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A ratiometric electrochemical biosensor for sensitive detection of Hg^{2+} based on thymine–Hg^{2+}–thymine structure

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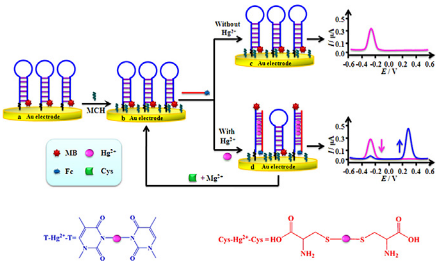
HIGHLIGHTS

- A ratiometric electrochemical biosensor based on thymine–Hg^{2+}––thymine structure.
- Hg^{2+} was sensitively detected by the developed electrochemical biosensor.
- The ratiometric electrochemical biosensor shows excellent analytical performance.

GRAPHICAL ABSTRACT



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ABSTRACT

In this paper, a simple, selective and reusable electrochemical biosensor for the sensitive detection of mercury ions (Hg^{2+}) has been developed based on thymine (T)-rich stem–loop (hairpin) DNA probe and a dual-signaling electrochemical ratiometric strategy. The assay strategy includes both “signal-on” and “signal-off” elements. The thiolated methylene blue (MB)-modified T-rich hairpin DNA capture probe (MB-P) firstly self-assembled on the gold electrode surface via Au–S bond. In the presence of Hg^{2+}, the ferrocene (Fc)-labeled T-rich DNA probe (Fc-P) hybridized with MB-P via the Hg^{2+}-mediated coordination of T–Hg^{2+}–T base pairs. As a result, the hairpin MB-P was opened, the MB tags were away from the gold electrode surface and the Fc tags closed to the gold electrode surface. These conformation changes led to the decrease of the oxidation peak current of MB (I_{MB}), accompanied with the increase of that of Fc (I_{Fc}). The logarithmic value of I_{Fc}/I_{MB} is linear with the logarithm of Hg^{2+} concentration in the range from 0.5 nM to 5000 nM, and the detection limit of 0.08 nM is much lower than 10 nM (the US Environmental Protection Agency (EPA) limit of Hg^{2+} in drinking water). What is more, the developed DNA-based electrochemical biosensor could be regenerated by adding cysteine and Mg^{2+}. This strategy provides a simple and rapid approach for the detection of Hg^{2+}, and has promising application in the detection of Hg^{2+} in real environmental samples.

1. Introduction

As we all know, Hg^{2+} is the most stable modality of inorganic mercury [1]. It is a highly toxic heavy metal ion due to its accumulative character and toxic properties in the environment and biota [2,3]. Long-term exposure to high level of mercury ion can lead to a lot of severe health problems, such as brain damage, kidney failure and other chronic diseases [4]. Therefore, the sensitive, rapid, selective and cost-effective analysis of Hg^{2+} in the environment and food industry is very important. Till now, many kinds of analytical techniques for the detection of Hg^{2+} were reported, such as cold-vapor atomic fluorescence spectroscopy (CV-AFS) [5–7], cold-vapor atomic absorption spectrometry (CV-AAS) [5,8,9], inductively coupled plasma-mass spectrometry (ICP-MS) [10–12], inductively coupled plasma-atomic emission

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spectrometry (ICP-AES) [13,14]. However, these methods require either expensive instrument or complicated sample preparation processes.

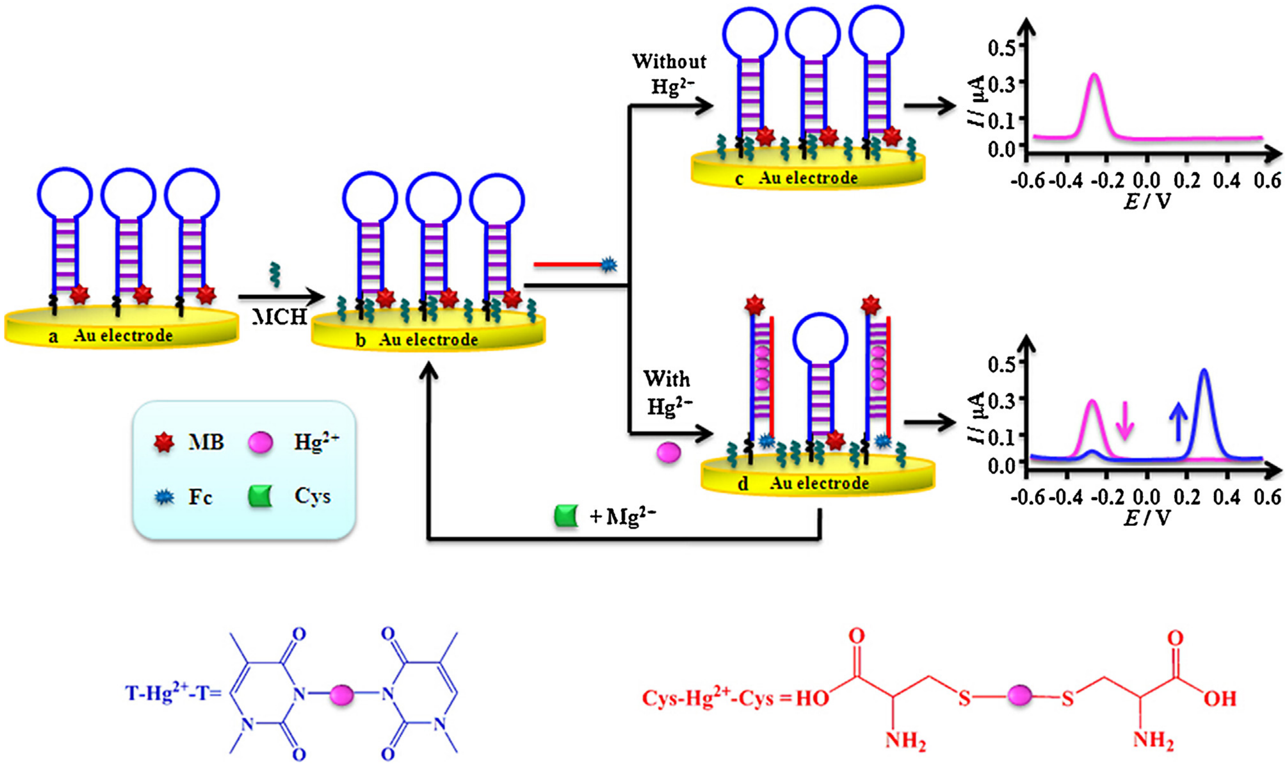
Electrochemical method is a general and classic technique in analytical chemistry because of its advantages of simplicity, high sensitivity, good selectivity and low cost [15–17]. It has been proved that Hg^{2+} ion can selectively bind between two DNA thymine (T) bases and promotes these T–T mismatches to form stable T–Hg^{2+}–T base pairs [18,19], and the binding constant (K_b) of T–Hg^{2+}–T is about $4.14 \times 10^6 \, L \, mol^{-1}$, higher than that of A (adenine)–T [20,21]. Based on this principle, many DNA-based electrochemical biosensors have been developed for the detection of Hg^{2+}. These electrochemical biosensors could be divided into “signal off” and “signal on” subgroups. In the past years, the DNA-based electrochemical biosensors with “signal off” architecture have gained substantial development. Yu and co-workers [22,23] reported the electrochemical Hg^{2+} biosensors based on the decrease of the electrochemical signal resulting from target-induced conformational changes. However, such “signal-off” sensors suffer from the limited signaling capacity, in which only a maximum of 100% signal suppression can be attained under any experimental conditions [24]. To circumvent this limitation, several “signal on” electrochemical Hg^{2+} biosensors have been reported in recent years. Han et al. [25] and Zhuang et al. [26] developed the “signal on” electrochemical Hg^{2+} biosensors based on the increase of the electrochemical signal resulting from the structural switching of Fc-tagged aptamers immobilized on the electrode surface in the presence of the target analyte. Nevertheless, these electrochemical biosensors were based on the single signal response mechanism. It is no doubt that combination of “signal off” and “signal on” strategies in one DNA-based electrochemical biosensor would have obvious advantages, such as high sensitivity, good selectivity and diversification of data analysis. Up to now, only a few works were reported for the detection of ATP [27], sequence of H5N1 virus [28], protein [29], approximate target mismatch location [30] based on this strategy. To the best of our knowledge, there are no works to address the Hg^{2+} detection based on the combination of “signal off” and “signal on” strategies.

On the other hand, ratiometric detection has been applied in fluorescence and electrochemiluminescence analysis of biomolecules extensively due to its good characteristics (such as self-calibration, low detection limit) [31–33]. In this work, a simple, selective and reusable electrochemical biosensor for the sensitive detection of mercury ions (Hg^{2+}) has been developed for the first time based on T-rich stem–loop (hairpin) DNA probe and a dual-signaling electrochemical ratiometric strategy (Scheme 1). The thiolated methylene blue (MB)-modified T-rich DNA capture probe (MB-P) self-assembled onto the gold electrode surface via Au–S bond. In the presence of Hg^{2+}, the ferrocene (Fc)-labeled T-rich DNA probe (Fc-P) hybridized with MB-P via the Hg^{2+}-mediated coordination of T–Hg^{2+}–T base pairs. As a result, the hairpin DNA probes (MB-P) were opened, the MB tags were away from the gold electrode surface and the Fc tags close to the gold electrode surface. These conformation changes led to a decrease of the oxidation peak current of MB (I_{MB}) and an increase of that of Fc (I_{Fc}). Based on the logarithmic value of I_{Fc}/I_{MB}, Hg^{2+} was detected sensitively and selectively. What is more, the developed DNA-based electrochemical biosensor could be regenerated by adding cysteine and Mg^{2+} based on the strong binding effect between cysteine and Hg^{2+} [34–36].

2. Experimental

2.1. Chemicals

The thiolated MB-modified T-rich hairpin DNA capture probe and the Fc-labeled T-rich DNA probe were designed and synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China). The base sequences of MB-P and Fc-P are 5′-SH-$(CH_2)_6$-



Scheme 1. Schematic illustration of the ratiometric electrochemical biosensor for Hg^{2+} detection.