Sensors and Actuators B 210 (2015) 290–296

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

A sensitive and selective electrochemical biosensor for detection of mercury(II) ions based on nicking endonuclease-assisted signal amplification

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ARTICLE INFO

Article history:
Received 20 September 2014
Received in revised form
16 December 2014
Accepted 28 December 2014
Available online 3 January 2015

Keywords:
Electrochemical DNA biosensor
Signal amplification
Nicking endonuclease
Hg^{2+} detection

ABSTRACT

A novel signal amplification method based on methylene blue (MB) and nicking endonuclease (NEase) was developed for Hg^{2+} detection. Hairpin-shaped probe A (PA) contains a thiol group at the 5′ end and methylene blue (MB) tag at the 3′ end. A NEase recognition sequence was embedded into the loop portion of the PA. PA was firstly immobilized on the Au electrode by a self-assembly approach through Au–S interaction. In the presence of Hg^{2+}, the loop of PA could hybridize with mismatched probe B through the stable T–Hg^{2+}–T linkage, forming the nicking recognition site, and PA was opened. Then NEase discerned the recognition site and nicked PA. After the dissociation of PA fragments, MB-labeled pieces dissociated from the Au electrode surface. The released probe B and Hg^{2+} could be reused to initiate the next cycle and more electroactive indicators dissociated from the electrode surface, resulting in a significant signal decrease. Under optimum conditions, this assay achieved a detection limit of 8.7×10^{-11} M (S/N = 3) and discriminated other metal ions from Hg^{2+} with a high selectivity. Moreover, the biosensor was used for the detection of Hg^{2+} in tap water samples with satisfactory results.

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1. Introduction

Mercury is a bioaccumulative and highly toxic heavy metal that would affect the immune and nervous systems, alter genetic expression, cause serious damage to mammals′ health, and lead to die even at low concentrations [1–5]. To protect public health, the US Environmental Protection Agency (EPA) has set the detection limit down to 10 nM Hg^{2+} in drinking water [6]. Therefore, the sensitive and selective detection of Hg^{2+} in the environment and food industry is in high demand. Traditional detection methods for Hg^{2+} detection, such as atomic absorption/emission spectroscopy (AAS/AES) [7,8], inductively coupled plasma mass spectrometry (ICP-MS) [9], cold vapor atomic absorption spectroscopy [10], and electrochemiluminescence [11,12] are very sensitive and selective. However, some shortcomings restrict their applications, such as expensive and sophisticated instrumentations, complicated design and synthesis, and poor aqueous solubility. Up to now, many electrochemical methods have been developed for routine and effective detection such as quartz crystal microbalance analysis [13], differential pulse stripping analysis [14,15], and chronopotentiometric stripping analysis [16]. However, due to the non-specific interaction between the electrode modifier and Hg^{2+}, few methods can meet the requirement of selectivity at the same time.

To solve the problem of lack of selectivity, thymine–thymine (T–T) mispairs could selectively capture Hg^{2+} in aqueous solution to form T–Hg^{2+}–T base pairs in DNA duplexes that was reported in 2006 by the Miyake and Tanaka group [17,18]. The Hg^{2+} ion-mediated T–Hg^{2+}–T pair was more stable than the Watson–Crick (WC) A–T pair [17]. More importantly, this T–Hg^{2+}–T interaction is highly specific, and the T–T base pair can only be stabilized by Hg^{2+} [18]. By utilizing the strong T–Hg^{2+}–T interaction, many interesting colorimetric [19–21], electrochemical [22,23], and fluorescent sensors [24–27] with excellent performance on selectivity against the interferences of other metal ions have been developed. However, most of these sensors are limited in their practical use due to poor detection capability in the lower nanomolar range. Thus, the development of highly sensitive, selective, and ready-to-use methods for aqueous Hg^{2+} remains a challenge [28].

Many efforts have been made to develop novel electrochemical biosensor with good sensitivity. A signal amplification strategy based on nicking endonuclease (NEase) has attracted more and more attentions because of the simple detection process and

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http://dx.doi.org/10.1016/j.snb.2014.12.114
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high selectivity toward environmental Hg^{2+} detection over other related heavy metal ions [29–32]. The NEase can recognize specific sequence in duplex DNAs and nick only one specific strand of the duplex [33]. In this work, a novel, selective, and sensitive electrochemical method based on NEase-catalyzed signal amplification was developed to detect Hg^{2+}. The signal amplification was achieved by the recycling of Hg^{2+} and probe B. The detection limit of this electrochemical biosensor using this method was 8.7×10^{-11} M. The electrochemical biosensor exhibited high sensitivity. Herein, we evaluate the performance of this novel assay and show that Hg^{2+} can be detected down to the EPA-acceptable concentration (10 nM) in drinkable water.

2. Experimental

2.1. Reagents and solutions

All oligonucleotides were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China), and their base sequences in detail are as follows: Hairpin-shaped capture probe A (PA): 5′-HS-$(CH_2)_6$–CCACGGGATCTTTGCAGCGTGG-Methylene blue-3′; Probe B: 5′-CTGCTTTGATCC-3′. Hairpin-shaped capture probe A was heated at 90 °C for 5 min and naturally cooled down to room temperature before use. The NEase (Nt.AlWI, 10,000 U μL^{-1}) and NE buffer 2 (10 mM Tris–HCl (pH 7.9) containing 50 mM NaCl, 10 mM $MgCl_2$, and 1 mM dithiothreitol (DTT)) were purchased from New England Biolabs Ltd. (Beijing, China). Disodium ethylenediaminetetraacetic acid (EDTA), mercaptohexanol (MCH), tris(2-carboxyethyl) phosphine hydrochloride (TCEP), potassium ferricyanide ($[K_3Fe(CN)_6]$) and potassium ferrocyanide ($[K_4Fe(CN)_6]$), $AgNO_3$, KCl, NaCl, $MgCl_2$, NaH_2PO_4, Na_2HPO_4, $HgCl_2$, $CoCl_2$, $ZnCl_2$, $CaCl_2$, $Pb(NO_3)_2$, $MnCl_2$, $CuSO_4$, $Ni(NO_3)_2$, $Ba(NO_3)_2$, and $CdCl_2$ chemicals were of analytical grade and used without further purification. A stock solution of Hg^{2+} (1 mM) was prepared in ultrapure water with 2 drops of concentrated nitric acid.

Hybridization buffer was a mixture of 100 mM NaCl and 10 mM TE buffer (Tris–hydrochloride containing 1.0 mM EDTA, pH 7.4). DNA immobilization buffer was a mixture of 10 mM TE, 100 mM NaCl, and 10 mM $MgCl_2$ (pH 8.0). $MgCl_2$ was added into the electrolyte to induce the formation of the hairpin structure of PA, as reported previously [34–37]. Washing buffer was a solution containing 100 mM NaCl and 0.1 M phosphate buffer solution (PBS, pH 7.4). All solutions were prepared with ultrapure water of resistivity 18.2 MΩ cm.

2.2. Electrochemical measurement

All electrochemical measurements were recorded with a CHI 660D electrochemical workstation (Shanghai CHI Instruments Co., China). The electrochemical system employed the conventional three-electrode configuration which consisted of a working electrode (an Au disk electrode modified with hairpin capture probe A), a platinum wire as the auxiliary electrode, and an Ag/AgCl (sat.KCl) reference electrode. Cyclic voltammetry (CV) data were carried out at a scan rate of 100 mV s^{-1}. Square wave voltammetric (SWV) measurements were registered in the potential interval of 0.1 to −0.5 V. Electrochemical impedance spectroscopy (EIS) measurements were performed in the presence of 5 mM $[Fe(CN)_6]^{4-/3-}$ solution (pH 7.4) and were recorded with the frequency changed from 0.1 Hz to 10 kHz.

2.3. Formation of the Hg^{2+} biosensor

An Au disk electrode (4 mm diameter) was firstly polished to obtain mirror surface with 0.05 μm alumina powder, followed by sonication in ethanol and ultrapure water for 3 min, respectively. The Au disk electrode was then pretreated electrochemically in 0.5 M H_2SO_4 aqueous solution by potential cycling in the potential range of −0.3 to 1.5 V at a scan rate of 100 mV s^{-1} until the CV characteristic for the clean Au electrode was obtained [38]. Then, the Au electrode was washed thoroughly with copious amounts of ultrapure water and dried under nitrogen gas. Prior to immobilization onto the Au electrode surface, the PA was dissolved in 10 mM pH 8.0 TE buffer containing 100 mM NaCl and 10 mM TCEP, and incubated in the dark for 1 h to reduce disulfide bonds. Then, 25 μL of 1 μM PA solution was dropped onto the cleaned Au electrode surface at 37 °C for 5 h to obtain the PA/Au electrode. Next, this electrode was immersed in 25 μL of 2 mM MCH at 37 °C for 2 h to remove the nonspecific PA adsorption and optimize the orientation of the capture probes to make hybridization easier. The obtained electrode was labeled as the MCH/PA/Au electrode. The MCH/PA/Au electrode was further incubated in 25 μL of hybridization solution containing 0.1 μM probe B and Hg^{2+} with various concentrations at 45 °C for 2 h. After hybridization, the electrode was thoroughly rinsed with washing buffer and dried under a stream of nitrogen gas. The electrode was then incubated in 25 μL solution of NEase (Nt.AlWI, 0.2 U μL^{-1}) in NE buffer 2 at 50 °C (such temperature could facilitate faster hybridization and subsequent dissociation of DNA in a nicking reaction). After a specific period of 2 h, the electrode was rinsed with the washing buffer and used for electrochemical measurements.

Scheme 1 shows the design of the Hg^{2+} biosensor. The PA contains a thiol group at the 5′ terminus, a MB tag at the 3′ terminus, and a T-rich sequence. At the same time, the nicking endonuclease recognition sequence is embedded into the loop portion of the hairpin capture PA. DNA nicking endonucleases are a special family of restriction endonucleases and occur either naturally or via gene engineering. NEase can cut one strand of a double-stranded DNA (ds-DNA) at a specific recognition nucleotide sequence known as a restriction site and produce DNA molecules that are nicked. The nicking endonuclease used in this design is Nt.AlWI (New England Biolabs), which can recognize the specific sequence 5′-GGATC-3′/3′CCTAG-5′ in ds-DNA and catalyze a single strand break 4 bases beyond the 3′ end of the recognition sequence 5′-GGATC-3′. The PA is covalently attached to the Au electrode through Au-S covalent bond. Then the PA/Au electrode is blocked with MCH. The coverage of MCH can effectively prevent the non-specific adsorption of hairpin capture PA on the electrode surface and displace the weaker adsorption contacts between the hairpin capture PA and the Au electrode.

As shown in Fig. 1, the Nt.AlWI cannot cut the hairpin capture PA in the absence of Hg^{2+}. So the formation of a hairpin-like conformation brings the MB group close to the surface of Au electrode and results in a high redox signal being observed (curve a, in Fig. 1). In the presence of Hg^{2+}, hairpin PA hybridizes with probe B to form stand-up duplex DNA strands with T–Hg^{2+}–T base pairs and provides recognition sites for Nt.AlWI and the MB tag is away from the electrode surface. While, the current signal decreased slightly (curve b, in Fig. 1). By introducing NEase assisted amplification strategy and adding Nt.AlWI into the reaction solution, Nt.AlWI can bind to and nick the recognition site. After nicking reaction, PA was cleaved into two pieces, HS-$(CH_2)_6$-CCACGGGATCTTTG and CAGCGTGG-MB. At the same time, the complex becomes unstable and MB tags dissociate from the Au electrode at appropriate temperature. The probe B and Hg^{2+} can then hybridize to another PA and initiate the second cycle of cleavage. Eventually, each probe B and Hg^{2+} can go through many cycles, resulting in the cleavage of many PA and a much lower current signal of MB, as shown in curve c of Fig. 1. Therefore, this biosensor has an ultrahigh sensitivity for the detection of Hg^{2+}. Furthermore, the nicking reaction is highly specific because it requires a specific recognition site which

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