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Sensitive detection of microRNAs using hemin/G-quadruplex concatamers as trace labels and RNA endonuclease-aided target recycling for amplification

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ABSTRACT

In this work, a novel electrochemical biosensor based on endonuclease-aided target recycling and hybridization chain reaction (HCR) was constructed for the sensitive detection of microRNAs (miRNAs). To construct the biosensor, the hairpin-like capture probe (CP) which contained the complementary RNA sequence of target miRNA was immobilized on gold nanoparticles (nano-Au) modified electrode through Au—S bond for the following hybridization. In the presence of target miRNA, a linear RNA–RNA hybrid duplex was formed instead of the hairpin structure, and ribonuclease A (RNase A) selectively cuts the RNA fragment of CP in hybrid duplex, leaving the miRNA strand intact for analyte recycling. Subsequently, the cleaved single-strand fragment on electrode surface could initiate HCR, which resulted in the hybridization reaction to form double-strand DNA concatamers on the electrode surface. Consequently, hemin could stack into the G-quadruplex-forming region and the hemin/G-quadruplex can be formed to give an amplified electrochemical signal by differential pulse voltammetry (DPV). Using microRNA-101 as a model, the resulting biosensor presented high sensitivity and a broad linear response from 1.0×10^{-13} to 1.0×10^{-8} M with the detection limit of 1.0×10^{-13} M under optimal assay conditions.

1. Introduction

MicroRNAs (miRNAs) are non-coding single-stranded RNAs with about 21–23 nucleotides in length that can regulate the expression of genes at both the translational and transcriptional levels [1]. miRNAs have been implicated in different biological processes such as cell differentiation, apoptosis, proliferation, motility, invasiveness, embryogenesis, inflammation and programmed cell death. An abnormal miRNA may produce a series of consecutive pathological changes including cancer [2], neurodegenerative diseases [3], diabetes [4], heart diseases [5], kidney diseases and liver diseases [6]. Since miRNAs are involved in disease origin and development and they are pathology-specific, changes in miRNA profiles have been proposed as biomarkers for early detection, classification, prognostic and predictive diagnostic of diseases. Therefore, discovering a sensitive, rapid and quantitative detection method for miRNAs is imperative.

Several approaches are commonly used for miRNAs detection, such as quantitative-reverse transcription PCR (qRT-PCR), hybridization-based methods and sequencing. qRT-PCR is one of the most common methods used to detect low levels of miRNAs with high sensitivity and specificity [7]. Circular exponential amplification based on strand displacement amplification can even discriminate single-nucleotide difference between miRNA family members and perform well in real sample analysis with a detection limit of 3.80×10^{-13} M [8]. Moreover, in combination with the quantum-dot technique, this method can be applied for multiplex miRNA assay with a detection limit of 0.1 aM [9]. However, miRNAs are difficult to amplify by PCR because the short length of mature miRNA [10]. Based on hybridization, traditional molecular biology techniques for miRNA detection, such as cloning and northern blotting assays, are time-consuming and not very sensitive [11]. Deep sequencing methods have been recently used to reveal the differential expression of miRNAs in cancers, but at the present deep sequencing methods are expensive for routine laboratory work and their main application is to detect qualitative and quantitative differences in miRNA expression between healthy and unhealthy individuals [12].

During the past decade, electrochemical methods have been applied for the detection of miRNAs [13]. Quantitative determination of miRNAs by electrochemical methods is based on changes in circuit properties such as capacitance, conductance and resistance that occur before and after target miRNA hybridization. Several electrochemical biosensors have been demonstrated in the detection of miRNA [13,14]. Although sensitive, the fabrication process of these miRNA biosensors is usually time-consuming and

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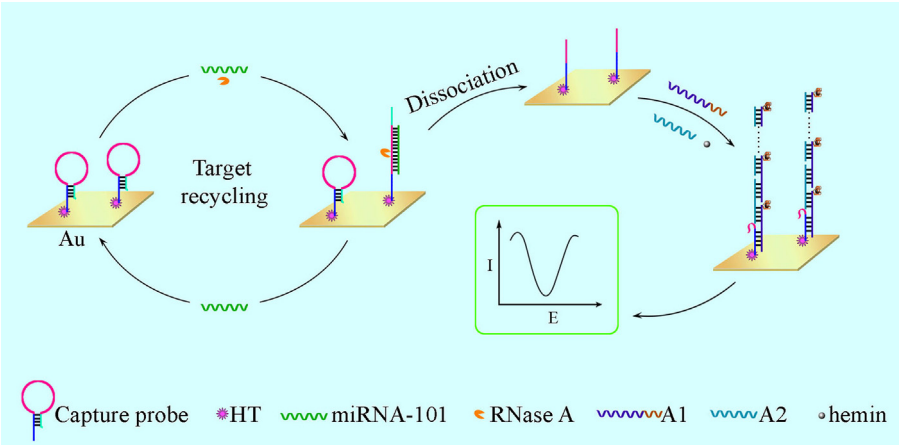

Fig. 1. The schematic illustration of the method for sensitive detection of miRNAs.

complicated. Meanwhile, electrochemical biosensors for detecting miRNA based on changes of current response are rarely reported. In addition, target recycling performed by enzymes has been demonstrated as an interesting and effective signal amplification route in some bioassay. For instance, DNAzymes [15], nicking enzymes [16] and exonucleases [17] have been applied in previous works of DNA biosensors. In view of miRNAs detection with higher sensitivity and better reliability, we designed a selective electrochemical sensor based on nuclease-assisted target recycling and hemin/G-quadruplex concatamers as trace labels.

In this study, target recycling was achieved by the formation of RNA-capture probe (CP) complex on the modified electrode surface in the presence of target miRNA and then release of miRNA from RNA-CP complex by RNase A digestion at a single phosphodiester bond bridging the ribonucleotides opposing the 10th and 11th nucleotide from the 5′ terminus of miRNA [18]. Subsequently, the single-strand fragment of CP on electrode surface can initiate hybridization chain reaction (HCR), which will result in forming hybridization double-strand DNA structure on the electrode surface. Furthermore, hemin stacks into the G-quadruplex-forming region and the hemin/G-quadruplex can be formed to give a current response. The proposed biosensor for miRNA detection also presented high sensitivity, assay simplicity and good stability.

2. Materials and methods

2.1. Reagents and materials

miRNA-101 (5′-UACAGUACUGUGAUAACUGAA-3′) was adopted in this research as a representative miRNA molecule, due to its important role in cell proliferation, harmonic secretion, and cancer development [19]. miRNA-155 (5′-UUAA UGCUAAUCGUGAUAGGGGU-3′) was used as an interfering substance. Dithiol-modified CP (5′-SH-$(CH_2)_6$-CATACTCGA*CGAAGT*UUCAGUUAUCACAGUACUGUA*ACTTCG*-3′) was used to modify the surface of the working electrode. CP contains two functional regions: (1) the underlined sequence is the complementary RNA sequence of miRNA-101 and the cleavage site of RNase A is between the ribonucleotides opposing the 10th and 11th nucleotide from the 5′ terminus of miRNA; (2) the italic are complementary sequences to ensure the finally formation of hairpin structure. Hybridization single-strand chain A1 (5′-GGGTAGGGCGGGTTGGGTTCATGCAACATCTAGACTTCGTCGAG TATG-3′) and A2 (5′-CTAGATGTTGCATGACA TACTCGACGAAGT-3′) were designed to initiate HCR. A hemin aptamer region (underlined showed) was added at the 5′ end of A1 for the purpose of making hemin easier recognize and form hemin/G-quadruplex. All oligonucleotides (HPLC-purified) were synthesized from Takara Biotechnology (Dalian, China). Ribonuclease A (RNase A) and hexanethiol (HT) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals and solvents were of analytical grade and were used as received. 0.1 M phosphate buffered solutions (PBS) containing 10 mM KCl and 2 mM $MgCl_2$ (pH 7.4) was employed as detection solution and 0.1 M PBS (pH 7.4) was served as washing solution. Buffer for preparation of miRNA-101 and DNA probes solutions was 20 mM Tris–HCl containing 140 mM NaCl, 1 mM $MgCl_2$, 5 mM KCl and 1 mM $CaCl_2$, pH 7.4. Double distilled water was used throughout this study.

2.2. Apparatus and characterization

Electrochemical measurements including cyclic voltammetric (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) were carried out using a CHI 660C electrochemistry workstation (Shanghai CH Instruments, China). A conventional three-compartment electrochemical cell comprised a platinum wire auxiliary electrode, a saturated calomel reference electrode (SCE) and modified glassy carbon electrode (GCE, Φ = 4 mm) as the working electrode.

2.3. Fabrication of the aptasensor

The schematic diagram of the stepwise fabrication procedure was shown in Fig. 1. Prior to surface modification, the GCE was polished repeatedly with 0.3 mm and 0.05 mm alumina to obtain a mirror-like surface, followed by successive sonication in ethanol and double distilled water each for 5 min and drying in air. Then, gold nanoparticles (nano-Au) were obtained by immersing the cleaning electrode in 1% $HAuCl_4$ solution and electrodeposited at −0.2 V for 30 s. Subsequently, 20 μL of 2.0 μM CP solution was dropped onto the modified GCE and incubated for 16 h at room temperature to form Au—S bond, and then washed with double distilled water. Next, the proposed electrode was incubated in 20 μL of HT solution (1 mM) for approximately 40 min in order to block the possible non-modified Au surface and eliminate any non-specific absorption. After washing, the modified electrode surface was added 10 μL of different concentrations of miRNA-101 solution and 10 μL of 1 unit/mL RNase A and incubating at 25 °C for 2 h. Following that, 10 μL of 2 μM A1 and A2 solution was added onto the electrode surface and incubated for 3 h. Finally, the obtained