Enantiospecific Cleavage of DNA using Copper(II) Chelated to the Periphery of a Ligand on a Chiral Tris-chelate of Ruthenium(II)

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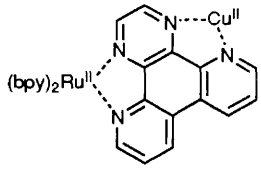
The Λ isomer of $[Ru(bpy)_2ppz^{2+}]$, but not the Δ, promotes cleavage of plasmid DNA in the presence of copper(II), 3-mercaptopropionic acid and hydrogen peroxide.

A number of transition metal complexes have been utilized[1–7] in probing nucleic acid structures. Several have been shown to combine the ability to bind to selective regions within the structure of polynucleotides, as determined by the shape of the complex and complementarity to the surface of the polymer as well as specific binding interactions, and affect cleavage at these sites *via* redox or photoredox mechanisms. For example, $[\Lambda\text{–}Co(dip)_3^{3+}]$ (dip = 4,7-diphenyl-1,10-phenanthroline) has been shown[1,2] to photocleave DNA in regions of Z-DNA structure. The racemic $[Rh(dip)_3^{3+}]$ has been shown[1,3] to target and photocleave cruciforms in supercoiled pBR322 DNA. The complex species $[Cu(phen)_2^{+}]$, usually generated *in situ* from the copper(II) complex (Cu/OP system), has been demonstrated[1,4,5] to cleave double-stranded DNA from a binding site within the minor groove with high efficiency in an almost sequence neutral fashion. Such activity has been utilized as a footprinting reagent in defining binding sites of proteins to DNA. We report here extensive cleavage of DNA in a Cu/OP related system, in which copper(II) is bound to a chelation site at the periphery of a ligand[6,7] (ppz) whose primary coordination is to ruthenium(II) as one ligand of a tris-chelate. This cleavage is almost exclusively limited to the Λ isomer of this complex and is therefore enantioselective.

We have reported[6,7] the enantioselective binding of tris-chelates of ruthenium(II) to DNA for complexes in which one of the ligands was multifunctional (*i.e.* possessed another chelation site, or monodentate binding site, or could be quaternized to provide positive charge at the periphery of the ligand). The complex $[Ru(bpy)_2ppz^{2+}]$ **1** (bpy = 2,2′-bipyridine; ppz = 4′,7′-phenanthrolino-5′,6′ : 5,6-pyrazine) was chromatographed on a DNA-hydroxylapatite column to provide samples of the Δ and Λ isomers which were enantiomerically pure.

Binding of copper(II) to $[Ru(bpy)_2ppz^{2+}]$ in aqueous solution is indicated by a shift of the visible MLCT absorption band associated with the ppz ligand from 475 nm in the complex to ~540 nm in the presence of copper(II). This shift is observable even at 1:1 molar ratio of Cu^{II} to complex. Investigation of the complex by Job's method indicates that the 1:1 complex forms exclusively. This may be due to the coulombic repulsion which would destabilize a 2:1 complex with two $[Ru(bpy)_2ppz^{2+}]$ units coordinated to one Cu^{II}.



We incubated pBR322 plasmid DNA from *E. coli* with the Δ and Λ isomers of $[Ru(bpy)_2ppz^{2+}]$(10 μmol dm^{-3}) and Cu^{II} (100 μmol dm^{-3}) at 37 °C for varying times in a system, which also included hydrogen peroxide and 3-mercaptopropionic acid. Fig. 1 shows the 1% agarose gel electrophoresis patterns for each enantiomer, and control with Cu^{II} but without Ru^{II} complex. Both the control and Δ isomer show residual activity with nicked (Type II) plasmid evident even after 5 min. The Λ isomer, however, demonstrates significant degradation of the DNA after 5 min, and by 30 min, no DNA is evident on the gel (lanes appear blank). The $[\Lambda\text{–}Ru(bpy)_2ppz^{2+}]$ is clearly much more efficient in cleaving DNA.

In order to assess the effectiveness of complexed ligand chelation of Cu^{II} on DNA cleavage efficiency, we followed cleavage in the same system with 10 μmol dm^{-3} Cu^{II} and varied the concentration of $[\Lambda\text{–}Ru(bpy)_2ppz^{2+}]$ to produce a [Cu]/[Ru] ratio of 10 [1 μmol dm^{-3} Ru^{II} complex] or 1 [10 μmol dm^{-3} Ru^{II} complex]. In Fig. 2 we can see that at this Cu^{II} concentration the absence of complex results in negligable cleavage, whereas, 1 μmol dm^{-3} complex produces Type II as well as Type III cleaved plasmid. When the complex concentration is raised to 10 μmol dm^{-3}, the DNA is almost completely degraded.

As our previous report[7] indicated, the enantioselective binding of $[Ru(bpy)_2ppz^{2+}]$ to DNA is assumed to be analogous to the binding of $[Ru(phen)_3^{2+}]$ as elucidated[1] by

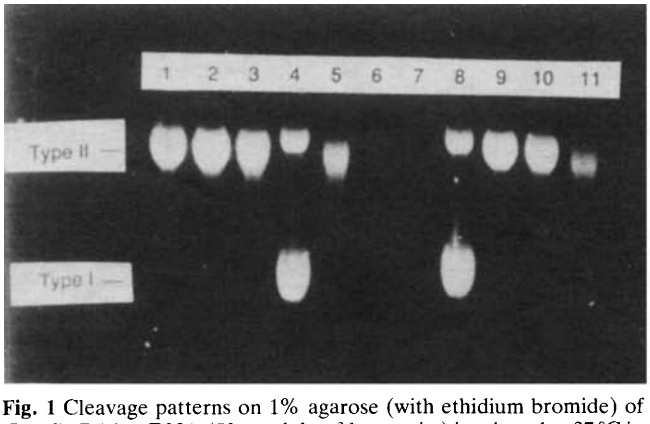


Fig. 1 Cleavage patterns on 1% agarose (with ethidium bromide) of *E. coli* pBR322 DNA (50 μmol dm^{-3} base pairs) incubated at 37 °C in pH 7.8 Tris-acetate buffer with 150 μmol dm^{-3} H_2O_2, 3 mmol dm^{-3} 3-mercaptopropionic acid, 100 μmol dm^{-3} copper(II). Lanes 4 and 8 are plasmid DNA as received. Lanes 1–3: no ruthenium(II) complex. Lanes 5–7: Λ-isomer, 10 μmol dm^{-3}. *Note*: lanes 6 and 7 appear empty. Lanes 9–11: Δ-isomer, 10 μmol dm^{-3}. Each series consists of 5, 15 and 45 min incubation times.

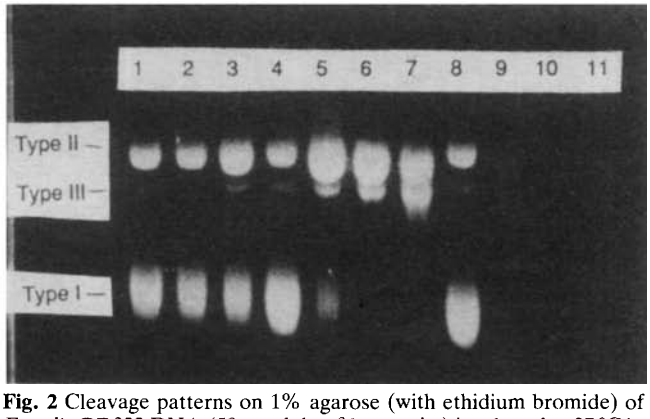


Fig. 2 Cleavage patterns on 1% agarose (with ethidium bromide) of *E. coli* pBR322 DNA (50 μmol dm^{-3} base pairs) incubated at 37 °C in pH 7.8 Tris-acetate buffer with 150 μmol dm^{-3} H_2O_2, 3 mmol dm^{-3} 3-mercaptopropionic acid, 10 μmol dm^{-3} copper(II). Lanes 4 and 8 are plasmid DNA as received. Lanes 1–3: no ruthenium(II) complex. Lanes 5–7: Λ-isomer, 1 μmol dm^{-3}, [Cu]/[Ru] = 10. Lanes 9–11: Λ-isomer, 10 μmol dm^{-3}, [Cu]/[Ru] = 1. Note: lanes 10 and 11 appear empty. Each series consists of 5, 15 and 45 min incubation times.

Barton *et al.* The cleavage observed here, by the Λ isomer only, is consistent with an interpretation in which this isomer binds preferentially within the minor groove of DNA and effects cleavage in a manner similar to the Cu/OP system. The major groove binding Δ isomer is ineffective in inducing cleavage, either because the major groove site does not allow access of the redox active species to the deoxyribose ring, or because the redox active species is not generated at this site as efficiently. Such enantioselective cleavage provides additional potential of coupling site-specific binding to the cleavage of polunucleotides.

Received, 7th April 1992; Com. 2/01850K

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