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Electrochemical DNA Biosensor for the Detection of Interaction between Di[azino-di(5,6-azafluorene)-κ^2-NN′] Dibromicoppers and DNA

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A new Cu(II) complex CuL_2Br_2 (L = azino-di(5,6-azafluorene)-κ^2-NN′) was synthesized, and a new method of electrochemical probe has been proposed for the determination of hepatitis B virus (HBV) based on its interaction with $[CuL_2]^{2+}$. This ligand, containing functional groups, as well as planar aromatic domains, is capable of binding to double-stranded DNA (dsDNA) more efficiently than to single-stranded DNA (ssDNA). Emphasis has been placed on the elucidation of the nature of the interaction by electrochemical techniques. The electroactive $[CuL_2]^{2+}$ could be employed as an electrochemical indicator to detect hybridization events in DNA biosensors. These biosensors have been constructed by immobilization of a probe DNA sequence from HBV onto glassy carbon electrode (GCE). After hybridization with the complementary target sequence, $[CuL_2]^{2+}$ was accumulated within the dsDNA layer. Electrochemical detection was performed by differential pulse voltammetry over the potential range. Using this approach, complementary target sequences of HBV can be quantified over the range of 1.74×10^{-9} to 3.45×10^{-7} M, with a detection limit of 8.32×10^{-10} M and a linear correlation coefficient of 0.9936. In addition, this approach is capable of detecting hybridization of complementary sequences containing one or three mismatched bases.

Introduction

Films of single-stranded DNA (ssDNA) immobilized on surfaces form the basis of a number of important biotechnology applications, including DNA microarrays (Wang, 2000; Pirrung, 2002) and biosensors (Su et al., 1994; Katz and Willner, 2003; Ostblom et al., 2005). The organization of ssDNA as a monolayer allows the investigation of various properties of DNA in a controlled manner (Pan and Sanche, 2005) and the use of DNA for analytical application (Nelson et al., 2001; Li et al., 2005) as well as for exploring futuristic schemes for molecular electronics (Tabata et al., 2003).

DNA biosensors are mostly based on optical, mechanical, and electrochemical signal transduction (Downs et al., 1988; Wang, 2002; Kuswand et al., 2005). Among these methods, electrochemical methods are especially well suitable for DNA biosensor since the electrochemical response could be directly characterized with an electrochemical signal, thus precluding the need for a transduction system. In addition, electrochemical techniques readily offer a lot of attractive advantages, such as good sensitivity, accurate specificity, simplicity, and low cost for converting nucleic acid hybridization events into useful analytical signals (Palecek et al., 1981; Lukasova et al., 1984).

The interaction between DNA and small molecules is a fundamental issue in life science, which relates to replication and transcription, mutation of genes and related variations of species in character, origins of some diseases, and action mechanisms of some DNA-targeted drugs. Some works have illustrated the interaction of some organic chemicals with DNA (Li and Zhu, 1998; Wang et al., 2002; Sun et al., 2004; Cusumano et al., 2006). Recently, the interaction between the components of traditional Chinese herbal medicine with DNA has also been well studied (Sun et al., 2004). It has been well explored that the binding event of these complexes with DNA is primary dependent upon the different central metal atoms, the ligands, and the coordination geometries (Maruyama et al., 2001; Fojta et al., 2002; Kizek et al., 2002). To date, the metal octahedral complexes with rigid bidentate ligands, such as 1,10-phenanthroline (phen) or 2,2′-bipyridyl (bipy), have been mostly investigated for the targeting of specific DNA sites by matching the shape, symmetry, and functionality

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of the metal complex to those of the DNA target (Onfelt et al., 2001; Maheswari and Palaniandavar, 2004; Wang et al., 2004). However, these metal complexes could not truly realize the selective discrimination of the hybridization events because of the prevalence of variable binding modes of these complexes with DNA. Thus, the development of a new redox active indicator of metal complexes to properly discriminate the hybridized electrode from the unhybridized ones was of great importance. In our laboratory, a lot of research efforts were also conducted to explore the interaction of these metal complexes with DNA (Zhang et al., 2005; Li et al., 2007). It has been found that the intercalating ability of these metal complexes with DNA increases with improvement in planarity of ligands (Kumar et al., 1985; Xu et al., 2003a). In addition, the coordination geometry and ligand donor atom also play key roles in determining the binding mode and extent of complexes formed with DNA (Mahadevan and Palaniandavar, 1997; Xu et al., 2003). The metal ion and its flexible valence, which are responsible for the geometry of complexes, also affect the intercalating ability of metal complexes to DNA (Chaires, 1998; Mozaffar et al., 2004).

In this work, the interaction of $[CuL_2]^{2+}$ with DNA was investigated by cyclic voltammetry (CV), differential pulse voltammetry (DPV), and fluorescence methods. Using $[CuL_2]^{2+}$ as an electrochemical indicator, the electrochemical DNA biosensor was developed for the detection of human hepatitis B virus (HBV) DNA fragment. The detection limit obtained in the present work with $[CuL_2]^{2+}$ as indicator is about 30 times lower than the limit of 2.40×10^{-8} M obtained for the DNA sequence related to HBV using traditional homogenous $Co(phen)_3^{3+}$ as indicator reported by Erdem et al. (Erdem et al., 1999). The new electroactive indicator and electrochemical DNA biosensor might have potential application in DNA detection, drug designing, and diagnosis of disease.

Materials and Methods

Materials

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide solution (EDC) and *N*-hydroxysuccinimide solution (NHS) from Sigma Chemical Company (St. Louis, MO) were used without further purification. Salmon sperm DNA was purchased from Shanghai Huashun Biological Engineering Company (Shanghai, China) (A260/A280 $>$ 1.8); the concentration was determined by the ultraviolet absorption at 260 nm (ε = 6600/M·cm).

All of the oligonucleotides related to HBV gene were supplied (as lyophilized powder) by SBS Genetech Company (Beijing, China), with the following sequences:

- DNA probe (21-mer base sequence S_1): 5′-AAT GTG CTC CCC CAA CTC CTC -3′,
- Target DNA (21-mer base sequence S_2): 5′-GAG GAG TTG GGG GAG CAC ATT-3′.
- Noncomplementary to S_1 (21-mer base sequence S_3): 5′-AAA AGG TGT AAG CGT TTG CCG-3′.
- One base mismatch to S_1 (21-mer base sequence S_4): 5′-GAG GAG TTG GGG GAG CAG ATT-3′.
- Three base mismatch to S_1 (21-mer base sequence S_5): 5′-GAC GAG TTG CGG GAG CTC ATT-3′.

All oligonucleotides and ethidium bromide (EB) were dissolved in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 (TE buffer), and kept frozen. Other chemicals employed were all of analytical grade and double distilled water (DDW) was used throughout the experimental work.

Synthesis of CuL_2Br_2

CuL_2Br_2 was prepared according to published methods (Cheng et al., 1988) with some modification. Detailed procedures are given in the following paragraphs.

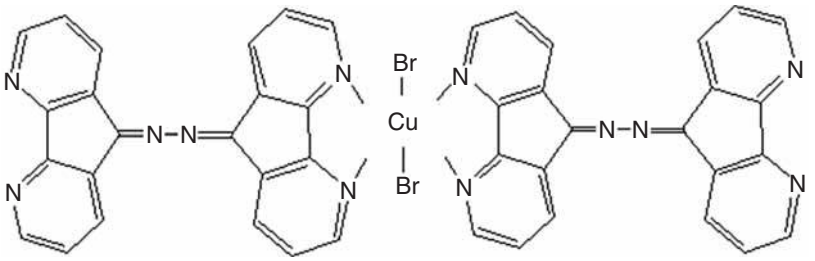
Dafone (4,5-diazafluoren-9-one). Phenanthroline (0.05 mol, 10.00 g) and KOH (0.09 mol, 5.20 g) were added to 400 mL of water and brought to reflux. Potassium permanganate (0.16 mol, 29.20 g) in 200 mL of water was added dropwise to the refluxing mixture. After addition, the solution was refluxed for 0.5 hour and filtered to remove MnO_2. Crude 4,5-diazafluoren-9-one was precipitated as yellow needle-shaped crystals after cooling the solution. Recrystallization once again from water yielded the desired dafone. Anal. calcd. (theoretical value) for $C_{11}H_6N_2O$ is C,72.57(72.94); H,3.32(3.22); N,15.36(15.41). The infrared (IR) spectra of the recrystallization precipitate were identical with the reported reference.

L (azino-di(5,6-azafluorene)-κ^2-NN′). 4,5-diazafluoren-9-one (6.1 mmol, 1.1 g) and 85% hydrazine hydrate (2.8 mmol, 0.14 mL) were placed in a 50 mL round flask. After addition, the solution was brought to reflux for 0.5 hour in 5 mL glacial acetic acid. After a natural cooling to room temperature and filtering, the final solid product was obtained by extensive washing with water and ethanol and then by recrystallization twice with dimethyl sulfoxide. Anal. calcd. (theoretical value) for $C_{22}H_{12}N_6$ is C,73.32(73.90); H,3.36(3.53); N,23.32(23.14). IR spectra of the recrystallization precipitate were identical with the ligand.

CuL_2Br_2. L (0.50 mmol, 0.17 g) was dissolved in 20 mL chloroform. $CuBr_2$ (1.00 mmol, 0.22 g) was dissolved in a mixture of acetone and water. By simply mixing the two solutions, the final product was precipitated and isolated by filtration. It was washed with water and dried under vacuum for 1 day (yield: 0.35 g). Anal. calcd.(theoretical value) for $C_{44}H_{24}N_{12}CuBr_2$ is Cu,10.32(9.74); C,43.86(43.31); H,2.16(2.31); N,13.96(13.31). IR spectra of obtained complex were also identical with the reported. The structural formula of CuL_2Br_2 is shown in Scheme 1.

Apparatus

All electrochemical measurements were carried out at room temperature, using a conventional three-electrode cell. Modified glassy carbon electrodes (GCEs) were used as working electrodes and Pt wire served as the auxiliary electrode. All potentials were reported with respect to an Ag/AgCl/KCl (sat) reference electrode. CV and DPV studies were carried out with a CHI 832b electrochemical analyzer (Shanghai Chenhua Instrument Company, China). A Hitachi F-4500 fluorescence spectrophotometer (Tokyo, Japan) equipped with 1-cm quartz cells was used for fluorescence measurements. The pH of all solutions was measured by a Model PHS-3D digital acidometer (Shanghai Leici Instrument



SCHEME 1. Structural formulae of CuL_2Br_2.

Factory, China). KUDOS ultrasonic cleaner was obtained from Shanghai Kedao Ultrasonic Instrument Company (Shanghai, China). The CJJ78-1 magnetic heating-up stirrer was obtained from Jiangsu Jintan Zhengji Instrument Factory (Jiangsu, China). The DF-101B magnetic stirrer with constant temperature controlling was obtained from Gongyi Yingyu Yuhua Instrument Factory (Henan, China).

Covalent immobilization of DNA on GCEs

Direct covalent modification of GCEs with DNA was conducted according to the following procedure (Pang et al., 1996). The pretreated GCE was first oxidized at +0.50 V for 1 minute in 50 mM phosphate buffer solution (PBS, pH 7.4). Modification of the electrode was achieved by dropping 20 μL of PBS containing 5 mM EDC and 8 mM NHS on the electrode surface. Finally, ssDNA (S_1) solution was dropped on the modified GCE surface. The electrode was then rinsed with DDW to eliminate the adsorbed DNA. The DNA-immobilized electrode was stored in 20 mM Tris-HCl buffer solution (pH 7.0).

Hybridization on oligonucleotide probe-modified electrode

The ssDNA-modified electrode was immersed in 20 mM Tris-HCl buffer (pH 7.0) containing ssDNA (S_2) for 1 hour at 38°C with shaking to form double-stranded DNA (dsDNA) at the electrode surface. After hybridization, the dsDNA electrodes were washed with Tris-HCl buffer and then water to remove the oligonucleotide bound nonspecifically.

Indicator binding to the hybrid

$[CuL_2]^{2+}$ was accumulated onto the hybridized surface by immersing the electrode into stirring Tris-HCl buffer solution containing 2.00×10^{-5} M $[CuL_2]^{2+}$ for 10 minutes at room temperature. After accumulation of $[CuL_2]^{2+}$, the electrode was rinsed with Tris-HCl buffer solution for 5 seconds and then washed with DDW to clean out the remnant $[CuL_2]^{2+}$ on it.

Electrochemical studies

CV and DPV experiments were carried out by immersing the desired electrode in 0.2 M Tris-HCl (pH 7.0) solution. For CV, the potential scanning range was from –0.40 to +0.40 V. For DPV, the initial potential was –0.40 V; the high potential was 0.4 V; the pulse amplitude was 50 mV, with a pulse period of 60 milliseconds; the step height was 2 mV; and the step time was 0.1 second (depicted in Fig. 1).

Determination of $[CuL_2]^{2+}$ binding parameters with DNA

All fluorescence measurements were conducted by using a Hitachi F-4500 fluorescence spectrophotometer. A quartz cuvette of 1 cm was used. Samples were excited at 500 nm, and the emission was monitored from 520 to 700 nm. Titrations of the complex and DNA system using EB solution were performed by adding different concentrations of EB solution. All solutions were allowed to be at equilibrium for 5 minutes before measurements were taken.

Results and Discussion

Electrochemical study on the interaction between CuL_2Br_2 and DNA in solutions

The precipitation of silver bromide was found with the addition of 4.50×10^{-8} M silver nitrate into 2.00×10^{-4} M CuL_2Br_2 aqueous solution, which indicated that CuL_2Br_2 complex could disassociate as $[CuL_2]^{2+}$ and Br^- in aqueous solution. To explore the application of $[CuL_2]^{2+}$ in the determination of dsDNA, electrochemical study on the interaction of $[CuL_2]^{2+}$ with DNA was performed at 25°C. Figure 2 shows the cyclic voltammograms of the $[CuL_2]^{2+}$-dsDNA reaction system. $[CuL_2]^{2+}$ showed a reversible redox voltammetric peak (curve a) at –0.085 and 0.012 V. The addition of dsDNA into $[CuL_2]^{2+}$ solution resulted in a decrease of the peak current and a slightly negative shift of peak potential (curve b), which indicated that an interaction between dsDNA and $[CuL_2]^{2+}$ took place in the Tris-HCl buffer solution.

The dependence of the cyclic voltammograms of the $[CuL_2]^{2+}$ on the scan rate (ν) in the absence and presence of dsDNA is shown in Figures 3A and 3B, respectively. As shown in Figure 3C, both I_p(a) and I_p(b) varied linearly with the square root of the scan rate ($\nu^{1/2}$) both in the absence and presence of dsDNA, as expected for a diffusion-controlled process (Bard and Faulkner, 1980). Furthermore, the slopes of plot (I_p vs. $\nu^{1/2}$) decreased after the addition of dsDNA, which indicated a reduction in the apparent diffusion coefficient of the complex in the presence of dsDNA. We can therefore interpret the change in current on addition of dsDNA in terms of the diffusion of an equilibrium mixture of free and dsDNA-bound complex to the electrode surface (Pang and Abruna, 1998).

Scatchard plot study of the effect of $[CuL_2]^{2+}$ on the interaction of EB and DNA

The curves in Figure 4A show the changes in fluorescence of 2.00×10^{-4} M DNA and 5.00×10^{-6} M EB with increasing

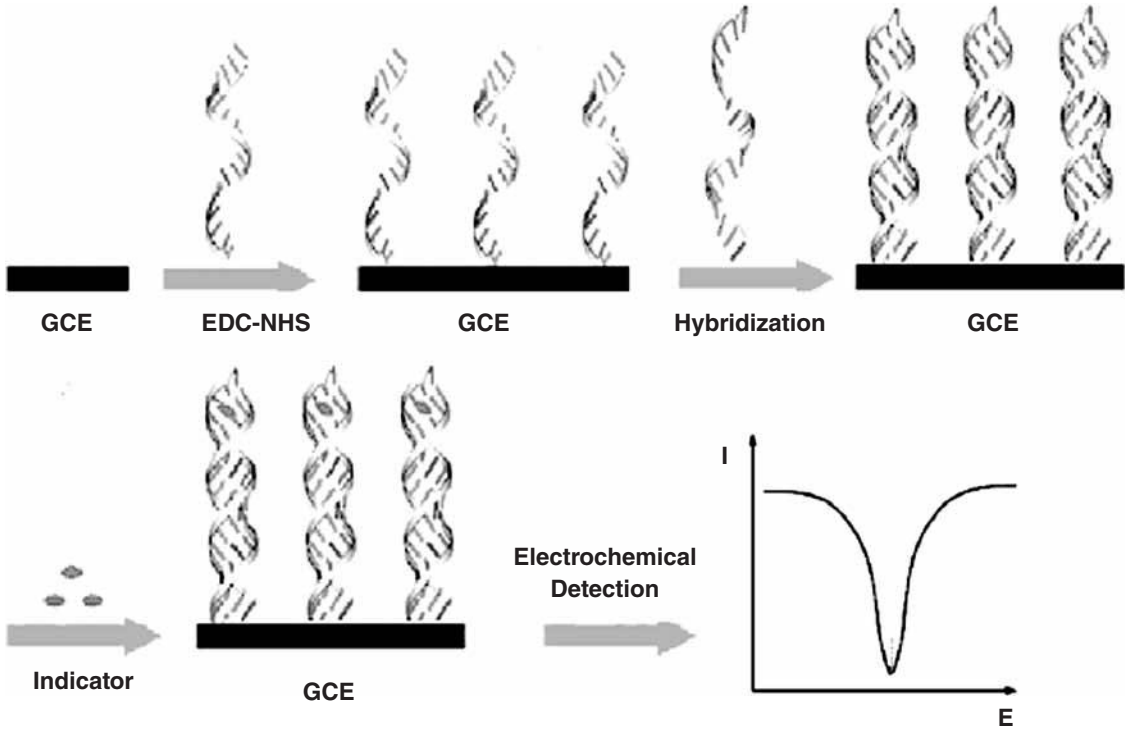


FIG. 1. Schematic diagram illustrating the target DNA detection based on CuL_2Br_2 as an indicator.

concentration of $[CuL_2]^{2+}$. The fluorescence intensity was found to decrease within the concentration range of $[CuL_2]^{2+}$ from 0 to 4.20×10^{-5} M, which corresponds to Rt (i.e., $C_{[CuL2]2+}/C_{DNA}$). This significant quenching of the fluorescence presumably indicates that the addition of $[CuL_2]^{2+}$ is not in favor of the interaction between DNA and EB. It suggests that this may be due to the conformational change of DNA caused by the cooperative interaction of DNA with $[CuL_2]^{2+}$ and charge screening, which decreases the binding strength of EB to DNA.

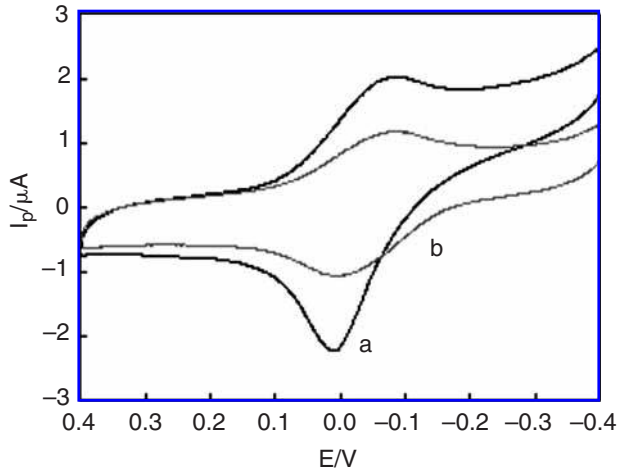


FIG. 2. Cyclic voltammograms of $[CuL_2]^{2+}$-dsDNA binding reaction solution. (a) 0.30 μM $[CuL_2]^{2+}$ + pH 7.0 Tris-HCl buffer solution; (b) (a) + 0.20 μM dsDNA.

By changing the fluorescence, the association constants K for $[CuL_2]^{2+}$ with DNA were calculated. The binding parameters have been calculated using the Scatchard's procedure (McGhee and von Hippel, 1974):

$$r/C_f = K \times n – K \times r$$

where K the intrinsic binding constant and n is the binding site numbering base pairs; r, the number of moles of bound complex per mole of DNA base pair; and C_f, the concentration of free complex. Using the Scatchard plot, the mode of interaction of $[CuL_2]^{2+}$ with DNA could be estimated. When an intercalative interaction of an electroactive indicator with DNA, namely the interaction of the complex and EB to DNA on the same situ, was competitive, the value of K would change while that of n would not change. In contrast, when an electrostatic interaction of an electroactive indicator with DNA, namely the interaction of the complex and EB to DNA on the same situ, was noncompetitive, the value of K would not change while that of n would change. When it has two types of binding mode, all the values of K and n would change. Figure 4B shows the Scatchard plot of r/C_f vs. r. It indicates that this system has two types of binding mode in the molar ratio range studied.

Interaction of ssDNA/GCE and dsDNA/GCE with $[CuL_2]^{2+}$

Figure 5 shows the cyclic voltammograms for $[CuL_2]^{2+}$ in solution at the bare GCE, ssDNA/GCE, and dsDNA/GCE. Curve a is the cyclic voltammogram of $[CuL_2]^{2+}$ at bare GCE in a 0.2 M Tris-HCl buffer solution of pH 7.0, which has a

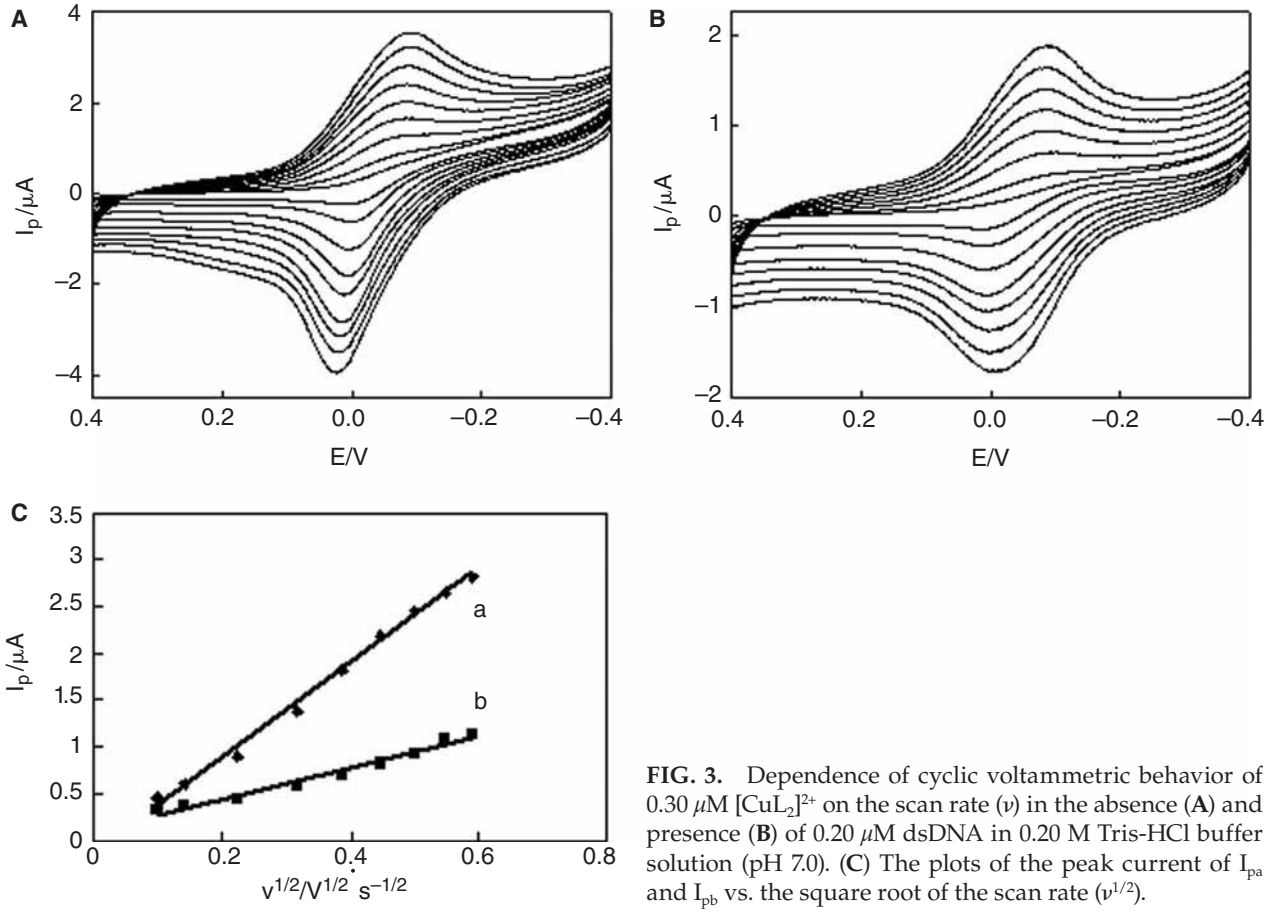


FIG. 3. Dependence of cyclic voltammetric behavior of 0.30 μM $[CuL_2]^{2+}$ on the scan rate (ν) in the absence (**A**) and presence (**B**) of 0.20 μM dsDNA in 0.20 M Tris-HCl buffer solution (pH 7.0). (**C**) The plots of the peak current of I_{pa} and I_{pb} vs. the square root of the scan rate ($\nu^{1/2}$).

couple of well-defined redox peaks in the potential range of 0.40 to −0.40 V and a scan rate of 100 mV/second. Curve b is the cyclic voltammogram of $[CuL_2]^{2+}$ at the ssDNA/GCE-modified electrode and curve c is the cyclic voltammogram of $[CuL_2]^{2+}$ at the dsDNA/GCE-modified electrode. Two couples of redox peaks also appear, but the peak currents decrease and the peak potential is almost the same, which indicates that the surface properties of the modified electrode have been significantly changed. Because there are abundant carboxyl groups on the surface of the GCE, $[CuL_2]^{2+}$ cation can be accumulated on the surface of the electrode through the electrostatic attraction, and this accumulation results in larger redox currents of $[CuL_2]^{2+}$. This decrease in the voltammetric signal obtained from the dsDNA-modified GCE was attributed to the accumulation of the indicator at the electrode surface as a result of the interaction of the planar ring between the dsDNA helix. The electrochemically active metal centers of $[CuL_2]^{2+}$ were enveloped by the bulky DNA molecule on the GCE surface and the signal of $[CuL_2]^{2+}$ was thus reduced (Pang et al., 1996). The changes in the current signal are more pronounced for the dsDNA than for the ssDNA because the copper complex intercalates only into the dsDNA not into the ssDNA (Kara et al., 2002). In addition, the shifts in the CV peak potentials for the electroactive indicators can indicate the mode of the interaction of DNA with the indicators (Carter et al., 1989). An electrostatic interaction of an electroactive indicator with ssDNA or dsDNA will make its reduction at the surface with negative charge more difficult and will thus result in a shift of the formal potential in the negative direction (Zhao et al., 1997; Pang and Abruna, 2000). In contrast, the intercalative interaction produces a positive shift of the formal potential of the intercalator. Thus, the binding properties of $[CuL_2]^{2+}$ to the immobilized ssDNA or dsDNA are not a pure electrostatic or intercalative interaction. The two contrary factors may lead to a constant E^0 value of $[CuL_2]^{2+}$ at the ssDNA/GCE and dsDNA/GCE.

The selectivity of the DNA sensor

The selectivity of this assay was explored by using the ssDNA/GCE to hybridize with different kinds of DNA sequence. Figure 6 shows DPV response of the ssDNA-modified electrode (curve a), ssDNA-modified electrode hybridized with noncomplementary sequence (S_3) (curve b), one-base mismatch sequence (S_4) (curve d), three-base mismatch sequence (S_5) (curve c), and 9.00×10^{-7} M of perfectly matched sequence (S_2) (curve e).

Typically, there is a low signal of $[CuL_2]^{2+}$ on the probe DNA-modified electrode, indicating that $[CuL_2]^{2+}$ has a little affinity for ssDNA and $[CuL_2]^{2+}$ on the GCE surface. Significant increases in the voltammetric signals were observed after hybridization with the complementary ssDNA. This may be attributed to more $[CuL_2]^{2+}$ accumulation on the dsDNA

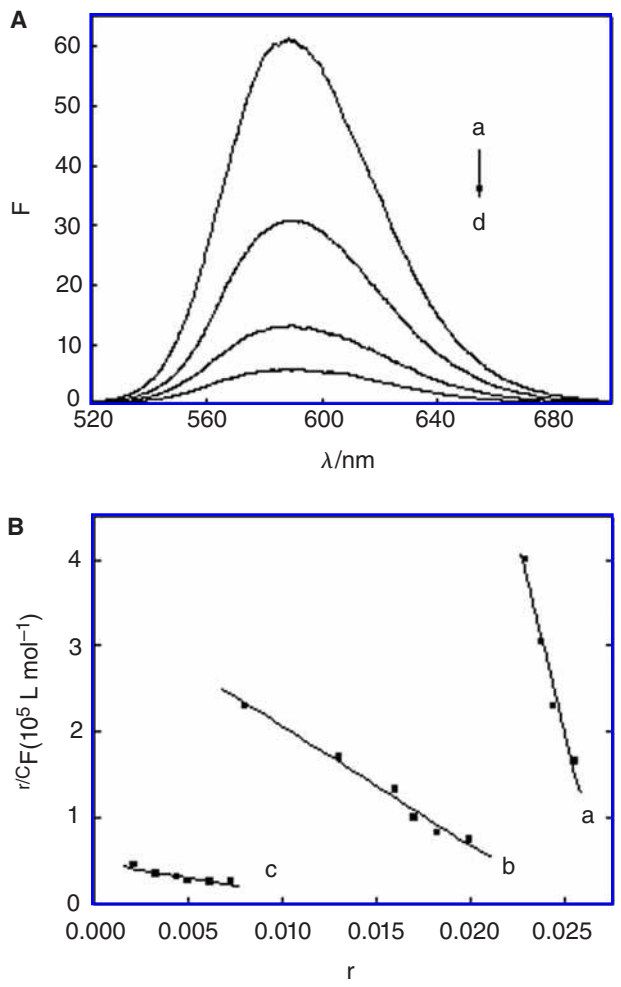


FIG. 4. (**A**) The effect of $[CuL_2]^{2+}$ on the fluorescent spectrum of DNA-EB. (a) Rt = 0; (b) Rt = 0.051; (c) Rt = 0.21; (d) Rt = 0.42. (**B**) Scatchard plots of DNA in $[CuL_2]^{2+}$. (a) Rt = 0; (b) Rt = 0.051; (c) Rt = 0.21.

caused by the bulky double helix of the DNA hybrids. The peak current almost did not decrease when the capture probe was exposed to the noncomplementary sequence in the control experiment. This result indicated that only a complementary sequence could form a double-strand DNA on the probe-modified GCE and cause a significant increase of the signal. No significant change in signal was observed for noncomplementary sequence, showing the high selectivity of the hybridization detection.

To further demonstrate the performance property of current DNA biosensor, a single-base-mismatched and a three-base-mismatched DNA sequences were chosen for differentiation study of base mismatches. As shown in Figure 6, the voltammetric signal for one-base-mismatched DNA sequences was about 85% of that for perfectly matched DNA of the same concentration, whereas only <15% of the signal for three-base-mismatched DNA comparable to that of perfectly matched DNA was obtained. This further indicated that $[CuL_2]^{2+}$ could be used as an effective indicator to

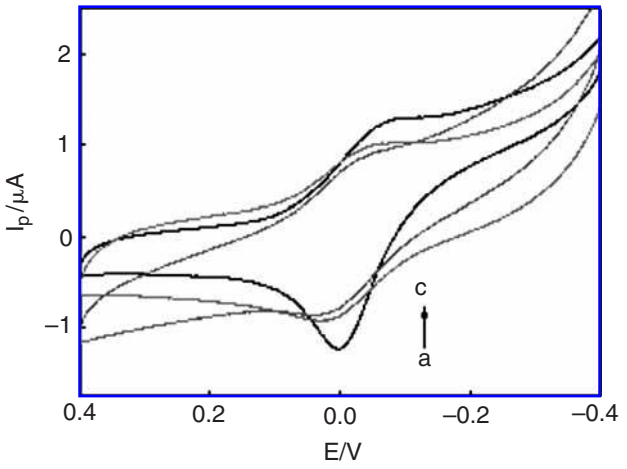


FIG. 5. Cyclic voltammograms of bare GCE (a), ssDNA/GCE (b) and dsDNA/GCE (c) in a 0.2 M Tris-HCl buffer (pH 7.0) containing 0.60 μM $[CuL_2]^{2+}$ at 100 mV·seconds^{-1}.

distinguish between hybrids, noncomplementary and mismatch oligonucleotides.

Calibration curve

To obtain the calibration curve, the peak current values of $[CuL_2]^{2+}$ were measured by DPV under optimum conditions after ssDNA probe was hybridized with the target DNA sequences of different concentrations according to the procedure described. As can be seen from Figure 7, the reduction peak current values were linear with the concentration of the complementary target DNA sequence over the range from 1.74×10^{-9} to 3.45×10^{-7} M, with a detection limit

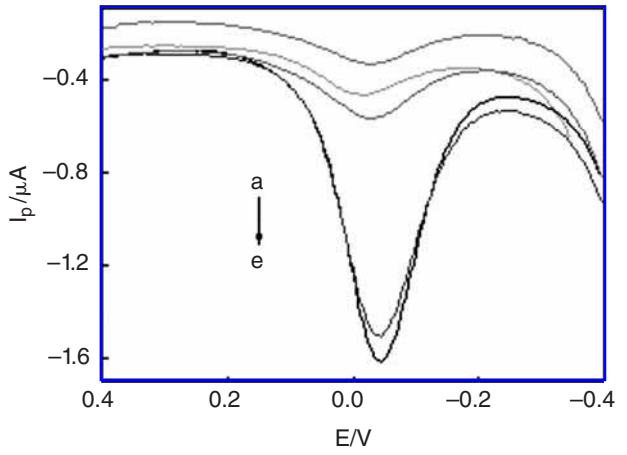


FIG. 6. DPVs of $[CuL_2]^{2+}$ on GCE with immobilized probe DNA (S_1) only (a) and hybridized with noncomplementary sequence (S_3) (b); complementary target DNA (S_2) (e); one-base-mismatch sequence (S_4) (d); and three-base-mismatch sequence (S_5) (c). The scan rate, pulse amplitude, and pulse period were 100 mV/seconds, 50 mV, and 60 milliseconds, respectively.

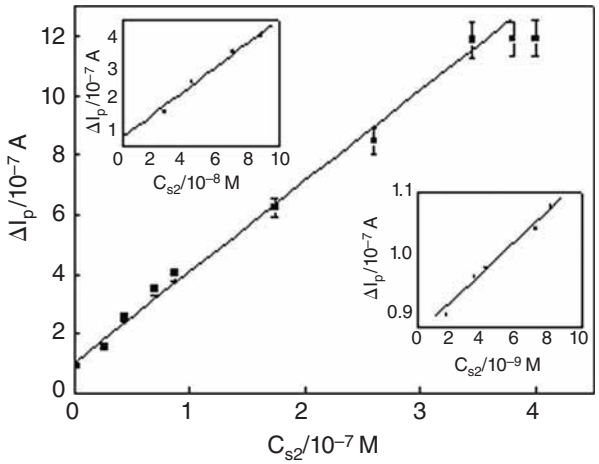


FIG. 7. The increase of peak current (ΔI_p) vs. concentration of target ssDNA (S_2).

of 8.32×10^{-10} M. The regression equation was $y = 3.0684x + 1.0049$, where y (10^{-7} A) was the reduction peak current value of $[CuL_2]^{2+}$ by DPV and x (10^{-7} M) was the concentration value of target DNA. The correlation coefficient was 0.9936, which is about 10 times lower than that of the mixed-component indicator in the reference (del-Pozo et al., 2005).

The detection limit obtained in the present work with $[CuL_2]^{2+}$ as indicator is about 30 times lower than the limit of 2.40×10^{-8} M obtained for the DNA sequence related to HBV using traditional homogenous $Co(phen)_3^{3+}$ as indicator reported by Erdem et al. (Erdem et al., 1999).

Reproducibility and stability of the DNA sensor

Reproducibility of the DNA sensor was tested by measuring 8.72×10^{-7} M of target DNA solution. Three DNA sensors, made independently, showed response current values of 5.02, 4.94, and 5.39×10^{-8} A with an acceptable variation coefficient of 4.69% ($n = 3$). It indicated that a satisfactory reproducibility could be obtained by this system.

The stability of the modified electrode is a key factor in the development of novel biosensor devices. No significant changes in cathodic and anodic peak current were observed for >30 complete CV cycles. When a modified electrode was stored in the 0.20 M Tris-HCl buffer solution (pH 7.0) for at least 1 week at 4°C, the electrode retained about 94% of its initial response to the reduction of DNA.

Conclusions

The results showed that $[CuL_2]^{2+}$ interacted dsDNA . The fluorescence results suggested that this system has two types of binding mode in the molar ratio range studied. Based on the significant difference between the voltammetric signals of $[CuL_2]^{2+}$ obtained with ssDNA and those with dsDNA on the electrode, an electrochemical DNA sensor was developed to study the hybridization specificity of HBV using $[CuL_2]^{2+}$ as a hybridization indicator, which was a simple and cost-effective method. With this approach, a sequence of the HBV could be quantified with a low detection limit of 8.32×10^{-10} M. The hybridization biosensor can be used for the detection of sequence-specific DNA with good selectivity and reproducibility.

Acknowledgments

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