Front. Chem. China 2008, 3(2): 138–142
DOI 10.1007/s11458-008-0026-z

RESEARCH ARTICLE

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Nuclease activity of copper(II) complex containing 1,10-phenanthroline-5,6-dione and L-phenylalanine ligands

Abstract The interaction between CT-DNA and a ternary copper (II) complex, [Cu(phendio)(L-Phe)(H_2O)](ClO_4)·H_2O (CuPP, phendio = 1,10-phenanthroline-5,6-dione, L-Phe = L-phenylalanine), has been conducted by electronic spectra, fluorescence spectroscopy and cyclic voltammetry. It has been found that the max. absorption peak from the electronic spectra is red shifted and the intensity is weakened and that the values of peak current from cyclic voltammetry are decreased significantly in the presence of DNA compared with that in the absence of DNA. At the same time, the complex can quench the emission intensity of EB-DNA system. The existence of the intercalation mode between the complex and DNA was proven. By submarine gel electrophoresis, we found that the copper(II) complex can cleave circular plasmid pBR322 DNA into nicked and linear forms in the presence of ascorbic acid and H_2O_2.

Keywords copper complex, DNA binding, DNA cleavage

1 Introduction

In the recent years, the interaction of transition metal complexes with nucleic acids has gained a lot of attention. These interactions are of great importance for understanding the pathogenesis of various diseases and designing new chemotherapeutic agents. Copper complexes containing 1,10-phenanthroline (phen) have received considerable interest in nucleic acid chemistry due to their various applications following the discovery of the 'chemical nuclease' activity of bis(phen)copper (I) complex in the presence of hydrogen peroxide and a reducing agent by Sigman et al. [1–3]. There are also some reports that *o*-quinones are highly redox-active molecules which can go into redox cycle with their semiquinone radicals, leading to the formation of reactive oxygen species (ROS), including superoxide, hydrogen peroxide, and ultimately, the hydroxyl radical. 1,10-phenanthroline-5,6-dione attracts our attention due to the presence of two coordinating functionalities in the same molecule (the quinonoid and the dimine).

Amino acids are the basic structural units of proteins and some copper complexes of amino acids were reported to exhibit potential antitumor and artificial nuclease activity [4]. In this context, we focused interests on the development of ternary copper (II) complexes of 1,10-phenanthroline-5,6-dione with amino acids and the investigation of their DNA cleavage activity. The results obtained from the present study may be helpful for the development of nucleic acid molecular probes and new therapeutic reagents for some diseases including cancer and inflammatory diseases.

Translated from *Acta Scientiarum Naturalium Universitatis Nankaiensis*, 2007, 40(1): 32–36 [译自: 南开大学学报(自然科学版)]

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2 Experimental

2.1 Materials and instrumentations

The UV/Vis spectra were measured on a JASCO V-570 Spectrophotometer. The fluorescence spectrum was measured on a Varian Cary Eclipse Fluorescence spectrophotometer. Cyclic voltammetry measurements were performed on a BAS Epsilon Electrochemical Workstation. All samples were purged with nitrogen prior to measuring. A standard three-electrode system consisting of platinum-disk working electrode, platinum-wire auxiliary electrode and a saturated calomel reference electrode (SCE) were used. All materials and

solvents were purchased commercially and no further purification was carried out, unless otherwise noted. The CT-DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 $mol^{-1}\cdot L\cdot cm^{-1}$) at 260 nm [5]. Agarose and ethidium bromide (EB) were from Sigma (USA). Tris-HCl/NaCl buffer solution was prepared by using deionized, sonicated triple-distilled water. Stock solutions were stored at 4°C and used after no more than 4 days. The stock solution of EB and the copper (II) complex were prepared by Tris-HCl/NaCl buffer.

2.2 Synthesis of complex

The complex was prepared as described previously [6].

2.3 DNA-binding and cleavage experiments

2.3.1 Electronic spectra

The electronic spectra of [Cu(phendio)(L-Phe)(H_2O)](ClO_4)·H_2O (CuPP) was recorded before and after the addition of CT-DNA in the 5 mmol/L tris-HCl/ 50 mmol/L NaCl buffer, pH 7.2. The intrinsic binding constant K_b for the interaction of the studied complex with CT-DNA was calculated by absorption spectral titration data using the following equation [7]: [DNA]/($\varepsilon_a - \varepsilon_f$) = [DNA]/($\varepsilon_b - \varepsilon_f$) + $1/K_b(\varepsilon_b - \varepsilon_f)$, where ε_a, ε_f and ε_b correspond to A_{obsd}/[Cu], the extinction coefficient for the free copper complex and the extinction coefficient for the copper complex in the fully bound form, respectively. The plot of [DNA]/($\varepsilon_a - \varepsilon_f$) vs. [DNA], K_b is then given by the ratio of the slope to intercept.

2.3.2 Fluorescence spectra

The fluorescence spectra were recorded at room temperature with excitation at 305 nm and emissions at 612 nm. The experiment was carried out by titrating CuPP (4.0×10^{-3} mol/L in 5 mmol/L tris-HCl/50 mmol/L NaCl buffer) into samples containing 2.0×10^{-4} mol/L of DNA and 1.0×10^{-5} mol/L ethidium bromide.

2.3.3 Cyclic voltammetric studies

The cyclic voltammetric experiments were carried out by using a solvent composition of 10 mmol/L tris/50 mmol/L NaCl buffer (pH 7.2) and DMSO (100:5 ratio). The experiments were carried out in 0.1 mmol/L complex solution in the absence and presence of CT- DNA varying from 0 to 40 μmol/L.

2.3.4 DNA cleavage

The reactions of DNA cleavage were carried out in a total volume of 10 μl 5 mmol/L Tris-HCl and 50 mmol/L NaCl (pH 7.4) buffer containing 0.4 μg pBR322 DNA, CuPP and ascorbic acid/H_2O_2 at 37°C for 1 h and stopped by adding of 2 μL quench buffer solution containing 0.25% bromophenol blue, 0.5 mmol/L EDTA and 50% (*V*/*V*) glycerol. The samples were immediately loaded on agarose gel (0.70%) containing 0.5 μg/mL of ethidium bromide. Electrophoresis was carried out at 90 V for 3 h in TAE buffer (40 mmol/L Tris, 20 mmol/L acetic acid, 1 mmol/L EDTA, pH 7.4). Images were captured by a bio-imaging system. The proportion of DNA in each fraction was estimated quantitatively from the volume of the bands using the UVITEC Gel Documentation System.

3 Results and discussion

3.1 Electronic spectroscopic studies

Hypochromism results from the contraction of DNA in the helix axis as well as from the change in DNA conformation. An intercalative binding of a complex to DNA generally results in hypochromism along with a red shift (bathochromic shift) of the electronic spectral band [8,9]. The absorption spectra of CuPP in the absence and presence of CT-DNA are shown in Fig. 1. In the UV region, the title complex mainly consists of one resolved band, 221 nm, which can be attributed to the π→π* transition of the ligand. The complex was found to show a minor bathochromic shift to 225 nm along with hypochromicity of ~20% at 221 nm after the addition of DNA which indicated the interactions between DNA and the complex. The intrinsic binding constant (K_b) of the complex with DNA was calculated to be 1.5×10^4 L/mol. The moderate binding for this complex is comparable to those observed for many copper complexes and ruthenium complexes [10,11].

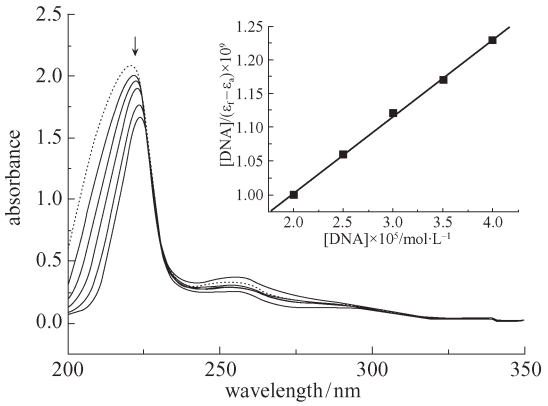

Fig. 1 Electronic spectra of 4.0×10^{-6} mol·L^{-1} CuPP in the absence (——) and presence (–) of increasing amount of CT-DNA at the ratio r = 5, 10, 15, 20, 25

The arrows indicate the directions of absorbance changes as a function of r.

3.2 Fluorescence spectroscopic studies

No luminescence was observed for this Cu(II) complex either with or without the presence of calf thymus DNA. So the binding of Cu(II) polypyridyl complex and DNA can not be directly presented in the emission spectra. Ethidium bromide (EB) is a weak fluorescent, but its emission intensity in the presence of DNA can be greatly enhanced because of its strong intercalation between the adjacent DNA base pairs [12]. It was previously reported that this enhanced fluorescence could be quenched, at least partly by addition of a second compound, which can be intercalated as well [13,14]. The fluorescence quenching assays of limited EB bound to excess of DNA is utilized to distinguish intercalating and nonintercalating ligands [15]. The emission spectra of EB bound to DNA in the absence and the presence of CuPP was shown in Fig. 2. The addition of the copper (II) complex to DNA pretreated with EB causes appreciable reduction in the emission intensity, indicating that the DNA-bound EB fluorophore is partially replaced by the complex.

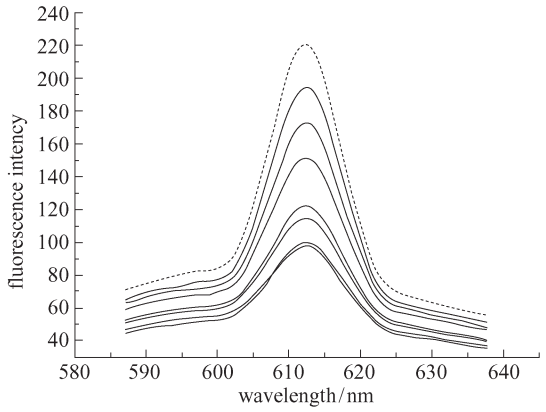

Fig. 2 Fluorescence emission spectra (excited at 305 nm) of the EB-CT-DNA system (2.4×10^{-6} mol·L^{-1}EB, 4.0×10^{-3} mol·L^{-1} CT-DNA) in the absence (——) and presence (–) of 4.0×10^{-3} mol·L^{-1} CuPP (10 μL per scan)

3.3 Cyclic voltammetric studies

The cyclic voltammetric experiments were carried out by using a solvent composition of 10 mmol/L tris/50 mmol/L NaCl buffer (pH 7.2). Typical cyclic voltammetry (CV) behaviors of CuPP in the absence and presence of CT-DNA are shown in Fig. 3. The cyclic voltammogram of CuPP in the absence of DNA featured reduction of the quinone species of phendio form dianion at a cathodic peak potential, E_{pc} of −0.052 V versus SCE. Oxidation of the Cu(II) occurred, upon scan reversal, at 0.197 V. The presence of DNA in the solution at the same concentration of CuPP caused a considerable decrease in the voltammetric current. The drop of the voltammetric currents in the presence of CT-DNA can be attributed to diffusion of the metal complex bound to the large, slowly diffusing DNA molecule.

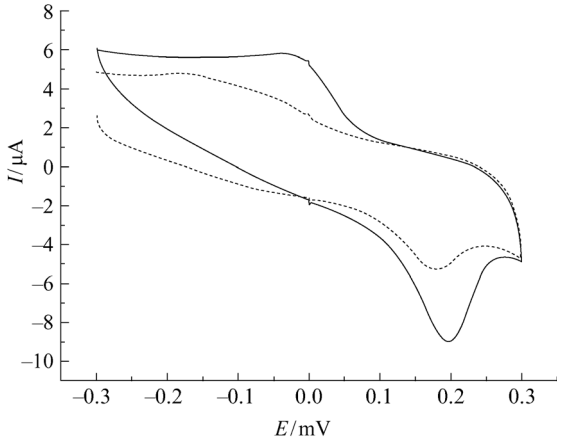

Fig. 3 Cyclic voltammograms of CuPP in absence (solid line) and presence (dash line) of CT-DNA in 10 mmol/L tris-HCl/50 mmol/L NaCl buffer (pH 7.2) at 50 mV/s

3.4 DNA cleavage study

Fig. 4 illustrates the gel electrophoretic separations showing the cleavage of plasmid pBR322 DNA induced by CuPP (40 μmol/L) under identical reaction conditions using H_2O_2/ascorbic acid activation, respectively. In the presence of H_2O_2/ascorbic acid the complex can convert supercoiled plasmid pBR322 DNA to a mixture of supercoiled (Form I) and nicked (Form II) DNA (lanes 5, 6). Interestingly, we have found that CuPP can cleave the supercoiled DNA to nicked and linear DNA at the same time H_2O_2 (10 mmol/L) was added together with ascorbic acid (100 μmol/L) (lane 11). However, control experiments using only the complex, ascorbic acid or H_2O_2/ascorbic acid did not show any apparent cleavage of DNA (lanes 0, 1, 2, 3, 4, 9, 10).

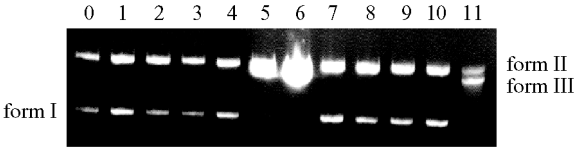

Fig. 4 Cleavage of pBR322 DNA (0.4 μg) by CuPP (40 μmol/L) in the presence of H_2O_2, ascorbic acid and mercaptoethanol in a buffer containing 5 mmol/L Tris-HCl and 50 mmol/L NaCl (pH 7.4) at 37°C

Hence it is clear that the complex can show nuclease activity in the presence of reducing agents. For comparison, reactions were carried out with mercaptoethanol (1 mmol/L). CuPP failed to show any apparent cleavage of DNA with mercaptoethanol (1 mmol/L) (lanes 7, 8).

Lane 0, DNA control; Lane 1, DNA + CuPP; lane 2, DNA + ascorbic acid (1 mmol/L); lane 3, DNA + ascorbic

acid (1 mmol/L) + CuPP; lane 4, DNA + ascorbic acid (1 mmol/L)/H_2O_2 (10 mmol/L); lane 5, DNA + ascorbic acid (1 mmol/L)/H_2O_2 (10 mmol/L) + CuPP; lane 6, DNA + ascorbic acid (2.5 mmol/L)/H_2O_2 (5 mmol/L) + CuPP; lane 7, DNA + mercaptoethanol (1 mmol/L); lane 8, DNA + mercaptoethanol (1 mmol/L) + CuPP; lane 9, DNA + ascorbic acid (100 μmol/L); lane 10, DNA + ascorbic acid (100 μmol/L) + CuPP; lane 11, DNA + ascorbic acid (100 μmol/L)/H_2O_2 (10 mmol/L) + CuPP

Figure 4 shows the cleavage of DNA at different concentration of the complex for 1 hour reaction time (pH 7.4, 37°C). With the increase of complex concentration, the supercoiled DNA decreased and was finally converted completely to nicked DNA. The cleavage efficiency of CuPP reaches about 100% at a concentration of 160 μmol/L (Fig. 4, lane 5) in converting Form I to Form II, which indicates that CuPP is a potent DNA cleavaging agent in the presence of ascorbic acid and H_2O_2 under the present experimental conditions.

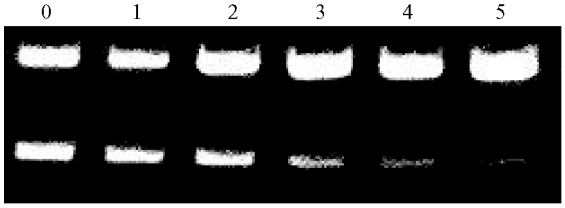

Fig. 5 Cleavage of pBR322 DNA (0.4 μg) by CuPP different concentrations of CuPP in the presence of ascorbic acid and H_2O_2 in a buffer containing 5 mmol/L tris-HCl and 50 mmol/L NaCl (pH 7.4) at 37°C

Lane 0, DNA control; Lane 1, DNA + ascorbic acid (75 μmol/L)/H_2O_2 (1.5 mmol/L); lane 2, DNA + ascorbic acid (75 μmol/L)/H_2O_2 (1.5 mmol/L) + CuPP (40 μmol/L); lane 3, DNA + ascorbic acid (75 μmol/L)/H_2O_2 (1.5 mmol/L) + CuPP (80 μmol/L); lane 4, DNA + ascorbic acid (75 μmol/L)/H_2O_2 (1.5 mmol/L) + CuPP (120 μmol/L); lane 5, DNA + ascorbic acid (75 μmol/L)/H_2O_2 (1.5 mmol/L) + CuPP (160 μmol/L)

In order to make clearer the cleavage mechanism, Fig. 6 illustrates a new assay using radical scavengers of the reactive oxygen species (ROS). The hydroxyl radical scavenger, DMSO (lane 3), diminished the nuclease activity of the compound that was indicative of the involvement of the hydroxyl radical in the cleavage process. Prior treatment of pBR322 DNA with ethanol or tert-butyl alcohol did not modify the DNA strand break (lane 5, 7). The data shows that the overall efficiency of the scavengers is low and in some cases not effective at all. This further confirmed that species exerting DNA cleavage were not the free diffusible hydroxyl radicals. Some copper-centered radicals, that are DNA-bound and not easily reached by the radical scavengers, should be responsible for cleavage [16–18]. Sodium azide viewed as singlet oxygen scavenger, shows a slight reduction in the activity (compare lanes 8 and 9 with lanes 0 and 1) which suggests that $^1O_2$ may participate in the oxidative cleavage.

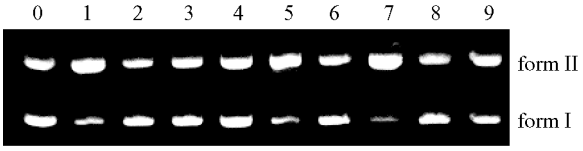

Fig. 6 Cleavage of pBR322 DNA (0.4 μg) by CuPP (80 μmol/L) in the presence of ascorbic acid (100 μmol/L)/H_2O_2 (1 mmol/L) in a buffer containing 5 mmol/L Tris-HCl and 50 mmol/L NaCl (pH 7.4) at 37°C, using inhibition reagents

Lane 0, DNA control; Lane 1, DNA + CuPP O; lane 2, DNA + DMSO; lane 3, DNA + CuPP + DMSO; lane 4, DNA + ethanol; lane 5, DNA + ethanol + CuPP; lane 6, DNA + tert-butyl alcohol; lane 7, DNA + CuPP + tert-butyl alcohol; lane 8, DNA + NaN_3 (1 mol/L); lane 9, DNA + CuPP + NaN_3 (1 mol/L)

4 Conclusions

The DNA binding behaviors of the copper (II) complex has been examined by electronic spectra, fluorescence spectroscopy and cyclic voltammetric measurements. It is suggested by the results that the copper (II) complex binds to DNA with an intercalative mode. The observed efficient nuclease activity of CuPP in the presence of ascorbic acid and H_2O_2 is interesting and may have further influences in the chemistry of copper-based nucleolytic agents.

Acknowledgements This work was supported by the National Natural Science Foundation of China (Grant No. 20331020).

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