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Synthesis, crystal structure, and DNA-binding properties of a new copper (II) complex containing mixed-ligands of 2,2′-bipyridine and *p*-methylbenzoate

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Abstract

A novel copper complex of $[Cu(bpy)(pba)_2 \cdot H_2O] \cdot 0.5H_2O$ (bpy = 2,2′-bipyridine, pba = *p*-methylbenzoate) was synthesized. The interaction of the complex to native fish sperm DNA was investigated through electrochemistry, electronic absorption spectroscopy and viscosity experiments. In the X-ray crystallography structure, the copper (II) ion is coordinated by two oxygen atoms of two *p*-methylbenzoate groups, two nitrogen atoms of 2,2′-bipyridine and one water molecule. The observed changes in the physicochemical features of the copper (II) complex on binding to DNA suggested that the complex bind to DNA with intercalation mode via 2,2′-bipyridine ring into DNA base pairs. Electrochemical studies revealed that the complex prefer to bind to DNA in Cu(I) form rather than Cu(II) oxidation state form. Additionally, the nuclease activity of the title complex was assessed by gel electrophoresis assay and the results shown that the copper complex can cleave pBR322 DNA effectively in the presence of ascorbic acid.

Keywords: 2,2′-Bipyridine ligand; Copper (II) complex; Crystal structure; DNA

1. Introduction

Copper (Cu) is a physiologically important metal element that plays an important role in the endogenous oxidative DNA damage associated with aging and cancer [1]. Some of copper (II) complexes are found to exhibit a variety of pharmacological activity and superoxide dismutase activity [2]. Complexes of copper (II) with carboxylate and imidazole ligands have been studied as model for copper protein that contains both functionalities in the side chain [3]. These potential and versatile applications are stimulating researchers to exploit new ligands and complexes with potential chemical, biological and catalytic properties.

Deoxyribonucleic acid (DNA) is the primary target molecule for most anticancer and antiviral therapies according to cell biology [4,5]. Investigations of the interactions of DNA with transition metal complex are basic work to design new types of the pharmaceutical molecules, to elucidate the mechanisms involved in the site specific recognition of DNA and to determine the principles governing the recognition [6,7]. Since Sigman et al. [8] discovered that copper ion complexed to 1,10-phenanthroline can cleave DNA in 1979, the copper complexes are widely used as footprint DNA binding molecules [9,10] and cleaving reagents [11–19] over the past two decades. Li et al. [19] have synthesized a Schiff base copper (II) complex, Cu(*o*-VANAHE)$_2$ (*o*-VANAHE = 2-(*o*-vanillinamino)-1-hydroxyethane) and assessed the interaction between this complex and DNA by

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viscosity, fluorescence spectroscopy, cyclic voltammetry and submarine gel electrophoresis. The results suggested that this copper complex can intercalate into helix DNA and cleave circular plasmid pBR322 DNA to nicked and linear forms. A new ligand L, 1-[3-(2-pyridyl)pyrazol-1-ylmethyl]naphthalene, and its copper complexe, $[Cu(L)_3](ClO_4)_2$ have been synthesized and characterized by Zhang et al. [20]. The interactions of the complex and the ligand L with calf thymus DNA were then investigated by thermal denaturation, viscosity measurements and spectrophotometric methods. The experimental results indicated that the copper complex bind to DNA by intercalative mode via the ligand L. The intrinsic binding constants are $1.8 \times 10^4\ M^{-1}$ and $2.8 \times 10^3\ M^{-1}$ for the complex and ligand L with DNA, respectively. Pasternack and his co-workers [21] have described the interactions of three cationic water soluble copper (II) porphyrins, differing in peripheral substituents, with calf thymus DNA using extinction spectroscopy, circular dichroism, RLS and resonance Raman spectroscopy. It was found that tetrakis(*N*-methylpyridinium-4-yl)porphyrin copper (II) behaves as a simple intercalator under the investigated conditions, whereas tetrakis(4-*N*,*N*′,*N*″-trimethylanilinium) porphyrin copper (II), binds externally, with some limited aggregation under high drug load conditions. In contrast, *trans*-bis(*N*-methylpyridinium-4-yl)diphenylporphyrin copper (II) (*t*-CuPagg), like the free-base *t*-H_2Pagg from which it is derived, is capable of forming extended electronically coupled arrays while bind to the DNA template.

In this paper, we synthesized and obtained the crystal structure of a new aqua-2,2′-bipyridine copper (II) bis(*p*-methylbenzoate) hydrate: $[(bpy)Cu(pba)_2 \cdot H_2O] \cdot 0.5H_2O$. The binding properties of the title complex to fish sperm DNA were carried out using voltammetry, absorption spectroscopy and viscosity experiment, and so on. The binding mode of the copper complex to DNA is accessed to be intercalation from the experimental results, which implicated that the copper (II) complex can be a candidate for DNA-binding reagents, as well as laying the foundation for the rational design of new useful DNA probes. The gel electrophoresis experiment shown that the copper complex can cleave pBR322 DNA effectively in the presence of ascorbic acid as an effective inorganic nuclease.

2. Experimental

2.1. Materials

Double-strand fish sperm DNA from Beijing Baitai Biochemistry Technology Company (China) was used as received. The stock solution of DNA was prepared by dissolving appropriate amount of DNA in H_2O and stored at 4 °C. The ratio of the absorbance at 260 and 280 nm (A_{260}/A_{280}) was checked to be ~1.89, indicating that the DNA is sufficiently free from protein [22]. The concentration of DNA in nucleotide phosphate [NP] was determined spectrophotometrically at 260 nm after 1:100 dilutions using the known molar extinction coefficient value of $6600\ M^{-1}\ cm^{-1}$ [23]. Supercoiled plasmid pBR322 DNA was purchased from TaKaRa Biotechnology Co., Ltd. (China). Ethidium bromide (EB) was obtained from Fluka Company (Switzerland). The other chemical reagents for synthesizing copper complex were all purchased commercially and used without further purification. Double distilled water was used for preparing all the solutions.

2.2. The preparation of the title complex

$(pba)_2Cu$ was prepared by mixing aqueous solutions of copper (II) sulfate and sodium *p*-methylbenzoate according to the literature procedures [24]. To a warm solution of 2,2′-bipyridine (0.16 g, 1 mmol) in EtOH (50 mL) was added with stirring $(pba)_2Cu$ (0.33 g, 0.1 mmol) and the mixture was refluxed for 2 h. The resulting solution was filtered and was left to stand overnight. Blue precipitation appeared. A blue crystal suitable for X-ray analysis was obtained by recrystallizing this blue solid from ethanol solution. The C, H and N contents were determined by elemental analysis. *Anal*. Calc. for $C_{26}H_{25}Cu_1N_2O_{5.5}$: C, 60.40; H, 4.87; N, 5.42. Found: C, 60.51; H, 4.95; N, 5.34%.

2.3. The physical measurement of the title complex and its DNA-binding experiments

Elemental analyses (C, H and N) were carried out on a Perkin–Elmer 1400C analyzer. Voltammetry was performed by using CHI 832 electrochemical analysis system (China) with three-electrode system consisted of glass carbon (GC) electrode ($\Phi = 3$ mm) as the working electrode, saturated calomel electrode (SCE) as the reference electrode, and a platinum wire as the auxiliary electrode. All the electrochemical measurements were carried out in a 10 mL electrolyte cell with 0.01 M pH 6.86 KH_2PO_4–Na_2HPO_4 buffer solution as electrolyte. Electronic absorption spectrum was measured on a Cary 50 probe spectrophotometer (Australia) using 0.01 M pH 6.86 KH_2PO_4–Na_2HPO_4 as blank solution. Viscosity experiments were carried out using an Ubbelodhe viscometer maintained at a constant temperature at 30 ± 0.1 °C in a thermostatic water-bath. 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 $(\eta/\eta_o)^{1/3}$ versus binding ratio of [Cu]/[DNA], where η is the relative viscosity of DNA in the presence of complex, and η_o is the relative viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solutions corrected for the flow time of KH_2PO_4–Na_2HPO_4 buffer solution alone (t_0), $\eta = t - t_0$ [23]. The cleavage of DNA by the title copper complex was monitored using agarose gel electrophoresis. Reactions using 10 μM supercoiled (SC) pBR322 plasmid DNA in 50 mM pH 7.1 Tris–NaCl buffer was treated with the copper complex and 100 μM ascorbic acid. The samples were incubated for 0.5 h at 37 °C. A loading buffer containing

25% bromophenol blue, 3 μL 30% glycerol was added and the electrophoresis was performed at 40 V in Tris–acetate–EDTA (TAE) buffer using 1% agarose gel containing ethidium bromide. Agarose gel electrophoresis of plasmid DNA was visualized by photographing the fluorescence of intercalated ethidium bromide under a UV illuminator.

2.4. X-ray structure determination

Single-crystal X-ray diffraction measurement for the title complex was carried out on a SMART CCD diffractometer with graphite monochromated Mo Kα ($\lambda = 0.071073$ nm) radiation at the temperature of 293(2) K. Intensities were corrected for Lorentz and polarization effects and empirical absorption, and the data reduction using SADABS [25] program. The structure was solved by direct methods using SHELXS-97 [26]. All the non-hydrogen atoms were refined on F^2 anisotropically by full-matrix least-squares method. All hydrogen atoms were placed in calculated positions assigned fixed isotropic thermal parameters at 1.2 times the equivalent isotropic U of the atoms to which they were attached and allowed to ride on their respective parent atoms. The contributions of these hydrogen atoms were included in structure-factor calculations. Atomic scattering factors and anomalous dispersion corrections were taken from International Table for X-ray Crystallography [27]. A summary of the key crystallographic information is given in Table 1.

Table 1
Summary of crystallographic results for the title complex

Empirical formula	$C_{26}H_{25}Cu_1N_2O_{5.5}$
Formula weight	517.02
T (K)	293(2)
λ (Å)	0.71073
Crystal system	triclinic
Space group	$P\bar{1}$
Unit cell dimensions	
a (Å)	7.0230(14)
b (Å)	11.167(2)
c (Å)	16.700(3)
α (°)	103.89(3)
β (°)	90.77(3)
γ (°)	104.28(3)
V (Å^3)	1228.4(4)
Z	1
D_{calc} (Mg/m^3)	1.398
Absorption coefficient (mm^{-1})	0.930
$F(000)$	536
θ Range for data collection (°)	1.26–24.50
Limiting indices	$0 \leqslant h \leqslant 8$, $-13 \leqslant k \leqslant 12$, $-19 \leqslant l \leqslant 19$
Reflections collected/unique (R_{int})	4403/4030 (0.0833)
Completeness to $\theta = 24.50°$ (%)	98.8
Refinement method	full-matrix least-squares on F^2
Data/restraints/parameters	4030/0/317
Goodness-of-fit on F^2	1.018
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0705$, $wR_2 = 0.1438$
R indices (all data)	$R_1 = 0.1920$, $wR_2 = 0.1916$
Largest difference in peak and hole (e Å^{-3})	0.590 and −0.504

3. Results and discussion

3.1. Crystal structure description

Fig. 1 shows a perspective view of the title complex with atomic numbering. Selected bond lengths and bond angles are presented in Table 2.

In the molecule, each Cu ion is coordinated by two oxygen atoms of two *p*-methylbenzoate groups, two nitrogen atoms of 2,2′-bipyridine and one water molecule as showed in Fig. 1. The coordination geometry of the copper center is essentially square pyramid with Cu(1)N(1)N(2)O(1)O(3)···O(1w) chromospheres. The *p*-methylbenzoate anion behaves as a monodentate carboxylate ligand. The Cu–O distances [Cu(1)–O(1) = 1.967(5) Å; Cu(1)–O(3) = 1.949(5) Å] in title complex are normal and are in close agreement with those found in a similar structure reported before [28]. The axial Cu(1)–O(1w) distance of 2.359(5) Å is in agreement with that found in a similar reported Cu–O(1w) bond [32], while, is significantly longer than those of the equatorial Cu(1)–O(1), O(3) bonds. The 13 atoms containing one Cu(1) atom and 12 atoms from 2,2′-bipyridine molecule are almost coplanar, the deviation of the Cu atom from the least-squares plane through the 13 atoms being 0.057(3) Å. The dihedral angle between the two *p*-methylbenzoate ligands planes is 61.71(2)°. The *p*-methylbenzoate ligand including O(1) made a dihedral of 87.90° with 2,2′-bipyridine ligand. The angles with the axial atom O(1w) at Cu(1), viz. O(1w)–Cu(1)–O(1), O(3), N(1), N(2) are 107.6(2)°, 91.1(2)°, 88.5(2)°, 93.7(2)°, respectively.

In the lattice, there are some intramolecular, intermolecular and potentially weak (C–H···Y hydrogen bonds, Y = O, N and Cl) interactions. For intramolecular hydrogen bonds, the donor and acceptor distances are 3.0257 Å [O(2W)···O(3)] and 2.6646 Å [O(1W)···O(4)]; for intermo-

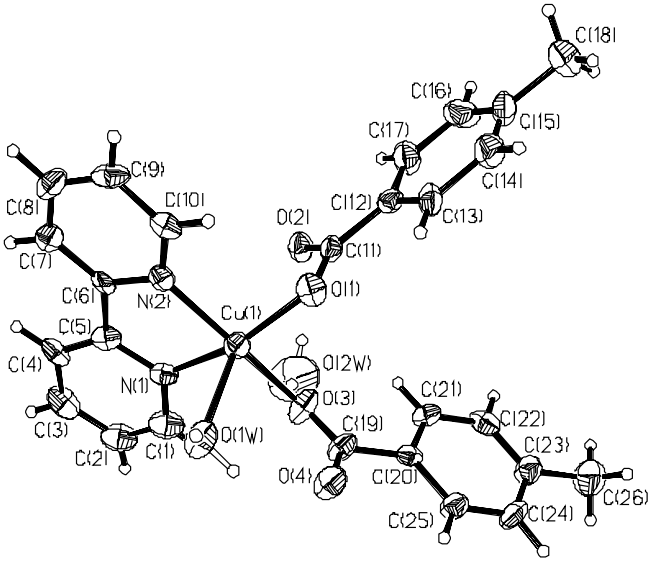

Fig. 1. Molecular structure of the title complex with the atomic numbering scheme.

Table 2
Selected bond lengths (Å) and bond angles (°)

Bond lengths (Å)		Bond lengths (Å)		Bond lengths (Å)	
Cu(1)–O(3)	1.949(5)	O(1)–C(11)	1.271(9)	N(1)–C(5)	1.357(9)
Cu(1)–O(1)	1.967(5)	O(2)–C(11)	1.267(8)	N(2)–C(10)	1.333(9)
Cu(1)–N(1)	2.001(6)	O(3)–C(19)	1.262(9)	N(2)–C(6)	1.350(9)
Cu(1)–N(2)	2.007(6)	O(4)–C(19)	1.235(9)		
Cu(1)–O(1W)	2.359(5)	N(1)–C(1)	1.313(10)		
Bond angles (°)		Bond angles (°)		Bond angles (°)	
O(3)–Cu(1)–O(1)	91.2(2)	O(1)–Cu(1)–N(2)	92.8(2)	N(1)–Cu(1)–O(1W)	88.5(2)
O(3)–Cu(1)–N(1)	93.8(3)	N(1)–Cu(1)–N(2)	80.5(3)	N(2)–Cu(1)–O(1W)	93.7(2)
O(1)–Cu(1)–N(1)	163.1(2)	O(3)–Cu(1)–O(1W)	91.1(2)	C(11)–O(1)–Cu(1)	105.5(5)
O(3)–Cu(1)–N(2)	172.5(2)	O(1)–Cu(1)–O(1W)	107.6(2)	C(19)–O(3)–Cu(1)	124.5(5)

lecular hydrogen bonds, the donor and acceptor distances are 2.7641 Å [O(1W)···O(2)] and 2.9672 Å [O(2W)···O(1w)], respectively; the O(2w) with C(8) atoms in methyl group form potentially weak C–H···O intermolecular interactions, the donor and acceptor distances are 3.2369(2) Å [C(8)···O(2w)]. All above hydrogen bonds in this structure form the three-dimensional hydrogen bonds network that stabilizes the crystal structure.

3.2. The studies on the binding of the title complex to DNA

3.2.1. Absorption spectroscopic studies

The application of electronic absorption spectroscopy in DNA-binding studies is one of the most useful techniques [29–31]. Complex binding with DNA through intercalation usually results in hypochromism and bathochromism, due to the intercalative mode involving a strong stacking interaction between an aromatic chromophore and the base pairs of DNA. The absorption spectra of the complex in the absence and presence of DNA are illustrated in Fig. 2. In the UV region, the two conjoint peaks of the complex at 300 and 310 nm, respectively, may attributed to intraligand π–π* transition of the coordinated groups. Addition of DNA results in hypochromism of both the absorption peaks and moderate bathochromic shift (ca. 2 nm) of the peak at 310 nm. These spectral characteristics are consistent with a mode of interaction that involves a stacking interaction between the complex and the base pairs of DNA, which means that the titled complex can intercalate into the double helix structure of DNA.

3.2.2. Viscosity studies

Optical photophysical probes are necessary, but not sufficient clues to support a binding mode of small molecules to DNA. Further clarification of the interaction mode between the copper complex and DNA is carried out by viscosity measurements. Hydrodynamic measurements that are sensitive to length change (i.e., viscosity and sedimentation) of DNA are regarded as the least ambiguous and the most critical tests of binding mode in solution in the absence of crystallographic structural data [32,33]. A classical intercalation mode results in lengthening the DNA helix, as base pairs are separated to accommodate the binding ligand, leading to the increase of DNA viscosity. The effects of the complex together with EB on the viscosity of DNA are shown in Fig. 3. It is found that the viscosity of DNA

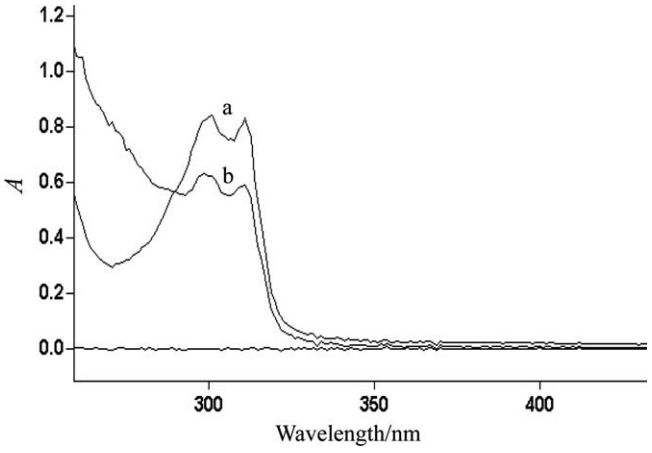

Fig. 2. Absorption spectra of 2.00×10^{-4} M title copper (II) complex in the absence (a) and presence of 1.02×10^{-4} M DNA (b) in 0.01 M pH 6.86 KH_2PO_4–Na_2HPO_4 buffer solution.

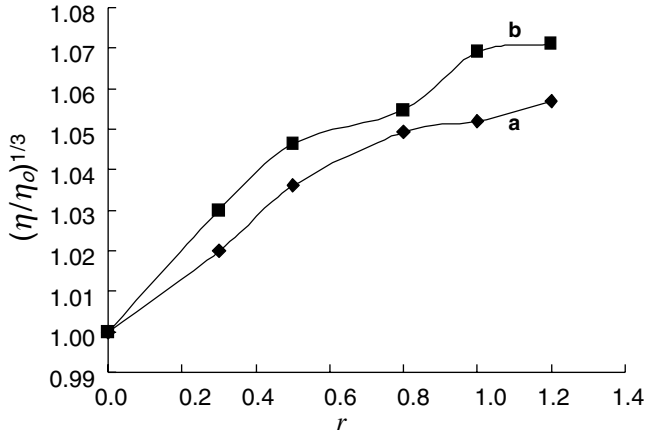

Fig. 3. Effect of increasing amounts of copper (II) complex (◆) and EB (■) on the relative viscosities of DNA at 30 ± 0.1 °C. r = [Cu]/[DNA] or [EB]/[DNA], [DNA] = 1.02×10^{-4} M.

increases steadily with the increase of the concentration of the complex (Fig. 3a), which is similar to that of a classical intercalator EB [34] (Fig. 3b). This result demonstrates that the copper complex and EB bind to DNA through the same way, i.e., the classical intercalation mode, which also parallels the pronounced hypochromism and spectral red shift of the complex in the absorption spectrum experiment.

3.2.3. Electrochemical studies

Cyclic and differential pulse voltammetric techniques was employed to study the interaction of the present redox active metal complex with DNA with a view to further explore the DNA binding modes assessed from the above spectral and viscometric studies.

Typical cyclic voltammetry curves for the title complex in 0.01 M pH 6.86 KH_2PO_4–Na_2HPO_4 buffer solution, in the absence and presence of DNA are shown in Fig. 4. In the absence of DNA, the complex has a cathodic peak at −0.335 V and an anodic peak at −0.115 V, respectively, corresponding to the electrochemical process of Cu^{II}/Cu^{I} [35]. The separation of the cathodic and anodic peak potential, $\Delta E = 0.240$ V, indicates that the electrochemical behavior of the copper complex on glass carbon electrode is a quasi-reversible process. After addition of 2.72×10^{-5} M DNA, the value of ΔE in the presence of DNA decreases to 0.179 V, showing that the reversibility of electron-transfer process of the complex is enhanced after interaction with DNA. The formal potential of the complex, $E^{0\prime}$ (or voltammetric $E_{1/2}$), taken as the average value of cathodic peak and anodic peak potentials, is −0.235 V and −0.225 V, respectively, when DNA is absent and present in the solution. Obviously, $E^{0\prime}$ undergoes a positive shift (10 mV) after forming aggregation with DNA, suggesting that the copper complex bind to DNA mainly by intercalation binding mode [36], and this result also proves the results obtained from viscosity and absorption spectrum studies again.

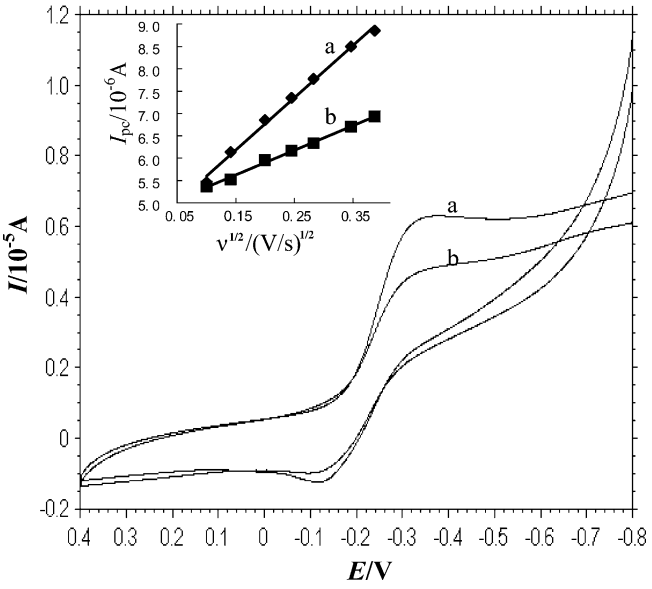

Fig. 4. Cyclic voltammetry of 3.00×10^{-4} M Copper (II) complex in the absence (a) and presence 2.72×10^{-5} M DNA (b) in 0.01 M pH 6.86 KH_2PO_4–Na_2HPO_4 buffer solution, Scan rate: 0.04 V/s. Inset: the plots of the cathodic peak currents of $Cu(bpy)(pba)_2$ in the absence (a) and presence (b) of DNA vs. the square root of the scan rate ($v^{1/2}$).

In addition to changes in formal potential upon addition of DNA, the voltammetric current decrease significantly as shown in Fig. 4. The dependences of cathodic currents of the copper (II) complex on scan rate (v) are investigated and the results are shown in the inset of Fig. 4. It is observed that the cathodic currents of the copper complex both with and without DNA are linear to the square root of the scan rate ($v^{1/2}$), which are expected as the diffusion-controlled processes [37]. While, the slop of the I_{pc}–$v^{1/2}$ plot decreases distinctly after the mixing with DNA, indicating the reduction in the apparent diffusion coefficient of complex in the presence of dsDNA [36]. So, we can interpret the change in current upon DNA addition in terms of the diffusion of an equilibrium mixture of free and dsDNA-bound complex to the electrode surface.

The net shift in $E^{0\prime}$ can be used to estimate the ratio of equilibrium constants for the binding of the bivalent and univalent ions to DNA. This is analogous to the treatment of the association of small molecules with micelles. For a Nernstian electron transfer system, in which both the oxidized and reduced forms associate with a third species in solution (DNA), differential pulse voltammetry (DPV) can be used to calculate the corresponding equilibrium constants for each oxidation state binding to DNA according to the following equation [37]:

$$E_b^{0\prime} - E_f^{0\prime} = 0.059 \log(K_I/K_{II}),$$

where $E_b^{0\prime}$ and $E_f^{0\prime}$, the formal potentials of Cu(II)/Cu(I) complex couple in the binding and free forms, respectively, are determined by the formula: $E^{0\prime} = E_p + \Delta E_p/2$, ($E_p$, the DPV peak potential; ΔE_p, the pulse amplitude); K_{II} and K_I are the binding constants of oxidized and reduced forms to DNA, respectively.

Fig. 5 shows the differential pulse voltammograms of 3.00×10^{-4} M copper complex in the absence and presence of DNA. It is observed that the reduction current of Cu(II) decrease obviously and its potential shifts positively after reacting with DNA, which is consistent with the results of the cyclic voltammetry (Fig. 4). Thus, for a limiting potential shift of +12 mV after adding excess DNA, the ratio of the binding constants (K_I/K_{II}) of the Cu(I) and Cu(II) complex to DNA was calculated to be 1.6, which indicates that the intensity of Cu(I) binding to DNA is stronger than that of Cu(II).

3.3. Cleavage of pBR322 plasmid DNA by copper (II) complex

The cleavage ability of the title copper (II) complex to DNA was investigated by gel electrophoresis using supercoiled pBR322 DNA in 50 mM Tris–NaCl buffer (pH 7.1). The cleavage efficiency was measured by determining

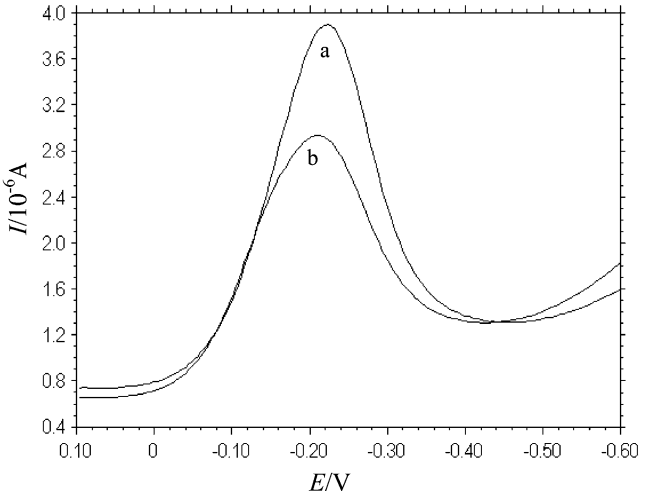

Fig. 5. Differential pulse voltammograms of 3.0×10^{-4} M copper (II) complex in the absence (a), and presence of 2.72×10^{-5} M DNA (b) in 0.01 M pH 6.86 KH_2PO_4–Na_2HPO_4 buffer solution. Pulse amplitude, 50 mV; Pulse width, 50 ms; Pulse period, 200 ms; Increasing potential, 4 mV.

the ability of the complex to convert the supercoiled DNA (form I) to nicked circular form (form II) and linear form (form III). Control experiments using only copper complex or ascorbic acid (H_2A) failed to show any apparent cleavage of DNA (Fig. 6, lanes 1 and 2). While, the copper complex can cleave the supercoiled plasmid DNA (form I) to nicked circular DNA (form II) in the presence of H_2A, and even to linear DNA (form III) when the concentration of the copper complex increased (Fig. 6, lanes 3–5), suggesting that the copper complex has obvious nuclease activity after mixing with ascorbic acid. It has been established that copper (II) complexes are catalytically active in the oxidation of ascorbic acid by dioxygen involving copper (I) intermediate species [38], so, according to the DNA cleavage mechanism proposed by Sigman et al. [39] for $[Cu(phen)_2]^{2+}$ complex, the cleavage of DNA by the title copper (II) complex can be expatiated by the following procedures: the copper (II) is first reduced by ascorbic acid to form the Cu(I) species, which then binds to DNA forming a Cu(I) complex–DNA adduct, then reacts with dioxygen to form a 'copper-oxene' radical, that is the species responsible for the cleavage of DNA.

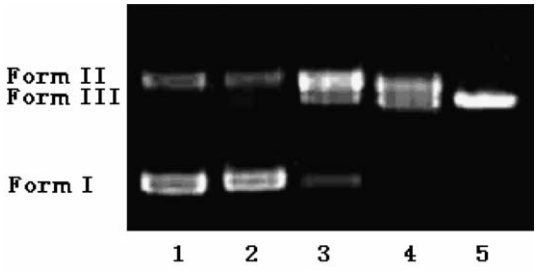

Fig. 6. Cleavage of 10 μM supercoiled pBR322 DNA by the title copper (II) complex in a buffer containing 50 mM pH 7.1 Tris–NaCl in the presence of 100 μM ascorbic acid (H_2A) at 37 °C. Lane 1, DNA + H_2A; lane 2, DNA + 20 μM $Cu(bpy)(pba)_2$; lanes 3–5, DNA + H_2A + $Cu(bpy)(pba)_2$, the concentration of the copper complex is 10, 20, 50 μM, respectively. Forms I, II and III are supercoiled, nicked circular and linear forms of DNA, respectively.

4. Conclusions

In summary, a new copper complex, $[Cu(bpy)(pba)_2 \cdot H_2O] \cdot 0.5H_2O$, was synthesized and its structure and physicochemical properties were characterized by X-ray crystallography, elemental analysis, electronic absorption spectrum and electrochemistry. The binding properties of the copper complex to native DNA were studied by electrochemical techniques, electronic absorption spectrophotometry and viscosity experiment, and so on. The experimental results show that the title copper complex can intercalate into the double helix structure of the native DNA. The cleaving ability of the title complex to DNA was assessed by gel electrophoresis, and the result shows that it has obvious nuclease activity after mixing with ascorbic acid.

Acknowledgements

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Appendix A. Supplementary data

CCDC-257520 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1222 336 033; e-mail: deposit@ccdc.cam.ac.uk]. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ica.2005.12.035.

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