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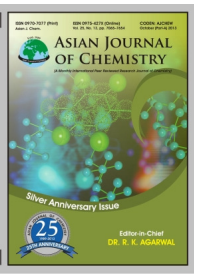

DNA Binding and Oxidative Cleavage Studies of Mixed-ligand Copper(II) Complex

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A mixed-ligand copper(II) complex ((4'-ferrocenyl-2,2':6',2"-terpyridine-k^3,N',N") (1,1'-phenanthroline-k^{2}N,N')-copper(II) *bis*(perchlorate)) has been synthesized. Binding interactions of the complex with calf thymus DNA (CT-DNA) were investigated by UV-visible and CD spectroscopies. Results show that the copper complex mainly adopt electrostatic attraction binding mode with DNA and the binding constant is $(3.22 \pm 0.05) \times 10^3$ mol^{-1} L. The complex shows appreciable chemical nuclease activity in the presence of ascorbic acid. The DNA cleavage by the complex were inhibited strongly in the presence of DMSO and *tert*-butyl alcohol, which suggests that hydroxyl radicals are reactive oxygen species for the cleavage.

Key Words: Mixed-ligand copper(II) complex, DNA binding, Nuclease activity.

INTRODUCTION

The DNA cleavage can occur by hydrolytic or oxidative way. Hydrolytic cleavage directly breaks the phosphodiester bond but does not result in sugar damage[1,2]. The oxidative cleavage involves either oxidation of the deoxyribose moiety by abstraction of sugar hydrogen or oxidation of nucleobases[3]. Transition metal complexes stand out as candidates for artificial nucleases due to their diverse structural features and reactivities[4-6]. The copper complexes have been extensively used as chemical nucleases because they possess biologically accessible redox potential and relatively high nucleobase affinity[7,8]. Since $[Cu(OP)_2]^{2+}$ has been reported in breaking DNA chain in the presence of H_2O_2 (OP = 1,10-phenanthroline)[3,9,10], many mononuclear[11-21] and multi-nuclear copper(II) complexes[22-25] with single ligands have been widely synthesized and some of them did show efficient oxidative cleavage activities. Recently, many mononuclear copper(II) complexes with mixed ligands 1,10-phenanthroline and L-ornithine[26], dipeptide[27], 2-[(2-(2-hydroxyethylamino)ethylimino)methyl]phenol[28,29], L-proline[30], L-leucine or L-isoleucine[31] and *o*-phthalate[32] have been recently investigated and revealed efficient DNA cleavage activities in the presence of reducing agents. Guo *et al.*[33] synthesized a copper complex with mixed-ligand 4'-*p*-tolyl-2,2':6,2"-terpyridine and glycine, which exhibited better oxidative cleavage activities in the presence of reducing agents.

In the present work, a copper complex with mixed-ligand 4'-ferrocenyl-2,2':6',2"-terpyridine and 1,1'-phenanthroline was prepared. We further investigated DNA binding ability and 'chemical nuclease' activity of the complex in the presence of ascorbic acid.

EXPERIMENTAL

Distamycin and SOD were purchased from Sigma-Aldrich Chemical Co., pBR 322 DNA was purchased from MBI, *tris*(hydroxymethyl)aminomethane (Tris) and catalase were purchased from BBI, CT-DNA and ethidium bromide were purchased from Sino-American Biotechnology, 2,2,6,6-tetramethyl-4-piperidone monohydrate (TEMP) was purchased from Acros organic. 4'-Ferrocenyl-2,2':6',2"-terpyridine was prepared by us[34]. Other reagents of analytical grades were obtained from domestic chemical corporations and used without further purification except that thionyl chloride was purified by distillation. Milli-Q water was used in all physical measurement experiments.

Synthesis of copper(II) complex: A solution of copper perchlorate hexahydrate (37 mg, 0.10 mmol), 4'-ferrocenyl-2,2':6',2"-terpyridine (42 mg, 0.10 mmol) and 1,10-phenanthroline (20 mg, 0.10 mmol) in acetonitrile (15 mL) was stirred for 4 h. The resulting solution was filtered off and the filtrate was diffused by diethyl ether evaporation to give dark-purple sheet crystals 50 mg (yield 55 %) of the present complex after 3 days.

CD spectroscopy: CD spectra of CT-DNA were recorded on a Jasco J-810 spectropolarimeter at room temperature in the absence or the presence of 20 μmol L^{-1} complex (r = 0.2, 0.4. r = [complex]/[DNA]). The concentration of DNA was 100 μmol L^{-1} and 5 mmol L^{-1} Tris-50 mmol L^{-1} NaCl solution (pH 7.5) was the buffer solution. Each test solution was scanned at a speed of 50 nm min^{-1} for four repetitions and the average spectra were used. The buffer background was subtracted automatically.

UV-visible spectroscopy: UV-visible spectroscopy was recorded on a Varian Cary 300 UV-visible spectrophotometer attached to a Cary Peltier temperature controller. The concentration of the copper complex was 20 μmol L^{-1} and the buffer solution was the same as that of CD spectroscopy.

DNA cleavage: The cleavage of pBR322 plasmid DNA (38 μmol L^{-1} bp) was performed in 20 mmol L^{-1} Tris buffer (pH 7.2) by treating DNA with a certain concentration of complex in the presence of 20-fold ascorbic acid. The total volume of 10 μL sample solution was incubated in a sealed plastic vessel and the cleavage was carried out at 37 ºC for 1 h, then 4 μL loading buffer (0.05 % bromophenol blue, 50 % glycerol and 2 mmol L^{-1} EDTA) was added. The sample was stored at -20 ºC for preservation, then was loaded onto a 0.9 % agarose gel and electrophoresed at a constant voltage of 120 V for 100 min in TBE buffer (100 mmol L^{-1} Tris-2 mmol L^{-1} EDTA-HBO_3, pH 8.3). DNA bands were visualized in an electrophoresis documentation and analysis system 120.

RESULTS AND DISCUSSION

The copper complex $[CuFe(C_5H_5)(C_{20}H_{14}N_3)(C_{12}H_8N_2)](ClO_4)_2^-\cdot C_2H_3N$ was reported by us[34]. The complex consists of a mononuclear $[Cu(C_{12}H_8N_2)\text{-}(C_{25}H_{19}FeN_3)]^{2+}$ cation, two ClO_4^- anions (one of which is disordered over two positions with equal occupancy) and one CH_3CN solvent molecule. The Cu(II) center has a distorted square-pyramidal coordination with three N atoms of the 4'-ferrocenyl-2,2':6',2"-terpyridine-k^3,N',N") (fctpy) ligand and one 1,10-phenanthroline (phen) N atom in the basal plane and a second phen N atom in the apical position with an axial distance of 2.254 (4) Å (Fig. 1).

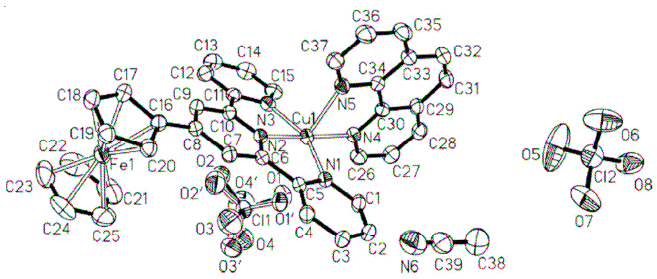

Fig. 1. Molecular structure of the copper complex

Interaction with CT-DNA: The interactions between both complexes and CT-DNA were evaluated by UV-visible spectroscopy titration and CD spectroscopy. UV-visible spectra were shown in Fig. 2. In the spectra of complex, hyperchromicity was observed with the addition of CT-DNA (0-9.0 × 10^{-5} mol L^{-1}). The hyperchromicity argued the binding mode of the complex towards CT-DNA to the 2.0 × 10^{-5} mol L^{-1} solution of complex should not be intercalation, since intercalation would

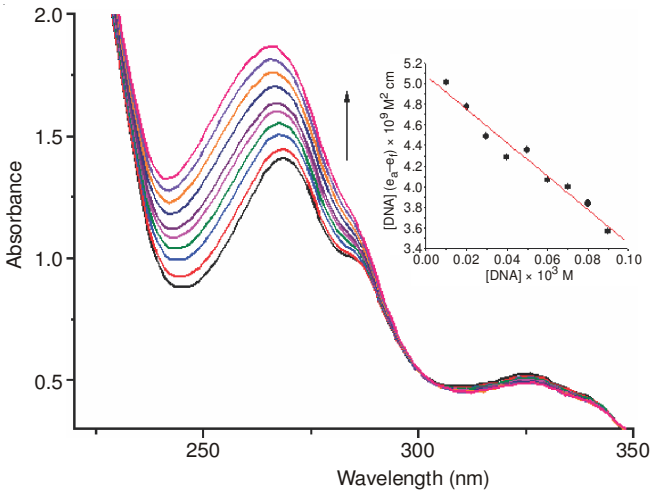

Fig. 2. Absorption spectra of 2.0 × 10^{-5} mol L^{-1} complex in the absence (dot line) and the presence (solid line) of increasing amount of CT-DNA (0-9.0 × 10^{-5} mol L^{-1}) in 5 mmol L^{-1} *Tris*-50 mmol L^{-1} NaCl buffer (pH 7.5) at 25 ± 0.1 ºC

bring on hypochromicity as a result of stacking effect of π electrons[35], which leads to the decrease of transition probability of π electrons and ultimately results in the decrease of absorption. The intrinsic binding constant (K_b) of the copper complex was calculated as (3.22 ± 0.05) 10^3 $mol^{-1}\cdot L$, which are much smaller than those reported for typical intercalators (*e.g.*, EB-DNA, *ca.* 10^6 mol^{-1} L)[36,37] and comparable with some reported non-intercalateors[38,39]. The quite low K_b values don't favour intercalation binding.

The interaction between the complex and CT-DNA was further studied by CD spectroscopy (Fig. 3). The CD spectrum of CT-DNA consists of a positive band at 277 nm due to base stacking and a negative band at 245 nm because of helicity, which is characteristic of DNA in the right-handed B form[40]. Intercalation of small molecules with DNA enhances the intensities of both bands, while groove binding and electrostatic interactions show little or no perturbations on the base stacking and helicity bands. With increasing concentration of the copper complex, both the intensities of positive and negative bands have no enhancement, which implied a non-intercalative mode between DNA and complexes. The cationic core of the complex could exert a strong electrostatic attraction to the anionic phosphate backbone of DNA, thus, the electrostatic binding mode is highly possible.

DNA cleavage studies: The oxidative DNA cleavage activity of the complex was studied in the presence of ascorbic acid. Fig. 4 exhibits the concentration dependent of the cleavage reactions after 1 h incubation (pH 7.2, 37 ºC). The amounts of form II DNA increased with the increase of complex concentration and form I DNA disappeared completely and form III started to appear when the complex concentration is up to 40 μmol L^{-1}. And at 40 μmol L^{-1}, form I DNA was converted to form II completely. Form III DNA was produced at 50 μmol L^{-1} complex. This suggested that complex exhibited appreciate nuclease activity.

The reactive oxygen species were also investigated (Fig. 5), the strong inhibitions of DNA cleavage to the complex were observed in the presence of hydroxyl radical scavengers DMSO and *tert*-butyl alcohol, indicating that hydroxyl radicals

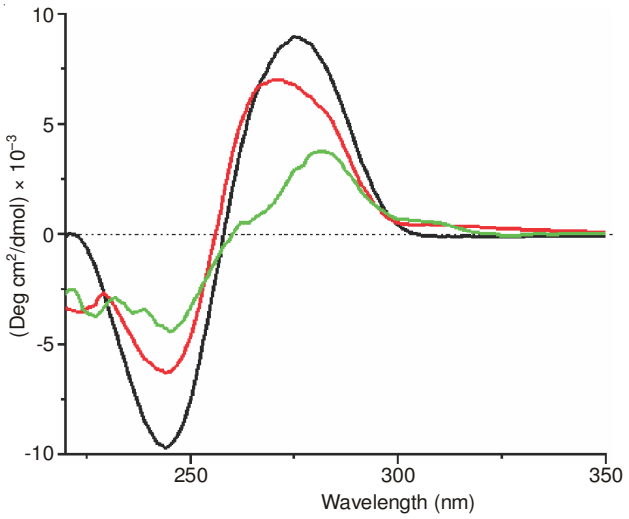

Fig. 3. CD spectra of CT-DNA (1.0×10^{-4} mol L^{-1}) in the absence (black) and the presence (red, green) of complex at ratio [complex]/[DNA] = 0.2, 0.4 in 5 mM Tris-50 mmol L^{-1} NaCl buffer (pH 7.5)

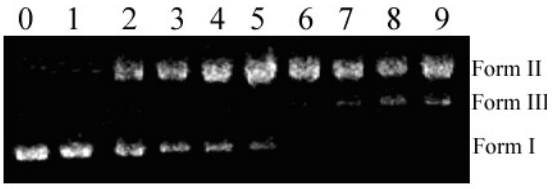

Fig. 4. Cleavage of pBR322 DNA bycomplex in the presence of ascorbic acid. DNA (38 μmol L^{-1} bp) was incubated with complex for 1 h in 20 mmol L^{-1} Tris-$HClO_4$/0.1 mol L^{-1} $NaClO_4$ aqueous buffer solution (pH 7.2) at 37 ºC. Lane 0, DNA control; Lane 1, DNA + ascorbic acid (2 mmol L^{-1}); Lane 2-9, DNA + complex (5, 10, 20, 30, 40, 50, 60, 70 μmol L^{-1} + ascorbic acid (20-fold of complex)

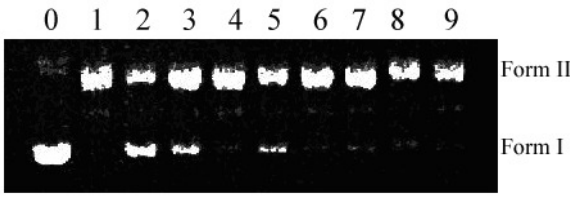

Fig. 5. Cleavage of pBR322 DNA by 40 μmol L^{-1} complex in the presence of different inhibitors with 20-fold excess of ascorbic acid. DNA (38 μmol L^{-1} bp) was incubated with complex for 1 h in 20 mmol L^{-1} Tris-$HClO_4$/0.1 mol L^{-1} $NaClO_4$ aqueous buffer solution (pH 7.2) at 37 ºC: Lane 0, DNA control; lane 1, no inhibitor; Lane 2, 0.5 mol L^{-1} DMSO; lane 3, 0.5 mol L^{-1} *t*-BuOH; lane 4, 0.5 mmol L^{-1} TEMP; lane 5, 500 U/mL catalase; lane 6, 500 U/mL SOD; lane 7, 100 μmol L^{-1} NaN_3; lane 8,10 μmol L^{-1} distamycin; lane 9, 10 μmol L^{-1} methyl green

are responsible for the cleavage. Singlet oxygen scavengers 2,2,6,6-tetramethyl-4-piperidone monohydrate and NaN_3 and superoxide anion radical scavenger SOD failed to inhibit cleavage, suggesting that singlet oxygen and superoxide anion radical are not likely to be the reactive oxygen species. A weak inhibition was observed by addition of hydrogen peroxide scavenger catalase, arguing hydrogen peroxide may play a role in the cleavage, but not a major active species.

The minor groove binding agent distamycin and major groove binding agent methyl green were used to probe the potential interacting site of the copper molecular complex with DNA. The major groove binding agent methyl green and the minor groove binder distamycin did not affect the cleavage activity of complex at all (lanes 9,10) (Fig. 5), which further indicated the electrostatic binding mode between the complex and DNA.

Conclusion

The present work mainly describes the copper complex with mixed-ligand 4'-ferrocenyl-2,2':6',2"-terpyridine and 1,1'-phenanthroline interacted with CT-DNA mainly by electrostatic attraction. The complex exhibited efficient nuclease activities in the presence of ascorbic acid, since the mixed-ligand complex contains tridentate terpyridine and phen. Control studies show the DNA cleavage are inhibited obviously in the presence of the hydroxyl radical scavengers DMSO and tert-butyl alcohol, which indicates the hydroxyl radicals are mainly the reactive oxygen species.

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REFERENCES

1. F. Mancin, P. Scrimin, P. Tecillab and U. Tonellato, *Chem. Commun.*, 2540 (2005).
2. C. Liu, M. Wang, T. Zhang and H. Sun, *Coord. Chem. Rev.*, **248**, 147 (2004).
3. Q. Jiang, N. Xiao, P.-F. Shi, Y.-G. Zhu and Z.-J. Guo, *Coord. Chem. Rev.*, **251**, 1951 (2007).
4. D.S. Sigman, A. Mazumder and D.M. Perrin, *Chem. Rev.*, **93**, 2295 (1993).
5. A. Pyle and J.K. Barton, *Prog. Inorg. Chem.*, **38**, 413 (1990).
6. G. Pratviel, J. Bernadou and B. Meunier, *Angew. Chem., Int. Ed. Engl.*, **34**, 746 (1995).
7. W.K. Pogozelski and T.D. Tullius, *Chem. Rev.*, **98**, 1089 (1998).
8. C.J. Burrows and J.G. Muller, *Chem. Rev.*, **98**, 1109 (1998).
9. D.S. Sigman, D.R. Graham, V.D. Aurora and A.M. Stern, *J. Biol. Chem.*, **254**, 12269 (1979).
10. D.S. Sigman, *Acc. Chem. Res.*, **19**, 180 (1986).
11. D.R. Graham, L.E. Marshall, K.A. Reich and D.S. Sigman, *J. Am. Chem. Soc.*, **102**, 5419 (1980).
12. S. Goldstein and G. Czapski, *J. Am. Chem. Soc.*, **108**, 2244 (1986).
13. B.K. Santra, P.A.N. Reddy, G. Neelkanta, S. Mahadevan, M. Nethaji and A.R. Chakravarty, *J. Inorg. Biochem.*, **89**, 191 (2002).
14. A.M. Thomas, G. Neelkanta, S. Mahadevan, M. Nethaji and A.R. Chakravarty, *Eur. J. Inorg. Chem.*, 2720 (2002).
15. A.M. Thomas, M. Nethaji, S. Mahadevan and A.R. Chakravarty, *J. Inorg. Biochem.*, **94**, 171 (2003).
16. P.A.N. Reddy, M. Nethaji and A.R. Chakravarty, *Eur. J. Inorg. Chem.*,1440 (2004).
17. S. Dhar, P.A.N. Reddy, M. Nethaji, S. Mahadevan, M.K. Saha and A.R. Chakravarty, *Inorg. Chem.*, **41**, 3469 (2002).
18. R. Pradhan, A.M. Thomas, A. Mukherjee, S. Dhar, M. Nethaji and A.R. Chakravarty, *Indian J. Chem.*, **44A**, 18 (2005).
19. M. Chikira, Y. Tomizawa, D. Fukita, T. Sugizaki, N. Sugawara, T. Yamazaki, A. Sasano, H. Shindo, M. Palaniandavar and W.E. Antholine, *J. Inorg. Biochem.*, **89**, 163 (2002).

20. X. Wang, H. Chao, H. Li, X. Hong, L. Ji and X. Li, *J. Inorg. Biochem.,* **98**, 423 (2004).
21. A.M. Thomas, M. Nethaji and A.R. Chakravarty, *J. Inorg. Biochem.,* **98**, 1087 (2004).
22. S.T. Frey, H.H.J. Sun, N.N. Murthy and K.D. Karlin, *Inorg. Chim. Acta,* **242**, 329 (1996).
23. K.J. Humphreys, K.D. Karlin and S.E. Rokita, *J. Am. Chem. Soc.,* **124**, 8055 (2002).
24. Y. Zhao, J. Zhu, W. He, Z. Yang, Y. Zhu, Y. Li, J. Zhang and Z. Guo, *Chem. Eur. J.,* **12**, 6621 (2006).
25. J.-W. Chen, X.-Y. Wang, Y. Shao, J.-H. Zhu, Y.-G. Zhu, Y.-Z. Li, Q. Xu and Z.-J. Guo, *Inorg. Chem.,* **46**, 3306 (2007).
26. P.R. Chetana, R. Rao, S. Saha, R.S. Policegoudra, P. Vijayan and M.S. Aradhya, *Polyhedron*, **48**, 43 (2012).
27. P.R. Reddy and N. Raju, *Polyhedron*, **44**, 1 (2012).
28. I.V. Rajendiran, R. Karthik, M. Palaniandavar, H. Stoeckli-Evans, V.S. Periasamy, M.A. Akbarsha, B.S. Srinag and H. Krishnamurthy, *Inorg. Chem.*, **46**, 208 (2007).
29. V. Rajendiran, M. Palaniandavar, P. Swaminathan and L. Uma, *Inorg. Chem.*, **46**, 10446 (2007).
30. R. Rao, A.K. Patra and P.R. Chetana, *Polyhedron*, **26**, 5331 (2007).
31. R. Rao, A.K. Patra and P.R. Chetana, *Polyhedron*, **27**, 1343 (2008).
32. A. Kellett, O. Howe, M. O'Connor, M. McCann, B.S. Creaven, S. McClean, A. Foltyn-ArfaKia, A. Casey and M. Devereux, *Free Radic. Biol. Med.,* **53**, 564 (2012).
33. W. Zhou, X.Y. Wang, M. Hu and Z.J. Guo, *J. Inorg. Biochem.*, **121**, 114 (2013).
34. S.P. Tang, D.Z. Kuang, Y.L. Feng and M.S. Chen, *Acta Cryst*., **E65**, m731 (2009).
35. E.C. Long and J.K. Barton, *Acc. Chem. Res*., **23**, 271 (1990).
36. M. Baldini, M. Belicchi-Ferrari, F. Bisceglie, P.P. Dall'Aglio, G. Pelosi, S. Pinelli and P. Tarasconi, *Inorg. Chem.*, **43**, 7170 (2004).
37. J.B. Le Pecq and C. Paoletti, *J. Mol. Biol*., **27**, 87 (1967).
38. J.-W. Chen, X.-Y. Wang, Y. Shao, J.-H. Zhu, Y.-G. Zhu, Y.-Z. Li, Q. Xu and Z.-J. Guo, *Inorg. Chem*., **46**, 3306 (2007).
39. J.-H. Li, J.-T. Wang, Z.-W. Mao and L.-N. Ji, *Inorg. Chem. Commun*., **11**, 865 (2008).
40. V. Uma, A. Castineiras and B.U. Nair, *Polyhedron*, **26**, 3008 (2007).