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Synthesis, Characterization and Electrochemical Studies on the Interaction Mechanism of Ternary Cu(II) Complex with DNA

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A new ternary complex of Cu(II) with Schiff base 2-hydroxy-1-naphthaldehyde-glycine (KHL) and 8-hydroxyquinoline (OHQ) was synthesized and characterized by elemental analysis, IR, TG-DTG and molar conductance. The composition of the complex was confirmed to be $[Cu(L)(OHQ)]\cdot 2H_2O$. The fundamental electrochemical characteristics of the Cu(II) complex have been studied and the interaction of the copper complex and DNA was also studied by cyclic voltammetry and fluorescence spectra. The results of the cyclic voltammetry showed that the Cu(II) complex had an oxidation peak with the oxidation potential at -0.394 V. After the DNA was added, the peak current declined with the peak potential shifting positively. This suggested that the complex combined with DNA in the form of intercalative binding, which was also proved by the fluorescence analysis. The binding ratio between the Cu(II) ternary complex and DNA was calculated to be 1:1 and the binding constant was 4.6×10^2 L mol^{-1}.

Key Words: Glycine, Schiff base, Cu(II) complex, Cyclic voltammetry, DNA.

INTRODUCTION

Some Schiff base complexes derived from amino acids are particularly active in biology. Recent, studies of such complexes of transition metal ions with Schiff base have been reported[1-4]. It is known that some copper(II) complexes with Schiff base possess antifungal and antibacterial properties[5,6]. DNA is important genetic material of organism and it plays an important role in the store, copy and transmission of genetic messages. Serving as a target molecule, the recognition of DNA for natural and artificial molecules in the inhibition of cellular disorders and in therapy of certain diseases is of paramount importance in inorganic biochemistry. In this paper the Cu(II) complex with glycine-2-hydroxy-1-naphthaldehyde and 8-hydroxyquinoline was synthesized and the interaction of the complex with DNA was studied by cyclic voltammetry and fluorescence spectra. The aim of this research is to investigate the interaction of complex with DNA, we synthesized the Cu(II) complex with glycine-2-hydroxy-1-naphthaldehyde and 8-hydroxyquinoline.

EXPERIMENTAL

Glycine was BR (Biochemical Reagent). The other reagents were AR (Analysis Reagent) grade and they 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 ($Z = 6600\ M^{-1}\ cm^{-1}$).

Preparation of the ligand: 2-Hydroxy-1-naphthaldehyde (10 mmol) was added (with constant stirring) to anhydrous ethanol (20 mL) to make a pellucid solution. Then, it was slowly dripped into the anhydrous ethanol solution (15 mL) containing 10 mmol glycine (containing 10 mmol 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 for later use. The yield of the reaction was 87.9 %. Anal. calcd. (%) For $C_{13}H_{10}NO_3K$ (267.2): C, 58.41; H, 3.77; N, 5.24; Found (%): C, 58.39; H, 3.73; N, 5.21. IR data (KBr pellets, ν_{max}, cm^{-1}): 1642 (C=N); 1590, 1360 (COO^-);1229 (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 8-hydroxyquinoline 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. (%) for $C_{22}H_{21}N_2O_6Cu$ (473.0): C, 55.86; H, 4.48; N, 5.92; Cu, 13.43. Found (%): C, 55.03%; H, 4.12; N, 6.13; Cu,12.97. IR data (KBr pellets, ν_{max}, cm^{-1}): 1633 (C=N); 1568, 1381 (COO^-); 1213 (Ar- O).

Physical measurements: Elemental analyses were carried out with a model 2400 Perkin-Elmer analyzer. Infrared spectrum was recorded in KBr pellets using a Nicolet 170SX spectrophotometer in the 4000-400 cm^{-1} region. Molar conductance was measured in 10^{-3} mol L^{-1} DMSO solution with a DDSJ-308A type conductivity meter at 25 ºC. The thermogravimetric measurements were made using a Perkin-Elmer TGA7 thermogravimeter. 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. The fluorescence spectra were recorded on a F-4500 fluorometer.

Electrochemical study on the interaction between the complex and DNA: A definite quantity of $[Cu(L)(OHQ)]\cdot 2H_2O$ solution was transferred into 5 mL colorimetric tubes containing 0.05 mol L^{-1} pH 6.82 B-R buffer solution and then DNA was added. The changes on characteristics of cyclic voltammetry were investigated. For cyclic voltammetry 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.

Fluorescent study on the interaction between Cu(II) complex and DNA: 2 mL B-R buffer solution (pH 6.82), 1 mL 5.02×10^{-4} mol L^{-1} DNA solution and 1 mL 2.0×10^{-5} mol L^{-1} EB (ethidium bromide) solution were added into 10 mL volumetric flask and they interacted about 2 h in the dark. Then a different quantity of the complex solution was added to the above solution and the solution was diluted with secondary distilled water to calibration tails. The fluorescence spectra were measured at the excitation wavelength at 530 nm and emission wavelength at 550-800 nm.

RESULTS AND DISCUSSION

Molar conductance and IR sprctra: The title complex is dark green powder, soluble in DMSO, DMF. The molar conductance value of the complex determined in DMSO is 14.0 S cm^2 mol^{-1}, which is expected for non-electrolyte[7].

The shift of ν(C=N) from 1642 cm^{-1} in the Schiff base ligand and 1636.1 cm^{-1} in 8-hydroxyquinoline to 1633 cm^{-1} in the complex, suggests that Cu(II) is bonded with N atom in Schiff base and 8-hydroxyquinoline. The shift of $\nu_{as}(COO^-)$ and $\nu_s(COO^-)$ from 1590 and 1360 cm^{-1} in the ligand to 1568 and 1381 cm^{-1} in the complex, respectively, suggests the coordination of the oxygen in the carboxylate group to the metal ion. The value of $\nu[\nu_{as}(COO^-)-\nu_s(COO^-)]$ =187 cm^{-1} indicates that the $-COO^-$ group is coordinated to the metal ion in a mono-dentate fashion[8]. A broad absorption band at the range of 3300-3000 cm^{-1} confirms the presence of water in the complex. The vibration of ν(Ar-O) of the ligand occurs at 1229 cm^{-1}. The shift to lower frequency by *ca.* 16 cm^{-1} in the metal complex indicates the coordination of hydroxyl oxygen to metal ion.

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 have decomposition temperature ranges of 25-140 ºC, with weight losses of 7.15 % (calcd. 7.62 %), which corresponds to the loss of two molecules of water. The fact that the water molecule was lost at a low temperature suggests that the water is crystal water. The second weight loss stage has decomposition temperature range of 140-1000 ºC, corresponding to the fractional loss of Schiff-base and 8-hydroxyquinoline. The exothermic peaks locate in 78 and 267 ºC. The weight percentage of 16.21 % (calcd. 16.82 %) of the original sample remained, CuO is the final residue.

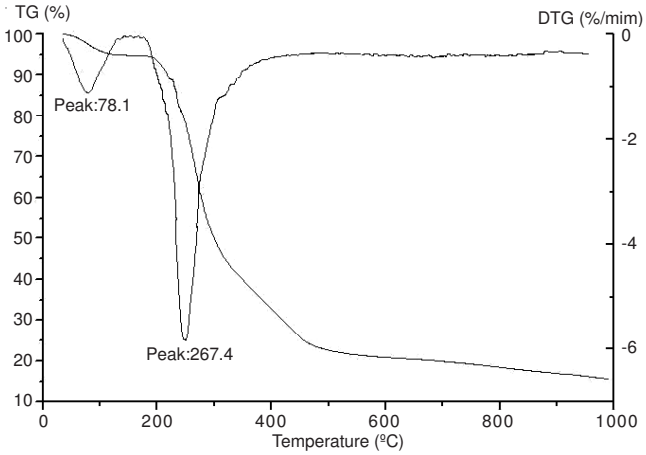

Fig. 1. Thermal analysis curves of the title compound

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

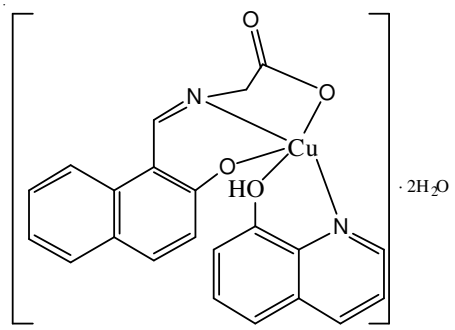

Fig. 2. Suggested structure of the complex

Cyclic voltammetry characteristics of the Cu(II) complex and its interaction with DNA: The cyclic voltammograms of Cu(II) complex are shown in Fig. 3. The curve 1 is a cyclic voltammogram of the title complex in the B-R (Britton-Robinson) buffer solution (pH = 6.82). The oxidation peak occurs at the potential -0.394 V. The curve 2 is cyclic voltammogram of the mixed solution of DNA and complex in the B-R buffer solution, the peak potential shifts to -0.354 V, 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

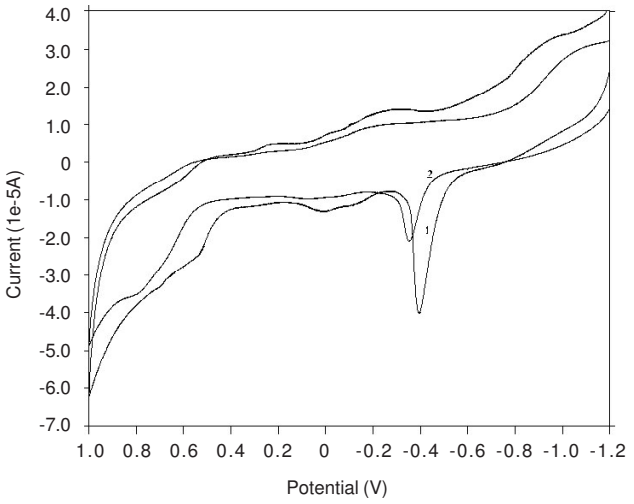

Fig. 3. Cyclic voltammograms of Cu(II) complex in B-R buffer solution (pH = 6.82). $C_{[Cu(L)(OHQ)]\cdot 2H2O}$: 2.00×10^{-4} mol L^{-1}, C_{DNA}: (1) 0 (2) 5.20 $\times 10^{-4}$ mol L^{-1}

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. Carter *et al.*[9] deemed that when the molecule inserts into the inner of DNA bi-helix structure, the peak currents of the oxidation of the voltammetry decreased with positive shifts of the peak potential. On the other way round, 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, we can infer that the complex interact with DNA in the form of intercalative binding.

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

$$\text{DNA} + n\text{ML} \rightleftharpoons \text{DNA-nML}$$

(n = 1, 2, 3, … or 1, 1/2, 1/3, …)

The equilibrium constant ß could be expressed as eqn. (1-7)

$$\beta = \frac{[\text{DNA} - n\text{ML}]}{[\text{DNA}][\text{ML}]^n} \quad (1)$$

$$\Delta I_{p,max} = KC_{DNA} \quad (2)$$

$$\Delta I_{p_a} = K[\text{DNA} - n\text{ML}] \quad (3)$$

$$[\text{DNA}] + [\text{DNA} - n\text{ML}] = C_{DNA} \quad (4)$$

$$\Delta I_{p,max} - \Delta I_{p_a} = K(C_{DNA} - [\text{DNA} - n\text{ML}]) \quad (5)$$

$$\Delta I_{p,max} - \Delta I_{p_a} = K[\text{DNA}] \quad (6)$$

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

C_{DNA} and [DNA] in the equations denote analysis concentration and equilibrium concentration of DNA, respectively, [ML] denotes equilibrium concentration of metal complexes, ΔI_{pa} and $\Delta I_{p,max}$ denote differencing and the maximum differencing of the reduction peak and the oxidation peak current of ML on account of DNA addition, respectively. According to the eqn. 7, different n might result in different curves of ΔI_{pa}^{-1} *versus* $[[Cu(L)(OHQ)]\cdot 2H_2O]^{-n}$. With the suitable n, the curve of ΔI_{pa}^{-1} *versus* $[[Cu(L)(OHQ)]\cdot 2H_2O]^{-n}$ should be a straight line if there was only one complex formed when [Cu(L)(OHQ)]·2H$_2$O 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(L)(OHQ)]·2H$_2$O 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}, $\Delta I_{pa} = I_{pa1} - I_{pa2}$) and the analytical concentration of [Cu(L)(OHQ)]·2H$_2$O was also displayed (curve 3).

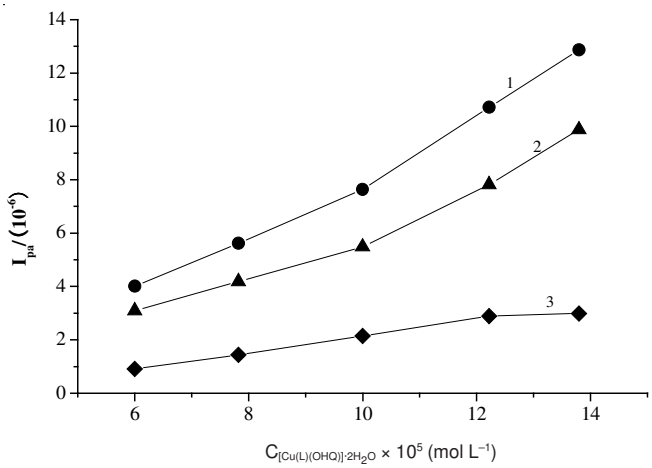

Fig. 4. Relationship curve of I_{pa1}, I_{pa2} and ΔI_{pa} *versus* C [Cu(L)(OHQ)]·2H$_2$O. 1. C_{DNA}: 0. 2. C_{DNA}: 1.4×10^{-4} mol L^{-1}, 3. $\Delta I_{pa} = I_{pa1} - I_{pa2}$

The curves of ΔI_{pa}^{-1} *versus* $[[Cu(L)(OHQ)]\cdot 2H_2O]^{-0.5}$, ΔI_{pa}^{-1} *versus* $[[Cu(L)(OHQ)]\cdot 2H_2O]^{-1}$ and ΔI_{pa}^{-1} *versus* $[[Cu(L)(OHQ)]\cdot 2H_2O]^{-2}$ were displayed in Fig. 5, where [Cu(L)(OHQ)]·2H$_2$O] represented the equilibrium concentration of [Cu(L)(OHQ)]·2H$_2$O and calculated from data in Fig. 4, for n = 0.5 and 2.0, the curves bent down and up, respectively. While for n = 1, the curve was a straight line (γ = 0.9992), indicating the forming of a 1:1 association between [Cu(L)(OHQ)]·2H$_2$O and DNA. From the slope and intercept of the straight line, the binding constant ß was calculated to be 4.6×10^2 L mol^{-1}.

Fluorescence spectra of the interaction between Cu(II) complex and DNA: Ethidium bromide (EB) is one of weak fluorescent reagent. When it inserts into the inner of DNA bi-helix structure, the system of EB-DNA can give off stronger fluorescence and hindrance the copy of DNA. If the molecule can insert DNA, the molecules compete with ethidium bromide at the bonding point of DNA, the ethidium bromide releases. As the ethidium bromide is free, the fluorescence intensity of the system become weak, through which we can determine if the molecule inserts into the inner of DNA bi-helix structure.

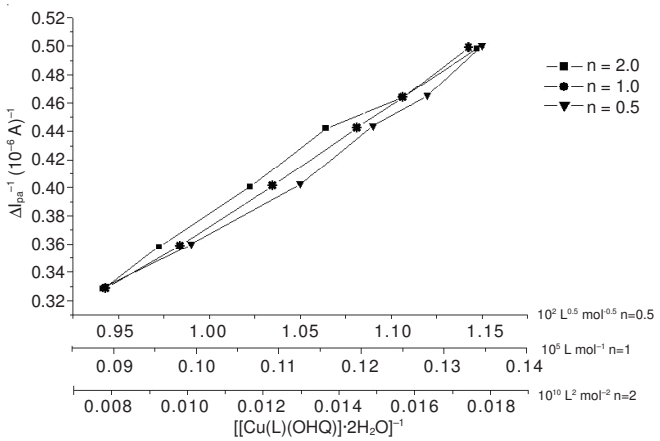

Fig. 5. Relationship curve of ΔI_{pa}^{-1} *versus* [[Cu(L)(OHQ)]·2H$_2$O]$^{-n}$

The fluorescence spectra of EB-DNA system with the complex of different concentration are shown in Fig. 6. Fig. 6 shows the fluorescence intensity of the EB-DNA system decreased obviously when the complex was added into the system of EB-DNA, which indicates the complex combined with DNA, competing with ethidium bromide and the ethidium bromide was replaced partly by the complex. The complex and ethidium bromide may combine with DNA at the same spot, which further proves the complex inserted into the inner of DNA bi-helix structure.

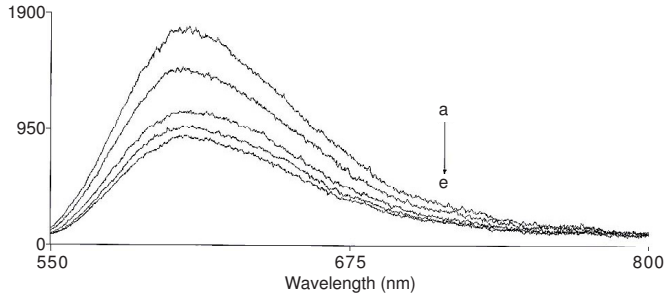

Fig. 6. Fluorescence spectra of EB-DNA system with the complex of different concentration. (a) C_{Comp} = 0 mol L^{-1}; (b) C_{Comp} = 3 × 10^{-5} mol L^{-1}; (c) C_{Comp} = 6 × 10^{-5} mol L^{-1}; (d) C_{Comp} = 8 × 10^{-5} mol L^{-1}; (e) C_{Comp} = 1 × 10^{-4} mol L^{-1}

Conclusion

The results presented here indicate that Cu(II) can form stable solid ternary complex with Schiff base glycine-2-hydroxy-1-naphthaldehyde (KHL) and 8-hydroxyquinoline (OHQ). The composition of the complex is confirmed to be [Cu(L)(OHQ)]·2H$_2$O. The interaction of the copper complex and DNA was also studied by cyclic voltammetry and fluorescence spectra. The results of the cyclic voltammetry showed that the copper complex has an oxidation peak with the peak potential at -0.394 V. After the DNA was added, the peak currents declined with the peak potential shifting positively. This suggested that the complex combined with DNA in the form of intercalative binding, which was also proved by the fluorescence analysis. The binding ratio between the Cu(II) ternary complex and DNA was calculated to be 1:1 and the binding constant was 4.6 × 10^2 L mol^{-1}.

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