Studies on the Interaction Mechanism of Morin-Co(II) with DNA and Preparation of Electrochemical DNA Biosensor

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Fabrication and electrochemical characterization of a sensitive and novel DNA electrochemical biosensor for the detection of the specific target DNA fragment with a new prepared indicator was presented. The sensor was based on multi-walled carbon nanotubes functionalized with a carboxylic acid group (MWNTs-COOH) as the signal hybridization detection enhancement element. The well dispersed MWNTs were dripped onto the glassy carbon electrode (GCE) surface firstly to prepare MWNTs/GCE. Oligonucleotides with the 5′-amino group were covalently bonded to the carboxyl group of carbon nanotubes. Cyclic voltammetry was used to characterized the modified electrode. The hybridization events were monitored by differential pulse voltammetry (DPV) analysis using a novel electroactive intercalator Morin-Co(II) as an indicator. The specific coliform DNA fragment was detected by the proposed biosensor with a good linear range and a lower detection limit. The linear range is from 2×10^{-9} M to 5×10^{-10} M with a detection limit of 8.1×10^{-11} M (3σ, n = 7). Moreover, the sensor presentes excellent stability and a high repeatability.

Keywords: DNA, Electrochemisitry, Multi-walled carbon nanotubes, Morin-Co(II).

INTRODUCTION

Over the past decades, there has been considerable interest for DNA hybridization biosensors for applications in many fields: DNA diagnostics, gene analysis, fast detection of biological warfare agents and environmental investigation[1,2]. The determination of the human genome, the need for detection of biological pathogens and the development of genetically modified organisms have all led to a demand for simple portable DNA detection tools, which has drawn great attention to electrochemical DNA hybridization biosensors[3].

Electrochemical DNA biosensor offers great promise for many DNA hybridization applications and lends itself to mass fabrication. There are several challenges that current research aims to overcome, including higher selectivity, higher sensitivity, shorter assay times and greater simplicity in performing the assay[4]. DNA can be immobilized on sensor surfaces with methods similar to those used for enzyme-based biosensors *i.e.*, adsorption, covalent immobilization and avidin (or streptavidin) biotin interaction[5].

Recently, the novel surfaces modified with nano-materials have presented an excellent prospect for biological recognition surfaces in order to develop a more selective and sensitive DNA biosensor technology[6]. Carbon nanotubes (CNT), which

can be categorize into multiwall (MWCNTs) and single-wall (SWCNTs) carbon nanotubes. In general, the high surface area, hollow geometry and the useful mechanical properties of CNTs combined with their electronic conductivity and ability to promote electron-transfer reactions[7]. The lower overvoltages and higher peak currents are observed in the voltammetric response of several molecules at electrodes modified with CNTs. Due to these unique properties, CNTs have received enormous attention for the preparation of ultrasensitive electrochemical biosensors. Besides, many other potential and practical applications of CNTs have been reported in the fields of chemical sensors[8-9], biosensors[10,11], cell counters[12], energy storage[13], field emission materials[14], catalyst supports[15], high-sensitivity nanobalances[16], *etc*.

On the other hand, the hybridization indicators, which are compounds that interact in measurably different ways with ssDNA and with dsDNA, are thus developed and are often employed in the detection of DNA hybridization. The binding mechanisms between DNA and various redox substances, such as metal complexes[17,18], anticancer or antivirus drugs[19] and organic dyes[20], have been extensively investigated. Morin hydrate (2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzo-pyran-4-one), a light yellowish natural plant dye, is known to have a broad pharmacological activity, such as

antitumour[21], antioxidation[22] and possibly even protective effects against chronic diseases[23]. In general, compared with the ligand, the morin complex is considered to have a better biological activity due to its cooperative effectivenes. Many different methods have been used to study the interaction with DNA, in particular the electrochemical analysis[24-25].

In this work, a convenient and effective strategy by using MWCNT-COOH as linker between GCEs and DNA is proposed. Firstly, MWCNT-COOH was mixed in DMF to form the suspension which was cast on a cleaned GCEs electrode surface to form MWCNT-COOH films. Subsequently, 5′ ends of DNA probes with amino groups was assembled onto the MWCNT films in the present of EDC by covalent bonds interaction between carboxyl of MWCNT and 5′-amino group. Morin was chosen as ligand for the synthesis of novel cobalt complex of $Co(C_{15}H_9O_7)_2 \cdot 3H_2O$ (abbreviated by morin-Co(II) or CoL_2). Due to the special molecular structure of morin, it was convinced that CoL_2 could perform a different binding role with DNA from other common metal complexes. The interaction of synthesized Morin-Co(II) with DNA was investigated with cyclic voltammetry (CV). Using Morin-Co(II) as electroactive indicator, a novel electrochemical DNA biosensor was developed with the accomplishment of hybridization of immobilized probe DNA with its complementary single-stranded DNA (ssDNA) on glassy carbon electrode (GCE) by differential pulse voltammetry (DPV). The specific coliform DNA fragment was detected by the proposed biosensor with a good linear range and a lower detection limit.

EXPERIMENTAL

Elemental analysis was performed on Elementar Vario EL III analyzer. All electrochemical measurements, including cyclic voltammetry (CV), differential pulse voltammetry (DPV) were performed with a CHI 832 Electrochemical Analyzer (Shanghai Chenhua Instrument Company, China). A three-electrode system was employed, including Pt wire as the auxiliary electrode, Ag/AgCl as the reference electrode and glassy carbon electrode (GCE) as the working electrode.

Salmon sperm DNA (dsDNA) was purchased from Shanghai Huashun Biological Engineering Company (≥ 95 %, $A_{260}/A_{280} > 1.8$, $\varepsilon_{260} = 6600$ M^{-1} cm^{-1}). EDC was purchased from Sigma and used without further purification. MWCNTs with a diameter of about 10-30 nm and a length of around 1-10 μm were obtained from Sun Nanotech Co. Ltd. (Jiangxi, China). Three 24-base oligonucleotides were purchased from SBS Genetech Company (Beijing, China) and purified using PAGE to obtain the purity of = 95.0 %. The base sequences were as follows:

ssDNA probe (S₁): 5'-NH₂-GAG CGG CGC AAC ATT TCA GGT CGA-3′

Complementary target ssDNA (S₂): 5′-TCG ACC TGA AAT GTT GCG CCG CTC-3′

Three-base mismatch target ssDNA (S₃): 5′-TCG TCC TGA AAC GTT GCG CCT CTC-3′

Single mismatch target ssDNA (S₄): 5′-TCG ACC TGA AAC GTT GCG CCG CTC-3′

non-complementary target DNA (S₅): 5′- GAG CGG CGC AAC ATT TCA GGT CGA -3′

The italicized bases in S₃ and S₄ indicate those mismatched with S₁.

All oligonucleotide stock solutions were prepared in TE solution (10 mM Tris-HCl plus 1mM EDTA, at pH 8). Other chemicals employed were all of analytical grade. Ultrapure water was used throughout.

Preparation of CoL₂: The complex of $Co(C_{15}H_9O_7)_2 \cdot 3H_2O$ was synthesized as described in the literature[26]. Elemental analysis: Calcd. (Theoretical Value) for $Co(C_{15}H_9O_7)_2 \cdot 3H_2O$, %: C 50.74 (50.35); H 3.35(3.36). The formula of the complex was shown in Fig. 1.
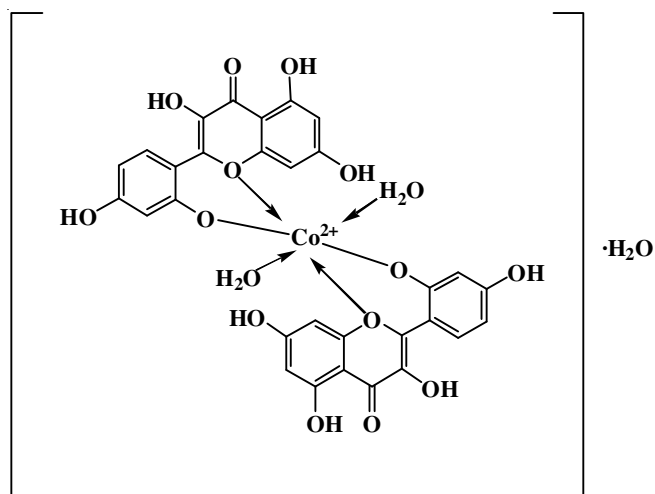

Fig. 1. Formula of $Co(C_{15}H_9O_7)_2 \cdot 3H_2O$

Electrochemical study on the interaction between CoL₂ and dsDNA: The glassy carbon electrode(GCE) was polished successively with 1.0 μm, 0.3 μm and 0.05 μm α-Al₂O₃ suspension, followed with extensively rinse in ultrapure water with sonication. Appropriate amount of dsDNA and CoL₂ were mixed and incubated in 0.2 M PBS buffer (pH 6.0) for 5 min. The interaction characterization of CoL₂ with DNA was performed by cyclic voltammetric method with the potential scan from 0.2 V to 0.8 V at the scan rate of 0.1 V·s⁻¹.

Preparation of MWCNTs-COOH: MWCNTs functionalized with carboxylic acid groups (MWCNTs-COOH) were prepared by heating at reflux with concentrated HNO₃ for approximately 10 h. The MWCNTs-COOH suspension was prepared by dispersing one milligram MWCNTs-COOH in 10 mL of N,N-dimethylformamide (DMF) with the aid of ultrasonic agitation.

DNA covalently immobilized to the MWCNTs-COOH modified GCE surface: The covalent immobilization of oligonucleotides to MWCNTs-COOH on GCE was conducted as follows: 10 μL of 1 mg mL⁻¹ suspension of MWCNTs-COOH was dropped onto a freshly smoothed GCE surface uniformly and had the solvent evaporated under an infrared lamp. The electrode was thoroughly rinsed, first with ethanol and then with ultrapure water to remove excessive nanotubes. For oligonucleotide immobilization, the MWCNTs modified electrode was immersed in a 30 μM acetate buffer (pH 5.2) containing 10 mM EDC for 1 h. The EDC-attached electrode was washed with acetate buffer and subsequently incubated in a 3.5 μM oligonucleotides/acetate buffer solution for 12 h at room

temperature. In this process the probe oligonucleotides were immobilized on the GCE through the covalent amide bonds formed by the carboxyl groups on the nanotubes and the amino groups on the oligonucleotides.

Hybridization on the ssDNA-immobilized electrode: The hybridization was carried out on S_1-immobilized electrode in 0.20 M *Tris*-HCl buffer solution (pH 7.0) containing complementary ssDNA segment (S_2), three-base mismatch target ssDNA (S_3), single mismatch target ssDNA (S_4) or non-complementary ssDNA segment (S_5), respectively. The solution was stirred for 1 h at 37 °C and then the electrode was dried at room temperature and rinsed with 0.20 M B-R buffer and ultrapure water successively to remove extra target ssDNA.

Intercalation of hybridization indicator: The accumulation of CoL_2 on the S_1-immobilized or hybridized surface was performed by immersing the electrode into 0.2 M PBS buffer (pH 6.0) containing 5.52×10^{-5} M CoL_2 for 10 min at room temperature and then rinsed with ultrapure water to remove the extra CoL_2.

Electrochemical detection: The electrochemical investigation of hybridization was carried out by differential pulse voltammetry (DPV) measurements in 0.2 M PBS buffer (pH 6.0), with potential scan from -0.1 V to 0.6 V. The peak related to the oxidation of CoL_2 at approximately 0.31 V was characterized as the electrochemical detection signal.

RESULTS AND DISCUSSION

Electrochemical behaviors of CoL_2 in presence of dsDNA at bare GCE: Electrochemical behaviors of CoL_2 and its interaction with double-stranded salmon sperm DNA (dsDNA) were investigated with GCE at 25 °C. The cyclic voltammograms were obtained at a concentration of 5.52×10^{-5} M CoL_2 for bare GCE in a 0.2 M pH 6.0 PBS buffer without dsDNA or containing 8.26×10^{-5} M dsDNA. It was observed that there were a couple of non-reversible redox peaks for CoL_2. The cathodic peak potential (E_{pc}) and the anodic peak potential (E_{pa}) were 0.238 V and 0.310 V respectively. The peak current decreased and its formal potentials shifted positively with the addition of dsDNA in PBS buffer, suggesting that an interaction between dsDNA and CoL_2 took place.

Bard *et al.*[27] had reported the discrimination of binding modes between small molecules and DNA. If formal potential ($E^{o'}$) shifted to more negative value, the interaction mode was electrostatic binding. On the contrary, if $E^{o'}$ shifted to more positive value, the interaction mode was intercalative binding. Therefore, in our studied system the interaction between CoL_2 and DNA can be attributed to the intercalative binding. When the concentration of dsDNA was fixed, the cathodic peak current(I_{pc}) decreased linearly with the increase of concentration of CoL_2 (plotted as $\log[\Delta I_{pa}/(\Delta I_{pa.max} - \Delta I_{pa})]$ to $\log\{[CoL_2]/M\}$ in Fig. 2. The binding constant between CoL_2 and dsDNA was calculated to be 3.59×10^9 L^2 mol^{-2} with a correlation coefficient of 0.9887.

Electrochemical characteristics of oligonucleotides/ MWCNTs-COOH/GCE: Electrochemical characteristics of oligonucleotides/MWCNTs-COOH/GCE scan from -0.4 V to +0.6 V was shown in Fig. 3. Curve a, b and c were the electrochemical response of bare GCE, MWCNTs-COOH/GCE and
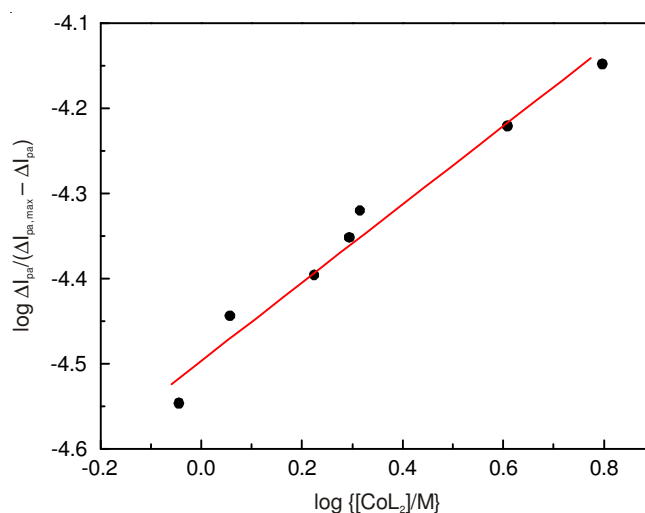

Fig. 2 Dependence of $\log \Delta I_{pa}/(\Delta I_{pa,\ max} - \Delta I_{pa})$ on $\log [CoL_2]$

oligonucleotides/MWCNTs-COOH/GCE in 0.1 M PBS (pH 7.0), respectively. As can be seen, after MWCNTs-COOH was deposited on the electrode, a pair of stable redox waves appeared with the cathodic peak potential of -0.05 V and the anodic peak potential of 0.01 V, which was related to the redox of carboxylic group of MWCNTs-COOH. Compared with the bare GCE, the background current of the MWCNTs-COOH/ GCE was apparently amplify, showing that modification of the electrode with MWCNTs-COOH could significantly enhance the effective electrode surface area as well as provide an active binding group for oligonucleotides derivation. As a result of oligonucleotides probe coupling to MWCNTs-COOH/GCE, the CV current of the electrode decreased significantly.
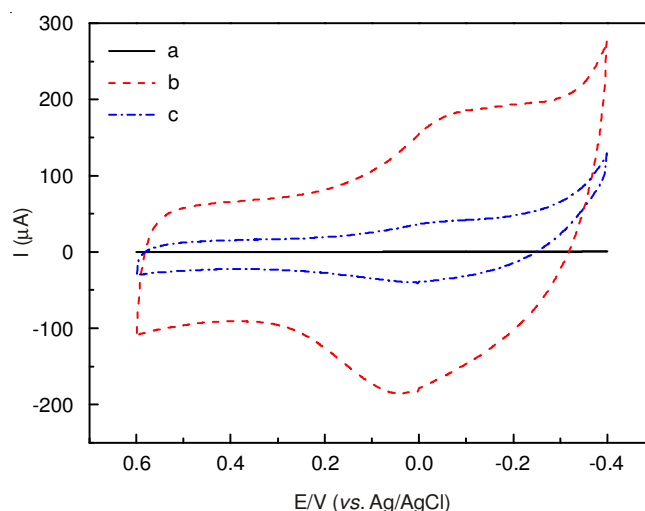

Fig. 3. Cyclic voltammograms of bare GCE (curve a); MWCNTs-COOH/ GCE (curve b) and oligonucleotide/MWCNTs/GCE (curve c) in 0.1 M PBS blank solution (pH 7.0)

Selectivity of the prepared electrochemical DNA biosensor: The CoL_2 complex was used as DNA hybridization indicator for the detection of a 24-mer oligonucleotide which is specific for colitoxin and the selectivity of this assay was investigated. Fig. 4 showed the DPV curves obtained in 0.2 M *tris*-HCl buffer (pH 6.0) on MWCNTs-COOH/GCE with

immobilized probe DNA solely (curve a), further hybridized with complementary DNA (curve e) and non-complementary target DNA (curve b), three-base mismatch target ssDNA (curve c) and single mismatch target ssDNA (curve d) respectively. Each measurement was performed after CoL_2 was accumulated. It was found that there was only a small DPV peak response of CoL_2 for S_1 modified MWCNTs-COOH/GCE (Curve a), indicating that only a negligible CoL_2 was bound to S_1 on modified MWCNTs-COOH/GCE. A significant increase in the DPV signal was observed after the ssDNA modified MWCNTs-COOH/GCE was hybridized with the complementary ssDNA, indicating that much more CoL_2 molecules were concentrated or bound to dsDNA helices. A good selectivity was thus obtained for the new electrochemical DNA biosensor.
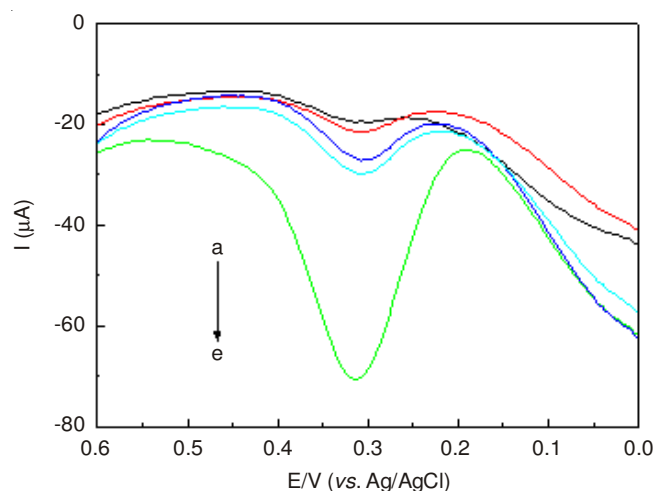

Fig. 4.   DPVs curves of CoL_2 on the different modified GCEs in 0.20 M *tris*-HCl buffer solution. (a) S_1/MWCNTs-COOH/GCE; (b) S_1-S_5/MWCNTs-COOH/GCE, (c) S_1-S_3/MWCNTs-COOH/GCE, (d) S_1-S_4/MWCNTs-COOH/GCE; (e) S_1-S_2/MWCNTs-COOH/GCE, where the concentration of S_2, S_3, S_4, S_5 was 36.2 nM respectively

Quantitative analysis of target ssDNA: The sensitivity of the prepared DNA electrochemical sensor was investigated by varying the concentration of target ssDNA, which was complementary to the probe ssDNA immobilized on MWCNTs-
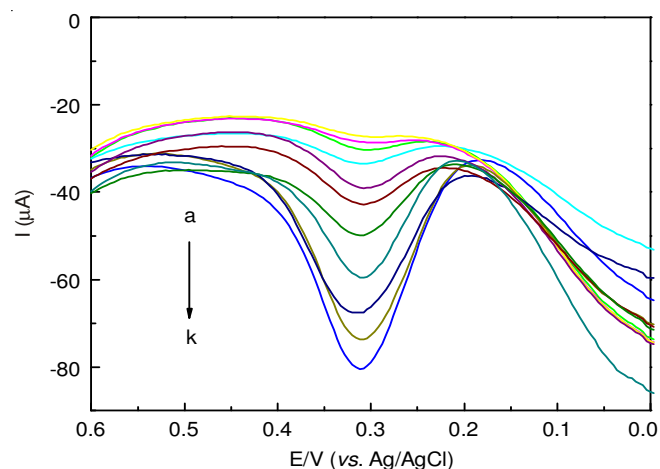

Fig. 5.   DPV curves of different S_2 concentrations, (a) 0; (b) 2.00×10^{-9} mol L^{-1}; (c) 4.00×10^{-9} mol L^{-1}; (d) 6.00×10^{-9} mol L^{-1}; (e) 8.00×10^{-9} mol L^{-1}; (f) 1.00×10^{-8} mol L^{-1}; (g) 2.00×10^{-8} mol L^{-1}; (h) 3.00×10^{-8} mol L^{-1}; (i) 4.00×10^{-8} mol L^{-1};(j) 5.00×10^{-8} mol L^{-1};
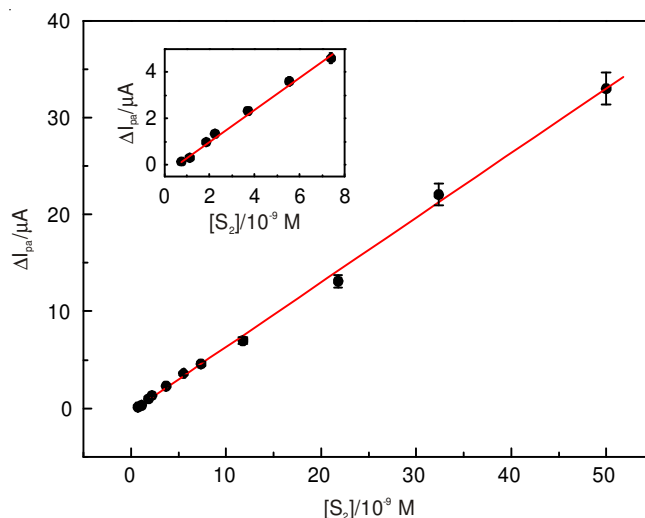

Fig. 6.   Relation curve obtained with different amounts complementary target DNA for hybridization

COOH/GCE. As shown in Fig. 5, the differentanodic peak currents obtained in DPV measurement increases with the increasing concentration of S_2. The value of peak current difference is linear with the concentration of target ssDNA ranging from 2.00×10^{-9} M to 5.00×10^{-8} M (Fig. 6). The regression equation was $\Delta I_{pa} = \{6.694 ([S_2]/nM) - 0.3645\} \times 10^{-7}$ A and the correlation coefficient was 0.9989. Thus, a detection limit of 8.2×10^{-11} M of the target DNA could be estimated using 3s (where s is the standard deviation of the blank solution, n = 7).

Conclusion

Interaction between CoL_2 and double-stranded salmon sperm DNA was studied by using cyclic voltammetry. Results showed that CoL_2 could intercalate into the base pairs of the dsDNA. Functionalized multiwall carbon nanotubes (MWCNTs-COOH) were used for covalent immobilization of ssDNA probe and for the enhancement of sensitivity of sequence-specific ssDNA detection. With CoL_2 as a novel electroactive indicator, ssDNA fragment could be selectively detected on the new electrochemical DNA biosensor with a detection limit of 8.2×10^{-11} M and a linear range from 2×10^{-9} M to 5×10^{-8} M.

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