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Voltammetric Determination of Adenovirus Specific DNA Sequence Using Self-Assembled Dimercapto-Succinic acid Monolayer on Gold Electrode

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A novel electrochemical DNA biosensor for the detection of adenovirus specific DNA sequence is presented in this paper. Dimercapto succinic acid was self-assembled on gold electrode surface and amino group ended single-strand DNA (NH_2-ssDNA) probe was immobilized covalently to carboxylic group of dimercapto succinic acid monolayer in the presence of 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide and N-hydroxysulfosuccinimide sodium salt as linker. The NH_2-ssDNA probe was hybridized with complementary ssDNA in solution. Meanwhile, $Co(phen)_3^{3+}$ as electrochemical hybridization indicator was intercalated into double stranded DNA (dsDNA) to form a dsDNA/$Co(phen)_3^{3+}$ system on the gold surface. Differential pulse voltammetry was utilized to detect an increase of redox peak current of $Co(phen)_3^{3+}$ on dsDNA/Au electrode. Using this method, an adenovirus specific DNA sequence could be quantified over the range from 1.03×10^{-9}-2.06×10^{-7} mol/L^{-1}. The detection limit is 5.23×10^{-10} mol/L^{-1}.

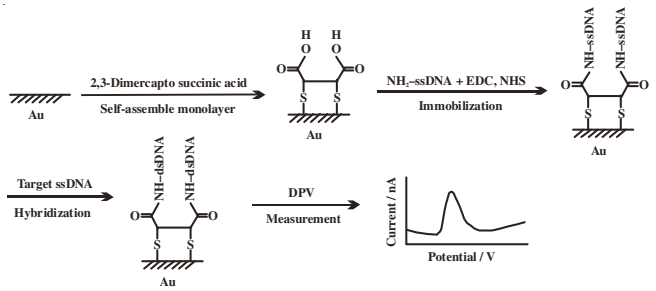
Key Words: DNA biosensor, 2,3-Dimercapto-succinic acid (DSA), NH_2-ssDNA probe, Adenovirus specific DNA sequence, Hybridization

INTRODUCTION

Sequence-specific detection of DNA targets associated with either genetic or pathogenic diseases has become increasingly important in molecular diagnostics[1,2]. Polymerase chain reaction (PCR) is employed toward these application widely[3-5], but its disadvantages include time-consuming, high cost and occasionally "false positive". Electrochemical DNA biosensor attracted much attention due to its low cost, rapid, sensitive and accurate[6-8]. There are many requests in different fields such as clinical diagnosis, food identification, environment monitoring and drug analysis[9-11]. The immobilization of oligonucleotide on the electrode surface is key to detection of hybridization procedure in electrochemical behaviour. Many methods have been reported for the immobilization of the ssDNA probe on an electrode surface, including direct adsorption[12], covalent bonding[13], nanoparticles modification[14], avidin-biotin system[15] and self-assembled monolayer[16] *etc*. Among them self-assembled monolayer (SAM) of alkane thiols on gold electrode has been verified to be an effective way for the DNA immobilization with the advantages of simplicity, rapidness and sequence-specific[17]. Millan and mikkelsen described a sequence-selective biosensor on self-assembled monolayer (SAM) glassy carbon electrode for DNA with $Co(bby)_3^{3+}$ as indicator[18]. Liu and Anzai self-assembled thiol-labeled ssDNA on the gold electrode surface to detect complementary sequence DNA[19].

Some experts had reported the binding capability of phosphate or carboxyl and amino groups cost less time than that between carboxyl and hydroxyl groups. Sun *et al.*[20], demonstrated it takes 3 h at 25 ºC to form a phosphoramidate bond between the phosphate group of ssDNA molecular and amino group of aminoethanethiol. Ying Xu *et al.*[21] immobilized NH_2-ssDNA onto carboxyl groups tips of multiwalled carbon nanotubes, which suggested it cost 2 h to immobilize the ss-DNA and hydroxyl groups. In this work, the NH_2-ssDNA probe was immobilized on dimercapto-succinic acid (DSA) self-assembled monolayer modified gold electrode surface by covalent binding with the help of water-soluble 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide and N-hydroxysulfo-succinimide sodium salt. Compare to covalent bonding between carboxyl group and hydroxy end, the method is tough and time-saving. The double thiol and amino group could link between ssDNA and electrode perfectly and toughly. $Co(phen)_3^{3+}$ is a traditional electrochemical indicator[22] for DNA biosensor, because of its different electrochemical responses to hybridization on ssDNA or dsDNA modified electrode. The

DNA biosensor was further used for the determination sequence-specific DNA of adenovirus at low level by different pulse voltammetry (DPV) and the results showed that the modified electrode was perfectly for the determination of sequence-specific DNA. The schedule1 for the preparation of DNA modified electrode was illustrated in **Scheme-I**.



Scheme-I: Whole detection scheme of this DNA biosensor. Step 1: DSA was self-assembled on a gold electrode. Step 2: In the presence of EDC, NH_2-ssDNA was immobilized covalently to the electrode surface. Step 3: Hybridization was carried out at ssDNA/DSA/AuE. Step 4: DPV was determinated electrochemical response

EXPERIMENTAL

Voltammetry measurements were performed with a CHI660A electrochemical system (Shanghai Chenhua, China). The three-electrode system consists of Au electrode (3 mm diameter) and modified electrode as working electrode, a Ag/AgCl electrode as reference electrode and a platinum wire electrode as counter electrode. An ultrasonic cleaning machine and thermostatic incubator and water bath were products of Ningbo Scientz Biotechonlogy Co., China and Shanghai Yuejin Medical Instruments Factory, China, respectively. The digital display pH-meter pHS-25 (Shanghai Presition & Scientific Instrument Co.) were used to control the pH of buffer solutions.

Adenovirus specific DNA sequences probe (5' NH_2-TCGAGCTCAAGATGTCCA CCCCATC 3', NH_2-ssDNA), its complementary target DNA (5' GATGGGGTGGACAT CTTGAGCTCGA 3') and the 3-base mismatched DNA (5' GATCGGGTGAACATCGTGA GCTCGA 3') with a length of 25 nucleotides were customer-designed and synthesized by Nanjing Jinsite Biotechnology Company (Nanjing, China). EDC, NHS and dimercapto-succinic acid (DSA) were purchased from Sigma. Electrochemical indicator $[Co(phen)_3](ClO_4)_3$ (phen = 1,10-phenanthroline) was prepared according to the reported procedure[23]. All other reagents were analytical grade and used without further purification and doubly distilled water was used throughout.

A gold electrode was polished carefully with 1.00, 0.30 and 0.05 μm alumina slurry on microcloth pads in turn. Then, it was dipped into a HNO_3 solution (1:1) and absolute ethanol for 15 min, respectively and sonificated in water for 2 min. Lastly it was electrochemically scanned in 0.5 mol/L H_2SO_4 solution by cyclic potential scanning between -0.2~1.7 V until a standard CV was obtained. The freshly pretreated gold electrode was rinsed with deionized water and dipped into 10^{-2} mol/L DSA/ethanol solution for about 10 h to get the DSA self-assembled gold electrode (denoted as DSA/Au electrode). After water washing thoroughly, the DSA/Au electrode was immersed in pH 7 PBS containing 5×10^{-3} mol/L EDC and 8×10^{-3} mol/L NHS for 0.5 h, then incubated the 2 × SSC buffer containing 5×10^{-7} mol/L NH_2-ssDNA probe solution for 2 h at room temperature (denoted as ssDNA/DSA/Au electrode). The ssDNA/DSA/Au electrode was immersed in 500 μL of the 2 × SSC buffer containing gradient concentration of target ssDNA for 1 h at 60 ºC. Then, the electrode would be rinsed by 2 × SSC containing 0.1 % SDS for 10 min. Before and after hybridization the modified electrode was in the tris-NaCl buffer of containing 5×10^{-5} mol/L $Co(phen)_3^{3+}$ for 2 min. Lastly the currents of working electrode was recorded by DPV in pH 7.05 tris-NaCl buffer, which potential was selected between +0.40 and -0.30 V. The experimented electrode was immersed in hot water at 95 ºC for 120 s. Then, it was transferred into ice-water for 120 s at once for reuse. When the electrodes were used for 6 times repeatedly measurement signals were 95 % of original measurement signals.

RESULTS AND DISCUSSION

When Au electrode surface was covered by self-assembled alkane thiol monolayer, the capacitance of electrode surface should be decreased[24]. Compare to mercaptoacetic acid (MAA), DSA has two thiol groups and is easier to form Au-S linkage. The self-assemble effects in same time were compared for MAA and DSA. With the increase of the self-assemble time, the capacitance deceased step by step. When the self-assemble time is 2 h or longer, the capacitance of self-assembled DSA film electrode surface is invariable (Fig. 1A). The analogical experiment was carried out on self-assembled MAA film electrode, but the capacitance of the electrode surface decreased after the self-assemble time exceeded 3 h (Fig. 1B). Fig. 1(A-B) showed the affinity of DSA for gold electrode is stronger than that of mercapto acetic acid.

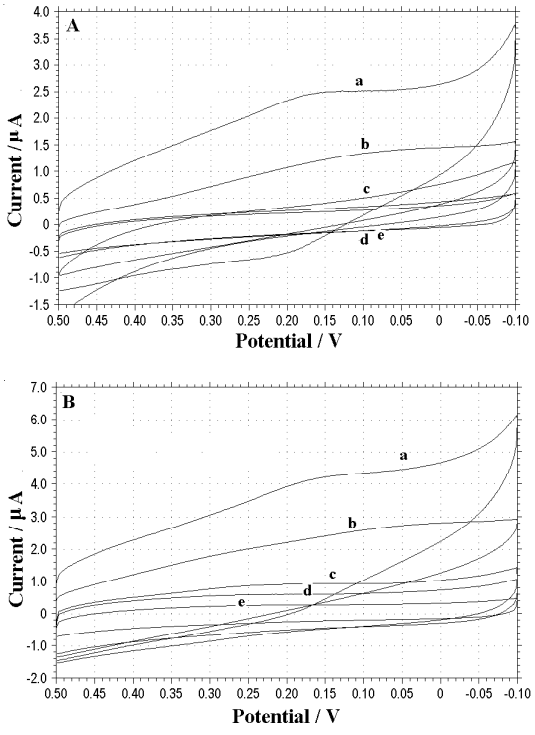


Fig. 1. Cyclic voltammograms of self-assembled DSA (A) and MAA (B) in 0.2 mol/L^{-1} KCl with different time with scan rate of 50 mv/s (from a-e T = 0, 0.5, 1.0, 2.0 and 3.0 h)

Cyclic voltammograms of different scanning rate on self-assembled DSA/Au electrode were recorded. Fig. 2 shows the results of different scanning rate on DSA/Au electrode. With the decrease of the scanning rate, the peak currents decreased. When scanning rate is 50 mV/s, the oxidate and redox peak current is invariable and symmetrical. Hence, 50 mV/L of scanning rate is concerned.

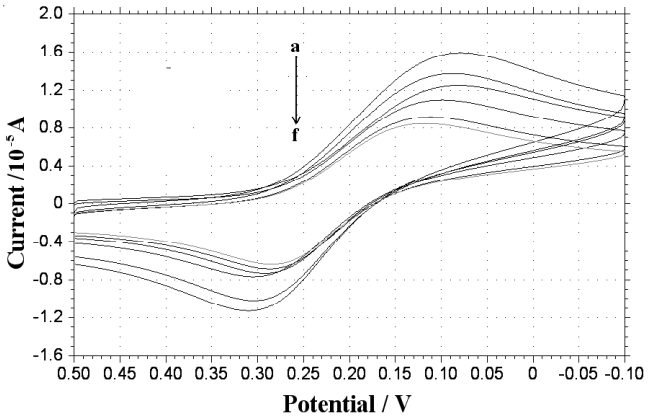


Fig. 2. Influence of scanning rate on peak current in 10^{-3} mol/L $K_3Fe(CN)_6$ (from a-e n = 200, 150, 100, 80, 60 and 50 mV/s^{-1})

There exists differential interaction between ssDNA or dsDNA and $Co(phen)_3^{3+}$, hence cyclic voltammogram will change. $Co(phen)_3^{3+}$ ion accumulated on the electrode surface is higher after hybridization than before hybridization, suggesting that much amounts of $Co(phen)_3^{3+}$ ion were bound to the dsDNA by intercalation[25]. An electrostatic force of attraction between phosphate anions in the ssDNA and $Co(phen)_3^{3+}$ ion suggested that was still adsorbed less effectively to ssDNA before hybridization. The electrochemical behaviour of $Co(phen)_3^{3+}$ was investigated by DPV. Concentration range of $Co(phen)_3^{3+}$ is between 50 and 400 μmol/L, the interaction of $Co(phen)_3^{3+}$ with dsDNA is stronger than with ssDNA and Δi_p become invariable while the indicator concentration is between 50 and 100 μmol/L (Fig. 3). Hence, 50 μmol/L $Co(phen)_3^{3+}$ solution is concerned.

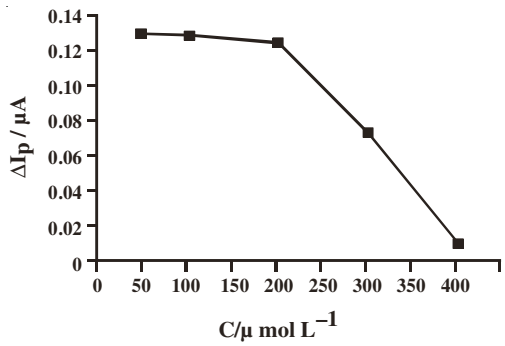


Fig. 3. Effect of indicator concentration in 50, 100, 200, 300 and 400 μmol/L $Co(phen)_3^{3+}$ solution

This ssDNA/DSA/Au electrode was used to monitor the target complementary strand DNA's concentration for its sensitivity assay. It was carried out by varying the concentrateion of complementary sequence to react with the ssDNA probe electrode under the same hybridization condition. As shown in Fig. 4, a linear response of the oxidate peak current was obtained from 1.03×10^{-9}-2.06×10^{-7} mol L^{-1} and a detection limit of 5.23×10^{-10} mol L^{-1}, with a regression equation of $\Delta i_p = 17.06C + 90.66$ (r = 0.9982, C in 10^{-8} mol L^{-1}, Δi_p in nA).

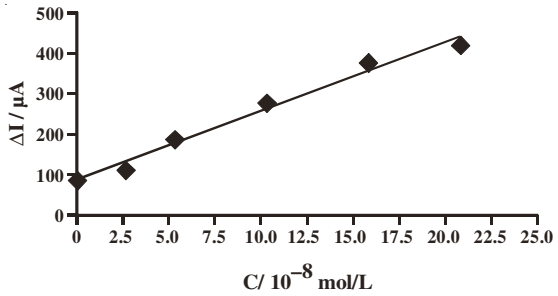


Fig. 4. Calibration curve for the complementry sequence detection

Complementary strand DNA and three-base mismatched was employed to evaluate the selectivity of the DNA biosensor. Compared to the complementary stranded DNA (Fig. 5A), the hybridization of the three-base mismatched sequence little increased in current response due to lower binding (Fig. 5). Hence, the DNA biosensor can diagnosis the complementary sequence ssDNA.

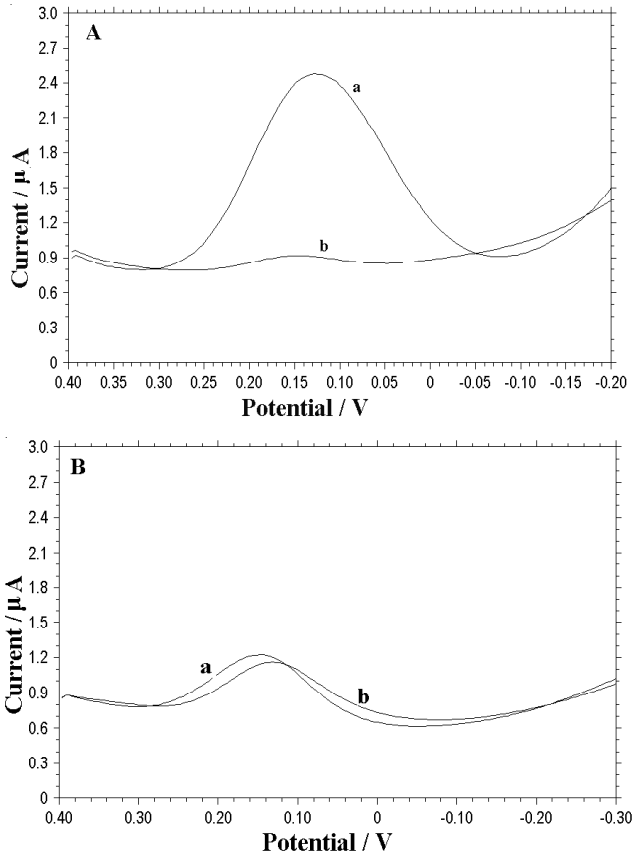


Fig. 5. (A) DPV response of the DNA biosensor after hybridization (a) and before hybridization (b) with 5.12×10^{-8} mol/L complementary sstrand DNA. (B) DPV response of the DNA biosensor after hybridization (a) and before hybridization (b) with 3-base mismatched sequence (csDNA concentration: 2.0×10^{-7} mol/L)

Reusability of the ssDNA/DSA/Au was performed by immersed the experimented dsDNA/DSA/Au electrode in water at 100 ºC for 120 s and in ice-water bath for 120 s

according to the reported procedure[15]. It is found that the DNA biosensor could be used for 6 times repeatedly after regeneration of the ssDNA/DSA/Au. RSD of 6 times was 1.54 % and the respond current was about 4.88×10^{-7} A.

Conclusion

In this study, Au electrode was self-assembled DSA and higher binding activity to NH_2-ssDNA. The developed assay was shown to be able to successfully determine adenovirus specific DNA sequence.

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