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Synthesis, Characterization and Electrochemical Studies on the Interaction Mechanism between Copper(II) Complex and DNA

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The Cu(II) Schiff base was synthesized and characterized by elemental analysis, molar conductivity, IR and thermal decomposition. Cyclic voltammetry was used to investigate the interaction between $[Cu(GNA)(H_2O)]\cdot H_2O$ (where GNA = glycine) and DNA. The complex had excellent electrochemical activity on the glassy carbon electrode (GCE) with a couple quasi-reversible redox peaks. In 0.05 M Britton-Robinson (B-R) buffer solution (pH 4.86), the binding ratio between $[Cu(GNA)(H_2O)]\cdot H_2O$ and DNA was found to be 1:1 and the binding constant was 1.67×10^4 L mol^{-1}.

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

INTRODUCTION

Deoxyribonucleic acid (DNA) is an important genetic substance in organism. As the basis of genetic expression, it plays an important role in the process of storing, copying and transmitting germ messages[1,2]. 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[3,4]. The knowledge of the structure of DNA and its interactions with other biological compound can lead to advances in pharmacology and diagnosis basis[5-9].

In this paper, the $[Cu(GNA)(H_2O)]\cdot H_2O$ was synthesized. The interaction between $[Cu(GNA)(H_2O)]\cdot H_2O$ and DNA has studied by cyclic voltammetry. The experimental result has proved that $[Cu(GNA)(H_2O)]\cdot H_2O$ could interact with DNA mainly by electrostatic binding.

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 (A260/A280 > 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 170SX spectrophotometer in the 4000-400 cm^{-1} region. Molar conductivity at room temperature was measured in 10^{-3} M DMSO solution using a DDS-11 A 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.

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 for later use. The yield of the reaction was 87.9 %. Anal. calcd. (%) for C, 58.41; H, 3.77; N, 5.24; Found: C, 58.39; H,3.73; N, 5.21. IR data (KBr, ν_{max}, cm^{-1}): 1642 (C=N); 1590, 1360 (COO-); 1229 (Ar-O).

Preparation of the complex: 0.5 mmol of the copper(II) 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. 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, 47.78; H, 4.01; N, 4.29; Cu, 19.45. Found: C, 47.64; H, 4.12; N,4.23; Cu,19.86. IR data (KBr, ν_{max}, cm^{-1}): 1632 (C=N); 1578, 1371 (COO-); 1219 (Ar-O).

Electrochemical study on the interaction between $[Cu(GNA)(H_2O)]\cdot H_2O$ and DNA: 25 µL of 6.00×10^{-5} mol L^{-1} $[Cu(GNA)(H_2O)]\cdot H_2O$ solution was transferred into 5 mL colorimetric tubes containing 0.05 mol L^{-1} pH 4.86 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 0.20 V to -0.40 V, the scanning rate was 0.062 V/s; the sample interval was 0.001 V and the quiet time was 2s.

RESULTS AND DISCUSSION

The title complex $[Cu(GNA)(H_2O)]\cdot H_2O$ is dark green powder, soluble in DMSO, 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[10].

The shift of ν(C=N) from 1642 cm^{-1} in the ligand to 1632 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 1578 and 1371 cm^{-1} in the complex, respectively, suggests the coordination of the oxygen in the carboxylate group to the metal ion. The value of $\nu[\nu_{as}(COO^-)-\nu_s(COO^-)]$ = 206.4 cm^{-1} indicates that the $-COO^-$ group is coordinated to the metal ion in a monodentate fashion[11]. 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 (1219 cm^{-1}) is lower than 1229 cm^{-1}, which exposes that Ar-O-Cu in the complex.

The TG and DTG curves of the complex are shown in Fig. 1, which indicates that the complex decomposes in three steps. The first weight loss step has a decomposition temperature range of 40-110 ºC with a weight loss of 5.13 %,which corresponds to the loss of one molecule of water (calcd. 5.51 %). The fact that the water molecule was lost at a low temperature suggests that the water is crystal water. The second weight loss step has a decomposition temperature range of 110-240 ºC with a weight loss of 5.13 %, which corresponds to the loss of one molecule of water (calcd. 5.51 %), suggesting that the water is coordinated with the metal ion. The third stage showed a continuous weight loss between 240 and 800 ºC, and 26.2 % of the original sample remained. With its calculated weight percentage of 24.5 %, CuO is the final product.

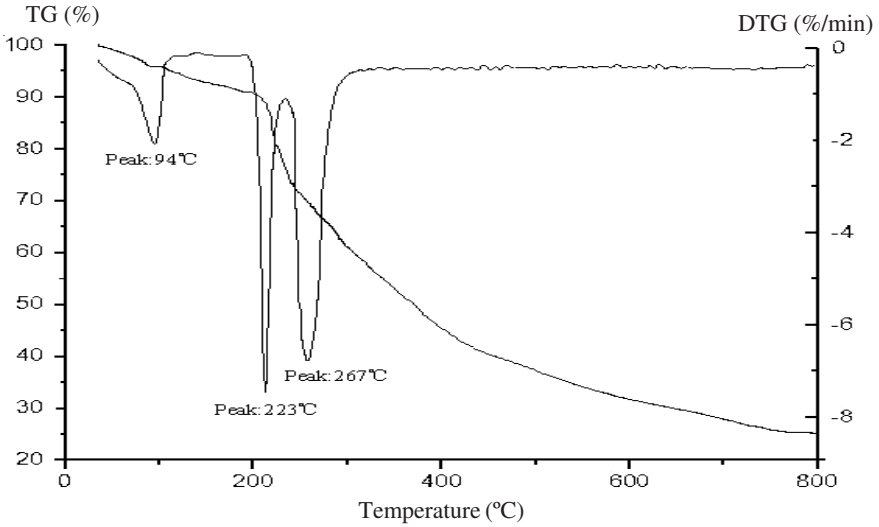

Fig. 1. TG-DTG curves of the complex

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

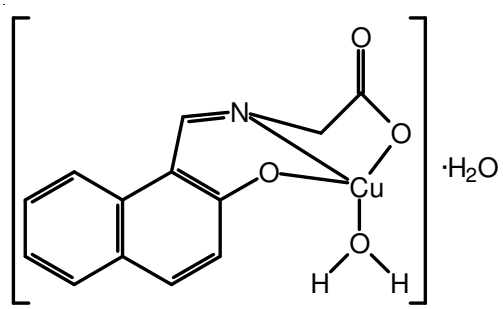

Fig. 2. Suggested structure of the complex

Electrochemical study on the interaction between [Cu(GNA)(H_2O)]·H_2O and DNA: Electrochemical study on [Cu(GNA)(H_2O)]·H_2O and its interaction with DNA were performed at 25 ºC. The cyclic voltammograms of [Cu(GNA)(H_2O)] ·H_2O in the absence and presence of DNA were shown in Fig. 3. The buffer used was 0.05 M pH 4.86 B-R solution. We could see that three a couple of quasi-reversible redox peaks for [Cu(GNA)(H_2O)]·H_2O (curve 1). The cathodic peak potential (E_{pc}) and the anodic peak potential (E_{pa}) were -0.194 V and -0.131 V, respectively. The peak potential separation(ΔE_p) was 63 mV at the scanning rate of 0.062 V S^{-1} as expected for a one-electron quasi-reversible redox process.

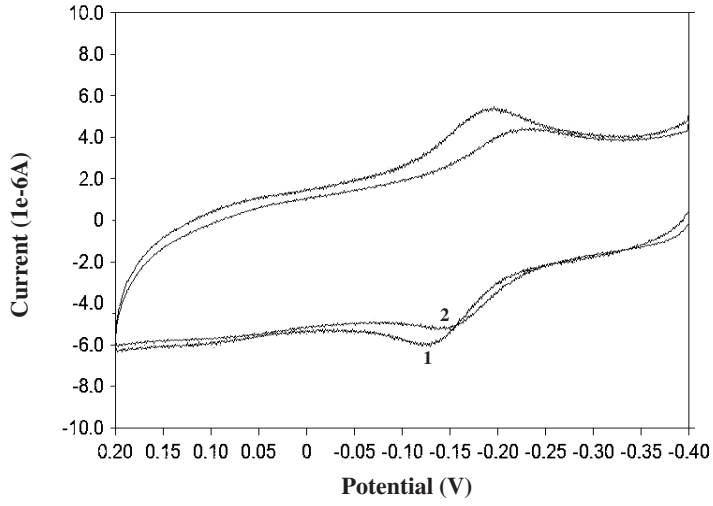

Fig. 3. Cyclic voltammograms of [Cu(GNA)(H_2O)]·H_2O and the interaction of the complex and DNA
$C_{[Cu(GNA)(H_2O)]\cdot H_2O}$:6.00 × 10^{-5} mol L^{-1}, C_{DNA}: (1)0(2) 1.40 × 10^{-4} mol L^{-1}

Curve 2 was the voltammogram obtained when $[Cu(GNA)(H_2O)]\cdot H_2O$ interacted with DNA for 7 min. The cathodic peak potential (E_{pc}) and the anodic peak potential (E_{pa}) were -0.238 and -0.138 V, respectively. The peak potential separation (ΔE_p) was 100 mV and its formal potential ($E_{½}$) was -0.188 V. As can be seen, both the cathodic and anodic peak currents (I_{pc} and I_{pa}) decreased and its formal potential ($E_{½}$) shifted to negative potentials. The phenomena indicated the forming of a new association complex. According to Carter and Bard[12], intercalative binding of small molecules to DNA might make $E_{½}$ shift to more positive value, while electrostatic binding might make $E_{½}$ shift to more negative value.

Binding ratio and binding constant between $[Cu(GNA)(H_2O)]\cdot H_2O$ and DNA: To study the binding ratio and binding constant between $[Cu(GNA)(H_2O)]\cdot H_2O$ and DNA, it was assumed the interaction of DNA and $[Cu(GNA)(H_2O)]\cdot H_2O$ only produced one single complex: DNA-nML, as shown in the following equation[13]:

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

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

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

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

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

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

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

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

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

According to the eqn. 7, different n might result in different curves of ΔI_{pa}^{-1} *vs.* $[[Cu(GNA)(H_2O)]\cdot H_2O]^{n-}$. With the suitable n, the curve of ΔI_{pa}^{-} *vs.* $[[Cu(GNA)(H_2O)]\cdot H_2O]^{n-}$ should be a straight line if there was only one complex formed when $[[Cu(GNA)(H_2O)]\cdot H_2O]^{n-}$ 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 $[Cu(GNA)(H_2O)]\cdot H_2O$ 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}, Ipa = I_{pa1} - I_{pa2}) and the analytical concentration of $[Cu(GNA)(H_2O)]\cdot H_2O$ was also displayed (curve 3).

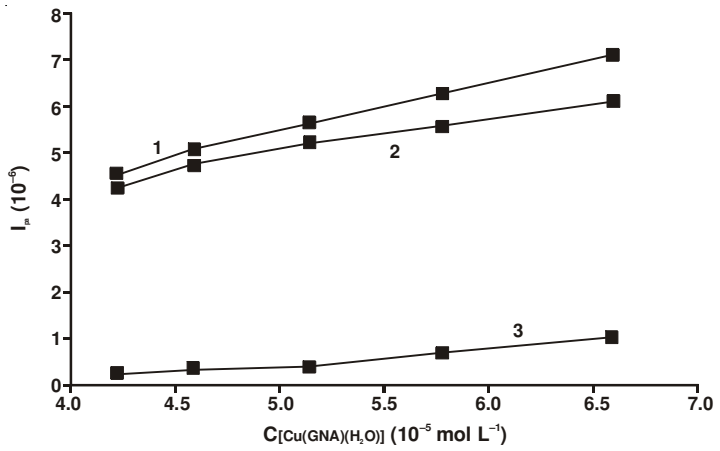

Fig. 4. Relationship curve of I_{pa1}, I_{pa2} and ΔI_{pa} *vs.* $C_{[Cu(GNA)(H2O)]\cdot H2O}$
1. C_{DNA}: 0, 2. C_{DNA}:1.4×10^{-4} mol L^{-1}, 3. $\Delta I_{pa} = I_{pa1} - I_{pa2}$

The curves of ΔI_{pa}^{-} *vs.* $[[Cu(GNA)(H_2O)]\cdot H_2O]^{-0.5}$, ΔI_{pa}^{-} *vs.* $[[Cu(GNA)(H_2O)]\cdot H_2O]^{-}$, ΔI_{pa}^{-} *vs.* $[[Cu(GNA)(H_2O)]\cdot H_2O]^{2-}$, were displayed in Fig. 5, where $[Cu(GNA)(H_2O)]\cdot H_2O$ represented the equilibrium concentration of $[Cu(GNA)(H_2O)]\cdot H_2O$ 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 ($\gamma = 0.9982$), indicating the forming of a 1:1 association between $[Cu(GNA)(H_2O)]\cdot H_2O$ and DNA. From the slope and intercept of the straight line, the binding constant β was calculated to be 1.67×10^4 L mol^{-1}.

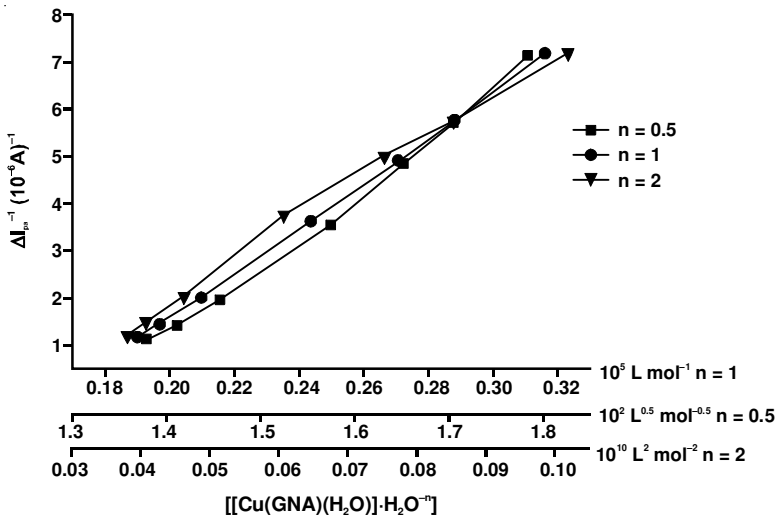

Fig. 5. Relationship curve of ΔI_{pa}^{-1} *vs.* $[[Cu(GNA)(H_2O)]\cdot H_2O]^{n-}$

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