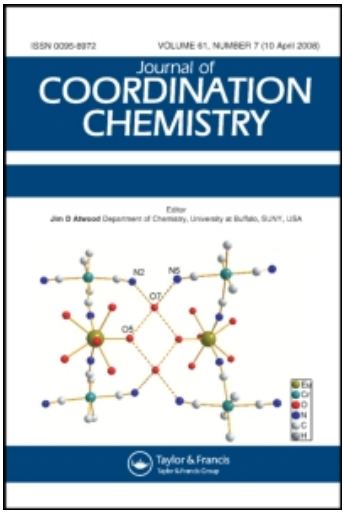


Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

CHARACTERIZATION OF COPPER(I) AND COPPER(II) COMPLEXES OF A BIS-DIIMINE COORDINATED TO RUTHENIUM(II) AND THEIR INTERACTION WITH CALF THYMUS DNA

Thomas C. Strekas[a]; A. David Baker[a]; Oma Harripersad-morgan[a]; Robert J. Morgan[b]
[a] Department of Chemistry and Biochemistry, Queens College of the City University of New York, Flushing, NY, USA [b] Department of Chemistry, Long Island University, Brooklyn, NY, USA

To cite this Article Strekas, Thomas C. , Baker, A. David , Harripersad-morgan, Oma and Morgan, Robert J.(1995) 'CHARACTERIZATION OF COPPER(I) AND COPPER(II) COMPLEXES OF A BIS-DIIMINE COORDINATED TO RUTHENIUM(II) AND THEIR INTERACTION WITH CALF THYMUS DNA', Journal of Coordination Chemistry, 34: 1, 77 — 85
To link to this Article: DOI: 10.1080/00958979508024305
URL: http://dx.doi.org/10.1080/00958979508024305

J. Coord. Chem., 1995, Vol 34, pp. 77–85
Reprints available directly from the publisher
Photocopying permitted by license only

CHARACTERIZATION OF COPPER(I) AND COPPER(II) COMPLEXES OF A BIS-DIIMINE COORDINATED TO RUTHENIUM(II) AND THEIR INTERACTION WITH CALF THYMUS DNA

THOMAS C. STREKAS,* A. DAVID BAKER,
OMA HARRIPERSAD-MORGAN

Department of Chemistry and Biochemistry, Queens College of the City University of New York, Flushing, NY 11367, USA

and ROBERT J. MORGAN

Department of Chemistry, Long Island University, Brooklyn, NY 11201, USA

(Received June 28, 1994; in final form September 14, 1994)

The DNA cleavage system which includes copper(II), sodium ascorbate and the complex ion $Ru(bpy)_2ppz(+2)$ (bpy = 2,2'-bipyridine; ppz = 4'7'-phenanthrolino-5',6':5,6-pyrazine), where coordinated ppz binds copper ion in the +1 and +2 oxidation states is investigated. Visible and electron paramagnetic resonance spectra are used to characterize the species with Cu(II) bound to coordinated ppz. The binding constant for Cu(II) in a 1:1 complex is determined spectroscopically as 1.7×10^4. Spectroscopic changes associated with binding of Cu(II), and reduction to Cu(I) are reported. Evidence that the Cu(I) species binds to a hydrophobic region of calf thymus DNA is presented. The cleavage of *E. coli* pBR322 DNA by the individual enantiomers is reassessed with ascorbate as reducing agent.

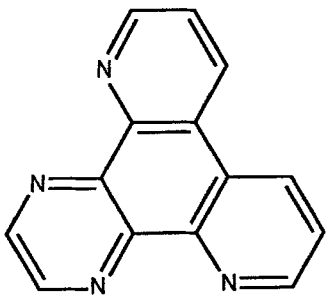
KEYWORDS: ruthenium tris-diimine, DNA cleavage, copper phenanthroline, 4',7'-phenanthrolino-5',6':5,6-pyrazine (ppz)

INTRODUCTION

The bis-bidentate ligand ppz (4',7'-phenanthrolino-5',6':6,-pyrazine(**1**)) is capable of binding metal ions at one or both of its diimine sites. We have shown[1–4] that the complex ion, $Ru(bpy)_2ppz(+2)$ (bpy = 2,2'-bipyridine) binds enantioselectively with DNA *via* a mode in which the ppz ligand partially intercalates and the degree of binding of the enantiomers is further influenced by the arrangement of the ancilliary bpy ligands.

We have reported[1] the enantiospecific cleavage of plasmid DNA utilizing purified enantiomers[2–4] of the complex $Ru(bpy)_2ppz(+2)$ as the "ligand" (i.e. chelator of added copper(II)), in a system which includes copper(II), peroxide and a thiol. This

* Author for correspondence.



1 ppz

is modelled after the copper/1, 10-orthophenanthroline (OP/Cu) system of Sigman[5,6] *et al.*, which is used extensively as a footprinting reagent for double-stranded DNA investigations. In this case, the ppz ligand provides a chelating site for the copper(II), in addition to inducing enantioselective binding[4] to the double-stranded DNA helix *via* an intercalative interaction within the major groove. We found that the Λ-Ru(bpy)$_2$ppz(+2) was much more active in cleaving DNA, possibly because the Δ isomer was more tightly bound to DNA within the major groove.

Previous reports involving the OP/Cu system[5–11] have indicated that access to the cleavage site is gained *via* the minor groove, and that a DNA bound copper(I) OP complex is involved in the mechanism of DNA cleavage. Evidence that the tetrahedral Cu(phen)$_2$+ complex and Cu(I) complexes of substituted phenanthrolines bind to DNA *via* hydrophobic and/or intercalative interactions has been presented.[5–11] Also, the use of ascorbate, rather than a thiol, as the reducing agent has been shown[8] to increase the cleavage efficiency of the system. Thiols were demonstrated to bind to copper(I) and inhibit the cleavage reaction.

Here we report the visible spectroscopic characterization of the copper(I) and copper(II) complexes with Ru(bpy)$_2$ppz(+2), including the effects of interaction with DNA in aqueous solution. We also report the 77K EPR spectrum of the Cu(II) species.

EXPERIMENTAL

Ru(bpy)$_2$ppz(+2) was prepared[3] as the chloride or hexaflurophosphate salt and resolved[1] using a hydroxylapatite/DNA column, as previously described.

Visible spectra were obtained with a Hewlett-Packard 8452 Diode Array Spectrometer. Fluorescence spectra were obtained with a Perkin-Elmer MPF-66 Spectrofluorimeter interfaced to a PE 7500 computer. Electron paramagnetic resonance spectra were obtained with an IBM-Brucker ER200-SCR EPR spectrometer.

Cleavage studies were carried out as previously described[1], using *E. coli* pBR 322 plasmid DNA purchased from Gibco-BRL Laboratories. Calf Thymus DNA was purchased from Sigma Chemical Co. and pre-treated as described previously.[4]

RESULTS

Characterization of Copper(II) and Copper(I) Complexes

The binding of Cu(II) to $Ru(bpy)_2ppz(+2)$ is studied by visible absorbance as shown in Figure 1. The observed color change from yellow to purple is due to the decrease in the 475 nm peak as the 530 nm peak increases. The 475 nm band is assigned as the Ru to ppz MLCT (metal to ligand charge transfer) band, and similar spectroscopic observations accompany protonation of the complexed ppz, dimer formation,[3] and binding of other metal ions. Construction of a Job's plot indicates that the complex has a 1:1 (Cu:Ru) stoichiometry, that is, one copper(II) ion binds per $Ru(bpy)_2ppz(+2)$ unit. Presumably the new species has the formula $Ru(bpy)_2ppzCu(+4)$, with perhaps four water molecules completing the copper coordination sphere for the expected tetragonally (Jahn-Teller) distorted, six coordinate copper(II). Steric hindrance, as well as a net charge of + 6 which would apply for two $Ru(bpy)_2ppz(+2)$ units binding to a single copper(II), apparently inhibit formation of the 1:2 complex. Also, the appearance of a clean isosbestic point at 498 nm supports an equilibrium involving only the two species. Analysis of the spectra in Figure 1 yields a formation constant, K_f, for the 1:1 complex of 1.7×10^4.

The 9.48 GHz EPR spectrum of the 1:1 complex at 77K is shown in Figure 2. The Cu(II) exhibits a rhombic signal with $g_{\|} = 2.28$ and $g_{\perp} = 2.07$. The ^{63}Cu hyperfine splitting is resolved in the parallel signal and $A_{\|} = 160$ Gauss. Such a signal is rather typical of a tetragonally distorted bis- or tris- chelate of Cu(II).[12]

The conversion to the Cu(I) complex is shown in Figure 3. At a Cu to Ru ratio of 9:1, sodium ascorbate was added to an N_2 purged solution of $Ru(bpy)_2ppz(+2)$ to give an ascorbate concentration of 0.13 millimolar. Conversion to the Cu(I) complex is evidenced by the shift of the MLCT peak from 530 nm to 510 nm.

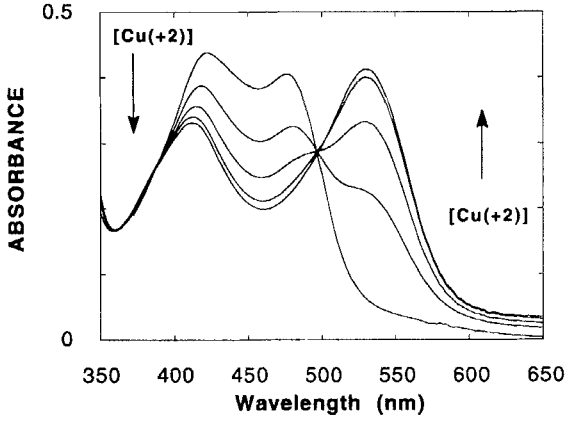


Figure 1 Absorption spectra in aqueous solution of $Ru(bpy)_2ppz$ (+2), 40 micromoles/L, with increasing concentrations of copper(II) sulfate: 68, 200, 640 and 1400 micromoles/L.

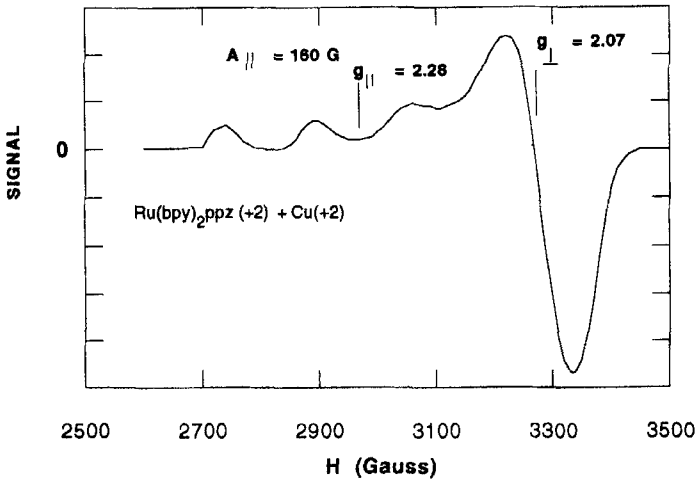


Figure 2 Electron spin resonance spectrum of $Ru(bpy)_2ppz(+2)$, 4.5 millimoles/L with copper(II) sulfate, 1.0 millimoles/L. Conditions: frozen aqueous solution at 77K; 9.48 GHz, 12.5 G modulation width, 2×10^4 gain.

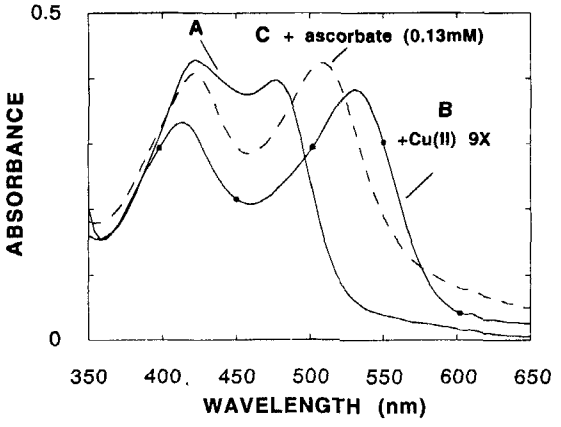


Figure 3 Absorption spectra in aqueous solution of: (A) $Ru(bpy)_2ppz(+2)$, 39 micromoles/L (—); (B) A + 344 micromoles/L copper(II) sulfate (—•—•—); (C) B + 0.13 millimoles/L sodium ascorbate after purging with nitrogen gas (- - -).

Effects of Added DNA

We studied the effect of added calf thymus DNA on the spectral properties of the isomer which is most effective in cleaving DNA in the Sigman type system. Addition of Cu(II) to Λ-$Ru(bpy)_2ppz(+2)$ in the presence of calf thymus DNA showed an unusual effect. In Figure 4, prior to Cu(II) addition, the presence of DNA at a [P]/[Ru] ratio of 25:1, results in the usual[4] hypochromic effect for the MLCT band at 475 nm, which shifts to about 490 nm as well. When Cu(II) is added at a Cu/Ru

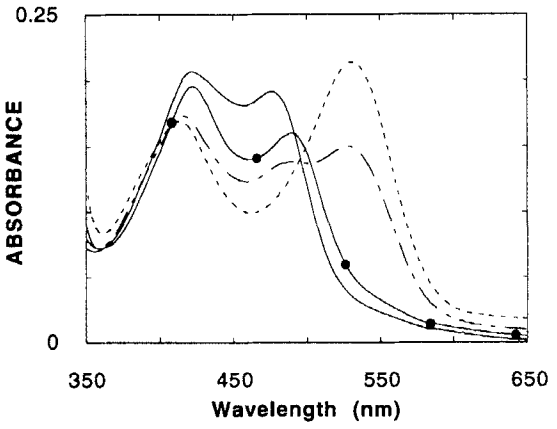


Figure 4 Absorption spectra in a aqueous solution: (A) Λ-Ru(bpy)$_2$ppz(+2), 19 micromoles/L(——); (B) A + calf thymus DNA, [P]/{Ru] = 22 × (—•—); (C) A + copper(II) sulfate, [Cu(II)]/[Ru] = 8 × (— — —); (D) B + copper(II) sulfate [Cu(II)]/[Ru] = 8 × (- - -).

ratio of 8.1, the conversion to the copper(II) complex is apparently complete in the presence of DNA, but obviously incomplete, as expected (see Figure 1), when no DNA is present. Figure 5 shows the spectra, with and without DNA, at the higher Cu/Ru ratio of 25X, where conversion to coordinated Cu(II) is essentially complete even in the absence of DNA. The only difference is a slightly higher absorbance in the presence of DNA. There is no observable wavelength shift. Thus there is no evidence for intercalation of the Λ-Ru(bpy)$_2$ppz(+2) when coordinated with Cu(II). The apparent improvement in binding of Cu(II) may be due to increased local concentrations of the two cations on, or near, the anionic DNA surface.

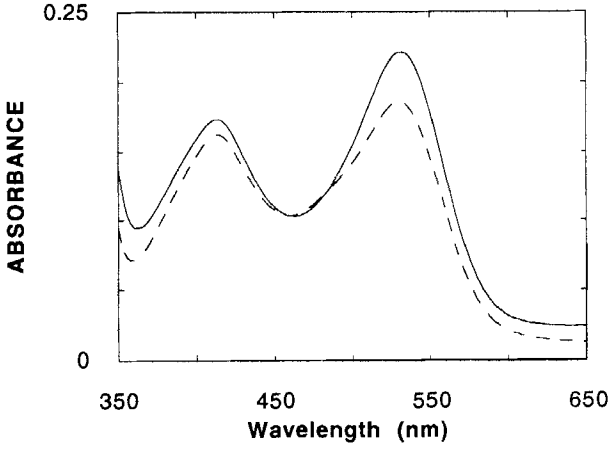


Figure 5 Absorption spectra in aqueous solution of: (A) Λ-Ru(bpy)$_2$ppz(+2), 19 micromoles/L + copper(II) sulfate, [Cu(II)]/[Ru] = 25 × (- - -); (B) A + calf thymus DNA, [P]/{Ru] = 21 × (——).

Evidence for interaction of the Cu(I) species with calf thymus DNA is presented in Figure 6. At a high Cu/Ru ratio (25:1) each solution was degassed with N_2, and made 0.13 millimolar in sodium ascorbate. In the solution with DNA, at a [P]/[Ru] ratio of 25:1, the peak is observed at 523 nm compared to 510 nm in the absence of DNA. This shift to lower energy of the ppz associated MLCT band is characteristic of binding of such complexed ligands to DNA.[4,8,9,13,14]

Cleavage Studies

We reinvestigated the stereospecificity of the cleavage of plasmid DNA when enantiomers of $Ru(bpy)_2ppz(+2)$ are substituted for 1,10-orthophenanthroline in the OP/Cu footprinting system. The results reported herein were obtained by substituting sodium ascorbate for thiol, and with molecular oxygen (i.e. in air) as the oxidizing agent. No hydrogen peroxide was added. Figure 7 shows the results for pBR 322 cleavage. Lanes 1–3 and 4–6 show results for the Λ- and Δ-isomers, respectively. The nearly complete digestion by the Λ-isomer in two minutes contrasts with the clear Type II and Type III bands still evident for the Δ-isomer. Cleavage by the Δ-isomer compares favorably with cleavage by the OP/Cu at a 1:1 ratio (lanes 8–10), which has been reported[7] to be considerably less effective than the 2:1 ratio usually employed. The Λ-isomer is considerably more effective in cleaving DNA.

DISCUSSION

The complexation of copper(II) to the remote chelating diimine site on coordinated ppz in the complex ion $Ru(bpy)_2ppz(+2)$ is shown by the purple color and ppz associated MLCT (from ruthenium (II)) peak at 530 nm (shifted from 475 nm).

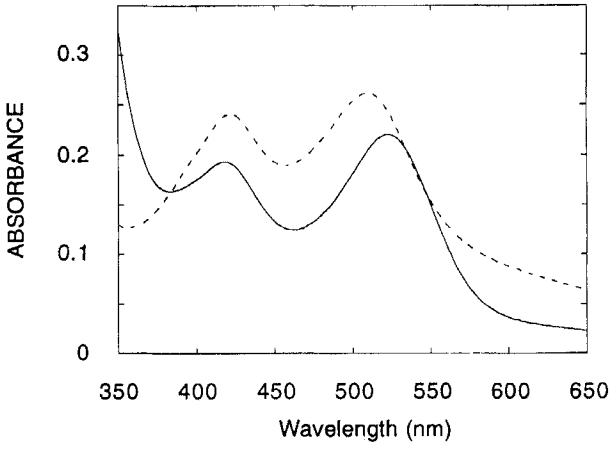


Figure 6 Absorption spectra in aqueous solution of: (A) Λ-$Ru(bpy)_2$ ppz(+2), 19 micromoles/L + copper(II) sulfate, [Cu(II)]/[Ru] = 25 × , + sodium ascorbate, 0.13 millimoles/L (- - -); (B) A + calf thymus DNA, [P]/{Ru] = 22 × (——).

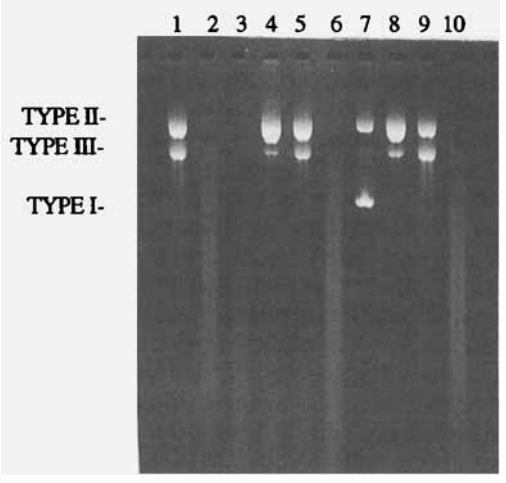


Figure 7 1% agarose gels, ethidium bromide stained and uv illuminated, of pBR 322 plasmid DNA, 50 micromoles/L base pairs; 5.0 micromoles/L copper(II) sulfate; and 5.0 millimoles/L sodium ascorbate. Lanes 1–3, 136 micromoles/L Λ-Ru(bpy)$_2$ppz(+2), quenched at 1, 2, and 5 minutes with EDTA. Lanes 4–6, 132 micromoles/L Δ-Ru(bpy)$_2$ppz(+2), quenched at 1, 2, and 5 minutes with EDTA. Lane 7, control plasmid DNA. Lanes 8–10, 1,10-orthophenanthroline, 10 micromoles/L, quenched at 1, 2, and 5 minutes with EDTA. Note: Type 1, unnicked DNA is evident only in lane 7 control (band nearest bottom). Lanes which may appear empty (e.g. 2 and 3) are extensively digested.

Note that the bpy associated MLCT peak near 422 nm shifts to about 410 nm upon copper(II) chelation. The shift of the MLCT peak at 475 nm to lower energy is characteristic of binding a charged species at the remote diimine site on the coordinated ppz ligand. It is notable that copper(II), in the presence of ppz alone, shows no visible absorption bands which could be attributed to MLCT transitions. As observed with 1,10-phenanthroline, only the *d-d* transitions which give rise to the blue color associated with copper(II) are observed in the visible region. By absorption spectroscopy, K_f for the 1:1 complex between copper(II) and the ruthenium(II) complexed ppz is approximately 1.7×10^4. This is much smaller than K_f for the 1:1 complex of copper(I) with 1,10-orthophenanthroline 2.0×10^{10}.[8] Binding copper(II) to another +2 ion probably results in a much smaller K_f in this case. Also, Cu(I) is expected to bind more strongly than Cu(II). The EPR spectrum of the 1:1 complex at 77K indicates a tetragonally distorted coordination with *g*-values and copper hyperfine coupling typical of diimine complexes of copper(II).

Conversion to a copper(I) complex upon reduction by sodium ascorbate is evidenced by a shift of the ppz associated MLCT band from 530 nm to 510 nm. Again, only the Ru(II) to ppz associated MLCT band is affected and in a way which clearly indicates that a change has occurred at the remote chelation site, *i.e* the oxidation state of the chelated copper has been changed. However, when ascorbate is added to N_2 degassed aqueous solutions of ppz ligand with copper(II), a visible absorption is observed in the 480 to 520 nm region. The absorption maximum shifts to shorter wavelengths as the ratio of ppz to Cu(II) increases from 1:2 to 4:1. The interpretation is complex because a single ppz ligand can bind up to two

copper(I) ions, giving species such as Cu(ppz)Cu (+2), and polymeric species, in addition to the expected monomeric species such as Cu(ppz)(+1) and $Cu(ppz)_2(+1)$. Nevertheless, the wavelengths observed are typical of copper(I) complexes with diimine ligands, and are reasonably assigned as MLCT transitions involving Cu(I) and ppz. In the spectrum of $Ru(bpy)_2ppz(+2)$ with copper(II) and ascorbate, there may be unresolved charge transfer transitions of this type in the same wavelength region as the Ru(II) to ppz CT transition centered at 510 nm.

The Cu(II) complex shows little or no evidence of a specific interaction with DNA. The apparent increase in K_f in the presence of DNA may be due to association of the cations present, including $Ru(bpy)_2ppz(+2)$ on the polyanionic DNA surface, but no wavelength shifts are observed. Only a modest increase in absorbance for the 530 nm band is observed when DNA is present.

For the Cu(I) species, however, the MLCT band shift from 510 to 523 nm in the presence of DNA provides evidence that the complex binds to DNA in such a way that the MLCT transition is affected by interaction of the ppz with the DNA. The corresponding DNA-induced shift for the MLCT transition in $Ru(bpy)_2ppz(+2)$ alone is from 475 to 490 nm. This lends support to the interpretation that the maximum at 510 is indeed not associated with a MLCT transition involving Cu(I), but rather Ru(II). Corresponding DNA-induced shifts in MLCT transitions involving Cu(I) and a diimine ligand are usually 4 to 6 nm, rather than the 13 nm seen here. Intercalation of the coordinated ppz ligand, with Cu(I) coordinated, seems less likely because of the anticipated steric interference of an additional two water molecules bound to the tetrahedral Cu(I) center (hence lying in a plane perpendicular to the plane of the ppz). Intercalation of apparently sterically-inhibited ligand systems has been proposed[13] as a possible interpretation of such spectroscopic observations previously. Whether the binding here is intercalative or not, the observed spectral perturbations indicate a strong non-covalent interaction between the Cu(I) species and DNA.

With respect to the DNA cleavage results, the efficiency of this system is improved by the use of ascorbate in place of thiols. Previously[1], addition of exogenous peroxide was required to observe significant cleavage. In the presence of ascorbate, no added peroxide is required. As previously reported, we find that the Λ-$Ru(bpy)_2ppz(+2)$ is significantly more active in cleaving DNA than is the Δ isomer, with activity in the ascorbate system significantly greater than that of the OP/Cu system at 1:1 ratio. Spectroscopic evidence that the complex with Cu(I) chelated binds to DNA indicates that such a species is present within the DNA structure and may participate in cleavage events in a fashion similar to $Cu(phen)_2^+$.

References

1. A.D. Baker, R.J. Morgan and T.C. Strekas, *J. Chem. Soc., Chem. Commun.* 1099 (1992).
2. A.D. Baker, R.J. Morgan and T.C. Strekas, *J. Am. Chem. Soc.* **113**, 1411 (1991).
3. Y. Fuchs, S. Lofters, T. Dieter W. Shi, R. Morgan, T.C. Strekas, H.D. Gafney, A.D. Baker, *J. Am. Chem. Soc.* **109**, 2691 (1987).
4. S.A. Tysoe, R.J. Morgan, A.D. Baker, T.C. Strekas, *J. Phys. Chem.* **97**, 1707 (1993).
5. D.S. Sigman and C.B. Chen, *Annu. Rev. Biochem.* **59**, 207 (1990).
6. D.S. Sigman, *Biochemistry* **29**, 9097 (1990).
7. J.M. Veal, K. Merchant, R.L. Rill, *Nucleic Acids Research* **19**, 3383 (1991).
8. J.M. Veal and R.L. Rill, *Biochemistry* **30**, 1132 (1991).
9. F. Liu, K.A. Meadows, D.R. McMillan, *J. Am. Chem. Soc.* **115**, 6699 (1993).

10. R.M. Everly, R. Ziessel, J. Suffert and D.R. McMillan, *Inorg. Chem.* **30**, 559 (1991).
11. R. Tamilarasan and D.R. McMillan, *Inorg. Chem.* **29**, 2789 (1991).
12. H.C. Allen, Jr., G.F. Kokoszka and R.G. Inskeep, *J. Am. Chem. Soc.* **86**, 1023 (1964).
13. A.M. Pyle and J.K. Barton, *Progress in Inorganic Chemistry: Bioinorganic Chemistry,* **Vol. 38**, (S.J. Lippard, Ed., John Wiley & Sons, New York, 1990) p. 143.
14. E.C. Long and J.K. Barton, *Acc. Chem. Res.* **23,** 273 (1990).