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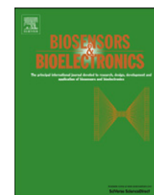

Short communication

Target-induced structure-switching DNA hairpins for sensitive electrochemical monitoring of mercury (II)

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A simple, sensitive and reusable electrochemical sensor was designed for determination of mercury (II) (Hg^{2+}) by coupling target-induced conformational switch of DNA hairpins with thymine–Hg^{2+}–thymine (T–Hg^{2+}–T) coordination chemistry. The hairpin probe consisted of a stem of 6 base pairs enclosing a 14 nucleotide (nt) loop and an additional 12 nt sticky end at the 3′ end. Each hairpin was labeled with ferrocene (Fc) redox tag in the middle of the loop, which was immobilized on the electrode *via* self-assembly of the terminal thiol moiety at the 5′ end. In the presence of target analyte, Hg^{2+}-mediated base pairs induced the conformational change from the sticky end to open the hairpins, resulting in the ferrocene tags close to the electrode for the increasing redox current. The strong coordination reaction of T–Hg^{2+}–T resulted in a good repeatability and intermediate precision down to 10%. The dynamic concentration range spanned from 5.0 nM to 1.0 μM Hg^{2+} with a detection limit of 2.5 nM at the $3s_{\text{blank}}$ level. The strategy afforded exquisite selectivity for Hg^{2+} against other environmentally related metal ions. Inspiringly, the developed sensor could be reused by introduction of iodide (I^-).

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1. Introduction

New analytical techniques require the ability to detect trace amounts of a wide variety of analytes with both high selectivity and sensitivity (Swiderska and Reymond, 2009). Design of metal ion sensors has been a focus of many research endeavors, since it can provide detection and quantification of metal ions in many fields of application including environmental bioinorganic chemistry, clinical toxicology, waste management, and bioremediation of radionuclides and metal ions (Liu and Lu, 2004). Routine protocols for the design of sensors are generally performed using fluorescence, surface plasmon resonance, electrochemistry, colorimetric sensors and other analytical techniques (Teresa Albelda et al., 2012; Zambelli et al., 2012; Schaferling, 2012; Gupta et al., 2011). Despite many advances in this field, there is still a quest for new schemes and strategies for improvement of the sensitivity, simplicity and selectivity of metal ion sensors.

Electrochemical sensors have attracted considered attention over the past few years due to their intrinsic advantages, such as good portability, low cost, simple instrumentation, and high sensitivity (Tang et al., 2010; Lost and Crespilho, 2012). Great efforts have been made worldwide to develop and improve the electrochemical sensors for probing trace of metal ions (e.g. Hg^{2+}) with the aim of manufacturing portable and affordable analytic device (Wu et al., 2010; Liu et al., 2009a; Guo et al., 2011). The Zhou group reported an electrochemiluminescent assay for highly sensitive detection of mercury (II) based isothermal rolling circular amplification on the thymine–Hg^{2+}–thymine (T–Hg^{2+}–T) biomimetic structure (Zhou et al., 2012). Guo et al. designed an amperometric "turn-on" sensor for mercury (II) based on the T–Hg^{2+}–T coordination chemistry, and the controlled assembly of carbon nanotubes on the electrode (Guo et al., 2011). The results demonstrated that T–Hg^{2+}–T coordination chemistry and the Hg^{2+}-stabilized hybridization of oligonucleotides with T–T mismatches can be considered as a selective and versatile scheme for Hg^{2+} detection. A fundamental mechanism for such sensors is the alternation of distance of the labeled redox tags (e.g. ferrocene and methylene blue) from the electrode by target-induced conformational change, strand dissociation, or surface hybridization. Unfortunately, most methods reported were based on the "signal-off" assay strategy (Wu et al., 2010; Liu et al., 2009a), which must demand a high background signal for the newly as-prepared sensors. Certainly, a few electrochemical sensors were constructed based on the "signal-on" assay mode. However, these methods usually involved in some nanomaterials (Guo et al., 2011), bioactive enzyme (Zhang et al., 2011), or auxiliary oligonucleotide strands (Li et al., 2010).

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Enzyme labels are usually employed for the amplification of measurable electrochemical signal (Zhang et al., 2011). Since for sterical reasons, there is, usually a 1:1 ratio of enzyme and DNA molecule, the obtainable signal amplification is always limited. There are ongoing efforts to develop new labels based on biofunctionalized nanoparticles (Guo et al., 2011). Unfavorably, the bioactivity of the conjugated biomolecules onto the nanoparticles is weakened to some extent. The reason might be the fact that the biomolecular conformation might be changed and/or the catalytic center of the enzyme was hindered due to the nanostructures (Tang et al., 2011). To tackle this issue, our motivation in this study is to design an enhanced "signal-on" electrochemical sensor for Hg^{2+} based on T–Hg^{2+}–T-mediated conformational switch on a DNA hairpins-modified electrode without the participation of other auxiliary strands, enzyme and nanomaterials.

Herein we describe a simple, sensitive and reusable electrochemical sensor for Hg^{2+} by coupling target-induced conformational switch of DNA hairpins with T–Hg^{2+}–T coordination chemistry. The DNA hairpins with a stem of 6-mer base pairs enclosing a 14 nt loop and an additional 12 nt sticky end at the 3′ end, are labeled with ferrocene molecular tags in the middle of the loop. The DNA hairpins are immobilized on the electrode through the Au–S bond. The assay principle is based on T–Hg^{2+}–T-mediated conformational change of the immobilized DNA hairpins, resulting in the labeled ferrocene far away or close to the electrode for the production of the electrochemical signal.

2. Experimental

2.1. Chemicals

Ferrocenecarboxylic acid, $HgCl_2$, $NaClO_4$, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and *N*-hydroxysulfosuccinimide (NHS) were purchased from Alfa Aesar®. Mecatohexanol (MCH) was purchased from Tokyo chemical industry Co., Ltd. (Japan). All the other chemicals were of analytical grade, and used without further purification. Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all runs. The synthesized oligonucleotides were purchased from Sangon Biotech. Co., Ltd. (Shanghai, China). The sequence is shown as follows (*Note*: In the hairpin sequence, loop is italicized while sticky end is underlined):

5′-SH-$(CH_2)_6$-*AAACGC*AATGAAT-3′-T($(CH_2)_6$-NH_2)-5′-AGTAAA-*GCGTTT*CGCTTTCGCTTT-3′.

2.2. Labeling of ferrocene to NH_2-modified DNA hairpins

The ferrocenecarboxylic acid was conjugated to the DNA hairpins with the NH_2-moiety using the succinimide coupling (EDC–NHS) method (Wu et al., 2010). Briefly, 1 mg of ferrocenecarboxylic acid was initially added into 1 mL of 0.3 M Tris–HCl buffer (pH 7.4) containing 0.1 M EDC and 0.1 M NHS, and then the resulting mixture was incubated for 60 min at room temperature (RT). Following that, 100 μL of the oligonucleotides (5 μM) was injected, and incubated for 4 h at RT with gentle stirring. Removal of unconjugated ferrocenecarboxylic acid was carried out by ultrafiltration for 12–15 times until the peak corresponding to ferrocene in the elution disappeared.

2.3. Fabrication and measurement procedure of electrochemical sensor

The electrochemical sensor was constructed *via* the self-assembly of the –SH group at the 5′ of DNA hairpins on a gold electrode (2 mm in diameter). Initially, 5 μL of 0.3 M Tris–HCl buffer (pH 7.4, 1 M NaCl) solution containing 5 μM ferrocene-tagged hairpins was dropped onto a cleaned gold electrode (held upside-down), and then kept in a water-saturated atmosphere at RT for 2 h. Afterwards, the electrode was rinsed thoroughly with 0.3 M Tris–HCl buffer (pH 7.4) containing 0.5 M NaCl and 0.1 M $NaClO_4$ 3 times. The resulting electrode was immersed into 2 mM mercaptohexanol for 60 min. Finally, the obtained electrochemical DNA sensor was stored in 0.3 M Tris–HCl buffer (pH 7.4) containing 1.0 M $NaClO_4$ at 4 °C for further use.

All electrochemical measurements were carried out with a CHI 630D Electrochemical Workstation (Shanghai, China) by using a modified gold electrode as the working electrode, a platinum wire as auxiliary electrode, and a saturated calomel electrode (SCE) as reference electrode. Before measurement, various concentrations of Hg^{2+} standards or samples were prepared by using 10 mM Tris–HCl buffer (pH 7.4) solution as sample matrix. To detect Hg^{2+} ions, the as-prepared sensor was initially immersed in 100 μL of sample solution, and incubated for 40 min at RT. After washing with a 10 mM Tris–HCl buffer (pH 7.4) solution containing 0.5 M NaCl and 0.1 M $NaClO_4$, square wave voltammetric (SWV) measurement from 150 mV to 500 mV (vs. SCE) (amplitude: 25 mV; frequency: 15 Hz; increase *E*: 4 mV) was carried out in 0.3 M Tris–HCl buffer (pH 7.4) containing 1.0 M $NaClO_4$. The SWV peak current was collected and registered as the sensor signal. After each run, the sensor was regenerated by immersing it into pH 7.4 Tris–HCl buffer containing 1.0 M NaCl and 1.0 M I^- for 10 min and washed with deionized water, prior to start the next run cycle. All incubations and measurements were conducted at room temperature. Analyses are always made in triplicate.

3. Results and discussion

3.1. Assay principle and characteristics of target-induced structure-switching DNA

In this work, the electrochemical signal was achieved by Hg^{2+}-induced conformational change of the designed DNA hairpins on the electrode. Hence, we designed a 38-base oligonucleotide sequence (as a model) for detection of Hg^{2+} ions. Prior to experiment, we used RNA folding prediction software to simulate the designed DNA structure for the formation of DNA hairpin (software name: RNA structure, version 5.3, University of Rochester Medical Center, Mathews Lab, http://rna.urmc.rochester.edu/software.html). The simulation results revealed that the proposed oligonucleotide sequence could form a DNA hairpin structure. To realize our design, the electrochemical properties of DNA hairpin-based sensor were first investigated by using cyclic voltammetry. As shown in Fig. 1A, the DNA hairpins contained a stem of 6 base pairs enclosing a 14 nt loop and an additional 12 nt sticky end. To acquire an electrochemical signal, the ferrocene redox tag was labeled in the middle of the loop through a 6-carbon-atom spacer. In the absence of Hg^{2+}, the DNA hairpins were in the closed form, which stood upright on the electrode due to the immobilized mercaptohexanol and the rigid structure of stem-loop DNA. The labeled ferrocene tags were far away from the electrode, resulting in a weak electrochemical signal (curve '*b*' in Fig. 1B). When Hg^{2+} ions were present in the incubation solution, however, it triggered the T bases at the 3′ end to prompt the T–Hg^{2+}–T formation. As a result of T–Hg^{2+}–T, A–T and G–C joint action, the 9 bases (TTTCGCTTT) at the sticky end readily paired with the neighbor AAAGCGTTT bases, resulting in the conformational change of the original hairpin, thus formed another new DNA hairpin. The new hairpin had a stem of 9 base pairs enclosing a 3 nt loop and an additional 17 nt sticky end. In this case, the labeled ferrocene tags were easily close to the electrode, thus producing a strong

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