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Short Communication

Label-Free Electrochemical Sensor for MicroRNAs Detection with Ferroceneboronic Acids as Redox Probes

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Electrochemical DNA (E-DNA) based biosensors have been shown to be a promising alternative to mass- and fluorescence-based sensors for the specific detection of oligonucleotide sequences. MicroRNAs (miRNAs) are believed to be important for cancer diagnosis and prognosis serving as reliable molecular biomarkers. In this work, we reported a label-free electrochemical sensor for miRNAs detection based on the difference in the structure of RNA and DNA. Specifically, miRNAs were captured by the pre-immobilized DNA probes on the electrode and recognized by electrochemically active ferroceneboronic acid (FBA) through the formation of boronate ester covalent bound between boronic acid group of FBA and ribose sugar in miRNAs. The results will be valuable for the design of new types of biosensor for label-free detection of miRNAs in a biological matrix.

Keywords: Electrochemical sensor; MicroRNAs; Ferroceneboronic acid; Label-free

1. INTRODUCTION

In the first decade of 21 century, electrochemical analysis of nucleic acids have been shown to be a promising alternative to mass- and fluorescence-based sensors for the specific detection of oligonucleotide sequences and small molecules in view of its high sensitivity, simplicity, rapid response, and compatibility with miniaturization [1,2]. Among kinds of electrochemical methods for the detection of nucleic acids, redox-labeled (most commonly methylene blue or MB and ferrocene or Fc) oligonucleotide probe site-specifically attached to an interrogating electrode is one of the most attractive approaches [3]. Hybridization-linked changes in the flexibility of this probe (due to specific change of the conformation or amount of double-helix) alter the rate with which electrons are transferred from the redox tags, leading to a readily detectable change in Faradaic current upon

voltammetric interrogation [4-12]. MicroRNAs (miRNAs) are small RNA molecules (typically 19−24 nucleotides long) that can regulate gene expression in plants and animals by translational repression or degradation of messenger RNAs [13]. Recently, a number of researchers have detected tumor-related miRNA in the body fluids [14-17] and the expression levels of individual miRNAs may serve as reliable molecular biomarkers for cancer diagnosis and prognosis [18]. Therefore, simple, cost-effective and rapid detection assays for miRNAs are in urgent demand.

The main difference in the structure of RNA versus DNA is the presence of a hydroxyl group at the 2’ position of the ribose sugar in RNA, which makes the RNA molecule contain cis-diol at the end of the chain. Based on this property, Gao’s group developed electrochemical biosensors for miRNAs detection with OsO_2 nanoparticles as tags for RNA labeling [19]. Paleček’s group demonstrated that the 3′-end of miRNAs can be selectively modified by Os(VI)2,2′-bipyridine [20]. Rahman et al. reviewed the advances in the development of particle-based nucleic acid purification and detection in view of the difference in the structure of DNA and RNA [21]. It is well known that phenylboronic acids can form boronate ester covalent bonds with cis-diols (e. g. catechol, sugar, nucleoside and glycoprotein) on substrate surface [22-39]. Electrochemically active ferroceneboronic acid (FBA) has also been used as redox probes for the recognition of catechol derivatives, sugars, nucleoside and glycated hemoglobin at electrodes based on the formation of boronate ester covalent bound [40-46]. Herein, we reported a label-free electrochemical sensor for miRNAs detection based on the formation of boronate ester covalent bond between miRNAs and FBA.

2. EXPERIMENTAL

2.1 Chemicals and reagents

Ferroceneboronic acid (FBA), 6-Mercapto-1-hexanol (MCH), KH_2PO_4, K_2HPO_4, and tris-(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) were obtained from Sigma–Aldrich. Cytidine-5'-monophosphate (CMP), 2'-deoxycytidine 5'-monophosphate (dCMP), diethylpyrocarbonate, anti-miRNAs probes 5'-SH-$(CH_2)_6$-GGG AAC TAT ACA ACC TAC TAC CTC A-3' (DNA-1) and 5'-AAC TAT ACA ACC TAC TAC CTC AGG G-$(CH_2)_6$-SH-3' (DNA-2) were purchased from Sangon Biotech. Co., Ltd. (Shanghai, China). The target miRNAs and its similar sequences were obtained from GenePharma Co., Ltd. (Shanghai, China), which have the following sequences: 5'-UGA GGU AGU AGG UUG UAU AGU U-3' (let-7a), 5'-UGA GGU AGU AGG UUG UGU GGU U-3’ (let-7b), 5'-UGA GGU AGU AGG UUG UAU GGU U-3' (let-7c), and 5'-UGA GGU AGU AGU UUG UGC UGU U-3' (let-7i). The anti-miRNAs solutions were prepared using TE buffer solution (10 mM Tris–HCl, 1 mM EDTA, pH 7.4) and kept at -18 °C. MiRNAs solutions were prepared and diluted to the desired concentrations with TNE buffer (TE + 0.1 M NaCl) treated with 0.1% diethylpyrocarbonate.

2.2 Binding of FBA to ribonucleotide

To investigate the interaction of FBA and CMP or dCMP with electrochemistry, FBA was incubated with CMP or dCMP for 1 min in the phosphate-buffered saline solution (PBS buffer, 100 mM, pH 7.4) containing 50 mM Na_2SO_4. Voltammetric determination was performed on a DY2013 electrochemical workstation (Digi-Ivy, Inc., Austin, TX) using a homemade plastic electrochemical cell. A glassy carbon (GC) disk electrode (3 mm diameter) was used as working electrode. A platinum wire and a Ag/AgCl electrode were used as the auxiliary and the reference electrodes, respectively. Electrospray ionization/time of flight mass spectrometry (ESI-TOF-MS) was conducted on a LCT Premier XE mass spectrometer.

2.3 Detection of miRNAs

The gold disk electrodes were polished with diamond pastes down to 3 μm and alumina pastes down to 0.3 μm, and then sonicated in ethanol and water. The anti-miRNAs probes-covered electrode was prepared by immersing the cleaned gold electrodes in a solution of 1.0 μM anti-miRNAs containing 10 μM TCEP in the darkness for 24 hours. This step was followed by washing the electrode thoroughly with water and soaking the electrode in 0.1 mM MCH for 10 min to block the unreacted gold surface. Then, 10 μL of TNE comprising a given concentration of miRNAs and 100 μM FBA was cast onto the electrode surface for the hybridization between miRNAs and pre-immobilized DNA probe at 60 °C for 30 min. The electrode was rinsed with buffer to rid any non-specifically adsorbed substance before voltammetric determination.

3. RESULTS AND DISCUSSION

3.1 Binding of FBA to ribonucleotide

The specific binding of phenylboronic acid and FBA to ribose sugar in ribonucleotide has been demonstrated previously [38,42,45,47-49]. To confirm the binding of FBA and nucleotide, we demonstrated the formation of the FBA-nucleotide complex with voltammetry and mass spectrometry. As shown in Fig. 1, the dominant mass peak at 516.0441 Da belongs to the complex of FBA and CMP. The formation of the complex was also characterized by cyclic voltammetry. As shown in the inset, the peak currents of FBA decrease apparently after addition of CMP to FBA solution. The result is acceptable since the formation of large size of FBA-CMP complex could decrease the electron transfer rate of FBA. No apparent change was observed after addition of dCMP to FBA solution, indicating that FBA binds to the ribose sugar of CMP through the formation of boronate ester covalent bound.

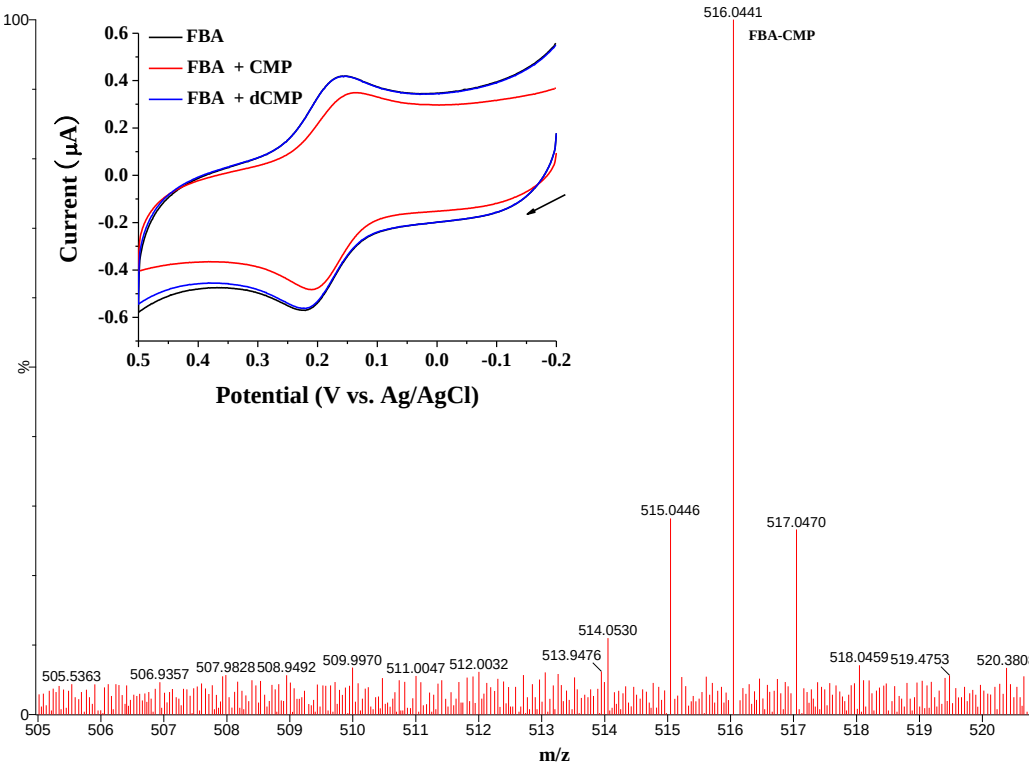

Figure 1. Mass spectrum of 20 μM FBA with the addition of 1 mM CMP. The inset shows the CVs of glass electrode in solutions of FBA, FBA/CMP and FBA/dCMP. The concentrations of FBA, CMP and dCMP were 0.05, 1 and 1 mM, respectively.

3.2 Principle for miRNAs detection with FBA

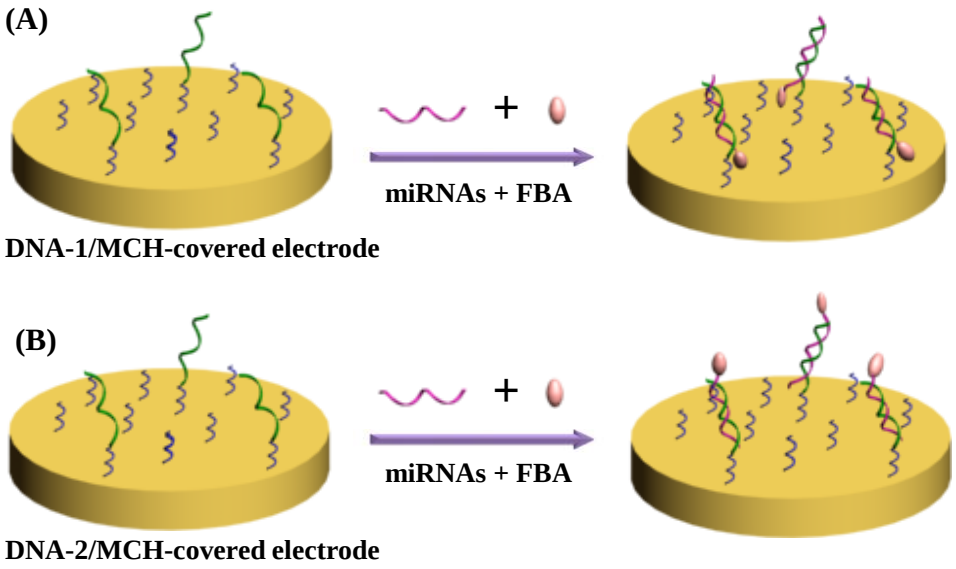

Figure 2. Schematic representation for miRNAs detection with FBA probe at gold electrodes covered with DNA-1 (A) and DNA-2 (B).

The DNA probes labeled with electrochemically active Fc or MB have been widely used for the sensing of DNA, protein, small biomolecules and metal ions [4-12]. In this context, the specific

binding of targets to the redox-labeled aptamers induces the conformational change of the aptamer probes and leads to the change in the distance between Fc and electrode, resulting in an apparent variation in the electrochemical signal. To prove the feasibility of our strategy, let 7 miRNAs under-expressed in lung cancer was tested as model analyte. The analysis principle of the present work is shown in Fig. 2. Probes 5'-SH-$(CH_2)_6$-GGG AAC TAT ACA ACC TAC TAC CTC A-3' (DNA-1) (Fig. 2A) and 5'-AAC TAT ACA ACC TAC TAC CTC AGG G-$(CH_2)_6$-SH-3' (DNA-2) (Fig. 2B) were used to capture let 7a. To fascinate the coupling of FBA and the hybridization of DNA/miRNAs, three G bases were included in the probes.

3.3 Feasibility for miRNAs detection

Differential pulse voltammogram (DPV) is an electrochemical technique that has been widely applied to monitor the current change of redox-labeled oligonucleotide probe. Therefore, DPV was performed to examine the feasibility and sensitivity of the sensor. Red curve in Fig. 3 is a representative DPV collected at an electrode modified with probe DNA-1 for let 7a capture in the presence of FBA. The peak with Ep = 0.178 V was attributed to the oxidation of FBA. The control DPV was acquired at an electrode modified with DNA-1 but without having exposed the electrode to the let 7a solution. In the absence of let 7a, the peak current dropped to a background level (black curve), indicating that the attachment of FBA is dependent on the captured let 7a. For comparison, the same procedure with that in the red curve was implemented with electrode covered with probe DNA-2 (blue curve). The current in the blue curve is apparently lower than that in the red curve, demonstrating that the signal intensity of FBA is related to the distance between FBA and electrode. Thus, the electrode modified with probe DNA-1 was employed for the detection of let 7a.

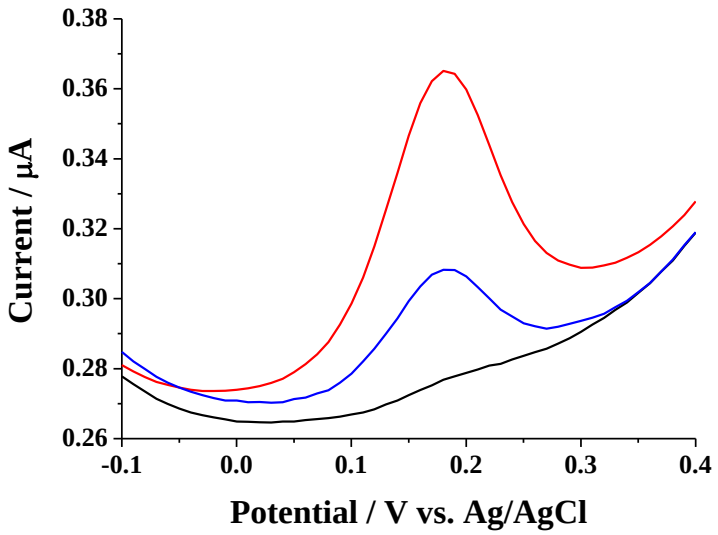

Figure 3. Differential pulse voltammogram (DPVs) acquired at mixed SAMs of DNA-1/MCH before (black curve) and after (red curve) hybridization with let 7a in the presence of FBA. Blue curve was obtained at DNA-2/MCH-covered electrode after capture of let 7a and FBA. The concentrations of let 7a and FBA were 2 and 100 μM, respectively.

3.4 Selectivity to miRNAs

The interference of mismatch miRNAs can be avoided by elevating the hybridization temperature. Herein, we assessed the selectivity of our method by incubating the DNA-1 probe modified electrode with base mismatch miRNAs at 60 °C. As shown in Fig. 4, the current in the case of complementary target let 7a was much higher than that in the case of the mismatch sequences (let 7b, let 7c and let 7i), indicating that the method is selective to let 7a detection.

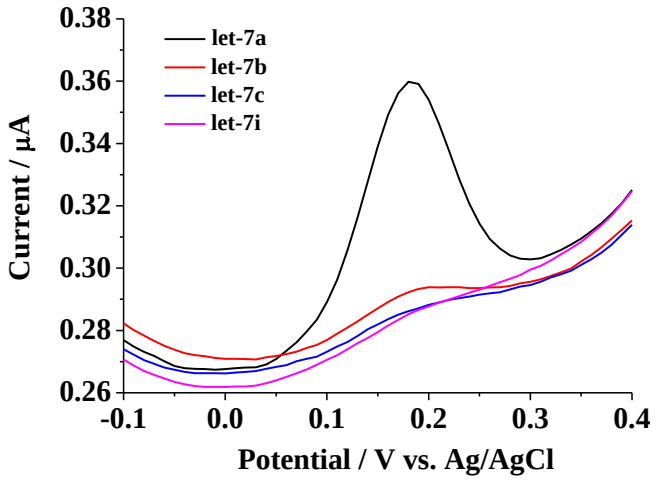

Figure 4. DPVs acquired at mixed SAMs of DNA-1/MCH after hybridization with let 7a, let 7b, let 7c, and let 7i in the presence of FBA. The concentrations of the four miRNAs were all 2 μM. The other experimental conditions were the same as those in Figure 3.

3.5 Sensitivity to miRNAs

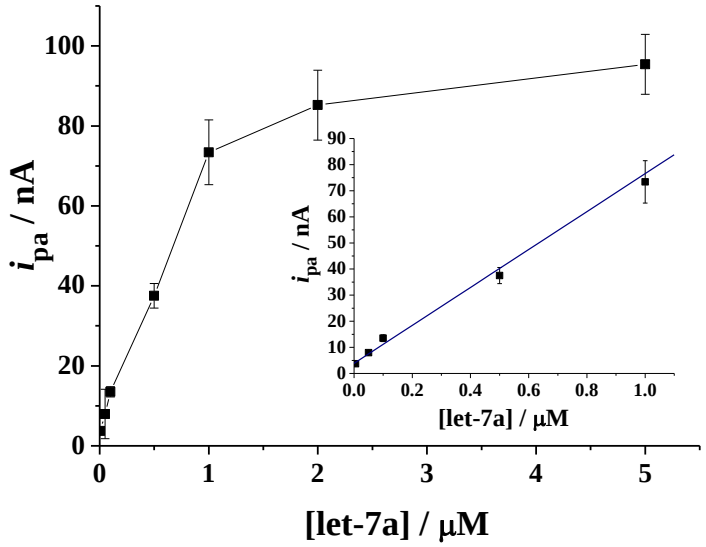

Figure 5. Dependence of the anodic peak current on let 7a concentration (0.005~5 μM). The inset shows the linear portion of the curve with the concentration ranging from 5 nM to 1 μM. The other experimental conditions were the same as those in Figure 3.

The dependence of the currents on the concentrations of let 7a is presented in Fig. 5. The currents increase linearly with the concentrations of let 7a ranging from 5 nM to 5 μM, and begin to level off beyond 1 μM. The linear regression equation is expressed as i_{pa} (nA) = 3.8 + 72.7 [let-a] (μM) (R^2 = 0.99). The detection limit of the method was estimated to be 1 nM. Although the method is less sensitive than the other electrochemical methods reported previously [19,50-61], it presents a new strategy for the detection of miRNAs based on the difference in the structure of RNA versus DNA. The content of miRNAs in biological samples is in femtomolar or even lower level [18]. Therefore, the proposed method currently cannot detect miRNAs in real body fluid samples. We believe that signal amplification with nanoparticles or enzymes would improve the sensitivity in such detection format.

4. CONCLUSION

In this work, we reported a strategy for the detection of miRNAs based on the difference in the structure of RNA versus DNA. MiRNAs were captured by DNA probes pre-immobilized onto the electrode. Cis-diols at the 3' end of miRNAs allowed them to be recognized by electrochemically active FBA through the formation of boronate ester covalent bound. The detection limit of the method was estimated to be 1 nM. The results will be valuable for the design of new types of biosensors for label-free detection of miRNAs in a biological matrix.

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