Biosensors and Bioelectronics 60 (2014) 218–223

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Electrochemical impedance spectroscopy aptasensor for ultrasensitive detection of adenosine with dual backfillers

Yitan Wang, Juanjuan Feng, Zhian Tan, Haiyan Wang*

Anhui Key Laboratory of Chemo-biosensing, College of Chemistry and Materials Science, Anhui Normal University, Wuhu 241000, PR China

ARTICLE INFO

Article history:
Received 27 January 2014
Received in revised form
3 April 2014
Accepted 15 April 2014
Available online 23 April 2014

Keywords:
Electrochemical impedance spectroscopy
Aptasensor
Dithiothreitol
6-mercaptohexanol
Adenosine

ABSTRACT

A highly sensitive and label-free electrochemical impedance spectroscopy (EIS) aptasensor for the detection of adenosine was fabricated by co-assembling thiolated aptamer, dithiothreitol (DTT) and 6-mercaptohexanol (MCH) on gold electrode surface, forming Au/aptamer-DTT/MCH. The interfacial electron transfer resistance (R_{et}) of the aptasensor using $[Fe(CN)_6]^{3-/4-}$ as the probe increased with adenosine concentration, and the change in Ret (ΔR_{et}) against the logarithm of adenosine concentration was linear over the range from 0.05 pM to 17 pM with a detection limit of 0.02 pM. Compared to that of aptasensors fabricated with MCH or DTT alone as the backfiller, the detection limit was improved dramatically (LOD was 0.03 nM and 0.2 pM for Au/aptamer/MCH and Au/aptamer-DTT, respectively), which was attributed primarily to the coupling of the cyclic- and linear -configuration backfillers. The coupling showed remarkably higher resistance to nonspecific adsorption, leading to low background noise and high response signal. The aptasensor reported herein is applicable for the detection of other kinds of aptamer-binding chemicals and biomolecules.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Aptamer is single-strand functional nucleic acid which can recognize various target molecules such as heavy metals, proteins, and even cells (You et al., 2011; Sun et al., 2011; Zhou et al., 2012; Zhu et al., 2013; Wu and Zhan et al., 2012) with high affinity and specificity. They are regarded as an attractive alternative to antibody with merits of simple synthesis, easy labeling, good stability and wide applicability. Due to their unique properties, aptamers have been extensively used as an important sensing element for the development of biosensing system.

Various aptamer-based biosensors, aptasensors, have been developed with different detection techniques including fluorescence (Xiang et al., 2010; Huang, Liu, 2010; Hu et al., 2012; Huang et al., 2011), electrochemiluminescence (ECL) (Ye et al., 2011; Chen and Cai et al., 2010; Wang et al., 2010; Zhu et al., 2011), surface-enhanced Raman scattering (Chen and Liu et al., 2008), and electrochemistry (Yan et al., 2011; Zhang et al., 2008; Yin et al., 2012; Yuan et al., 2012; Ding et al., 2012). Highly sensitive and selective these developed aptasensors, most of them, need labeling aptamers with redox compound. The labeling process is time-consuming and leads to the reduced bioaffinity. Therefore, it is necessary to develop the label-free aptasensor. Electrochemical impedance spectroscopy (EIS), a popular electrochemical method to measure interfacial molecular interactions, can be served as a promising "label-free" detection method (Bogomolova et al., 2009). Compared to other electrochemical methods, EIS-based biosensors possess unique advantages of ease of signal quantification, less destruction to the biological interactions being measured, and most importantly, redox marker-free (Li and Wang et al., 2008; Li et al., 2008; Kashefi-Kheyrabadi, Mehrgardi, 2012; Fan et al., 2013; Chen et al., 2013; Bogomolova et al., 2009).

Chen et al., (2013) developed the label-free and highly sensitive EIS aptasensor for the detection of potassium ion and a detection limit of 0.1 nM was obtained. Ease and reliable results could be achieved by EIS, however the problem of inability to discriminate between specific binding and nonspecific adsorption limits its sensitivity. Therefore, it is necessary to improve its sensitivity by decreasing the nonspecific adsorption via proper modification of electrode surface. Wu et al. (2010) proposed that dramatic improvements in the detection limits could be obtained for the electrochemical detection of DNA hybridization by designing a new ternary monolayer containing thiolated capture probe, dithiothreitol (DTT) and mercaptohexanol (MCH). The coupling of DTT and MCH backfillers minimized the nonspecific adsorption and resulted in a highly sensitive DNA biosensor with an ultrasensitive detection limit.

Adenosine is an endogenous nucleoside with potent biological activities including extension of the blood vessels and increment of the blood flow in the arteries. It plays vital functions in the

* Corresponding author. Tel./fax: +86 5533869303.
E-mail address: hywang@mail.ahnu.edu.cn (H. Wang).

http://dx.doi.org/10.1016/j.bios.2014.04.022
0956-5663/© 2014 Elsevier B.V. All rights reserved.

peripheral and central nervous system, as well as immune system. Moreover, it is an essential intermediate in the synthesis of ATP, adenine, adenylic acid and vidarabine (Huang et al., 2011; Wang et al., 2010). Thus, developing simple, sensitive, and specific methods for the detection of adenosine is of great value.

In this work, a remarkably sensitive and label-free electrochemical impedance spectroscopy (EIS) aptasensor for the detection of adenosine was demonstrated by co-assembling thiolated aptamer, dithiothreitol (DTT) and 6-mercaptohexanol (MCH) on gold surface. A compact and complete surface could be formed by the coupling of cyclic-(DTT) and linear-(MCH) configuration backfillers, resulting in dramatic improvement in the sensitivity compared to aptasensors with MCH or DTT alone as the backfiller. A linear relationship between the change in the electron transfer resistance (ΔR_{et}) and the logarithmic value of the adenosine concentration in the range from 0.05 pM to 17 pM, and a detection limit of 0.02 pM was obtained.

2. Experimental

2.1. Apparatus and reagents

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed with a CHI 760C electrochemical workstation (Shanghai, China). All electrochemical experiments were carried out with a conventional three-electrode system comprising a gold working electrode (2 mm in diameter), a platinum wire auxiliary electrode, and an Ag/AgCl (saturated KCl) reference electrode. EIS experiments were recorded in 5 mM $Fe(CN)_6^{3-/4-}$ solution containing 0.1 M KCl with the frequency range from 0.1 Hz to 10 kHz.

Ascorbic acid (AA) and thiolated adenosine aptamer was obtained from Sangon Biotechnology Co. Ltd. (Shanghai, China) and the sequence of the aptamer was: 5′–HS–$(CH_2)_6$–ACC TGG GGG AGT ATT GCG GAG GAA GGT CTA AGT AAC TCT-3′. 6-mercapto-1-hexanol (MCH), adenosine, dithiothreitol (DTT), and cytidine were purchased from J&K chemical. Dopamine (DA) was purchased from Sigma-Aldrich. Uridine, uric acid, and Tween-20 were purchased from Alfa Aesar. Other chemicals were of analytical grade and used as received. Doubly distilled water was used throughout the experiments.

2.2. Preparation of the aptasensor

The gold electrode was polished sequentially with 1.0, 0.3 and 0.05 μm alumina slurry, and then washed ultrasonically in water and ethanol, respectively, followed by potential scanning between −0.2 and 1.6 V in 0.5 M H_2SO_4 until a stable cyclic voltammogram was obtained. Afterwards, the gold electrode was washed thoroughly with water and dried under nitrogen stream.

The thiolated aptamer was immobilized on the gold electrode by dropping 6 μL 1.0 μM aptamer solution, with and without 200 μM freshly prepared DTT (in 10 mM Tris–HCl buffer, 1 M NaCl, pH=7.4) for ca. 16 h at 4°C, forming Au/aptamer or Au/aptamer-DTT. After rinsing extensively with Tris–HCl buffer (10 mM pH 7.4) to remove physically absorbed aptamer, the fabricated Au/aptamer or Au/aptamer-DTT was treated with 1 mM MCH for 1 h to block nonspecific sites, then thoroughly rinsed and dried under nitrogen stream. The obtained modified electrodes were denoted as Au/aptamer/MCH, Au/aptamer-DTT, Au/aptamer-DTT/MCH, respectively.

2.3. Measurement procedure

For the detection procedure, 6 μL droplet of various concentrations of adenosine was deposited onto the sensing interface and kept for appropriate time, followed by thoroughly rinsing with washing buffer (20 mM Tris–HCl+0.1 M NaCl+5 mM $MgCl_2$+1.0% (v/v) Tween-20 at pH=7.4) to remove unbound adenosine (Deng et al., 2009). In the control experiments, the method was the same as that of adenosine detection, but adenosine was replaced with the proper concentration of uric acid, ascorbic acid, dopamine, uridine, cytidine and glutathione.

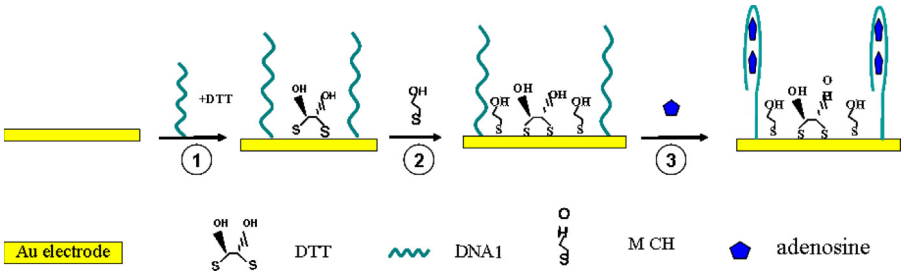
3. Results and discussion

3.1. Fabrication and sensing mechanism of the aptasensor

Scheme 1 shows a schematic representation of the aptasensor with fabrication steps and performance. The aptasensor was fabricated by co-immobilizing thiolated aptamer and dithiothreitol (DTT) on gold electrode (step 1), followed by treatment in 1 mM MCH for 1 h to block the nonspecific sites (step 2). On exposure of the anti-adenosine aptamer-modified electrode to a solution containing $[Fe(CN)_6]^{3-/4-}$ as a signaling transducer, the negatively charged aptamer acts as an electrostatic barrier that repelled $[Fe(CN)_6]^{3-/4-}$ probe and hinders its interfacial electron transfer reaction (Fan et al., 2013). After the aptasensor was incubated with different concentrations of adenosine (step 3), the aptamer was bounded to adenosine and folded, which hindered the electron transfer further and resulted in an increase in the electron transfer resistance.

3.2. Electrochemical characterization of the aptasensor

Electrochemical impedance spectroscopy (EIS) was performed to characterize the fabrication process of the proposed aptasensor. The diameter of the semicircle portion corresponded to the electron transfer resistance (R_{et}) and the increase of the diameter reflected the increase of the interfacial R_{et}. Fig. 1A shows the impedance spectra of the gold electrode at different modification steps which were recorded at the formal potential of $[Fe(CN)_6]^{3-/4-}$. It is observed that the bare gold electrode showed a very small



Scheme 1. Schematic illustration of the aptasensor fabrication and the procedure of adenosine detection.

Download English Version:

https://daneshyari.com/en/article/866436

Download Persian Version:

https://daneshyari.com/article/866436

Daneshyari.com