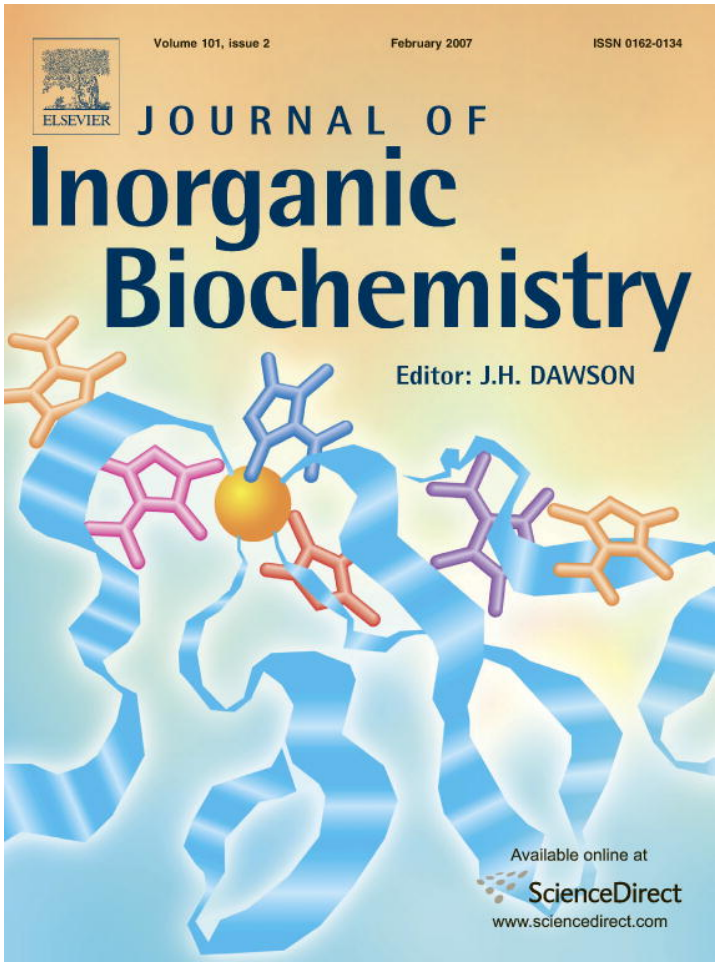

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DNA-binding and cleavage studies of novel binuclear copper(II) complex with 1,1′-dimethyl-2,2′-biimidazole ligand

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Abstract

DNA-binding properties of novel binulear copper(II) complex $[Cu_2(Dmbiim)_4(H_2O)_2](ClO_4)_4 \cdot 6H_2O$, where Dmbiim = 1,1′-Dimethyl-2,2′-biimidazole are investigated using electronic absorption spectroscopy, fluorescence spectroscopy, viscosity measurement and voltammetry. The results show that the copper(II) complex interacts with DNA through minor groove binding. The interaction between the complex and DNA has also been investigated by gel electrophoresis, interestingly, we found that the copper(II) complex can cleave circular plasmid pBR322 DNA efficiently in the presence of AH_2 (ascorbic acid) at pH 8.0 and 37 °C.

Keywords: Copper(II) complex; Biimidazole derivative; DNA-binding; DNA cleavage

1. Introduction

Binding studies of transition metal complexes have become a very important field in the development of DNA molecule probes and chemotherapeutics in recent years [1–10]. In order to find anticarcinogens that can recognize and cleave DNA, people synthesized and developed many kinds of complexes. Among these complexes, metals or ligands can be varied in an easily controlled way to facilitate the individual applications [11–14]. Copper is a bioessential element with relevant oxidation states. More than a dozen of enzymes that depend on copper for their activity have been identified; the metabolic conversions catalyzed by all of these enzymes are oxidative. Due to their importance in biological processes, copper(II) complexes synthesis and activity studies have been the focus from different perspectives. Among copper complexes explored so far, the copper(II) complexes of 1,10-phenanthroline and its derivatives attracts great attentions due to their high nucleolytic efficiency [15–22], which are able to break the DNA chain in the presence of H_2O_2 and reducing agents, these complexes have also been broadly used as foot printing agents of both proteins and DNA [23], probes of the dimensions of the minor groove of duplex structures [14], and identifiers of transcription starting sites [24]. However, most of the copper(II) complexes break the DNA chain rely to the presence of H_2O_2. And owing to the fact that all these complexes show their own selectivity for a cleavage mechanism or for DNA interaction, the design of new DNA cleavage agents is of great interest.

In addition, imidazoles are a common component of a large number of natural products and pharmacologically active molecules [25]. The imidazole ring functions as a ligand toward transition metal ions in a number of biologically important systems [25,26]. These facts make imidazole and its derivatives important target analytes. It was reported that the metal coordination compound of imidazole could inhibit tumor growth by interacting with DNA [27–29]. The presence of an imidazole moiety in biological molecules has the encouraged studies of H_2 biim-containing transition metal complexes [30,31]. However, few

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studies about the interaction between the transition metal complexes of 1,1′-dimethyl-2,2′-biimidazole (Dmbiim) and DNA have been reported, and most of the studies are limited in the structure characterization [32,33].

By these reasons, we aimed to discover a new DNA binding and cleavage reagent containing Dmbiim ligand. In this paper, the interaction of a novel binuclear copper(II) complex $[Cu_2(Dmbiim)_4(H_2O)_2](ClO_4)_4 \cdot 6H_2O$, where Dmbiim = 1,1′-Dimethyl-2,2′-biimidazole with DNA has been investigated using electronic absorption spectroscopy, fluorescence spectroscopy, viscosity measurement and voltammetry. These results show that the copper(II) complex interacts with DNA through minor groove binding. Agarose gel electrophoresis experiment showed that the binuclear copper(II) complex can effectively cleave plasmid DNA in the presence of AH_2. The results should be valuable in understanding the mode of the complex with DNA as well as laying a foundation for the rational design of novel, powerful agents for probing and targeting nucleic acids.

2. Experimental

2.1. Materials

All chemicals were of reagent grade and purchased from commercial vendors. They were used as purchased without further purification unless otherwise noted. Calf thymus DNA was obtained from Sigma, UV–Vis spectrometer was employed to check DNA purity (A_{260}: $A_{280} > 1.80$) and concentration ($\varepsilon = 6600\ M^{-1}\ cm^{-1}$ at 260 nm). All the measurements about interaction of the complex with CT DNA were conducted using solutions of the complex in Tris–HCl buffer (pH 7.2) containing 5 mM Tris–HCl and 50 mM NaCl. Plasmid pBR322 DNA was purchased from Shanghai Biologic Engineering Co. and used without purification. The crystal structure of $[Cu_2(Dmbiim)_4(H_2O)_2](ClO_4)_4 \cdot 6H_2O$ was reported in our previous study [34]. The crystal structure of $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ cation is shown in Fig. 1. The complex is cage-shaped, with two Cu(II) ions bridged by Dmbiim and each Cu(II) ion chelated by four nitrogen atoms of Dmbiim and one oxygen atom of H_2O.

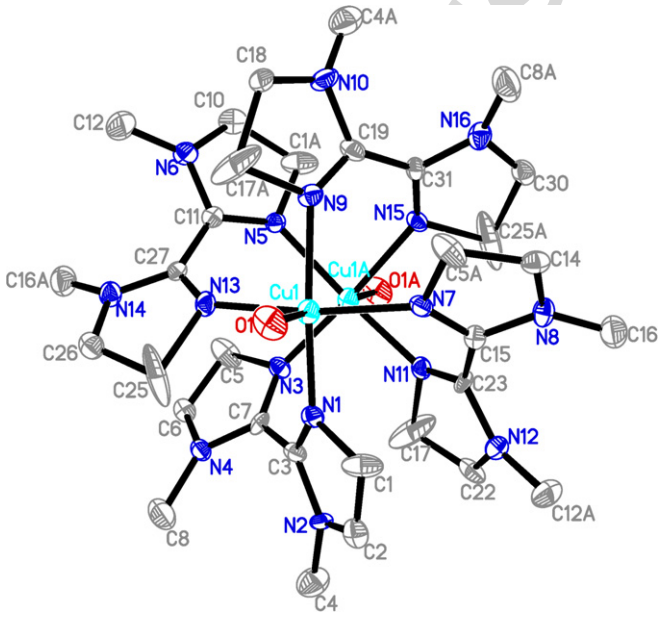

Fig. 1. The crystal structure of $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ cation.

2.2. Preparation of buffers

The buffer solution was prepared with double-distilled water. Buffer B-I, 5 mM Tris–HCl/50 mM NaCl, pH 7.2, for all spectroscopic studies, viscosity measurement and electrochemical experiments. Buffer B-II, 10 mM Tris–HCl/5 mM NaCl, pH 8, for plasmid pBR322 DNA and the Cu(II) complex in electrophoretic experiments. Buffer B-III, 40 mM Tris/19 mM acetate acid/1 mM EDTA, pH 7.4, for electrophoretic experiments.

2.3. Physical measurements

Electronic absorption spectra were recorded on a Hewlett–Packard HP-8453 Chemstation spectrometer. Fluorescene measurements were made with a Perkin–Elmer LS-50B fluorescence spectrophotometer.

Viscosity experiments were carried on an Ubbelodhe viscometer, immersed in a thermostated water-bath maintained at a constant temperature at 30.0 ± 0.1 °C. Flow time was measured with a digital stopwatch and each sample was measured three times and an average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ versus binding ratio [35], where η is the viscosity of DNA in the presence of complex, and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solutions ($t > 100$ s) corrected for the flow time of buffer alone (t_0), $\eta = t - t_0$.

Cyclic voltammetry were performed on a CHI 660B electrochemical analyzer (China) with three-electrode system consisted of a pyrolic graphite electrode (EPG) as the working electrode, a platinum wire as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode. All the electrochemical measurements were carried out in a 10 mL electrolytic cell. Solutions were deoxygenated by purging with N_2 prior to measurements. The freshly polished electrode was modified by transferring a droplet of 2 μL of 7.77×10^{-3} M of DNA solution onto its surface., followed by air-drying. Then the electrode was rinsed with distilled water. Thus, a DNA-modified EPG electrode was obtained. This was denoted as DNA/EPG throughout.

The cleavage of plasmid DNA was determined by agarose gel electrophoresis. The gel electrophoresis experiments were performed by incubation at 37 °C for 1.5 h of 0.014 mg mL^{-1} pBR322 DNA, 0.25 mM Cu(II) complex and 0.125 mM AH_2 (ascorbic acid) in buffer B-II. In some

tests, either 0.4 M DMSO, 0.4 M glycerol or 2.5 M MeOH was added in buffer B-II. Concentration of the Cu(II) complex was also varied from 50 to 500 μM in some tests. After incubation, samples were electrophoresed for 3 h at 75 V on 0.8% agarose gel using buffer B-III. The gel was then stained using 1 mg mL^{-1} ethidium bromide (EB) and photographed under ultraviolet light. All the experiments were performed at room temperatures (20–23 °C) unless otherwise noted.

2.4. Molecular modeling

The complex-DNA interactions were also studied by molecular modeling. All calculations were performed in SGI workstation with Insight II software package. The main calculating program was DISCOVER 98. Default settings for that program were used. The system studied contained DNA and two Cu atoms. ESFF force field could deal with the system efficiently and could offer more output information for analysis, so this force field was used with its default parameters. At the beginning of optimization, the Steepest Descent method was used until the root-mean-square (RMS) derivation was less than 5 kcal/mol. Then it was switched to Conjugate Gradient method automatically by the DISCOVER 98 program. When the RMS derivation was less than 0.5 kcal/mol, optimization was stopped. Calculations of all systems containing DNA were carried out in aqueous solution, while other systems in vacuum.

As a starting point, the structure of the metal complex was generated from the X-ray structure. Because the CT-DNA used in the experimental work was too large for current computational resources to model, the structure of the DNA $d(CCGTCGACGG)_2$ (a familiar sequence used in oligodeoxynucleotide study) was constructed in BIOPOLYMER Module of Insight II package to study the DNA binding characters of the complex. Both metal complex and DNA were optimized in ESFF force field before the interactions were investigated. The DNA-complex interactions were examined by comparing the potential energy differences among different binding sites of both minor and major grooves. As the DNA sequence used is symmetric, the same sequences in two end of DNA were not modeled repeatedly.

3. Results and discussion

3.1. Electronic spectral studies

In order to examine the binding mode of DNA with $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$, ethidium bromide (EB) was employed in this experiment, as EB interacts presumably with DNA as a typical indicator of intercalation [36]. Fig. 2 showed that the maximal absorption of EB at 470 nm decreased and shifted to 479 nm in the presence of DNA, which was characteristic of intercalation. Fig. 2(c) was the absorption of the mixture solution of

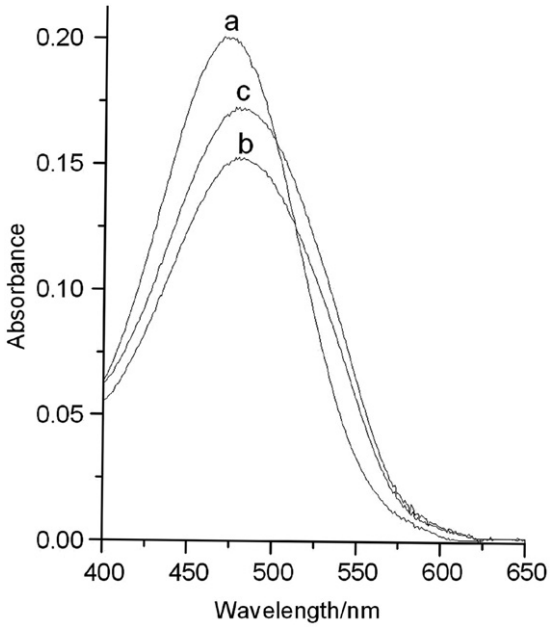

Fig. 2. The visible absorption spectra of 4.72×10^{-5} M EB (a); (a) $+6.73 \times 10^{-5}$ M DNA (b); (b) $+6.76 \times 10^{-5}$ M $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ (c).

EB, $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ and DNA. It was found that the absorption at 479 nm increased comparing with Fig. 2(b). It could result from two reasons: (1) EB bound to $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ strongly, resulting in the decrease of the amount of EB intercalated into DNA; (2) The binding mode of DNA with $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ is may be the minor groove binding, so releasing some free EB from DNA–EB complex [37]. However, the former reason could be precluded because there was not new absorption peak appeared.

3.2. Fluorescence spectroscopic studies

EB emits intense fluorescent light in the presence of DNA due to its strong intercalation between the adjacent DNA base pairs. It was previously reported that the enhanced fluorescence could be quenched by the addition of another molecule [38,39]. In fact, the extent of fluorescence quenching for EB bound to DNA is used to determine the extent of binding between another molecule and DNA. The emission spectra of EB bound to DNA in the absence and presence of $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ complex are shown in Fig. 3. The addition of the complex to DNA pretreated with EB causes an appreciable reduction in fluorescence intensity, indicating that $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ complex competes with EB to bind with DNA. According to the classical Stern–Volmer equation [39]:

$$I_0/I = 1 + K_{sq}r, \quad (1)$$

where I_0 and I represent the fluorescence intensities in the absence and presence of the complex, respectively, and r is the concentration ratio of the complex to DNA. K_{sq} is

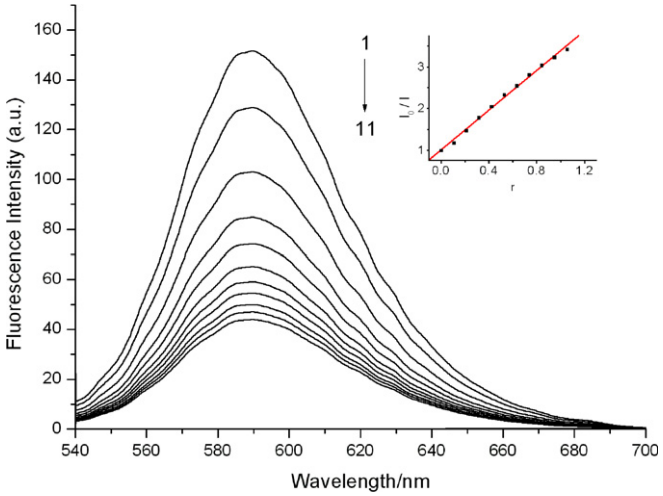

Fig. 3. Fluorescene spectra of the binding of EB to DNA in the absence and presence of increasing amounts of $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$, $\lambda_{ex} = 525$ nm, $C_{EB} = 5.08$ μ M, $C_{DNA} = 50.8$ μM, C_{Cu} (μM): (1) 0, (2) 5.34, (3) 10.68, (4) 16.02, (5) 21.36, (6) 26.7, (7) 32.04, (8) 37.38, (9) 42.72, (10) 48.06, (11) 53.4. The inset is Stern–Volmer quenching plots. $r = [[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}]/[DNA]$.

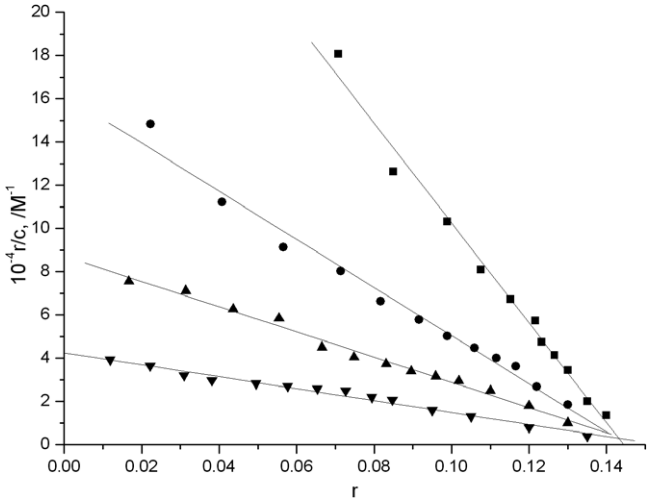

Fig. 4. Scatchard plots for the binding of EB to DNA in the absence (■) and presence of 4 μM (●), 8 μM (▲), 16μM (▼) $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ complex.

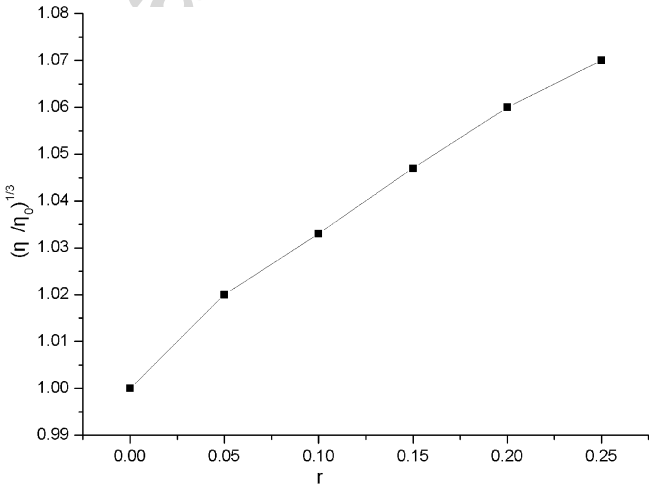

Fig. 5. Effect of increasing amounts of $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ on the relative viscosity of CT DNA at 30 ± 0.1 °C. [DNA] = 0.5 mM, $r = [[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}]/[DNA] = 0$, 0.05, 0.1, 0.15, 0.2, 0.25, respectively.

a linear Stern–Volmer quenching constant dependent on the ratio of the bound concentration of EB to the concentration of DNA (seen in Fig. 3). The K_{sq} value is obtained as the slope of I_0/I versus r linear plot. From the inset in Fig. 3, the K_{sq} value for $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ complex is 2.39. Such a value of quenching constant suggests that the interaction of the complex with DNA is strong [40]. It may be due to the complex interacts with DNA through minor groove binding, so releasing some free EB from DNA–EB complex [37], which is consistent with the above absorption spectral results.

In order to get a better insight into the nature of complex-DNA binding, we have carried out a fluorescence study of EB to DNA in the presence of a competing metal complex. The characteristics of the binding of EB to DNA can be expressed by Scatchard equation [41].

$$r/C = K(n - r) \quad (2)$$

Here, r is the ratio of bound EB to total nucleotide concentration, C is the concentration of free EB, n is the number of binding sites per nucleic acid, K is the intrinsic binding constant for EB. Fluorescence Scatchard plots for the binding of EB to DNA (1.6×10^{-5} M) in the presence of $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ are shown in Fig. 4. The K value in the Scatchard plot change but the n value no change, which indicates that the complex binds to DNA by competitive inhibition [42]. The competitive inhibition is probably due to the complex interacts with DNA through minor groove binding, blocking potential intercalation sites of EB.

3.3. Viscosity measurements

As a means for further clarifying the binding of $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ complex with DNA, viscosity of DNA solutions containing varying amount of added complex were measured. The results indicate that the presence of the complex increases the viscosity of the DNA solutions, as illustrated in Fig. 5. As general viewpoint, drug molecules can increase the viscosity of DNA when they intercalate into the double-stranded DNA or bind to the phosphate group of DNA backbone [43]. The configuration of the complex inhibits its intercalation into the double-stranded DNA. Therefore, we can conclude that the complex maybe electrostatically bind to phosphate group of DNA backbone.

3.4. Electrochemical studies

Fig. 6 shows the cyclic voltammograms of $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ at a bare EPG (solid line) and DNA/

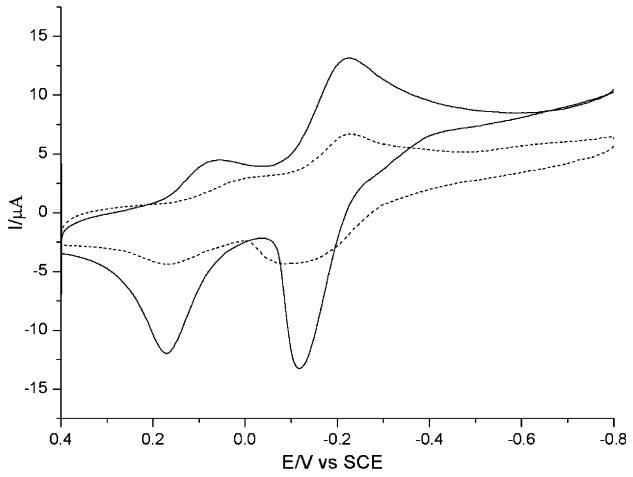

Fig. 6. Cyclic voltammograms of 0.5 mM $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ at a bare EPG (solid line) and DNA/EPG (dash line), scan rate 100 mV s^{-1}.

EPG (dash line). The cyclic voltammograms of $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ at a bare EPG featured two anodic peaks ($E_{pa1} = -0.118$ V and $E_{pa2} = 0.171$ V vs SCE) and two cathodic peaks ($E_{pc1} = -0.226$ V and $E_{pc2} = 0.056$ V vs SCE). The oxidation of peak E_{pa1} belongs to Cu(0)/Cu(I), and reduction of Cu(I) occurred, upon scan reversal at −0.226 V. The separation of the anodic and cathodic peak potential is 108 mV, and the ratio of anodic to cathodic peak currents, $i_{pa}/i_{pc} = 1.34$, indicating a quasi-reversible redox process. The oxidation of peak E_{pa2} belongs to Cu(I)/Cu(II), and reduction of Cu(II) occurred, upon scan reversal at 0.056 V. The separation of the anodic and cathodic peak potential is 115 mV, and the ratio of anodic to cathodic peak currents, $i_{pa}/i_{pc} = 3.51$, indicating a quasi-reversible redox process. The formal potential $E_{1/2}$, taken as average of E_{pc} and E_{pa} is −172 mV and 113.5 mV, are observed at the bare EPG, respectively. However, the formal potential $E_{1/2}$ is −164.5 mV and 92.55 mV, are observed at the same concentration at the DNA/EPG electrode, respectively. Compared to the peak currents for $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ in solution at the same concentration at the bare EPG electrode, the peak currents are lower at the DNA/EPG electrode coupled with a obvious shift in the peak (Cu(I)/Cu(II)) potential to negative and a slight shift in the peak (Cu(0)/Cu(I)) potential to positive. Among the three kinds of binding modes for small molecules to DNA, Bard has reported [44] that if $E_{1/2}$ shifted to more negative value when small molecules interacted with DNA, the interaction mode was electrostatic binding. On the contrary, if $E_{1/2}$ shifted to more positive value, the interaction mode was intercalative binding. Therefore, the conclusion that the $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ could bind to DNA by electrostatic binding was drawn.

3.5. Molecular modeling

The modeling results were tabulated in Table 1. In the table, Groove means which groove the complex interacts; Location means the binding site; Internal is the sum of items (bond = bond stretch, angle, torsion, out-of-plane) about bond interactions; Non-B means non-bond interaction, it compose of Van de Waals energy (VDW) and electrostatic energy (Elect); Total means total energy. As the table s hows, the values of total energy indicate that the minor groove binding of the complex in C_5G_6 region is the most preferential binding modeling of complex-DNA interactions (as shown in Fig. 7). It is worthy of note that the energies about bond items in minor groove binding is usually larger than that in major groove binding, while those of non-bond items in minor groove binding are much lower than that in major groove binding sometimes. In the non-bond energies, the VDW items varied not so essential in different site; it is the enormous decrease in electrostatic items, however, that finally decrease the total energy of the minor groove binding of the complex in C_5G_6 region to the minimum, which was consist with the results our previous modeling study in complex-DNA interactions that the results will be determined by electrostatic interactions [45–48] and closely correspond the experimental results mentioned above.

3.6. Cleavage of plasmid pBR322 DNA

It is known that DNA cleavage is controlled by relaxation of supercoiled circular conformation of pBR322 DNA nicked circular and/or linear conformations. When electrophoresis is applied to circular plasmid DNA, a fastest migration will be observed for DNA of closed circular conformations (Form I). If one strand is cleaved, the supercoil will relax to produce a slower-moving nicked conformation

Table 1
The energy distribution of the complex–DNA interactions

Groove	Location	Inter	Bond	Angle	Torsion	Out	Non-B	VDW	Elect.	Total
Major	C_2G_3	984.5	31.2	532.5	351.2	69.6	775.8	−2.5	778.3	1760.3
	G_3T_4	968.1	30.7	526.1	343.6	67.7	566.5	−6.9	573.4	1534.6
	T_4C_5	966.8	30.6	523.9	343.5	68.8	495.1	−5.3	500.4	1461.9
	C_5G_6	972.6	30.8	527.2	347.8	66.8	591.8	−7.9	599.7	1564.4
Minor	C_2G_3	984.0	29.1	539.7	347.9	67.2	637.3	5.4	631.9	1621.3
	G_3T_4	979.8	31.0	542.0	350.0	56.8	570.1	−5.5	575.6	1549.9
	T_4C_5	999.1	30.4	564.3	345.2	59.2	398.8	5.8	393.0	1397.9
	C_5G_6	997.2	31.0	550.3	347.8	68.0	390.5	10.9	379.6	1387.7

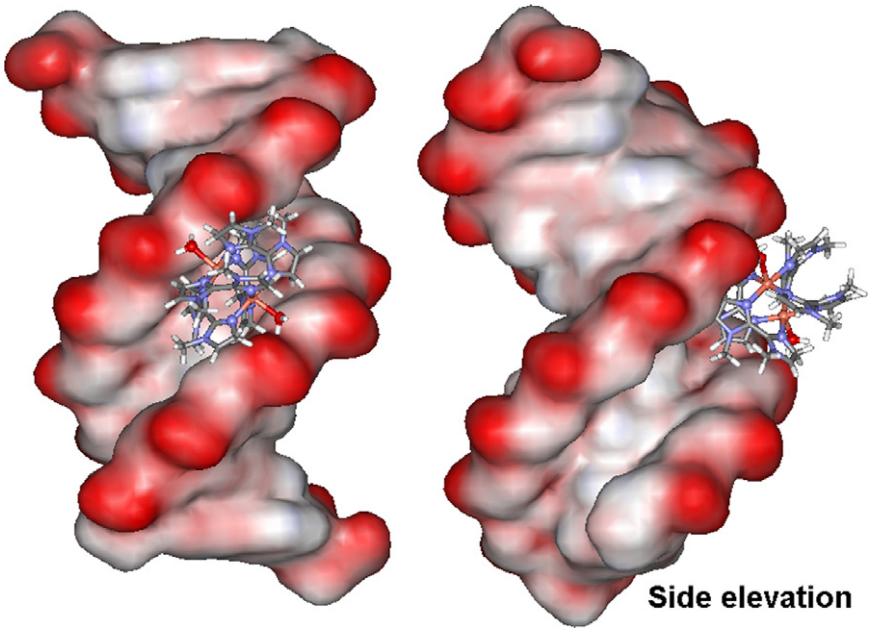

Fig. 7. The optimized binding model of $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$-DNA interactions (left picture) and its side elevation (right picture), it is clear that the complex inserts and stays at the DNA minor groove.

(Form II). If both strands are cleaved, a linear conformation (Form III) will be generated that migrates in between [49,10].

Fig. 8 shows the results of gel electrophoretic separations of plasmid pBR322 DNA induced by an increasing amount of $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ in the presence of AH_2 (ascorbic acid). Under the same conditions, free AH_2 produced no cleavage of pBR322 (lane 2). All supercoiled (form I) DNA was cleaved to form the mixture of form II and form III in the concentration of 2.5×10^{-4} M (lane 6). The conversion

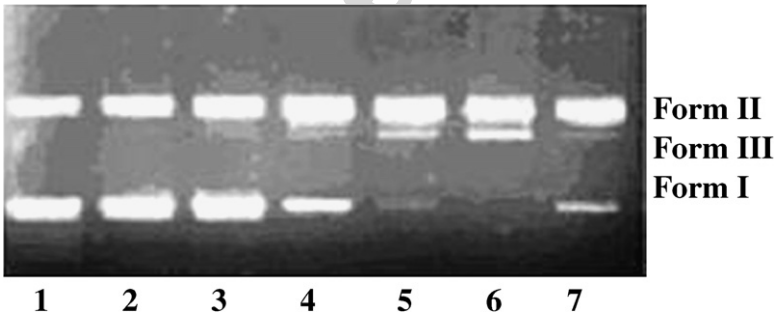

Fig. 8. Cleavage of pBR322 plasmid DNA (0.014 mg mL^{-1}) induced by $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ in the presence of AH_2 (0.125 mM), lane: (1) DNA alone, (2) DNA + AH_2, C_{Cu} (μM): (3) 50, (4) 75, (5) 100, (6) 250, (7) 500.

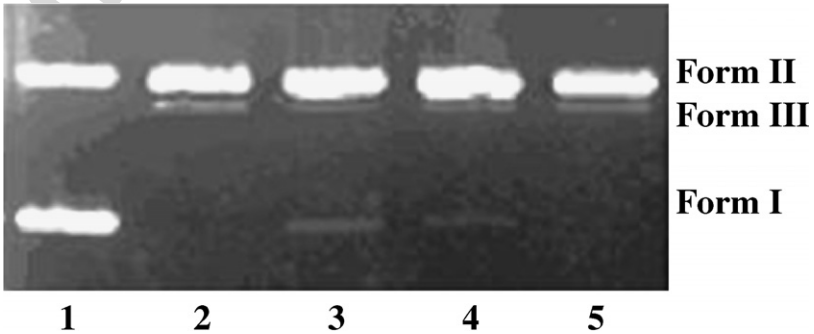

Fig. 9. Cleavage of pBR322 plasmid DNA (0.014 mg mL^{-1}) by $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ (250 μM) in the absence or presence of radical scavengers, lane: (1) DNA alone, (2) DNA + $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ + AH_2, (3) DNA + $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ + AH_2 + 0.4 M DMSO, (4) DNA + $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ + AH_2 + 0.4 M glycerol, (5) DNA + $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ + AH_2 + 2.5 M MeOH.

of form I to form II and form III were observed with the increase in concentration of the complex when concentration was lower than 2.5×10^{-4} M, the effect of cleavage was the best when the concentration was 2.5×10^{-4} M (lane 6), and the effect of cleavage was better than that of the complexes as 5×10^{-4} M (lane 7). The formation of form III began to appear in the presence of the complexes as 7.5×10^{-5} M (lane 4). These phenomena imply that $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ complex induces intensively the cleavage of plasmid pBR322 DNA in the presence of AH_2.

In order to clarify the cleavage mechanism of pBR322 DNA induced by $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ complex, further investigation was carried out. When pBR322 DNA was incubated with the complex in the presence of either 0.4 M DMSO, 0.4 M glycerol or 2.5 M MeOH as hydroxyl radical scavengers, only slight inhibition of the DNA cleavage was observed (Fig. 9). These observations suggested that $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$-mediated cleavage reaction did not proceed via radical cleavage.

In summary, $[Cu_2(Dmbiim)_4(H_2O)_2](ClO_4)_4 \cdot 6H_2O$ is able to perform an efficient cleavage of pBR DNA. Further studies on the reaction mechanism as well as on sequence selectivity of the copper(II) complex are in progress.

4. Conclusions

The properties of DNA binding and cleaving by a novel binuclear Cu(II) complex with 1,1′-Dimethyl-2,2′-biimidazole ligand have been studied. From this study, the following conclusions are derived.

1. $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ complex could interact with DNA through minor groove binding.
2. $[Cu_2(Dmbiim)_4(H_2O)_2]^{4+}$ complex are capable of cleaving pBR322 DNA in the presence of AH_2.

5. Abbreviations

AH_2	ascorbic acid
CT DNA	calf-thymus DNA
Dmbiim	1,1′-Dimethyl-2,2′-biimidazole
DMSO	dimethyl sulfoxide
EB	ethidium bromide
EDTA	ethylenediamine tetraacetic acid
EPG	pyrolic graphite electrode
SCE	saturated calomel electrode
Tris	tris (hydroxymethyl) aminomethane

Acknowledgements

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