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A regenerative ratiometric electrochemical biosensor for selective detecting Hg^{2+} based on Y-shaped/hairpin DNA transformation

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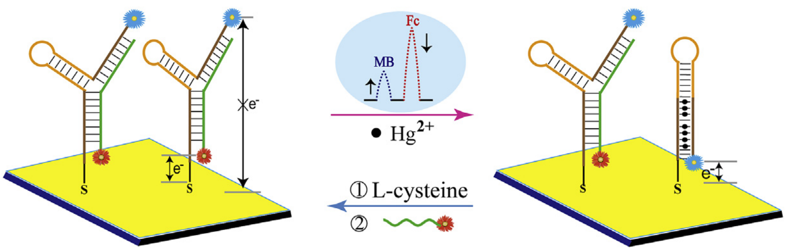
HIGHLIGHTS

- The dual-signaling ratiometric method improved the precision and sensitivity.
- The fabrication of biosensor is less time-consuming and simpler.
- The biosensor shows excellent selectivity and repeatability.
- This biosensor can be easily regenerated by using L-cysteine.

GRAPHICAL ABSTRACT



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ABSTRACT

Inspired by dual-signaling ratiometric mechanism which could reduce the influence of the environmental change, a novel, convenient, and reliable method for the detection of mercury ions (Hg^{2+}) based on Y-shaped DNA (Y-DNA) was developed. Firstly, the Y-DNA was formed via the simple annealing way of using two different redox probes simultaneously, omitting the multiple operation steps on the electrode. The Y-DNA was immobilized on the gold electrode surface and then an obvious ferrocene (Fc) signal and a weak methylene blue (MB) signal were observed. Upon addition of Hg^{2+}, the Y-DNA structure was transformed to hairpin structure based on the formation of T-Hg^{2+}-T complex. During the transformation, the redox MB gets close to and the redox Fc gets far away from the electrode surface, respectively. This special design allows a reliable Hg^{2+} detection with a detection range from 1 nM to 5 μM and a low detection limit down to 0.094 nM. Furthermore, this biosensor exhibits good selectivity and repeatability, and can be easily regenerated by using L-cysteine. This study offers a simple and effective method for designing ratiometric biosensors for detecting other ions and biomolecules.

1. Introduction

Mercury ion (Hg^{2+}) has been widely recognized as one of the most toxic heavy metals due to its accumulative and toxic properties in the environment and mercury-mediated toxicity also has permanent damage to the human central nervous system and other organs [1–4]. To reduce the damage of Hg^{2+} contamination, the US Environmental Protection Agency (EPA) has set the detection limit of Hg^{2+} down to 10 nM in drinking water [5]. Therefore, the exploration of new approaches for detecting Hg^{2+} in disease prevention and environmental protection has attracted intense attention. To date, various analytical techniques for sensing Hg^{2+} have been developed, such as atomic absorption/emission

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spectroscopy (AAS/AES) [6,7], inductively coupled plasma-mass spectrometry (ICP-MS) [8], inductively coupled plasma-atomic emission spectrometry (ICP-AES) [9], cold vapor atomic absorption spectroscopy [10], and electrochemiluminescence [11]. Although these methods are very sensitive and selective, creating new strategies with high sensitivity, selectivity and low background interference, and requiring inexpensive instruments as well as simple operation processes still remain a challenge.

Electrochemical biosensor has received much attention for its advantages of high sensitivity, good selectivity, low cost, and simplicity for target detection [12]. It has been widely applied in the detection of meaningful analytes, including specific proteins [13], nucleic acids [14], and small-molecule targets [15]. It has been proved that the thymine–thymine (T–T) mispairs could selectively capture Hg^{2+} to form T-Hg^{2+}-T base pairs, and Hg^{2+}-mediated T-Hg^{2+}-T pair is more stable than the Watson–Crick (WC) A-T pair [16]. By utilizing the strong T-Hg^{2+}-T interaction, many electrochemical biosensors with excellent performance on selectivity have been developed.

Based on the decrease or increase of the signal, the electrochemical biosensors are divided into “signal-off” and “signal-on” subgroups, respectively [17]. In a “signal-off” biosensor, target binding restricts collisions between the redox species and the electrode surface thereby reducing the response signal. This signal-off mechanism suffers the difficulty in adopting signal amplification strategies because the biosensor can never suppress more than 100% of the original signal [18]. In a “signal-on” biosensor, it not only pushes the detected background signal toward zero in the absence of the target, but also employs a lot of signal amplification methods to achieve enormous signal gain without limit in theory [19]. As false positive or negative errors may occur during the process of target detection due to some test-environmental changes, the single-signaling (“signal-off” or “signal-on”) biosensors are subjected to some limitations for practical applications [20]. There is no doubt that the use of dual-signaling response as the signal readout enables the detection method to be relatively reliable and convincing, improving the accuracy of target detection in the complicated environments. Besides the recent advances, almost all of the mechanisms of biosensors were based on “signal-off” or “signal-on” alone. Now a growing number of studies focus on the ratiometric electrochemical sensors due to the advantages of the dual-signaling strategies [20–24], such as diversification of data analysis, good selectivity, high sensitivity, and good repeatability.

Nucleic acids have become a common material in developing novel electrochemical biosensors for their high selectivity in molecular recognition [25]. The most widely used structures of nucleic acids in electrochemical sensing platforms are single-strand and double helical conformations. Furthermore, the abundant secondary structures of nucleic acids such as hairpin [26], G-quadruplex [27], hand-in-hand [28], cruciform [29], and pseudoknot [30] have been widely applied in sensing platforms because of the roles they played in transcription regulatory mechanisms. Among various secondary structures, Y-DNA is composed of three single-strand DNAs (ssDNAs), each of which is partially complementary to the other two ssDNAs and it could be achieved by stepwise as well as all-in-one (one-pot) synthesis methods [31]. Y-DNA can grow to supramolecular nanoarchitectures such as dendrimers with multiple functions as its branch DNA can be designed to contain different functional moieties by designing different branch sequences [32]. The dendrimers are potentially conducive to widespread in vivo applications such as gene and drug delivery, tissue repair, and imaging [33].

Moreover, some efforts have gone into developing the Y-DNA-based biosensors due to the advantages of conformational stability and structural flexibility of Y-DNA. For example, Chen and coworkers reported a Y-shaped probe electrochemical biosensor [34], which is simple, sensitive, and stable, and has excellent discrimination ability for single-base mismatch. His group also provided a novel and simple approach for the genotyping/detection of single-nucleotide polymorphisms (SNPs) in trace salivary DNA of oral cancer based on a Y-shaped probe [35].

In an effort to combine the advantages of dual-signaling strategy and Y-DNA, herein, we demonstrated a novel and simple ratiometric biosensor for Hg^{2+} detection via target-induced transformation between Y-DNA and hairpin DNA. This biosensor is easy to be fabricated without time-consuming operation on the electrode. It also shows high selectivity and good repeatability, and can be easily regenerated by using L-cysteine. Moreover, this study offers a simple and effective method for designing ratiometric biosensors for detecting other ions and biomolecules.

2. Materials and methods

2.1. Chemicals and reagents

L-cysteine (L-cys) and HPLC-purified oligonucleotides were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China), and their base sequences are as follows: Y1 probe: 5′–SH–$(CH_2)_6$-CTG TTT TCT TTC GGA CGA CCC CCC TCG TCC GTT TGT TTT CAG-MB^+-3′; Y2 probe: 5′-AAA ACA AAA AAG AAA AGC C-Fc-3′. Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), 6-mercaptohexanol (MCH), and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The buffer solutions are as follows: A-buffer solution was 10 mM TE-10 mM TCEP-1 M NaCl solution (pH 8.0); B-buffer solution was 10 mM TE-1.0 M NaCl solution (pH 8.0); electrochemical measurement buffer solution was 10 mM phosphate buffer (pH 7.4) containing 500 mM NaCl and 2.7 mM KCl; washing buffer solution was 10 mM phosphate buffer containing 0.1 M NaCl (pH 7.4). All solutions were prepared with ultrapure water (specific resistance of 18.2 MΩ cm). $Hg(NO_3)_2$, $Ca(NO_3)_2{\cdot}4H_2O$, $Fe(NO_3)_3$, $ZnCl_2$, $Pb(NO_3)_2$, $Co(NO_3)_2$, $Al(NO_3)_3{\cdot}9H_2O$, $Cd(NO_3)_2{\cdot}4H_2O$, $Cr(NO_3)_3{\cdot}9H_2O$, $Cu(NO_3)_2$, $Ba(NO_3)_2$, $Mg(NO_3)_2{\cdot}6H_2O$, $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]$, Na_2HPO_4, and NaH_2PO_4 chemicals were of analytical reagent grade and were used without further purification.

2.2. Apparatus

All electrochemical measurements were monitored on an electrochemical workstation (CHI 660D, CH Instruments, Chenhua Corp, Shanghai, China) at room temperature. The experiment was performed with a typical three electrode system, in which the working electrode was the functionalized gold electrode, the reference electrode was an Ag/AgCl electrode with saturated KCl, and the counter electrode was a platinum electrode.

Ferricyanide solution (5.0 mM $[Fe(CN)_6]^{3-/4-}$) used as the supporting electrolyte medium was obtained by dissolving potassium ferrocyanide and potassium ferricyanide (1:1) with phosphate buffer (0.1 M, pH 7.4). Cyclic voltammetry (CV) was performed within the potential range from −0.3 to 0.7 V at 0.1 V s^{-1}. Electrochemical impedance spectra (EIS) were performed in a frequency range from 0.1 Hz to 10 kHz with 5 mV as the amplitude.

2.3. Electrode pretreatment

The gold electrode (4.0 mm in diameter) was polished carefully with alumina powder (1.0, 0.3, and 0.05 μm in diameter) on a micro cloth pad in sequence until a mirror surface was formed, followed by ultrasonic cleaning with ultrapure water, ethanol, and ultrapure

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