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Electrochemical and Spectroscopic Studies on the Interaction Between Tetracoordinate Macrocyclic Copper(II) Complex and DNA

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The crystal structure of tetracoordinate macrocyclic copper(II) complex ([CuL]$I_2 \cdot H_2O$, CuL) was determined by X-ray crystallography. The interaction of tetracoordinate macrocyclic copper(II) complex (CuL) with DNA in 0.1 mol L^{-1} Na_2HPO_4—NaH_2PO_4 buffer solution (pH 6.5) was studied by differential pulse voltammetry, cyclic voltammetry and UV/Vis spectroscopy. The observed oxidative peak from differential pulse voltammogram was shown at the glassy carbon electrode. It has been found that the oxidative peak current decreased significantly in the presence of DNA compared with that in the absence of DNA. At the same time, the absorbance of CuL at its absorption peaks also decreased with increasing concentration of DNA, but no obvious shift was observed. All experimental results indicate that CuL could interact with DNA mainly by electrostatic interaction and form a 1 : 1 DNA-CuL association complex with a binding constant of 1.02×10^4 mol^{-1} L.

Key Words: Tetracoordinate macrocyclic copper(II) complex, DNA, differential pulse voltammetry, Cyclic voltammetry, UV/Vis spectroscopy, Electrostatic interaction.

INTRODUCTION

Deoxyribonucleic acid (DNA) is the most important germplasm of most organisms. It plays an important role in the process of storing, copying and transmitting germ messages. The recognition that DNA serves as a target for natural and artifical molecules in the inhibition of cellular disorders and in therapy of certain diseases is of paramount importance in inorganic biochemistry. The binding of small molecules, especially transition metal complxes to DNA and molecular identification are important research subjects in life science. There are many articles on the interaction between small molecules and DNA since 1960s. Gradually this research has become a field of general interest[1], because it is helpful to understand the way of the interaction between small molecules and DNA. What is more, this is very important to expound the action mechanism of anticancer drugs, the external selection of drugs and carcinogenesis of the

carcinogenic compounds. It has been reported that many metal complexes have anticarcinogenesis. Among these compounds, people have paid much attention to the complexes, such as $Fe[EDTA]^{2-}$, $Cu(phen)_2^{2+}$, RuNi binuclear complex, dicyclopentadienyl iron, etc. They have the ability of splitting DNA and distinguishing DNA[2-5]. In addition, copper complexes including $Cu(II)_2(salicylate)_4$ and $Cu(II)_2(3,5\text{-}DIPS)_4$ having SOD-mimetic activity lead to conclusions that copper complexes might have anticancer activity[6]. These complexes decreased tumour growth, metastasis and increased survival of tumour-bearing mice. An action mechanism of copper complexes as anticancer has been suggested[7] to involve glutathione oxidation and accumulation of H_2O_2.

However, there are no reports on the action mechanism of anti-AIDS drugs. Moreover, macrocyclic copper complexes have activity of chemical nuclease[8] and anti-AIDS[9], so it is significant for further research and complete understanding of the interaction of macrocyclic copper(II) complex with DNA to expound the action mechanism of anti-AIDS drugs. In this paper, we have conducted experiments on the interaction of CuL with DNA by electrochemical and spectroscopic methods with CuL prepared according to the literature[10]. The experimental results have proved that CuL could interact with DNA mainly by electrostatic interaction. This conclusion would surely bring detailed insight into the action mechanism of macrocyclic copper(II) complexes as anti-AIDS drugs and provide useful message for designing new and efficient anti-AIDS drugs.

EXPERIMENTAL

CHI832 electrochemical analyzer was produced by Shanghai Chenhua Instrument Company of China; the three-electrode system was composed of a glassy carbon electrode (GCE) as working electrode, an Ag/AgCl electrode as the reference electrode and a platinum electrode as auxiliary electrode; Cary50 UV/Vis spectrophotometer was produced by Nicolet Company of United States; pH-25 pH-meter was produced by Shanghai Leici Instrument Factory of China.

Salmon sperm DNA was purchased from Shanghai Huashun Biologic Engineering Company used without further purification. Its concentration was determined by the ultraviolet absorption at 260 nm ($\varepsilon = 6600$ L mol^{-1} cm^{-1}), used without further purification. 1.07×10^{-2} mol L^{-1} solution of CuL was prepared by dissolving 0.0676 g of CuL in 10 mL doubly deionized water. 0.1 mol L^{-1} Na_2HPO_4-NaH_2PO_4, pH 6.5, was used as buffer solution. The other reagents were all analytical reagents prepared with doubly deionized water.

Preparation of CuL Single Crystal: 13 mL anhydrous ethylenediamine and 10 mL anhydrous alcohol were mixed in a round-bottom flask, which was put into a container of frozen water, then 36 mL hydriodic acid and 30 mL acetone were added into this flask; the resulting solution was allowed to stand overnight at this condition. The solid that appeared was separated by decompressing filtration, followed by washing with acetone and then white crystals of macrocyclic ligand were obtained.

0.10 mol macrocyclic ligand and 0.01 mol copper acetate were dissolved in 40 mL anhydrous alcohol completely. After refluxing for 1 h, the mixture was

filtered. The filtrate was heated in hot water until solid began to form, followed by being cooled in frozen water for several hours. The solid that appeared was separated by decompressing filtration and dissolved in anhydrous alcohol. The amaranthine solution was filtered and the filtrate was left to stand undisturbed. Upon slow evaporation at room temperature, purple crystals suitable for detection appeared a week later.

Electrochemical studies of the interaction between CuL and DNA: Different quantityies of 1.07×10^{-2} mol L^{-1} CuL were added to 5 mL of 0.1 mol L^{-1} Na_2HPO_4—NaH_2PO_4 buffer solution. The differential pulse voltammetry and cyclic voltammetry curves of CuL were recorded on CHI832 electrochemical analyzer with the three-electrode system above. Then different quantities of DNA were added to the solution followed by recording the figure. Cyclic voltammetry instrument parameters: the potential scanning range is from 1.2–1.0 V; the scanning rate is 0.6 V s^{-1}; the sample interval 0.001 V and the quiet time 2 s. Differential pulse voltammetry instrument parameters: the potential scanning range is from 0.40–0.05 V; the increasing potential is 0.004 V; the pulse width is 0.06 s; the pulse period is 0.2 s and the quiet time 2 s. Figs. 3–7 were recorded by cyclic voltammograms. Figs. 4, 8 and 9 were recorded by differential pulse voltammograms.

UV/Vis studies of the interaction between CuL and DNA: 2 mL of 1.07×10^{-2} mol L^{-1} CuL and different volumes of 4.68×10^{-2} mol L^{-1} salmon sperm DNA solution were in turn added to 10 mL colorimetric tubes respectively, then diluted to the desired scale with 0.1 mol L^{-1} Na_2HPO_4—NaH_2PO_4 buffer solution. The solutions were set for 9 min at room temperature. The UV/Vis spectra were recorded on a Cary 50 spectrophotometer in 1 cm quartz cuvettes. The range of the scanning wavelengths is from 200 to 800 nm.

RESULTS AND DISCUSSION

X-ray crystal structure of the title compound ($[CuL]I_2 \cdot H_2O$)

The asymmetric unit of the title compound ($[CuL]I_2 \cdot H_2O$, L = 5,7,7,12,14,14-hexamethyl-1,4,8,11-tetraazacyclotetradeca-4,11-diolefin) consists of one monomeric $[CuL]^{2+}$ cations, two iodine anions and one free water molecules linked by electrostatic forces and hydrogen bonds. The $[CuL]I_2 \cdot H_2O$ crystallizes in the triclinic system, space group P-1, with cell dimensions of a = 7.0968 (15) Å, b = 8.8896 (19) Å, c = 10.533 (2) Å, α = 103.304 (4)°, β= 100.684 (4)°, γ = 109.959 (3)°, and Z = 1. The copper(II) ions have a square-planar geometry. In the crystal packing, the water molecule plays important roles in the structure acting as a bridge between the cation and the anion, two iodine anions are between regular lamination forming by the macrocyclic complex ion. The whole complexes forming a 3-dimensional network structure. Crystal data and structure refinement for the title compound are shown in Table-1. Selected bond lengths

and angles are presented in Table-2. Fig. 1 shows a perspective view of the molecular structure of $[CuL]I_2 \cdot H_2O$ with the atomic numbering scheme.

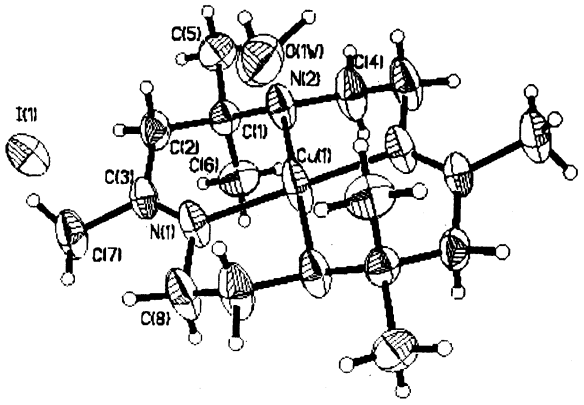

Fig. 1. The molecular structure of $[CuL]I_2 \cdot H_2O$ with the atomic numbering scheme

TABLE-1
CRYSTAL DATA AND STRUCTURE REFINEMENT PARAMETERS FOR $[CuL]I_2 \cdot H_2O$

Formula	$C_{16}H_{34}CuI_2N_4O_2$
Formula weight	631.81
Colour/shape	Purple/column
Crystal system	Triclinic
Space group	P-1
a (Å)	7.0968 (15)
b (Å)	8.8896 (19)
c (Å)	10.533 (2)
α (°)	103.304 (4)
β (°)	100.684 (4)
γ (°)	109.959 (3)
V (Å^3)	582.0 (2)
Z	1
$D_{(calcd.)}$ (g cm^{-3})	1.803
μ (mm^{-1})	3.607
Crystal size/mm	0.40 × 0.30 × 0.10
Temp. (K)	293 (2)
θ ranges (°)	2.08–23.26
h/k/L	–6, 7/–9, 6/–9, 11
Reflections collected	1868
Independent reflections	1540
Absorption correction	multi-scan
Final *R* indices [$I > 2\sigma(I)$]	0.0388

TABLE-2
SELECTED BOND DISTANCES (NM) AND BOND ANGLES (°) OF $[CuL]I_2 \cdot H_2O$

Cul-N1	1.970 (5)	C1-C6	1.514 (9)
Cul-N1	1.970 (5)2	C1-C2	1.530 (8)
Cul-N2	2.01ᴢ (5)	C1-C5	1.531 (8)
Cul-N2	2.012 (5)2	C2-C3	1.499 (9)
N1-C3	1.278 (8)	C3-C7	1.494 (8)
N1-C8	1.464 (8)	C4-C8	1.504 (10)
N2-C4	1.480 (8)	C8-C4	1.504 (10)
N2-C1	1.501 (8)		
N1-Cu1-N1	180.0 (4)	N2-C1-C6	112.3 (5)
N1-Cu1-N2	85.4 (2)	N2-C1-C2	107.0 (4)
N1-Cu1-N2	94.6 (2)	C6-C1-C2	110.5 (5)
N1-Cu1-N2	94.6 (2)	N2-C1-C5	109.8 (5)
N1-Cu1-N2	85.4 (2)	C6-C1-C5	109.2 (5)
N2-Cu1-N2	180.0 (2)	C2-C1-C5	107.9 (5)
C3-N1-C8	121.4 (5)	C3-C2-C1	119.5 (5)
C3-N1-Cu1	128.0 (4)	N!-C3-C7	123.5 (6)
C8-N1-Cu1	110.5 (4)	N1-C3-C2	121.3 (5)
C4-N2-C1	115.3 (5)	C7-C3-C2	115.2 (5)
C4-N2-Cu1	104.8 (4)	N2-C4-C8	107.6 (5)
C1-N2-Cu1	118.7 (4)	N1-C8-C4	107.7 (6)

Electrochemical behaviour of CuL on the glassy carbon electrode

There are shapely oxidation or reduction peaks of CuL from cyclic voltammograms or differential pulse voltammograms in the base solution of 0.1 mol L^{-1} Na_2HPO_4-NaH_2PO_4, 0.2 mol L^{-1} B—R, 0.05 mol L^{-1} tris-HCl or 0.1 mol L^{-1} NaOAc—HOAc. Among them, the peaks in 0.1 mol L^{-1} Na_2HPO_4-NaH_2PO_4 are the best. Therefore, 0.1 mol L^{-1} Na_2HPO_4-NaH_2PO_4 as the base solution is selected.

The cyclic voltammogram (CV) of CuL was recorded in 0.1 mol L^{-1} $Na_2HPO_4 \cdot NaH_2PO_4$ buffer solution (pH 6.5). A couple of redox peaks for the ligand of CuL were observed at the glassy carbon electrode. The cathodic peak potential ($E_{pc.}$) and the anodic peak potential ($E_{pa.}$) are 0.453 and 0.539 V respectively, the separation of the cathodic and the anodic peak potentials (ΔE_p) is 86 mV, indicating a quasi-reversible redox process; its formal potential ($E_{1/2}$) is 0.496 V. In addition, the other anodic peak for the central copper ion was also observed, its peak potential ($E_{pa.}$) is –0.084 V.

Effect of scan rate on the oxidation peak current of CuL (by CV)

I_{pa} is directly proportional to the square root of the scanning rate in the range from 0.01 to 0.25 V s^{-1}, with a regression equation: $y = 14.351x + 0.3955$ and a correlation coefficient: $\gamma = 0.9989$, where y is the $I_{pa.}$ value, x is the scanning rate.

The plot of $I_{pa.}$ *vs.* $v^{1/2}$ (v is the scanning rate), showed a straight line (Fig. 3), indicating the electro-oxidation process of CuL is controlled by the diffusion of CuL.

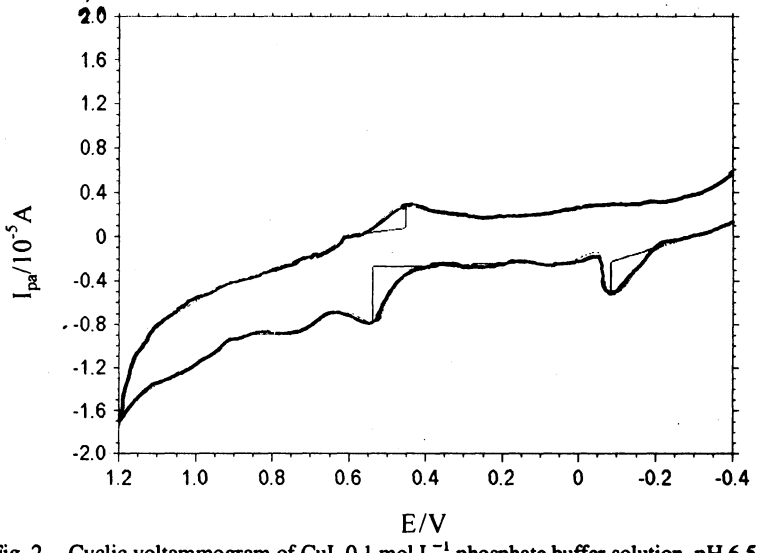

Fig. 2. Cyclic voltammogram of CuL 0.1 mol L^{-1} phosphate buffer solution, pH 6.5

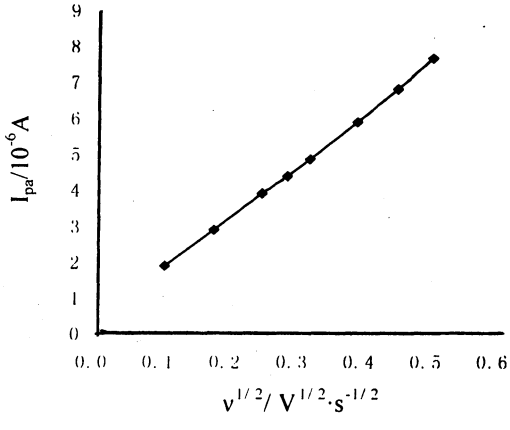

Fig. 3. The relationship between the oxidation peak current of CuL and the scan rate

Electrochemical studies of the interaction between CuL and DNA

The differential pulse voltammograms of CuL at the glassy carbon electrode in 0.1 mol L^{-1} Na_2HPO_4-NaH_2PO_4 buffer solution (pH 6.5) are shown in Fig. 4. The curve 1 is the differential pulse voltammogram of CuL in the absence of DNA, while the curve 2 is the differential pulse voltammogram of CuL in the presence of DNA. It was observed that the peak current of CuL greatly decreased with the addition of DNA. No new oxidation-reduction peaks appeared after adding DNA. So, CuL interacting with DNA forms electrochemically non-active complex[11] and results in a decrease of CuL concentration as well as the oxidation

peak current. So the initial conclusion can be drawn that CuL can interact with DNA.

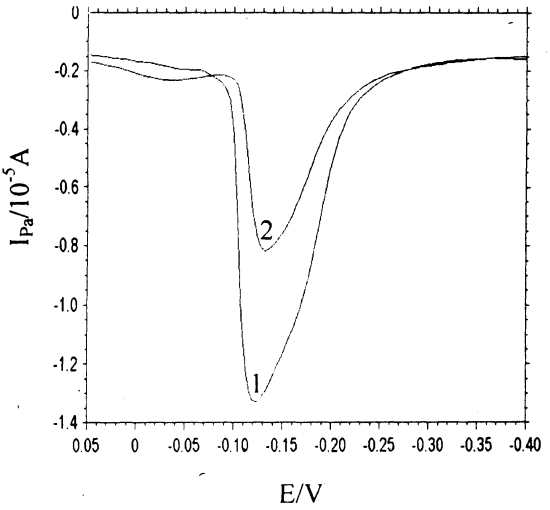

Fig. 4. Differential pulse voltammogram of CuL in the absence or presence of DNA. Scan rate: 0.06 V s^{-1}; C_{CuL} : 8.58×10^{-5} mol L^{-1}; C_{DNA}: (1) 0; (2) 2.81×10^{-4} mol L^{-1}

In addition, CV of CuL before and after adding DNA was also investigated. The cyclic voltammogram (CV) of CuL without DNA was the same as shown in Fig. 2. The formal potential ($E_{1/2}$) is 0.496 V. In the presence of DNA, the formal potential shifts slightly to more positive value. It is generally accepted that there are three kinds of binding modes for small molecules to DNA, which referred to intercalative binding, groove binding and electrostatic binding. Bard reported[12] that if the formal potential shifts to more negative value when small molecules interact with DNA, the interaction mode is electrostatic binding. On the contrary, if the formal potential shifts to more positive value, the interaction mode is intercalative binding. According to the molecular structure of CuL and literature[13], the initial conclusion can be drawn that CuL may intercalate into DNA, with partial insertion of the ligands between adjacent base pairs on the duplex strand. At the same time, the remaining ligands are disposed along the major groove of the DNA molecule and, therefore, the complex can also interact electrostatically with the sugar-phosphate backbone.

Effect of the reaction time on the interaction of CuL with DNA

The relationship between the variation of the oxidation peak current of CuL (by CV) and the reaction time of CuL with DNA at room temperature was experimented. Fig. 5 is the plot of ΔI_{pa} *vs.* t (ΔI_{pa} is the variation of the oxidation peak current of CuL in the absence or presence of DNA, t is the reaction time). The value of $\Delta I_{pa.}$ increases with the increase of reaction time and reaches a constant value after about 9 min, which indicates that the reaction of CuL with DNA has reached equilibrium state. Consequently, 9 min was chosen as the reaction time.

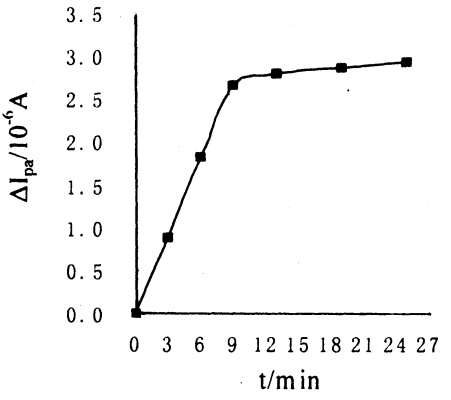

Fig. 5. Effect of time on the variation of the oxidation peak current of CuL C_{CuL} : 1.29×10^{-4} mol L^{-1}; C_{DNA} : 3.74×10^{-4} M L

Effect of pH on the interaction of CuL with DNA

Fig. 6 shows the relationship between the variation of the oxidation peak current of CuL (by CV) before and after adding DNA and the pH value. During the experiment, the value of ΔI_{pa} increases firstly and then reaches a maximum when pH is 6.5. After that, it decreases slowly. Consequently, 6.5 was chosen as the best pH of the reaction.

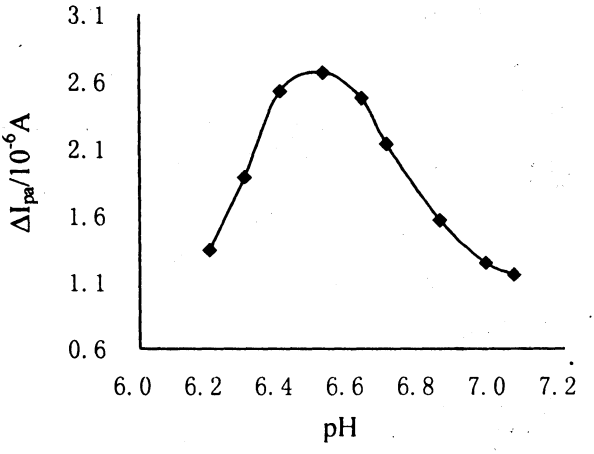

Fig. 6. The relationship between pH and the variation of the oxidation peak current of CuL C_{CuL} : 1.29×10^{-4} mol L^{-1}; C_{DNA} : 3.74×10^{-4} mol L

Effect of DNA concentration on the oxidation peak current of CuL (by CV)

The experiment that the concentrations of both ds DNA and ss DNA increased gradually while the concentration of CuL was unchanged was done. Fig. 7 shows the relationship between the oxidative peak current of CuL and DNA concentration. At the beginning, the peak current decreased obviously. When the concentration of DNA increased to a certain degree, the peak current reached a constant

value. Eventually, the peak current decreased no longer, suggesting that the interaction of CuL with DNA was saturated.

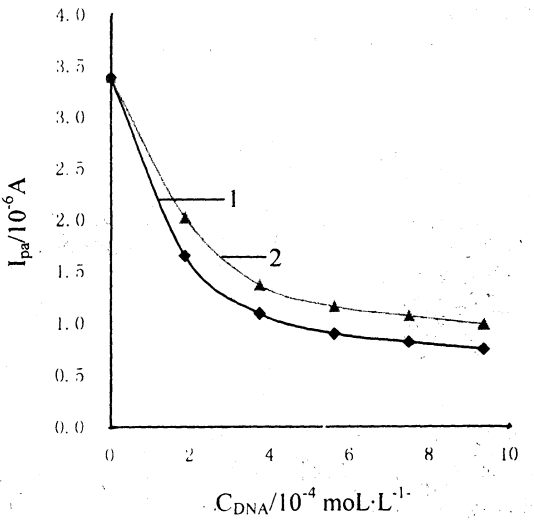

Fig. 7. Effect of the concentration of ds DNA (curve 1) or ss DNA (curve 2) on the oxidation peak current of CuL C_{CuL} : 1.29×10^{-4} mol L^{-1}

The relationship between the peak current (I_p) and the diffusion coefficient (D) can be deduced having the formula[14] $I_p = 269\ n^{3/2}\ AD^{1/2}v^{1/2}C_0$. After adding DNA, the diffusion coefficient of the association complex (CuL-DNA) is much smaller than that of the free complex (CuL) when CuL is bound to DNA, which results in the reduction of the diffusion velocity and the peak current.

According to literature[15], for the intercalative binding in which the intercalator can provide a planar aromatic heterocyclic molecule surface for efficient intercalation into ds DNA strand, the reduction effect of ds DNA concentration on the peak current is very obvious, while ss DNA has almost no reduction effect on it[16]. According to Fig. 7, the concentration of both ds DNA and ss DNA had reduction effect on the peak current of CuL, indicating that the binding mode of CuL to DNA is mainly electrostatic binding.

Binding ratio and binding constant of DNA-CuL complex

It is assumed that DNA and CuL only produce a single complex DNA-n CuL according to the reference[17]:

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

The equilibrium constant can be expressed as follows:

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

and the following equations can be deduced as

$$\Delta I_{max} = K'C_{DNA} \quad (2)$$

$$\Delta I = K'[DNA - nCuL] \quad (3)$$

$$[DNA] + [DNA - nCuL] = C_{DNA} \quad (4)$$

$$\Delta I_{max} - \Delta I = K'(C_{DNA} - [DNA - nCuL]) \quad (5)$$

$$\Delta I_{max} - \Delta I = K'[DNA] \quad (6)$$

$$\frac{1}{\Delta I} = \frac{1}{\Delta I_{max}} + \frac{1}{\beta \Delta I_{max}[CuL]^n} \quad (7)$$

According to equation (7), the curve between ΔI^{-1} and $[CuL]^{-n}$, with the suitable n, should be a straight line if only one complex was formed when CuL is bound to DNA. From the slope and intercept of the best line, the binding constant β can be calculated.

The relationship between the oxidation peak current of CuL (CV signals) and CuL concentration before and after adding DNA is shown in Fig. 8. By calculating different ΔI^{-1} and $[CuL]^{-n}$, the plot of ΔI^{-1} *vs.* $[CuL]^{-n}$ was obtained (Fig. 8). As for n = 1, the curve is a straight line (γ = 0.9991) (Fig. 9), while for n = 1/3 and 1/2, the curve bends up and down respectively. From the slope and intercept of the best line, the binding constant β can be calculated to be 1.02×10^4 L mol^{-1}, which corresponds to the equation

$$DNA + CuL \rightleftharpoons DNA - CuL$$

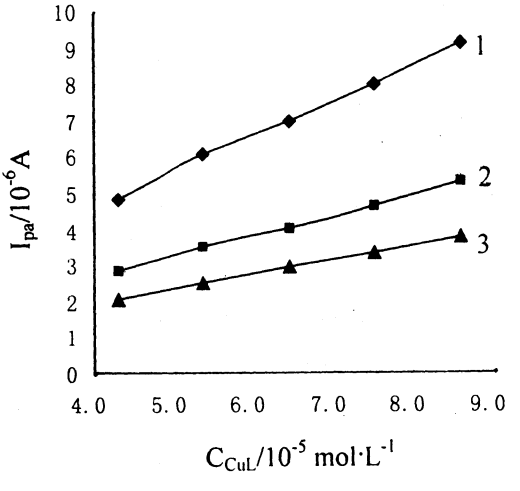

Fig. 8. The relationship between I_{pa1}, I_{pa2}, ΔI_{pa} and C_{CuL} (1) C_{DNA} : 0; (2) C_{DNA} : 1.87×10^{-4} mol L^{-1}; (3) $\Delta I_{pa} = I_{pa1} - I_{pa1}$

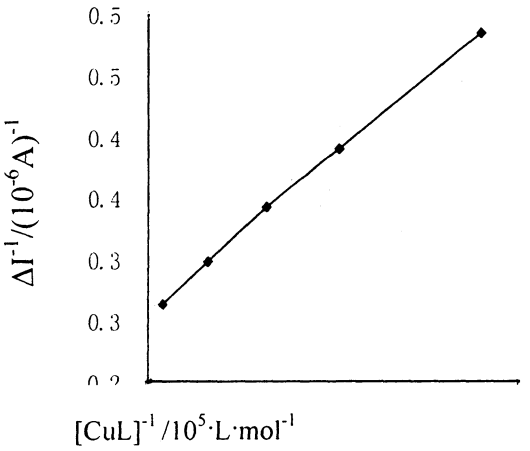

Fig. 9. The relationship curve of ΔI^{-1} *vs.* $[CuL]^{-1}$

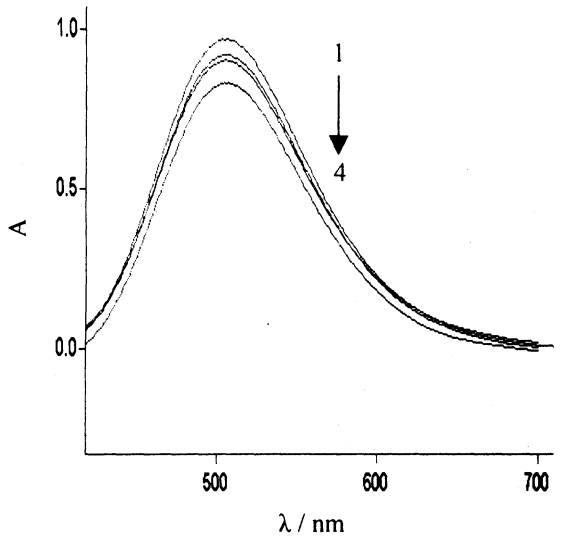

Fig. 10. Absorption syectra of CuL in different concentrations of DNA C_{CuL} : 1.07×10^{-2} mol L^{-1}; 0.1 mol L^{-1} Na_2HPO_4-NaH_2PO_4 buffer, pH 6.5; C_{DNA} : (1) 0, (2) 1.17×10^{-4} mol L^{-1}, (3) 5.78×10^{-4} mol L^{-1}, (4) 1.03×10^{-3} mol L^{-1}

UV/Vis spectroscopic studies of the interaction between CuL and DNA

Hypochromism and red shift of the absorption bands were used to characterize the binding of small molecules to DNA[18]. The variation of CuL spectra in the presence of different concentrations of DNA are shown in Fig. 10. It was observed that the absorbance of CuL at 506.1 nm greatly decreased with increasing concentrations of DNA, but no obvious red shift was observed. It is recognized that the red shift of the absorption band is an important evidence for the intercalation of small molecules into DNA base stack, while the phenomena of hypochromic effect without shift are evidence for electrostatic binding[19]. There-

fore, it can be deduced that the interaction mode of CuL with DNA is mainly electrostatic binding.

Conclusions

The interaction between CuL and DNA was studied by cyclic voltammetry, differential pulse voltammetry and UV/Vis spectroscopy. In the presence of DNA, the oxidative peak current of CuL decreased. At the same time, the absorbance of CuL at its absorption peaks also decreased with increasing concentrations of DNA, but no obvious shift of absorption peaks was observed. The conclusion can be drawn that CuL could interact with DNA mainly by electrostatic binding and form a 1 : 1 DNA-CuL complex with a binding constant of 1.02×10^4 mol^{-1} L.

ACKNOWLEDGEMENTS

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