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Studies on the Interaction Mechanism Between Cu(II) Ternary Complex and DNA

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A new complex of Cu(II) with N-(2-hydroxy-1-naphthalidene)glycine Schiff base and 1,10-phenanthroline was synthesized and characterized by elemental analysis, molar conductivity, IR and thermal decomposition. The fundamental electrochemical characteristics of a Cu(II) ternary complex have been studied and the interactivity of copper complex with DNA was also studied by cyclic voltammetry and fluorescence spectrum. The results suggest that the binding ratio between the Cu ternary complex and DNA was calculated to be 1:1 and the binding constant was 6.05×10^4 L mol^{-1}.

Key Words: Schiff base, Cyclic voltammetry, DNA.

INTRODUCTION

The numerous biological experiments performed so far suggest that DNA is the primary intracellular target of anticancer drugs because the interaction between small molecules and DNA can cause DNA damage in cancer cells, blocking the division of cancer cells and resulting in cell death[1-3]. Of those studies, the interaction of transition metal complexes with DNA has gained much attention, owing to their possible applications as new therapeutic agents and their photochemical properties that make them potential probes of DNA structure and conformation[4-10].

It is necessary to understand the binding properties in developing new potential DNA targeting antitumor drugs. Basically, metal complexes interact with double helix DNA in either non-covalent or covalent way. The former way includes three binding modes, *i.e.*, intercalation, groove binding and external static electronic effects. Among these interactions, intercalation is one of the most important DNA binding modes. It was reported that the intercalating ability appeared to increase with the planarity of ligands[11,12]. Additionally, the coordination geometry and ligand donor atom type also play key roles in determining the binding extent of complexes to DNA[13,14]. The metal ion type and its flexible valence, which are responsible for the geometry of complexes, also affect the intercalating ability of metal complexes to DNA[15,16].

In this paper, the Cu ternary complex was synthesized. The interaction between the complex and DNA has studied by cyclic voltammetry. The experimental results the binding ratio between [Cu (GNA) (1,10-phen)] $\cdot 2H_2O$ and DNA was calculated to be 1:1 and the binding constant was 6.05×10^4 L mol^{-1}.

EXPERIMENTAL

Glycine was biochemical reagent (BR) the other reagents were analysis reagent (AR) grade and were used without further purification. Salmon sperm DNA was purchased from Shanghai Huashun Biological Engineering Company ($A_{260}/A_{280} > 1.8$). The concentration was determined by the ultraviolet absorption at 260 nm ($Z = 6600\ M^{-1}\ cm^{-1}$).

Elemental analyses were carried out with a model 2400 Perkin-Elmer analyzer. Infrared spectrum was recorded in KBr pellets using a Nicolet 170 SX spectrophotometer in the 4000-400 cm^{-1} region. Molar conductivity at room temperature was measured in 10^{-3} M DMSO solution using a DDS-11A type conductivity meter at 25 ºC. The thermogravimetric measurements were made using a Perkin-Elmer TGA7 thermo gravimeter. The heating rate was programmed to be 10 ºC/min with the protecting stream of N_2 flowing at 40 mL/min. All electrochemical measurements were carried out with Model CHI 832 voltammetric analyzer. A three-electrode, Ag/AgCl/KCl(salt) as reference electrode and glass carbon electrode (GCE) as working electrode.

Preparation of the ligand: 2-Hydroxy-1-naphthaldehyde was added (with stirring) to anhydrous ethanol (20 mL) to make a pellucid solution. Then, it was slowly dripped into the anhydrous ethanol solution (15 mL) containing 0.01 mol glycine (containing 0.01 mol KOH) at 65 ºC (pH = 6.0~6.5), a mass of yellow grain was separated out which was collected by filtration and washed several times with anhydrous ethanol, recrystallized with methanol and then dried under vacuum (Yield 87.9 %). Anal. Calcd. (found) %: C 58.41 (58.41), H 3.77 (3.73), N 5.24 (5.21). IR (KBr, cm^{-1}): 1642 ν(C=N), 1590, 1360 ν(COO^-), 1229 ν(Ar-O).

Preparation of the complex: 0.5 mmol of the cupric acetate in 15 mL of anhydrous ethanol was added dropwise into the solution of Schiff base (0.5 mmol) in 15 mL of anhydrous ethanol and was stirred at 70 ºC. After 2 h, 10 mL of anhydrous ethanol containing 0.5 mmol 1.10-phen was dripped into the mixture, continuously to be stirred at the same temperature for 8 h. The dark green solution obtained was filtered and the dark green powder was dried under vacuum. The C, H and N contents were as follows: Anal. Calcd. (found) %: C 59.22 (61.13), H 4.17 (4.12), N 8.29 (8.45), Cu 12.53 (12.42). IR (KBr, cm^{-1}): 1628 ν(C = N), 1579, 1384 ν(COO^-), 1217 (Ar-O).

Electrochemical study on the interaction between the complex and DNA 25 μL of 1.08×10^{-4} mol L^{-1} [Cu(GNA)(1,10-phen)]·$2H_2O$ solution was transferred into 5 mL colorimetric tubes containing 0.05 mol L^{-1} pH 6.54 B-R buffer solution and then DNA was added. The changes on characteristics of CVs were investigated. For CV scanning, the potential scanning range was from 1.0 to -1.2 V, the scanning rate was 0.062 V/s; the sample interval was 0.001 V and the quiet time was 2 s.

RESULTS AND DISCUSSION

The title complex is dark green powder, soluble in DMSO and DMF. The molar conductivity of the complex is 11.65 Ω^{-1} cm^2 mol^{-1} in DMSO. Low molar conductivity for the complex in DMSO corresponds to non-electrolytes[17].

The shift of ν(C=N) from 1642 cm^{-1} in the ligand to 1628 cm^{-1} in the complex, suggests that Cu ion is bonded with N atom in Schiff-base. The shift of $\nu_{as}(COO^-)$ and $\nu_s(COO^-)$ from 1590 and 1360 cm^{-1} in the ligand to 1579 and 1384 cm^{-1} in the complex, respectively, suggests the coordination of the oxygen in the carboxylate group to the metal ion. The value of $\nu[\nu_{as}(COO^-)-\nu_s(COO^-)]$ = 194 cm^{-1} indicates that the -COO^- group is coordinated to the metal ion in a monodentate fashion[18]. A broad absorption band at the range of 3300-3000 cm^{-1} confirms the presence of water in the complex. The appearing of Ar-O frequency (1217 cm^{-1}) is lower than 1229 cm^{-1}, which exposes that Ar-O-Cu in the complex. Vibrations of 1,10-phen at 1545 cm^{-1}splits into 1510 and 1482 cm^{-1}.

Thermal decomposition studies: The TG and DTG curves of the complex are shown in Fig. 1, which indicate that complex decomposes in two steps. The first weight loss stage has decomposition temperature ranges of 25-140 ºC, with weight losses of 6.15 % (calcd. 7.10 %), which corresponds to the losses of two molecules of water. The second weight loss stage has decomposition temperature range of 140-1000 ºC, corresponding to the fractional losses of Schiff base, 1,10-phen.The exothermic peaks locate in 78.1 and 267.4 ºC. The weight percentage of 16.21 % (calcd. 15.78 %) of the original sample remained, CuO is the final residue.

According to the characterizations enumerated above, the possible structure of the complex is shown as Fig. 2.

Electrochemical study on the interaction between [Cu(GNA) (1,10-phen)] ·$2H_2O$ and DNA: Electrochemical study on [Cu(GNA) (1,10-phen)] ·$2H_2O$ and its interaction with DNA were performed at 25 ºC. CVs of [Cu(GNA) (1,10-phen)]·$2H_2O$ in the absence and presence of DNA were shown in Fig. 3. The buffer used was 0.05 M pH 6.54 B-R solution. The curve 1 is a cyclic voltammetry of the title complex in the B-R buffer solution (pH = 6.54). The curve 2 is cyclic voltammetry of the mixed solution

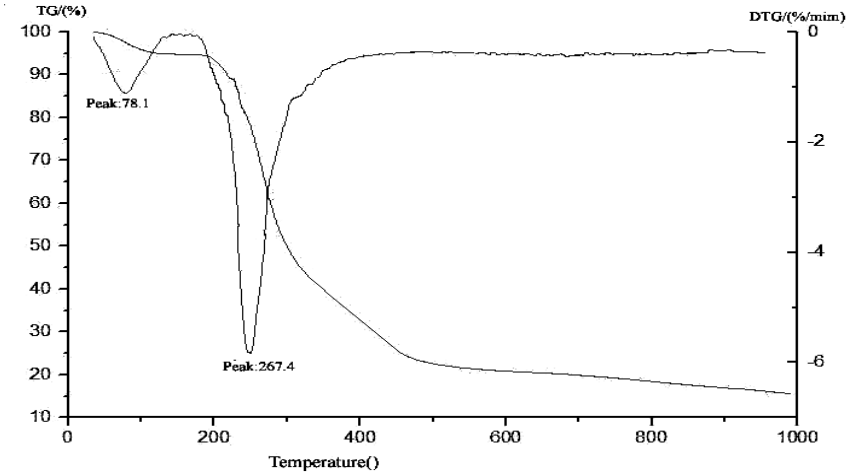

Fig. 1. Thermal analysis curves of [Cu(GNA) (1,10-phen)] ·2H_2O

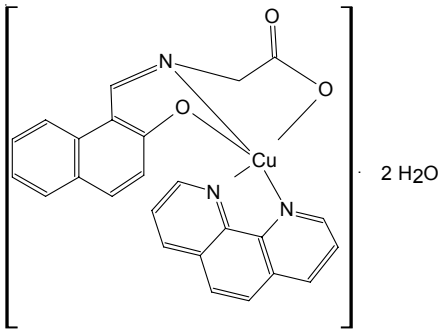

Fig. 2. Suggested structure of the complex

of DNA and complex in the B-R buffer solution, from which we can induce that the electric current of oxidation peak decreased and had no new oxidation peak occur, through which we can presume DNA and complex formed the new compound that wasn't electric activity compound. Due to the new compound didn't conducted, the concentration of the complex reduced so that the number of the molecule of the complex moved to the surface of electrode declined, which lead to the electric currents became weak. It is generally accepted that there are three kinds of binding modes for small molecules to DNA, which refer to intercalative binding, groove binding and electrostatic binding. Bard[19] suggested that when the molecule inserts into the inner of DNA double helix structure, the peak currents of the oxidation of the voltammetry decreased with positive shifts of the peak potential.

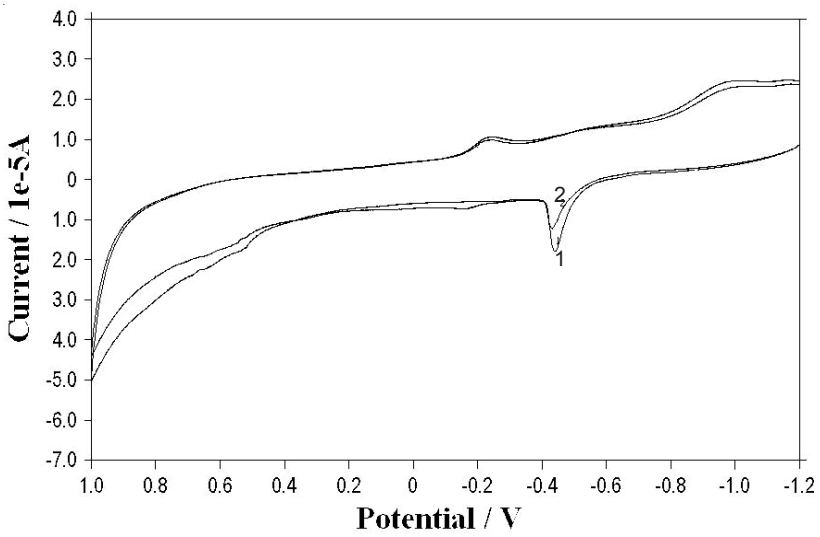

Fig. 3. Cyclic voltammetry of Cu(II) ternary complex and the interaction of the complex and DNA; $C_{[Cu(GNA)\ (1,\ 10\text{-phen})]\cdot 2H_2O}$: 1.08×10^{-4} mol L^{-1}, C_{DNA}:(1)0(2) 5.20×10^{-4} mol L^{-1}

On the other way around, when the molecule interacted with DNA in the form of static effect, the peak currents of the voltammetry curves of the oxidation of the voltammetry decreased with negative shifts of the peak potential. In summary, one can infer that the complex interact with DNA in the form of intercalative binding.

The binding ratio and the binding constant between [Cu(GNA) (1,10-phen)]·2H$_2$O and DNA: To study the binding ratio and binding constant between [Cu(GNA) (1,10-phen)]·2H$_2$O and DNA, it was assumed the interaction of DNA and [Cu(GNA) (1,10-phen)]·2H$_2$O only produced one single complex: DNA-nML, as shown in the following equation[20]:

$$DNA + nML \rightleftharpoons DNA - nML \quad (n = 1, 2, 3, \ldots, 1/2, 1/3, \ldots)$$

The equilibrium constant β could be expressed as eqns. 1-7

$$\beta = \frac{[DNA - nML]}{[DNA][ML]^n} \tag{1}$$

$$\Delta I_{p,max} = KC_{DNA} \tag{2}$$

$$\Delta I_p = K[DNA - nML] \tag{3}$$

$$[DNA] + [DNA - nML] = C_{DNA} \tag{4}$$

$$\Delta I_{p,max} - \Delta I_p = K[C_{DNA} - (DNA - nML)] \tag{5}$$

$$\Delta I_{p,max} - \Delta Ip = K[DNA] \tag{6}$$

$$\frac{1}{\Delta I_p} = \frac{1}{\Delta I_{p,max}} + \frac{1}{\beta \Delta I_{p,max}[ML]^n} \quad (7)$$

According to the eqn. 7, different 'n' might result in different curves of ΔI_{pa}^{-1} *vs.* $[[Cu(GNA)(1,10\text{-phen})]\cdot 2H_2O]^{n-}$. With the suitable n, the curve of ΔI_{pa}^{-1} *vs.* $[[Cu(GNA)(1,10\text{-phen})]\cdot 2H_2O]^{n-}$ should be a straight line if there was only one complex formed when $[[Cu(GNA)(1,10\text{-phen})]\cdot 2H_2O]^{n-}$ bound to DNA. From the slope and intercept of the straight line, the binding constant β could be calculated and the n could be regarded as the binding ratio.

The dependence of the oxidation peak current (I_{pa}) on the analytical concentration of $[Cu(GNA)(1,10\text{-phen})]\cdot 2H_2O$ in the absence (curve 1) and presence (curve 2) of DNA was shown in Fig. 4. The relationship between ΔI_{pa} (the difference of I_{pa1}, I_{pa2}, $I_{pa} = I_{pa1} - I_{pa2}$) and the analytical concentration of $[Cu(GNA)(1,10\text{-phen})]\cdot 2H_2O$ was also displayed (curve 3).

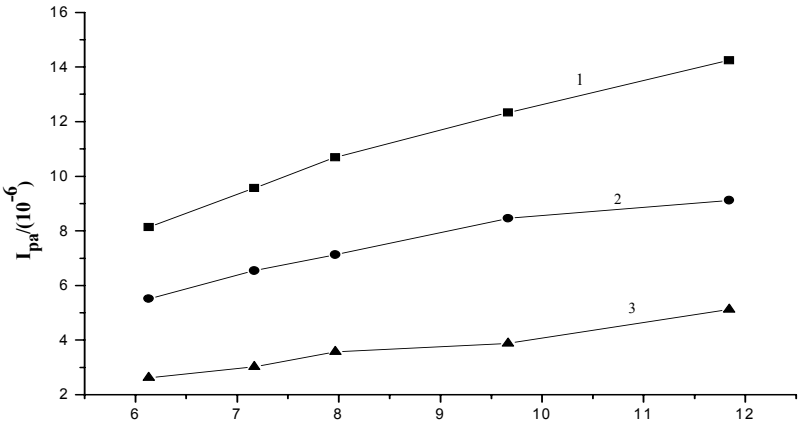

Fig. 4. Relationship curve of I_{pa1}, I_{pa2} and ΔI_{pa} *vs.* $C_{[Cu(GNA)(1,10\text{-phen})]\cdot 2H_2O}$
1. C_{DNA}: 0; 2. C_{DNA}: 1.4×10^{-4} mol L^{-1}; 3. $\Delta I_{pa} = I_{pa1} - I_{pa2}$

The curves of ΔI_{pa}^{-1} *vs.* $[[Cu(GNA)(1,10\text{-phen})]\cdot 2H_2O]^{0.5-}$, ΔI_{pa}^{-1} *vs.* $[[Cu(GNA)(1,10\text{-phen})]\cdot 2H_2O]^{1-}$, ΔI_{pa}^{-1} *vs.* $[[Cu(GNA)(1,10\text{-phen})]\cdot 2H_2O]^{2-}$, were displayed in Fig. 5, where $[Cu(GNA)(1,10\text{-phen})]\cdot 2H_2O$ represented the equilibrium concentration of $[Cu(GNA)(1,10\text{-phen})]\cdot 2H_2O$ and calculated from data in Fig. 4, For n = 0.5 and 2, the curves bent down and up, respectively. While for n = 1, the curve was a straight line (γ = 0.999), indicating the forming of a 1:1 association between $[Cu(GNA)(1,10\text{-phen})]\cdot 2H_2O$ and DNA. From the slope and intercept of the straight line, the binding constant β was calculated to be 6.05×10^4 L mol^{-1}.

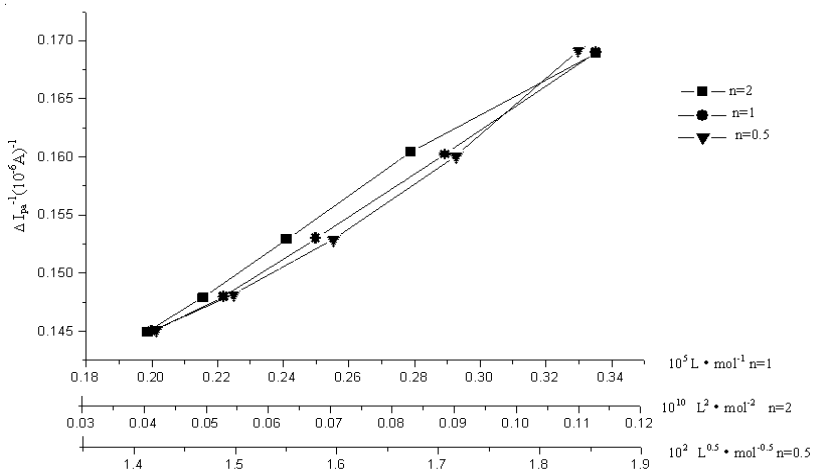

Fig. 5. Relationship curve of ΔI_{pa}^{-1} *vs.* $[[Cu(GNA)\ (1,10\text{-phen})]\cdot 2H_2O]^{n-}$

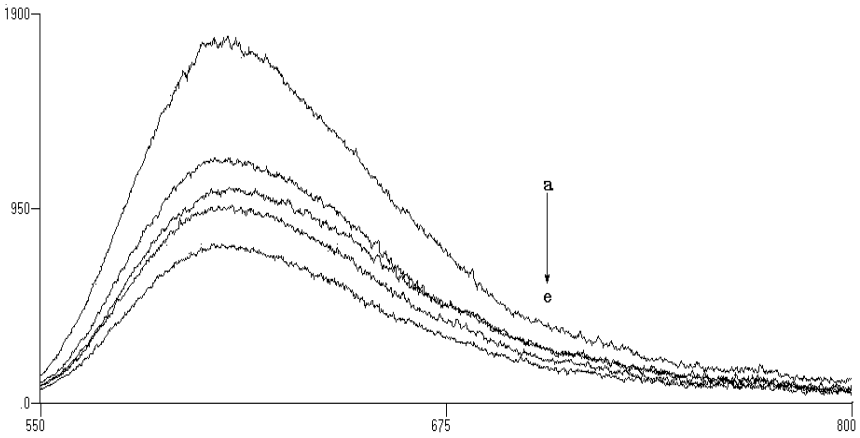

Fig. 6. Fluorescence spectra of different concentration complex interaction with EB-DNA system; (a) $C_{Comp} = 0$ mol/L; (b) $C_{Comp} = 1.5 \times 10^{-5}$ mol/L; (c) $C_{Comp} = 3.0 \times 10^{-5}$ mol/L; (d) $C_{Comp} = 6.0 \times 10^{-5}$ mol/L; (e) $C_{Comp} = 8 \times 10^{-5}$ mol/L

Fluorescence spectrum of the interactivity of the complex and DNA: The EB is one of fluorescent reagent. When it inserts into the inner of DNA double helix structure, the system of EB-DNA can give off the fluorescence and hindrance the copy of DNA. If the molecule can insert DNA, the molecules compete with EB at the bonding point of DNA, the EB

releases. As the EB is free, the fluorescence intensity of the system become weak, through which we can determine if the molecule inserts into the inner of DNA double helix structure.

Fig. 6 shows different concentration of complex interacted with EB-DNA (excitation wavelength:540 nm pH = 6.54). From Fig. 6, it is concluded that when added the complex into the system of EB-DNA, the fluorescence of the system of EB-DNA obviously trailed off, that is these indicated complex combined with DNA, competing with EB. When added the complex in the system of EB-DNA, the EB replace by the complex. According, the complex and EB may be combined with DNA at the same spot and proved the complex inserted into the inner of DNA double helix structure[21].

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