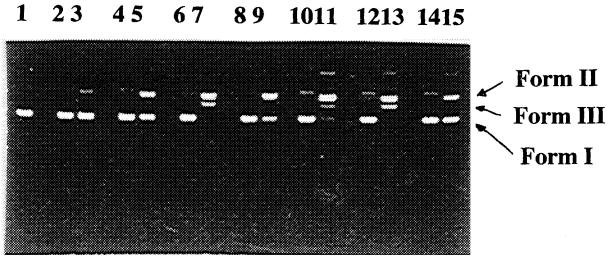
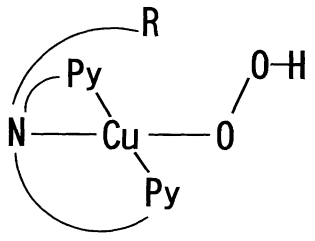


Figure 2. Relaxation of pBR322 form I DNA by copper(II) complex in the presence of hydrogen peroxide. Lane 1, DNA alone; lane 2, $CuCl_2$; lane 3, $CuCl_2$ and H_2O_2; lane 4, Cu(dpa)Cl; lane 5, Cu(dpa)Cl and H_2O_2; lane 6, Cu(tpa)Cl^+; lane 7, Cu(tpa)Cl^+ and H_2O_2; lane 8, Cu(tfpy)Cl^+; lane 9, Cu(tfpy)Cl^+ and H_2O_2; lane 10, Cu(fpy)Cl^+; lane 11, Cu(fpy)Cl^+ and H_2O_2 ; lane 12, Cu(mopy)Cl^+; lane 13, Cu(mopy)Cl^+ and H_2O_2 ; lane 14, Cu(pipy)Cl^+; lane 15, Cu(pipy)Cl^+ and H_2O_2.

(pipy)(lane 15 in Figure 2). In contrast to this, some complexes, such as (mopy)(lane 13), (fpy)(lane 11) and (tpa)(lane 7), can effect the conversion of form I(supercoiled) DNA to form II(relaxed circular) and form III(linear duplex).[13] These are indicating that the relaxation of the form I DNA is highly dependent on the complexes used, but not on the charge of a complex, and the structural features of the chelate give a remarkable effect on the degradation of DNA.

It is well known that metal complexes bind to DNA through both covalent and noncovalent modes.[2] In the present case, a copper(II) complex with cationic charge may bind to the sugar-phosphate backbone electrostatically. Based on the fact that ESR spectral features(77 K) of the solutions containing the copper(II) complex and hydrogen peroxide are quite similar to those of the mononuclear copper(II) complexes with tetragonal symmetry, it seems quite likely that the addition of hydrogen peroxide gives a copper(II)-hydroperoxide adduct(see **Scheme-1**), through the coordination of hydroperoxide anion at the site of chloride ion.



Scheme 1.

Several authors have pointed out the importance of hydroxyl radical in the DNA damage,[6,8] however, it is hard to imagine that free hydroxyl radicals generated in cytoplasma actually react with DNA, since their diffusion distance is very short. The present results, 1) free copper(II) ion cannot effect the degradation of DNA in the presence of hydrogen peroxide under our experimental conditions, 2) relaxation of DNA is highly dependent on the chelate structure of the copper(II) complex used, and 3) a hydroperoxide adduct of the metal complex has recently been shown to exhibit high electrophilic nature,[14-16] may lead to a proposal that a copper(II)-hydroperoxide formed near the DNA may attack the sugar site, to nick the DNA. The origin for the higher activity of the (mopy) than that of the (pipy) complex is now under progress in our laboratory.

References and Notes

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9 The ligands used in this study were obtained according to the published methods. Y. Nishida, T. Okuno, S. Ito, A. Harada, H. Matsushima, and T. Tokii, *Chem. Lett.*, **1995**, 885.
10 Crystal data of Cu(mopy)$ClClO_4$: monoclinic, $P2_1/n$, a=9.282(3), b=11.592(4), c=19.731(3) Å, β=90.76(2)°, V=2122.8(9) $Å^3$, Z=4, λ(MoKα)=0.71073 Å, μ=1.320 mm^{-1} Dx=1.598 Mgm^{-3}, R=0.0684 for 4105 observde reflections.
11 Crystal data of Cu(pipy)$ClPF_6$: monoclinic, $P2_1/c$, a=11.855 (3), b=15.258(2), c=13.186(3) Å, β=105.14(2)°, V=2302.2(7) $Å^3$, Z=4, μ=1.20 mm^{-1}, Dc=1.60 Mgm^{-3}, R=0.062 for 3434 observed reflections.
12 DNA(supercoiled pBR322 and λ-form) were purchased from Wako Chemicals. In a typical run, a copper(II) complex(4 μl of 0.1 mM solution), DNA(4 μl of 0.1μg/μl solution), Tris buffer(3 μl of 0.1 M solution), and hydrogen peroxide(4 ml of 10 mM solution) were mixed and kept to stand for an hour at 25 C°. The extent of DNA cleavage was assessed by analysis on 0.9% agarose gel containing Ethidium bromide.[13] The bands were photographed with Polaroid 667 film.
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