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Short communication

Highly sensitive microRNA quantification with zero background signal from silver nanoparticles

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ABSTRACT

MicroRNAs are a class of noncoding RNAs, which play vital roles in numerous cellular processes. Recent studies have confirmed their significance in the theranostics of various diseases. We herein fabricate an electrochemical approach for microRNA quantification. DNA/microRNA/DNA hybridization and electrochemical signals from silver nanoparticles (AgNPs) are employed in this work. DNA1 immobilized on a gold electrode interacts with target microRNA, along with amino group labeled DNA2, to form the sandwich hybrid. The adjacent DNA1 and DNA2 are then ligated, which can keep DNA2 on the electrode surface during the denaturation. Amino group modified at the 5′ end of DNA2 captures AgNPs on the electrode surface, which provide sharp stripping peaks for microRNA quantification. This electrochemical approach offers a simple and sensitive platform for the detection of microRNA, which shows great utility in biomedical research and clinical diagnosis.

1. Introduction

MicroRNAs are a class of small endogenous noncoding RNAs, which act as important regulators of gene expression [1]. Currently, prominent roles of microRNAs have been uncovered in various cellular processes such as embryonic differentiation, cardiac hypertrophy, apoptosis, immunological response and tumor development [2–4]. Recent studies have revealed that microRNAs exert significant effects on various diseases (neurodegeneration, lymphoma, lung cancer, colon carcinoma, etc.) and can be used as noninvasive biomarkers for disease diagnosis [5–7]. Moreover, by modulating specific microRNA expression through the delivery of inhibitors, they can also be novel therapeutic targets for many diseases [8]. Therefore, there are urgent demands for flexible microRNA quantification approaches with high sensitivity and selectivity [9,10].

Currently, northern blotting technique is predominantly employed for RNA analysis. Due to some features of microRNA like low content, short sequences and high sequence homology among family members, northern blotting is not competent for the detection of microRNA, which stimulates the development of novel biosensors and signal amplification strategies [11,12]. For example, real-time reverse transcription polymerase chain reaction [13], size-coded ligation chain reaction [14], endonuclease cleavage cycles [15] and hybridization chain reaction [16] have been employed for the sensitive detection of microRNA. Many of these methods involve the application of electrochemical techniques due to some unique advantages of electroanalysis for microRNA (fast response, low cost, high sensitivity, etc.) [17–19]. However, labor-intensive operation procedures may be required. Herein, we have developed a simple and sensitive electrochemical method for microRNA assay, which may be more promising for practical applications. In this work, we have subtly combined DNA/microRNA/DNA hybridization and ligation/denaturation procedures to achieve the microRNA-induced selective capture of silver nanoparticles (AgNPs). The nanoparticles then exhibit a highly characteristic solid-state Ag/AgCl reaction and provide sharp silver stripping peaks. A signal-on electrochemical approach for microRNA quantification is thus developed, which can detect target microRNA as low as 10 fM. This method offers a concept for a simple and integrated electrochemical device for microRNA assay and disease diagnostics.

2. Experimental section

2.1. Materials and chemicals

Sodium borohydride ($NaBH_4$), Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), diethypyrocarbonate (DEPC), ethylenediaminetetraacetic acid (EDTA) and mercaptohexanol (MCH) were purchased from Sigma-Aldrich (USA). Silver nitrate was ordered from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). T4 DNA ligase

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was purchased from New England Biolabs Ltd. (Beijing, China). Human serum samples were supplied by local hospital (Suzhou, China). Other reagents were of analytical grade and were used as received. All solutions were prepared with double-distilled water, purified with a Milli-Q purification system (Branstead, USA). Two DNA probes (DNA1 and DNA2) and target microRNA were synthesized and purified by Takara Biotechnology Co., Ltd. (Dalian, China). DNA1 was labeled with phosphate group at the 5′ end and thiol group at the 3′ end. DNA2 was modified with amino group at the 5′ end. The sequences were listed as follows. The italic and underlined parts were complementary sequences, respectively.

DNA1 : 5′-P-*CTGATAAGCTA*TTTT-SH-3′
DNA2 : 5′-NH_2-TTTTTCAACATCAGT-3′
microRNA (miRNA-21) : 5′-*UAGCUUAUCAG*ACUGAUGUUGA-3′

2.2. Preparation of AgNPs

AgNPs were prepared by borohydride reduction of $AgNO_3$ [20]. Briefly, 100 mL of $AgNO_3$ (0.25 mM) and trisodium citrate (0.25 mM) solution was prepared, which was then mixed with 3 mL of $NaBH_4$ (10 mM) under vigorous stirring for 30 min. The formed AgNPs were left overnight and purified by three cycles of centrifugation at 12 000 rpm for 20 min.

2.3. Hybridization of DNA1/microRNA/DNA2 on the electrode

The substrate gold electrode was treated with piranha solution (98% H_2SO_4: 30% H_2O_2 = 3:1) for about 5 min. Then it was polished with P3000 silicon carbide paper and 1, 0.3 and 0.05 μm alumina slurry. After sonication in ethanol and then water for 5 min each, the electrode was immersed in HNO_3 (50%) for 30 min. Subsequently, it was electrochemically cleaned with 0.5 M H_2SO_4. The pretreated electrode was soaked in 1 μM DNA1 solution (10 mM Tris–HCl, 1 mM EDTA, 10 mM TCEP, 0.1 M NaCl, pH 7.4) at room temperature for 8 h. Then it was incubated with 1 mM MCH for 30 min [21]. microRNA solutions were prepared for the incubation of DNA1 modified electrode for 1 h. The concentrations of microRNA were from 10 fM to 5 nM. Then the electrode was washed and further treated with 1 μM DNA2 solution for 1 h to form the hybrid of DNA1/microRNA/DNA2.

2.4. DNA ligation/denaturation and AgNPs capture

The nick between DNA1 and DNA2 in the hybrid was linked by 5 unit/mL T4 DNA ligase (50 mM Tris–HCl, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, pH 7.5) at 22 °C for 1 h. Afterward, the electrode was immersed in 10 mM Tris–HCl (pH 7.4) at 95 °C for 5 min and then cooled down. The electrode was rinsed and incubated with 10 nM AgNPs for 4 h. Through the interaction between Ag and amino group on DNA2, AgNPs were thus localized on the electrode surface.

2.5. Electrochemical measurements

All electrochemical experiments were performed by using the electrochemical analyzer (CHI660D, CH Instruments, China) at room temperature. Three electrode system was employed, consisting of the Ag/AgCl reference electrode, the platinum auxiliary electrode and DNA modified electrode (gold area: 3.142 mm^2) as the working electrode. Electrochemical impedance spectroscopy (EIS) experiments were carried out in 5 mM $[Fe(CN)_6]^{3-/4-}$ with 1 M KNO_3. The parameters were as follows: bias potential, 0.231 V; amplitude, 5 mV; frequency range, 0.1–100000 Hz. Linear sweep voltammetry (LSV) experiments were performed in 0.1 M KCl, and the scan rate was 100 mV/s.

3. Results and discussion

Fig. 1 represents the scheme of the electrochemical approach for microRNA quantification. DNA1 with 3′ end thiol group is immobilized on the electrode surface via gold-sulfur chemistry [22]. Afterward, the electrode is treated with the spacer thiol molecules, MCH [23]. In the presence of target microRNA, hybridization reaction occurs and DNA1/microRNA/DNA2 hybrid forms, which brings DNA2 close to DNA1. The adjacent two DNA probes are then ligated, which can keep DNA2 on the electrode during the denaturation. The procedure of denaturation removes microRNA and unligated DNA2, retaining a monotonous electrode surface, which exhibits zero background signal. Since DNA2 is labeled with amino group, AgNPs are thus localized on the electrode surface via silver-amino chemistry [24] and can be detected by a highly characteristic solid-state Ag/AgCl reaction [25]. MicroRNA levels can be calculated accordingly.

The stepwise modification of the gold electrode is recorded by EIS. The negatively charged phosphate groups of the oligonucleotides decelerate electron transfer for $[Fe(CN)_6]^{3-/4-}$ redox probe, which is reflected by the semicircle portion in the impedance spectrum [26]. As shown in Fig. 2A, a large semicircle is observed in the case of DNA1 modified electrode, which becomes larger after the hybridization of DNA1/microRNA/DNA2. Since ligation/denaturation procedures keep DNA2 and release microRNA back into the solution, the diameter of the semicircle declines. Afterward, the further attached AgNPs exhibit unique electrical properties and the semicircle becomes even smaller. The EIS experiments results confirm the stepwise modification processes.

Quantification of microRNA-induced sandwich hybridization and further AgNPs adsorption is then studied by LSV [27]. As depicted in Fig. 2B, DNA1 modified electrode cannot exhibit a significant current peak unless the subsequent incubation with microRNA, DNA2 and AgNPs, demonstrating the fact that incomplete DNA1/microRNA/DNA2 hybrid cannot effectively capture AgNPs on the electrode surface. Fig. 2C displays the sensitivity of this method. With larger amount of microRNA, the stripping silver peak rises, which is in linear relationship with the logarithmic microRNA concentration. The detection range is from 10 fM to 5 nM, with the fitting equation of $y = 90.27 + 6.44x$, where x is the logarithmic microRNA concentration, y is the peak current, $R^2 = 0.994$ and $n = 3$. The detection limit is calculated to be 10 fM ($S/N = 3$). To check the selectivity of this microRNA assay, 1 pM single-base mismatch microRNAs are employed in the electrochemical measurements and the results are compared in Fig. 2D. Tiny stripping silver peaks are observed in the case of mismatch microRNAs, which confirms the high selectivity towards target microRNA. To further verify the practical utility of this approach, human serum samples are used. As shown in Table 1, detection of microRNA is satisfactorily achieved and recoveries have substantiated the fine accuracy of this method in biological samples.

4. Conclusions

In summary, we have offered a simple and sensitive electrochemical approach for the determination of microRNA levels based on the DNA/microRNA/DNA hybridization and electrochemical signals from AgNPs localized on the electrode surface. Highly sensitive and selective detection of target microRNA is achieved by analyzing the stripping silver peaks. Zero background signal is attributed to the ligation and denaturation procedures. Moreover, due to the advantages of convenient operation and high stability, one might expect the design of an integrated electrochemical device for microRNA quantification based on the proof-of-concept approach described here.

Conflict of interest

The authors declare no competing financial interests.