Electrochemistry Communications 12 (2010) 1294–1297

Contents lists available at ScienceDirect

Electrochemistry Communications

journal homepage: www.elsevier.com/locate/elecom

Label-free oligonucleotide detection method based on a new L-cysteine-dihydroartemisinin complex electroactive indicator

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ARTICLE INFO

Article history:
Received 28 May 2010
Received in revised form 4 July 2010
Accepted 5 July 2010
Available online 9 August 2010

Keywords:
L-cysteine-dihydroartemisinin complex
Electroactive indicator
Detection and recognition of base-mismatched oligonucleotide

ABSTRACT

A novel base-mismatched oligonucleotide assay method based on label-free electrochemical biosensor was developed, in which the L-cysteine (Cys)-dihydroartemisinin (DHA) complex was used as a new electroactive indicator. In DNA sensor, Cys-DHA complex was initially formed on electrode surface by cathodic scanning, and target oligonucleotide was conjugated with Cys-terminated DHA indicator through electrostatic interaction under optimal pH. The subsequent sequence assay was responsive to hybridization recognition, which target oligonucleotide was captured by the surface-anchored DNA/Cys-DHA probe. The electrochemical signals of biosensor before and after hybridization were compared basing the measurements of semi-derivative linear scan voltammetry (SDLSV) and electrochemical impedance spectroscopy (EIS). On the basis of signal amplification of electroactive indicator and specific recognition of DNA probe, five target oligonucleotides with different mismatched bases were assayed, and a detection limit reached 0.3 nM. Furthermore, atomic force microscopy (AFM) was used to visually characterize specific recognition spots of biosensor at nanoscale. This study demonstrated a new electroactive molecule-based, biomolecule-involved electroactive indicator and its application in recognition and detection of complementary and base-mismatched oligonucleotide.

1. Introduction

The detection of base-mismatched oligonucleotides, based on the hybridization recognition of specific DNA sensor, is of central importance in the diagnosis and treatment of genetic disease, detection of infectious agents, and in drug screening [1,2]. Compared with other conventional techniques, electrochemical method for detecting DNA hybridization has received considerable attention because of their high sensitivity, low cost, and/or independence of sample turbidity or optical pathway [3,4]. The key step for developing a novel electrochemical biosensor is to fabricate an electroactive indicator that can provide sensitive electrochemical signal and high immobilization capacity of capture oligonucleotides.

Various label-free electrochemical biosensors have been used for transducing electronically DNA hybridization events based on oligonucleotide-bearing redox indicators or nanoparticles [5,6]. However, these chemosynthesis of electroactive indicators, such as $Ru(bpy)_3^{3+}$ [7], ferrocene [8] and methylene blue [6], suffer from potential drawbacks that might impair the bioactivity of capture oligonucleotides even by mild intercalative binding. Alternatively, functionalized nanoparticles, such as a secondary oligonucleotide bearing nanocrystal tags [9], are employed, but the toxicity of CdS-nanoparticles hinders further use for *in vivo* analysis. In addition, traditional label-free biosensor often requires multiple self-assembled steps for the probes with indicator molecules, which prolongs the assay time and increases the assay cost. In our protocol, formation of a new electroactive molecule-based, biomolecule-involved electroactive indicator on electrode surface can avoid external chemosynthesis process and decrease fabricating steps, which can be directly used in label-free electrochemical biosensor.

Here dihydroartemisinin-based, L-cysteine-involved electroactive complex is used as a new electroactive indicator to be assayed on base-mismatched oligonucleotide. Dihydroartemisinin (DHA), an analogue of artemisinin which is isolated from the traditional Chinese herb *Artemesia annua L.* (Scheme 1), exhibits a sensitive electrochemical signal because of its endoperoxide bridge [10,11]. L-cysteine (Cys), being rich in amino groups (Scheme 1), firmly provides an ideal platform for conjugating capture oligonucleotides [12,13]. In this paper, semi-derivative linear scan voltammetry (SDLSV) and electrochemical impedance spectroscopy (EIS) were used to characterize the electrochemical behaviors of DNA sensor before and after hybridization recognition. Nanoscale atomic force microscopy (AFM) was employed to measure the nanostructures changed according to specific binding spots of hybridization recognition.

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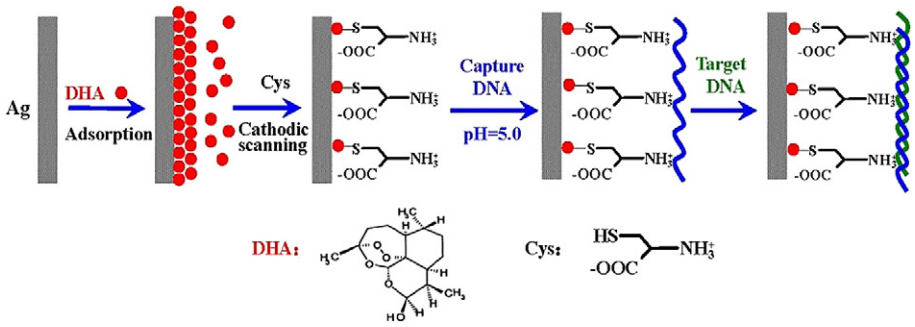
doi:10.1016/j.elecom.2010.07.003



Scheme 1. A schematic illustration of DNA/Cys-DHA electrochemical biosensor using a Cys-DHA complex as electroactive indicator.

2. Materials and method

2.1. Materials

Dihydroartemisinin (DHA) was purchased from PIDI Co. (Chongqing, China). All 10-base oligonucleotides and L-cysteine (Cys) were purchased from Sangon Co. (Shanghai, China). The following oligonucleotide sequences were used: Probe: 3′-G-T-A-T-C-G-A-A-T-C-5′, complementary: 5′-C-A-T-A-G-C-T-T-A-G-3′, 3-base mismatch: 5′-A-A-T-A-C-C-T-T-A-A-3′, 5-base mismatch: 5′-A-C-C-A-C-C-T-T-A-A-3′, 7-base mismatch: 5′-A-C-C-G-C-A-T-T-A-A-3′, noncomplementary: 5′-A-C-C-G-C-A-C-G-T-A-3′. Other chemicals were of analytical reagent grade. All samples and buffers were prepared using deionized water obtained from a Mill-Q water purification system.

2.2. Fabricating of DNA/Cys-DHA electrochemical biosensor

For electrochemical experiments, a freshly polished silver electrode was first immersed in 0.2 M Britton-Robinson buffer (20% ethanol, pH 7.0) [11] containing 0.8 mM DHA and 0.1 mM Cys. The Cys-DHA complex was then formed on electrode surface under cathodic scanning from −0.3 V to +1.3 V (*versus* SCE). Second, Cys-DHA/Ag electrode was immersed in a acetate buffer solution (0.2 M, pH 5.0) containing 1.0 μM capture oligonucleotide for 1 h at room temperature, and washed with phosphate buffer (pH 7.0) to remove unconjugated DNA. Finally, voltammetric measurements were performed in phosphate buffer hybridization solution containing target oligonucleotide with DNA/Cys-DHA sensor at room temperature.

Before AFM imaging, the substrate was prepared on glass slide by gold film deposition. The gold substrate was rinsed carefully with deionized water and ethanol, respectively. The following steps of indicator fabricating, probe immobilization, and hybridization recognition were same with above electrochemical experiments.

2.3. Measurement of electrochemical behaviors of DNA sensor

Semi-derivative linear scan voltammetry (SDLSV) and electrochemical impedance spectroscopy (EIS) were performed with a CHI 660A electrochemical workstation (CH Instrument Co., USA). A three-electrode system consisted of the silver working electrode, a saturated calomel electrode and a platinum wire, which served both as the reference and an auxiliary electrode, respectively. For SDLSV measurement, a 0.2 M Britton-Robinson buffer containing 20% ethanol was used as the supporting electrolyte. For EIS measurement, the supporting electrolyte solution was 0.1 M KCl containing 5 mM $[Fe(CN)_6]^{3-/4-}$. The frequency range investigated was from 100 kHz to 1 Hz at the formal potential of 250 mV using an alternate voltage of 5 mV.

2.4. AFM imaging of DNA sensor recognition spots

AFM images were performed with an AutoProbe CP Research atomic force microscope (Thermo Microscopes Inc., presently named Veeco, USA). Commercially available silicon cantilevers with a length of 100 μm and a resonance frequency of around 100 KHz (Veeco, Santa Barbara, USA) was used. AFM images were carried out in uncontact mode in air to perform the topography images at room temperature. Image processing and data analysis were performed using Proscan Image Processing Software Version 2.1 equipped with the instrument.

3. Results and discussion

3.1. Characterizing electrochemical behaviors of the DNA/Cys-DHA biosensor

As illustrated in Scheme 1, DNA probe was initially formed on electrode surface through electrostatic interaction between target oligonucleotide and Cys-terminated DHA at pH 5.0. The subsequently sequence recognition was responsive to hybridization reaction by the surface-anchored DNA/Cys-DHA probe. As shown in Fig. 1A, DHA exhibited an irreversible reduction peak at −0.69 V (curve a), characteristic of the reduction of adsorption-controlled DHA [14]. The subsequent addition of Cys to the DHA solution caused the appearance of a new irreversible reduction peak at −1.10 V (curve b), demonstrating that a new electroactive complex, Cys-DHA, was formed on the electrode surface which avoided the external chemosynthesis process. The formation of Cys-DHA complex is involved in a two-step process of initially cathodic scanning-mediated cleavage of the endoperoxide bridge of DHA [14], and subsequently a S–C bond of sulfhydryl group of Cys with C-centered primary radicals of DHA, which is supported by the binding mechanism of artemisinin with thiol compounds, such as glutathione [15]. It is worthwhile to point out that the peak current of the Cys-DHA complex is higher than that of DHA, and the peak-to-peak potential separation reaches 0.3 V, indicating that Cys-DHA complex can act as a new electroactive indicator, which is not interfered by the electrochemical signal of DHA alone. Introduction of capture oligonucleotide on Cys-DHA electroactive indicator induced negative shift of peak potential along with a decrease in peak current (curve c), demonstrating that negative charged oligonucleotide was immobilized with positively charged Cys-terminated DHA through electrostatic interaction under the isoelectric point of Cys (pH 5.0). Curve d and e were the representative SDLSV curves of DNA/Cys-DHA electrochemical biosensor with its complementary oligonucleotides recognition, showing that peak current gradually decreased along with the increase of target oligonucleotide concentration. This indicates that DNA/Cys-DHA electrochemical biosensor can specifically recognize target oligonucleotides and the