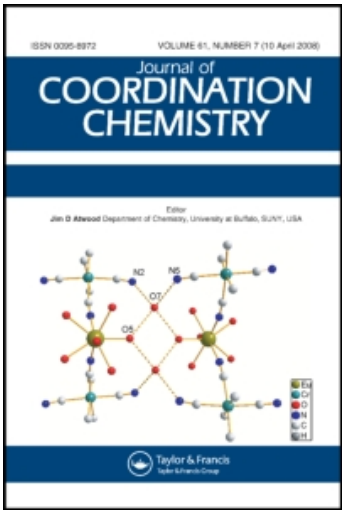


Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

High nuclease activity of a copper(II)-bipyridyl complex containing cytosine pendants

Jin-Tao Wang[a]; Xiao-Hui Zheng[a]; Liang-Nian Ji[a]; Zong-Wan Mao[a]
[a] MOE Key Laboratory of Bioinorganic and Synthetic Chemistry, School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou 510275, P.R. China

First published on: 14 June 2010

To cite this Article Wang, Jin-Tao , Zheng, Xiao-Hui , Ji, Liang-Nian and Mao, Zong-Wan(2010) 'High nuclease activity of a copper(II)-bipyridyl complex containing cytosine pendants', Journal of Coordination Chemistry, 63: 14, 2440 — 2449, First published on: 14 June 2010 (iFirst)
To link to this Article: DOI: 10.1080/00958972.2010.492216
URL: http://dx.doi.org/10.1080/00958972.2010.492216

Journal of Coordination Chemistry
Vol. 63, Nos. 14–16, 20 July–20 August 2010, 2440–2449

High nuclease activity of a copper(II)-bipyridyl complex containing cytosine pendants†

JIN-TAO WANG, XIAO-HUI ZHENG, LIANG-NIAN JI and ZONG-WAN MAO*

MOE Key Laboratory of Bioinorganic and Synthetic Chemistry, School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou 510275, P.R. China

(*Received 1 February 2010; in final form 11 March 2010*)

A new copper(II) complex bearing 2,2′-bipyridine (bpy) derivatives with cytosine nucleobases $[Cu(L)Cl_2]\cdot 2H_2O$ has been synthesized and characterized, where L = 5,5′-di[(4-amino-2-oxo-1(2H)-pyrimidinyl)methyl]-2,2′-bipyridine. Circular dichroism spectra reveal interactions between ligand and pBR322 DNA and showed that the local DNA structure was perturbed by the ligand. Cleavage of pBR322 DNA by the title complex was carried out in 20 mmol L^{-1} HEPES (pH 7.5) at 37°C. The calculated pseudo-Michaelis–Menten kinetic parameter (k_{cat}) of the complex was 8.80 h^{-1}. The cleavage efficiency was 17-fold higher than that of its simple analog $[Cu(bpy)Cl_2]$ ($k_{cat} = 0.50\ h^{-1}$). Hydrolytic mechanisms involved in DNA cleavage were explored using radical scavengers and T4 ligase. Competitive experiments with special binding agents showed that the complex could preferentially bind to the minor groove of double-stranded DNA, suggesting specific DNA binding characteristics. High DNA cleavage reactivity and selectivity of the complex could be attributed to the synergic effects of the metal center and the cytosine pendants.

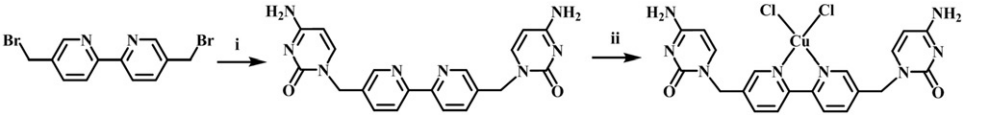
Keywords: Copper(II) complex; Cytosine; Artificial nuclease; Hydrolytic DNA cleavage

1. Introduction

DNA is a significant hydrolysis substrate and there has been considerable interest in development of various transition metal complexes serving as artificial nucleases [1–5]. Hydrolytic cleavage of double-stranded (ds) DNA through hydrolysis of the phosphodiester bond exhibits more advantages in the cell in comparison with the oxidative DNA cleavage targeting the deoxyribose sugar moiety or the guanine base [6–8]. Inspired by structures of natural nucleases, introduction of organic functional groups has become a widely used and an important strategy for design of artificial nucleases. The organic group may promote interaction between the metal complexes and targeted DNA, thus facilitating DNA cleavage. Many metal complexes containing organic functional ligands (e.g., thiophenyl [9], amino group [10–13], guanidino group [14], hydroxyalkyl [15–17], and nucleobases [18–21] etc.) have been reported

*Corresponding author. Email: cesmzw@mail.sysu.edu.cn
†Contributions for the van Eldik tribute.

Journal of Coordination Chemistry
ISSN 0095-8972 print/ISSN 1029-0389 online © 2010 Taylor & Francis
DOI: 10.1080/00958972.2010.492216



Scheme 1. Synthesis of the ligand L and the complex. Reagents and conditions were: (i) Cytosine, K_2CO_3, CH_3CN/H_2O 2 : 1, 80°C; (ii) $CuCl_2 \cdot 2H_2O$, H_2O, 80°C.

to act as artificial nucleases or hydrolases. Our group also has designed several metal complexes of 2,2′-bipyridyl-based ligands containing organic functional groups, including tetraalkylammonium [22], guanidine [23], and nucleobases [24]; these complexes can bind and cleave duplex DNA with high reactivity and structural selectivity.

It is a promising strategy that small molecules containing both nucleobases and DNA intercalators could selectively recognize complementary base pairs in single or ds polynucleotides [25], so it is possible to utilize nucleotide recognition to promote DNA–drug interaction. Only a few metal complexes with nucleobase pendants were synthesized, which acted as artificial nucleases. Very recently, we synthesized three copper(II) complexes bearing 2,2′-bipyridine (bpy) derivatives with adenine, thymine, and uracil nucleobases and the one with thymine is the most effective artificial nuclease to catalyze ds DNA hydrolytic cleavage. In this study, we designed and synthesized another copper(II) complex bearing 2,2′-bipyridine derivatives with cytosine, $[Cu(L)Cl_2] \cdot 2H_2O$ (L = 5,5′-di[(4-amino-2-oxo-1(2H)-pyrimidinyl)methyl]-2,2′-bipyridine) (scheme 1). Furthermore, the interaction between ds DNA and the complex, as well as the cleavage of pBR322 DNA by the complex, was investigated.

2. Experimental

2.1. *Chemicals and reagents*

5,5′-dimethyl-2,2′-bipyridine, ethidium bromide (EB), methyl green, and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma. pBR322 DNA was obtained from MBI. Agarose gel, buffer reagents (TRIS and HEPS), and cytosine were purchased from Sangon. T4 DNA ligase and *Eco*RI endonuclease were purchased from Toyobo Co., Ltd. Other reagents were of analytical grade and obtained from domestic chemical corporations and used without further purification. Milli-Q water was used in all physical measurement experiments.

2.2. *Physical methods*

Microanalyses (C, H, and N) were carried out using an Elementar Vario EL elemental analyzer. ESI-mass spectra were recorded using a LCQ DECA XP liquid chromatography-mass spectrometer (Thremo Co.). ^{1}H-NMR and ^{13}C-NMR spectra were collected on a Varian Mercury-plus 300 NMR spectrometer. Circular dichroism (CD) spectra were recorded on a Jasco J-810 spectropolarimeter.

2.3. *Synthesis*

The synthetic route of the ligand and complex is shown in scheme 1, while the 5′-dibromomethyl-2,2′-bipyridine was synthesized according to the previous method [22]. ^{1}H-NMR and ^{13}C-NMR spectra of ligand, ESI-MS spectra of ligand and complex are presented in "Supplementary material" (figures S1–S4).

2.3.1. 5,5′-Di[(4-amino-2-oxo-1(2H)-pyrimidinyl)methyl]-2,2′-bipyridine (L). Potassium carbonate (0.276 g, 2 mmol) was added to a stirred solution of excess cytosine (8.888 g, 8 mmol) in 30 mL CH_3CN/H_2O (2 : 1), followed by slow addition of acetonitrile solution of 5,5′-dibromomethyl-2,2′-bipyridine (0.342 g, 1 mmol) in drops. The reaction mixture was stirred for 1 h at 80°C until TLC analysis indicated that very little 5,5′-dibromomethyl-2,2′-bipyridine was left. The excess solvent was removed by rotary evaporation under reduced pressure. Water (40 mL) was added to the residue and the solution was neutralized to pH 7.0 by slowly adding 1% HCl. Then the mixture was heated to 60°C and further stirred for 30 min. Finally, white powder of L (0.240 g, 59.6%) was obtained after filtration and drying. ^{1}H-NMR (DMSO-d_6, 300 MHz): δ 8.590 (s, 2H, bpy-H); 8.295 (d, 2H, $J = 8.4$; bpy-H); 7.785 (d, 2H, $J = 8.4$; bpy-H); 7.785 (d, 2 H, $J = 6.0$; cytosine-H); 7.078 (s, 4H, cytosine-NH_2); 5.680 (d, 2H, $J = 6.0$; cytosine-H); 4.912 ppm (s, 4H; CH_2). ^{13}C-NMR (75 MHz, D_2O): δ 161.50, 151.04, 148.39, 147.36, 144.51, 136.27, 125.52, 97.24, 51.79, and 39.49 ppm; ESI–MS: m/z (%): 403.1 (100) $[L + H]^+$; Anal. Calcd for $C_{20}H_{18}N_8O_2$ (%): C, 59.69; H, 4.51; and N, 27.85. Found: C, 59.52; H, 4.75; and N, 27.64.

2.3.2. $[Cu(L)Cl_2]\cdot 2H_2O$. L (0.201 g, 0.5 mmol) was suspended in H_2O (5 mL) at 80°C, followed by addition of aqueous solution of $CuCl_2\cdot 2H_2O$ (0.102 g, 0.6 mmol) in drops. Green precipitate was observed after stirring overnight. Then the solid was isolated and washed with ethanol several times and dried *in vacuo* to give the title complex (0.256 g, 89.4%) as green powder. ESI–MS: m/z (%): 524.07 (100) $[Cu(C_{20}H_{17}N_8O_2)Cl + Na]^+$; Anal. Calcd for $C_{20}H_{22}N_8O_4CuCl_2$ (%): C, 41.93; H, 3.87; N, and 19.56. Found: C, 42.81; H, 3.98; and N, 19.44.

2.4. *Molecular modeling*

The Gaussian 03 suite of programs [26] was used to obtain preliminary information about the structure of the title complex. Frequency calculations were included to confirm the true minimum and correct thermal and zero-point energies. Complex was optimized at the B3LYP/6-31G (d, p) level.

2.5. *CD spectroscopy*

CD measurements were recorded using a cell of 1-cm path length. Titrations were performed by incrementally adding aliquots of the ligand to 25 μmol L^{-1} pBR322 DNA in 20 mmol L^{-1} HEPES buffer containing 10 mmol L^{-1} NaCl, pH 7.5 at 37°C. After each addition, an average CD spectrum from 220 to 400 nm (20 accumulations) was recorded. The concentration of each ligand ranged from 0 to 20 μmol L^{-1}. The CD

spectrum of pBR322 DNA alone (25 μmol L^{-1}) was recorded as the control. The background of HEPES buffer was subtracted automatically.

2.6. *DNA cleavage and k_{obs} calculation*

The rates of DNA cleavage at various catalyst concentrations were determined in 20 mmol L^{-1} HEPES, pH 7.5 at 37°C for different intervals of time according to previous reports [27–29]. Briefly, after incubation of pBR322 DNA with the complex for a certain period of time, 4 μL of loading buffer (0.05% bromophenol blue, 50% glycerol, and 2 mmol L^{-1} Na_2H_2edta) was added to the mixture. Control reactions were carried out using the same conditions as the cleavage reactions, but lacking copper(II) complexes. Then, the samples were loaded directly onto a 0.9% agarose gel and electrophoresed in TBE buffer (89 mmol L^{-1} tris-borate, 2 mmol L^{-1} Na_2H_2edta) at a constant voltage of 70 mV for 120 min. The gels were visualized in the electrophoresis documentation and analysis system 120. Densitometric calculations were performed using Image Tools 3.00. Intensities of supercoiled pBR322 DNA were corrected by a factor of 1.22 as a result of its lower staining capacity by EB [30]. The cleavage of DNA was fitted to a single exponential decay curve (pseudo-first-order kinetics) by equation 1(a) or 1(b), where y_0 is the initial percentage of a form of DNA, y the percentage of a specific form of DNA at time t, a the percentage of uncleaved DNA, and k_{obs} is the hydrolysis rate constant, or apparent rate constant.

$$y = (y_0 - a)\exp(-k_{obs}x) + a, \tag{1a}$$

$$y = (100 - y_0)(1 - \exp(-k_{obs}x)), \tag{1b}$$

3. Results and discussion

3.1. *Molecular modeling of the complexes*

The final optimized structure of the complex, together with the atomic labeling scheme, is shown in figure S5 and the corresponding structural data are presented in table S1. The result shows that copper(II) had a distorted square-planar CuN_2Cl_2 configuration in the complex. The angle between cytosine and bpy was 112.74°, very close to our previous report (the angles between three nucleases and bpy were 113.35°, 112.87°, and 112.97°, respectively) and another copper(II) complex (the angle between adenine and phenyl was 113.00°) [18].

3.2. *Interaction with pBR322 DNA*

CD is a useful technique to assess whether nucleic acids undergo conformational changes as a result of complex formation or changes in the environment. Herein, the interaction between the ligand and pBR322 DNA was studied by CD spectroscopy. Figure 1 shows the CD spectrum of free DNA in HEPES buffer at pH 7.5, harboring a positive peak at ~273 nm and a negative peak at ~244 nm. In some degree,

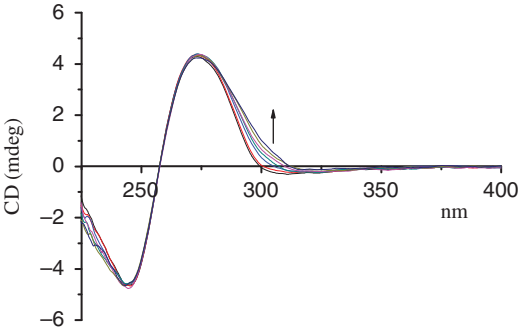


Figure 1. CD spectra of pBR322 DNA (25×10^{-6} mol L^{-1} b.p.) modified by L at different ligand-to-DNA ratios (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6).

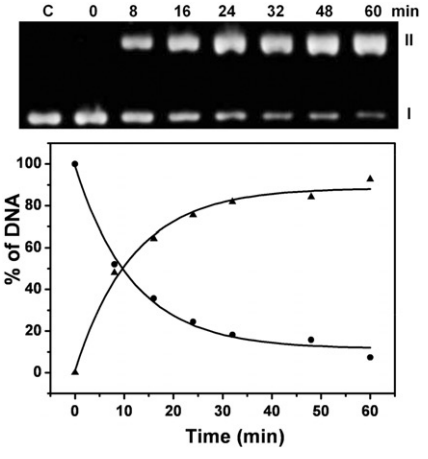


Figure 2. Electrophoresis pattern and time course plot showing the time-dependent cleavage of pBR322 DNA (38 μmol L^{-1} b.p.) promoted by the complex (70 μmol L^{-1}) in 20 mmol L^{-1} HEPES buffer (pH 7.5) at 37°C.

the positive band at ~300 nm was broadened toward the redshift direction, which indicated that the structure of pBR322 DNA was perturbed by the ligand. However, the simple analog (bpy) did not induce any regular and apparent changes of the CD spectra of pBR322 DNA [24]. This result suggested that the introduced cytosine played important roles during the interactions between ligand and pBR322 DNA.

3.3. *Nuclease activity*

3.3.1. DNA cleavage by the complex. pBR322 DNA cleavage by the copper(II) complex was investigated in the absence of ascorbic acid, H_2O_2, or any reducing agent. The reaction leads to the formation of open circular DNA (form II) from supercoiled form I. A typical agarose gel electrophoresis pattern and time course for DNA cleavage by the complex are shown in figure 2. We found that most supercoiled DNA (form I)

Figure 3. Ionic strength dependence of the plasmid DNA (38 μmol L^{-1} b.p.) cleavage by the complex (70 μmol L^{-1} for 25 min) at 37°C in 20 mmol L^{-1} HEPES at pH 7.5. Lane 1, DNA control; lanes 2–11, DNA + complex with 0, 10, 20, 40, 70, 100, 150, 200, 300, and 400 mmol L^{-1} NaCl.

was cleaved in the presence of 70 μmol L^{-1} complex within 1 h. Thus, the title complex showed high cleavage activity toward pBR322 DNA.

3.3.2. Effect of ionic strength on DNA cleavage reactivity. The effect of ionic strength on DNA cleavage in which pBR322 DNA was treated with the complex under increasing ionic strength is shown in figure 3. The pBR322 DNA cleavage reactivity decreased as ionic strength increased, indicating that the copper bound not only with the phosphate of DNA but also with the DNA bases *via* electrostatic interactions. Excess salt partially neutralizes the phosphate backbone, which in turn decreases the local concentration of these cationic complexes and leads to a decrease in DNA cleavage [31]. Therefore, ionic strength played an important role in the regulation of DNA binding in our study. Electrostatic interactions between copper(II) complexes and DNA are very common [22–24, 31b, 32] because cationic molecules can interact with DNA by neutralizing the negatively charged phosphate backbone of DNA [33]. Here the title complex is neutral in the solid state, but in aqueous solution, the Cu–Cl bond is labile and Cl^- exchanges readily with water to give the cationic active species, $[Cu(L)(Cl)(H_2O)]^+$ or $[Cu(L)(H_2O)_2]^{2+}$, which can interact with pBR322 DNA through the electrostatic binding.

3.3.3. Pseudo-Michaelis–Menten kinetics of DNA cleavage. Under hydrolytic condtions, DNA can be cleaved from supercoiled (form I) into nicked (form II) by the title complex at various concentrations. A series of DNA cleavage experiments was performed, and time-dependent and concentration-dependent cleavages were observed (figure S6). The time-dependent decrease of form I and increase of form II followed pseudo-first-order kinetic profiles and could be fit well with a single exponential function. The kinetics of DNA cleavage were studied using increased concentrations of the title complex (15–250 μmol L^{-1}); a plot of k_{obs} *versus* concentrations is shown in figure 4.

Plots of the rate constants against the concentrations of the complexes gave $k_{cat} = 8.8 \pm 0.4\,h^{-1}$, $K_m = 5.14 \times 10^{-5}$ mol L^{-1} for the complex. The obtained hydrolysis rate constant showed that the title complex had high nuclease activities and gave 2.4×10^8 fold rate enhancement compared with unhydrolyzed ds DNA (for unhydrolyzed ds DNA [6], first-order hydrolysis rate constant $k = 3.6 \times 10^{-8}\,h^{-1}$). Recently, we reported a similar complex with thymine groups, which was the most active artificial nuclease that could hydrolytically cleave ds DNA with hydrolysis rate constant $k = 40.4\,h^{-1}$ [24]. Copper(II) complexes that cleavage ds DNA were widely reported but most of them promote DNA cleavage through an oxidative

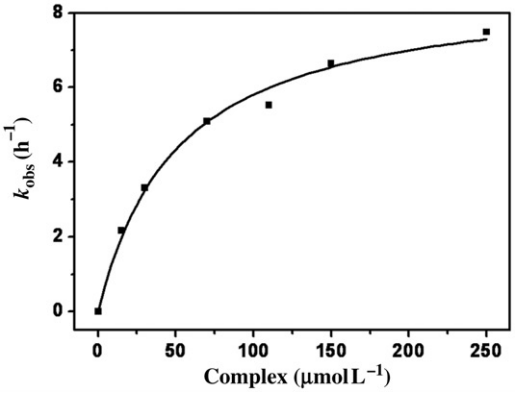


Figure 4. Kinetics for the cleavage of plasmid pBR322 DNA by the complex in 20 mmol L^{-1} HEPES, pH 7.5 at 37°C. The samples were run on a 0.9% agarose gel and stained with EB.

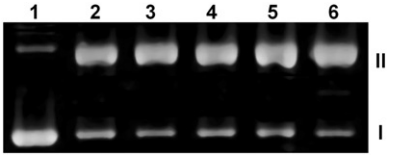


Figure 5. Agarose gel showing cleavage of 38 μmol L^{-1} b.p. pBR322 DNA incubated with the complex (70 μmol L^{-1}) in 20 mmol L^{-1} HEPES, pH 7.5 at 37°C. Lane 1, DNA control; lane 2, DNA + complex; lanes 3–6, DNA + complex + 1 mol L^{-1} DMSO, 1 mol L^{-1} *t*BuOH, 500 μmol L^{-1} TEMP, and 500 μmol L^{-1} KI for 25 min, respectively.

DNA damage pathway. Even the title complex was not as efficient as the analog with thymine group, but shows higher reactivity than most other complexes that hydrolytically cleave ds DNA [27, 34].

3.3.4. DNA cleavage mechanism. The cleavage of supercoiled ds DNA by the title complex was studied in the absence of reducing agents. In order to investigate the possible cleavage mechanism, pBR322 DNA cleavage by the copper(II) complex was investigated in the presence of hydroxyl radical scavengers (DMSO and *t*BuOH) [35], hydrogen peroxide scavengers (KI), and singlet oxygen scavengers (2,2,6,6-tetramethyl-4-piperidone, TEMP). As shown in figure 5, all the scavengers showed little effect on DNA cleavage. This result potentially rules out the possibility of DNA cleavage by hydroxyl radicals, singlet oxygen, and hydrogen peroxide. Therefore, DNA cleavage by the title complex undergoes a hydrolytic degradation pathway. Further direct evidence of DNA hydrolysis was obtained from ligation experiments of pBR322 DNA linearized by the complex. It is well known that during DNA hydrolytic cleavage 3′-OH and 5′-OPO_3 (5′-OH and 3′-OPO_3) fragments remain intact and these fragments can be enzymatically ligated and end-labeled [36]. We tried to recover the linear DNA from a low melting point agarose gel by cutting off the gel fragment and extracting DNA using the DNA gel extraction kit. The recovered linear DNA was subjected to overnight ligation reaction with T4 DNA ligase. The result of electrophoresis (figure 6) showed

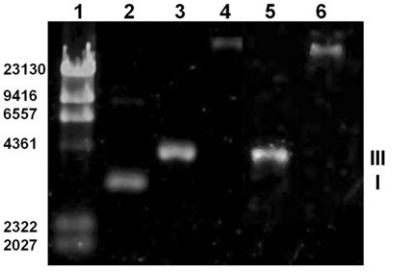


Figure 6. Agarose gel for ligation of pBR322 DNA linearized by the complex. Lane 1, λ*Hind*III DNA markers; lane 2, DNA control; lanes 3 and 4, pBR322 DNA linearized by *Eco*RI without and with T4 DNA ligase; lanes 5 and 6, pBR322 DNA linearized by the complex without and with T4 DNA ligase.

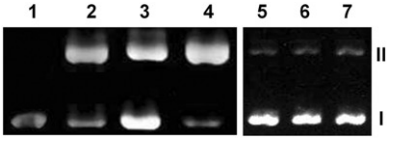


Figure 7. Agarose gel electrophoresis patterns for the cleavage of pBR322 plasmid DNA (38 μmol L^{-1} b.p.) by title complex (70 μmol L^{-1}) in the presence of DNA minor binding agent DAPI (10 μmol L^{-1}), major binding agent methyl green (10 μmol L^{-1}), Cu^{2+} (200 μmol L^{-1}), and ligand (100 μmol L^{-1}) only at 37°C (20 mmol L^{-1} HEPES per 10 mmol L^{-1} NaCl buffer, pH 7.5). Lane 1, DNA control; lane 2, DNA + complex (1 h); lane 3, DNA + complex + DAPI (1 h); lane 4, DNA + complex + MG (1 h); lane 5, DNA + $CuCl_2$ (2 h); lane 6, DNA + bpy (1 h); lane 7, DNA + L (1 h).

that the linear DNA fragment cleaved by the title complex could be religated by T4 ligase just like linear DNA mediated by *Eco*RI endonuclease. Hence, this result indicated that DNA cleavage by the complex was through hydrolysis, similar to the action of the natural enzyme *Eco*RI.

3.3.5. DNA recognition and interaction by the complex. Minor groove binding agent DAPI [37] and major groove binding agent methyl green [38] were used to probe the potential binding sites of the complexes with supercoiled plasmid DNA. The pBR322 DNA was treated with DAPI or methyl green prior to the addition of the title complex. As shown in figure 7 (lane 3), DNA cleavage was slowed in the presence of DAPI, suggesting that minor grooves were preferred binding sites for the complex. Only the ligand (lane 7) and free copper(II) (lane 5) did not show any cleavage activity to pBR322 DNA. Thus, the introduced cytosine played a crucial role in promoting interactions between copper(II) complex and the target DNA as well as in facilitating DNA cleavage. The ligand could perturb the supercoiled DNA rapidly, reducing DNA stability, and making DNA more vulnerable, thus facilitating attack of the phosphodiester backbone of nucleic acid by copper complex.

4. Conclusion

A new copper(II) complex bearing 2,2′-bipyridine derivatives with cytosine was synthesized and could hydrolytically cleave ds DNA effectively. The hydrolytic

mechanisms involved in DNA cleavage were testified using radical scavengers and T4 ligase. The complex showed efficient DNA minor groove binding. The high DNA cleavage reactivity and selectivity must be attributed to the introduced cytosine pendant. The general strategy in this study may be extended to the design of models of other metalloenzymes.

Supplementary material

^{1}H-NMR and ^{13}C-NMR spectra of the ligand, ESI–MS spectra of ligand and complex, the optimized structures of the complex, agarose gel electrophoresis, and the corresponding time course of DNA cleavage by the complexes.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (20821001, 20831006, 20725103, 30770494), the Guangdong Provincial Natural Science Foundation (9351027501000003), and 973 Program (2007CB815306).

References

[1] (a) P.U. Maheswari, S. Roy, H. den Dulk, S. Barends, G. van Wezel, B. Kozlevcar, P. Gamez, J. Reedijk. *J. Am. Chem. Soc.*, **128**, 710 (2006); (b) P.U. Maheswari, M. van der Ster, S. Smulders, S. Barends, G.P. van Wezel, C. Massera, S. Roy, P. den Dulk Gamez, J. Reedijk. *Inorg. Chem.*, **47**, 3719 (2008); (c) F. Mancin, P. Scrimin, P. Tecilla, U. Tonellato. *Chem. Commun.*, 2540 (2005).
[2] (a) C. Liu, L. Wang. *Dalton Trans.*, 227 (2009); (b) Q. Jiang, N. Xiao, P.F. Shi, Y.G. Zhu, Z.J. Guo. *Coord. Chem. Rev.*, **251**, 1951 (2007); (c) C. Liu, M. Wang, T.L. Zhang, H.Z. Sun. *Coord. Chem. Rev.*, **248**, 147 (2004); (d) J.A. Cowan. *Curr. Opin. Chem. Biol.*, **5**, 634 (2001).
[3] (a) T.A. Shell, D.L. Mohler. *Curr. Org. Chem.*, **11**, 1525 (2007); (b) S.J. Franklin. *Curr. Opin. Chem. Biol.*, **5**, 201 (2001).
[4] N.H. Williams, B. Takasaki, M. Wall. *Chin. J. Acc. Chem. Res.*, **32**, 485 (1999).
[5] M. Komiyama, J. Sumaoka. *Curr. Opin. Chem. Biol.*, **2**, 751 (1998).
[6] (a) A. Sreedhara, J.A. Cowan. *J. Biol. Inorg. Chem.*, **6**, 337 (2001); (b) J.A. Cowan. *Chem. Rev.*, **98**, 1067 (1998); (c) E.L. Hegg, J.N. Burstyn. *Coord. Chem. Rev.*, **173**, 133 (1998); (d) B. Meunier. *Chem. Rev.*, **92**, 1411 (1992).
[7] (a) K.E. Erkkila, D.T. Odom, J.K. Barton. *Chem. Rev.*, **99**, 2777 (1999); (b) D.S. Sigman, T.W. Bruice, A. Mazumder, C.L. Sutton. *Acc. Chem. Res.*, **26**, 98 (1993); (c) D.S. Sigman, A. Mazumder, D.M. Perrin. *Chem. Rev.*, **93**, 2295 (1993); (d) D.S. Sigman. *Acc. Chem. Res.*, **19**, 180 (1986).
[8] (a) W.K. Pogozelski, T.D. Tullius. *Chem. Rev.*, **98**, 1089 (1998); (b) D.R. McMillin, K.M. McNett. *Chem. Rev.*, **98**, 1201 (1998); (c) C.J. Burrows, J.G. Muller. *Chem. Rev.*, **98**, 1109 (1998).
[9] R. Breslow, D. Berger, D.L. Huang. *J. Am. Chem. Soc.*, **112**, 3686 (1990).
[10] S. Liu, A.D. Hamilton. *Tetrahedron Lett.*, **38**, 1107 (1997).
[11] G. Feng, J.C. Mareque-Rivas, R. Torres Martín de Rosales, N.H. Williams. *J. Am. Chem. Soc.*, **127**, 13470 (2005).
[12] M. Livieri, F. Mancin, G. Saielli, J. Chin, U. Tonellat. *Chem. Eur. J.*, **13**, 2246 (2007).
[13] (a) E. Kövári, R. Krämer. *Chem. Ber.*, **127**, 2151 (1994); (b) E. Kövári, J. Heitker, R. Krämer. *J. Chem. Soc., Chem. Commun.*, 1205 (1995); (c) E. Kövári, R. Krämer. *J. Am. Chem. Soc.*, **118**, 12704 (1996).
[14] (a) F. Folmer-Andersen, H. Ait-Haddou, V.M. Lynch, E.V. Anslyn. *Inorg. Chem.*, **42**, 8674 (2003); (b) S.L. Tobey, E.V. Anslyn. *J. Am. Chem. Soc.*, **125**, 14807 (2003); (c) H. Ait-Haddou, J. Sumaoka, S.L. Wiskur, F. Folmer-Andersen, E.V. Anslyn. *Angew. Chem. Int. Ed. Engl.*, **41**, 4014 (2002).

[15] M. Livieri, F. Mancin, G. Saielli, J. Chin, U. Tonellat. *Chem. Eur. J.*, **13**, 2246 (2007).
[16] J.W. Chen, X.Y. Wang, Y.G. Zhu, J. Lin, X.L. Yang, Y.Z. Li, Y. Lu, Z.J. Guo. *Inorg. Chem.*, **44**, 3422 (2005).
[17] Y.B. Fan, Y.Q. Gao. *J. Am. Chem. Soc.*, **129**, 905 (2007).
[18] Q. Jiang, Z.Y. Wu, Y.M. Zhang, A.C.G. Hotze, M.J. Hannonb, Z.J. Guo. *Dalton Trans.*, 3054 (2008).
[19] J.L. García-Giménez, G. Alzuet, M. González-Álvarez, A. Castiñeiras, M. Liu-González, J. Borrás. *Inorg. Chem.*, **46**, 7178 (2007).
[20] C.Q. Xia, N. Jiang, J. Zhang, S.Y. Chen, H.H. Lin, X.Y. Tan, Y. Yue, X.Q. Yu. *Bioorg. Med. Chem.*, **14**, 5756 (2006).
[21] S.G. Srivatsan, M. Parvez, S. Verma. *J. Inorg. Biochem.*, **97**, 340 (2003).
[22] (a) J.H. Li, J.T. Wang, L.Y. Zhang, Z.N. Chen, Z.W. Mao, L.N. Ji. *Inorg. Chim. Acta*, **362**, 1918 (2009); (b) J.H. Li, J.T. Wang, P. Hu, L.Y. Zhang, Z.N. Chen, Z.W. Mao, L.N. Ji. *Polyhedron*, **27**, 1898 (2008); (c) J.H. Li, J.T. Wang, Z.W. Mao, L.N. Ji. *Inorg. Chem. Commun.*, **11**, 865 (2008); (d) J.H. Li, J.T. Wang, Z.W. Mao, L.N. Ji. *J. Coord. Chem.*, **62**, 446 (2008); (e) J.H. Li, J.T. Wang, L.Y. Zhang, Z.N. Chen, Z.W. Mao, L.N. Ji. *J. Coord. Chem.*, **62**, 1775 (2008); (f) Y. An, Y.Y. Lin, H. Wang, H.Z. Sun, M.L. Tong, L.N. Ji, Z.W. Mao. *Dalton Trans.*, 1250 (2007); (g) Y. An, M.L. Tong, L.N. Ji, Z.W. Mao. *Dalton Trans.*, 2066 (2006); (h) Y. An, S.D. Liu, S.Y. Deng, L.N. Ji, Z.W. Mao. *J. Inorg. Biochem.*, **100**, 1586 (2006).
[23] (a) J. He, J. Sun, Z.W. Mao, L.N. Ji, H.Z. Sun. *J. Inorg. Biochem.*, **103**, 851 (2009); (b) J. He, P. Hu, Y.J. Wang, M.L. Tong, H.Z. Sun, Z.W. Mao, L.N. Ji. *Dalton Trans.*, 3207 (2008).
[24] J.T. Wang, Q. Xia, X.H. Zheng, H.Y. Chen, H. Chao, Z.W. Mao, L.N. Ji. *Dalton Trans.*, 2128 (2010).
[25] (a) C.Q. Xia, X.Y. Tan, S.Y. Chen, Y. Yue, X.Q. Yu. *Arkivoc.*, **ii**, 68 (2006); (b) L.-M. Tumir, I. Piantanida, I. Juranović, Z. Meić, S. Tomić, M. Žinić. *Chem. Commun.*, 2561 (2005); (c) L.-M. Tumir, I. Piantanida, I.J. Cindrić, T. Hrenar, Z. Meić, M. Žinić. *J. Phys. Org. Chem.*, **16**, 891 (2003); (d) I. Juranović, Z. Meić, I. Piantanida, L.-M. Tumir. M. Žinić. *Chem. Commun.*, 1432 (2002); (e) A. Martelli, J.-F. Constant, M. Demeunynck, J. Lhomme. P. Dumy. *Tetrahedron*, **58**, 4291 (2002).
[26] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery Jr, T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople. *Gaussian 03, Revision D.01*, Gaussian, Inc., Wallingford, CT (2004).
[27] A. Sreedhara, J.D. Freed, J.A. Cowan. *J. Am. Chem. Soc.*, **122**, 8814 (2000).
[28] X. Sheng, X.M. Lu, J.J. Zhang, Y.T. Chen, G.Y. Lu, Y. Shao, F. Liu, Q. Xu. *J. Org. Chem.*, **72**, 1799 (2007).
[29] (a) S.J. Franklin, J.K. Barton. *Biochemistry*, **37**, 16093 (1998); (b) R.H. Terbrueggen, T.W. Johann, J.K. Barton. *Inorg. Chem.*, **37**, 6874 (1998).
[30] R.P. Hertzberg, P.B. Dervan. *Biochemistry*, **23**, 3934 (1984).
[31] (a) A. Nomura, Y. Sugiura. *J. Am. Chem. Soc.*, **126**, 15374 (2004); (b) K.J. Humphreys, A.E. Johnson, K.D. Karlin, S.E. Rokita. *J. Biol. Inorg. Chem.*, **7**, 835 (2002).
[32] (a) Y.T. Li, C.Y. Zhu, Z.Y. Wu, M. Jiang, C.W. Yan. *J. Coord. Chem.*, **62**, 3795 (2009); (b) J. Sun, S.Y. Deng, L. Zhang, J. He, L. Jiang, Z.W. Mao, L.N. Ji. *J. Coord. Chem.*, **62**, 3284 (2008).
[33] L. Strekowski, B. Wilson. *Mutat. Res.*, **623**, 3 (2007).
[34] (a) X. Sheng, X.M. Lu, Y.T. Chen, G.Y. Lu, J.J. Zhang, Y. Shao, F. Liu, Q. Xu. *Chem. Eur. J.*, **13**, 9703 (2007); (b) V. Rajendiran, R. Karthik, M. Palaniandavar, V.S. Periasamy, M.A. Akbarsha, B.S. Srinag, H. Krishnamurthy. *Inorg. Chem.*, **46**, 8208 (2007); (c) S. Dhar, P.A.N. Reddy, A.R. Chakravarty. *Dalton Trans.*, 697 (2004); (d) T. Itoh, H. Hisada, T. Sumiya, M. Hosono, Y. Usui, Y. Fujii. *Chem. Commun.*, 677 (1997).
[35] O.I. Aruoma, B. Halliwell, M. Dizdaroglu. *J. Biol. Chem.*, **264**, 13024 (1989).
[36] (a) M.E. Branum, A.K. Tipton, S. Zhu, L. Que Jr. *J. Am. Chem. Soc.*, **123**, 1898 (2001); (b) B. Weiss, A. Jacquemin-Sablon, T.R. Live, G.C. Fareed, C.C. Richardson. *J. Biol. Chem.*, **243**, 4543 (1968).
[37] E. Trotta, N. Del Grosso, M. Erba, M. Paci. *Biochemistry*, **39**, 6799 (2000).
[38] P. Wittung, P. Nielsen, B. Norden. *J. Am. Chem. Soc.*, **118**, 7049 (1996).