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Oligonucleotide probes applied for sensitive enzyme-amplified electrochemical assay of mercury(II) ions

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ABSTRACT

We developed a novel electrochemical sensor for Hg^{2+} detection using two mercury-specific oligonucleotide probes and streptavidin-horseradish peroxidase (HRP) enzymatic signal amplification. The two mercury-specific oligonucleotide probes comprised a thiolated capture probe and a biotinated signal probe. The thiolated capture probe was immobilized on a gold electrode. In the presence of Hg^{2+}, the thymine–Hg^{2+}–thymine (T–Hg^{2+}–T) interaction between the mismatched T–T base pairs directed the biotinated signal probe hybridizing to the capture probe and yielded a biotin-functioned electrode surface. HRP was then immobilized on the biotin-modified substrate via biotin–streptavidin interaction. The immobilized HRP catalyzed the oxidation of hydroquinone (H_2Q) to benzoquinone (BQ) by hydrogen peroxide (H_2O_2) and the generated BQ was further electrochemically reduced at the modified gold electrode, producing a readout signal for quantitative detection of Hg^{2+}. The results showed that the enzyme-amplified electrochemical sensor system was highly sensitive to Hg^{2+} in the concentration of 0.5 nM to 1 μM with a detection limit of 0.3 nM, and it also demonstrated excellent selectivity against other interferential metal ions.

1. Introduction

Mercury(II) ions (Hg^{2+}) are highly toxic environmental pollutants and harmful to human health. Excessive accumulation of Hg^{2+} in the human body has adverse effects on the nervous and immune systems as well as other organs, causing a number of diseases (Korbas et al., 2008; Morel et al., 1998). Accurate quantitative analysis of Hg^{2+} is of critical importance in environmental monitoring and clinical toxicology. Previously reported Hg^{2+} detection methods, including optical assays using fluorophores, chromophores or conjugated polymers (Huang and Chang, 2006; Kim and Bunz, 2006; Liu et al., 2007; Nolan and Lippard, 2003, 2007; Prodi et al., 2000; Yang et al., 2005; Zhu et al., 2005, 2006), and electrochemical detection by anodic stripping voltammetry (Nolan and Kounaves, 1999), have some limitations of poor selectivity, low sensitivity or the need of using organic media. The US environmental protection agency (EPA) defined a tolerable limit of Hg^{2+} in drinkable water as 10 nM, much lower than the detection limit of most available assays. Therefore, it is still desirable to develop special and highly sensitive methods for Hg^{2+} detection.

As trace level of Hg^{2+} often coexist with much excesses of other metal ions, selectivity is a critical issue for Hg^{2+} detection. To achieve highly selective assay of Hg^{2+}, the coordination chemistry involving specific thymine–Hg^{2+}–thymine (T–Hg^{2+}–T) interaction has recently been applied to design Hg^{2+} sensors. These T–Hg^{2+}–T based Hg^{2+} detection systems include fluorescent biosensors (Chiang et al., 2008; Guo et al., 2005, 2009; Wang and Liu, 2008), gold nanoparticle-based colorimetric and electrochemical amplifying sensors (Kong et al., 2009; Lee et al., 2007; Li et al., 2008; Liu et al., 2008a; Wang et al., 2010; Xue et al., 2008), electrochemiluminescent biosensor (Zhu et al., 2010), anodic stripping voltammetry (Zhu et al., 2009), amperometric biosensor based on electrically contacted enzyme (Mor-Piperberg et al., 2010) and electrochemical DNA structure switching sensors (Liu et al., 2009; Wu et al., 2010). All of these sensors demonstrate excellent selectivity for Hg^{2+} detection owing to the high specificity of T–Hg^{2+}–T interaction. In particular, the electrochemical Hg^{2+} sensors show significant advantage of high sensitivity over other detection systems. With signal amplification of nanoparticle-labeled reporters, Hg^{2+} concentration as low as 1 nM has been reported to be sensitively detected by these T–Hg^{2+}–T based electrochemical methods (Kong et al., 2009; Zhu et al., 2009).

Enzyme-amplified assay is a representative strategy of signal amplification for highly sensitive detection in modern bioanalytic science (Bakker, 2004). For example, immobilized enzyme architecture directed by DNA hybridization has been well established as a highly sensitive platform for DNA detection (Hwang et al., 2005; Liu et al., 2008b; Lucarelli et al., 2006; Mao et al., 2008;

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Table 1
Capture and signal probe sequences.

Name	Sequence
Capture probe S1	HS $(CH_2)_6$-5′-AAAGTCGCTTCGCTC-3′
Signal probe S2	3′-CAGCGTTGCGAGAAA-5′-Biotin

Miranda-Castro et al., 2007; Patolsky et al., 2003; Zhang et al., 2008, 2010a,b). Through converting the DNA hybridization events into the highly sensitive enzymatic signals, target DNA concentration of very low level has been reported to be successfully detected. Inspired by the excellent performance of these enzyme-amplified DNA biosensors, we developed an enzyme-amplified electrochemical Hg^{2+} sensor in this study. Similar to the enzyme-amplified DNA biosensors, we exploited the T–Hg^{2+}–T coordination chemistry dominated hybridization event between the biotinated DNA signal probe and the surface-bound DNA capture probe and biotin–streptavidin interaction to capture HRP enzyme on the sensor interface. The immobilized HRP enzyme catalyzed oxidation of hydroquinone (H_2Q) to benzoquinone (BQ) by hydrogen peroxide (H_2O_2) and the further electrochemical reduction of BQ at the sensor interface produced a readout signal for quantitative analysis of Hg^{2+}. The results demonstrated that the developed enzyme-amplified electrochemical Hg^{2+} sensor was highly sensitive and selective for Hg^{2+} detection.

2. Experimental

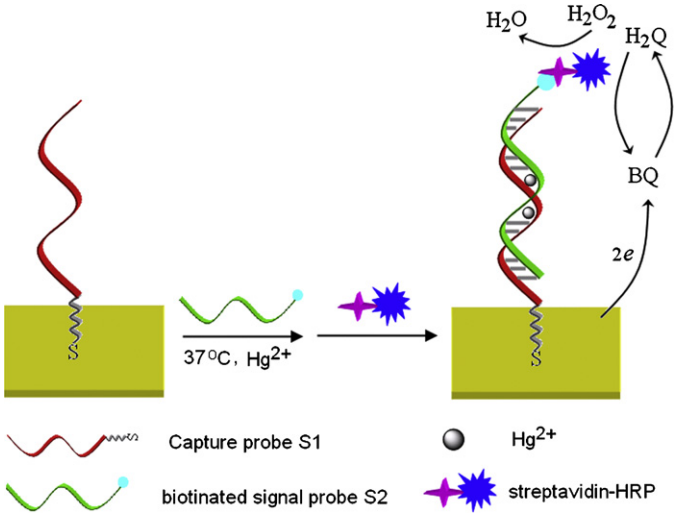
2.1. Chemicals and materials

HPLC-purified oligonucleotides were obtained from Sangon Biotechnology Co. Ltd (Shanghai, China). The sequences are listed in Table 1. Bovine serum albumin (BSA), Tween-20, 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), streptavidin-labeled horseradish peroxidase (HRP), 6-mercaptohexanol (MCH) and 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) were purchased from Dingguo Biologic Products (Beijing, China). Hydroquinone (H_2Q), H_2O_2 and the used metal salts ($Hg(NO_3)_2$, $Zn(NO_3)_2$, $MgCl_2$, $Ca(CH_3COO)_2$, $Pb(NO_3)_2$, $CdCl_2$, $Mn(CH_3COO)_2$, $Cu(NO_3)_2$, $NiCl_2$, $FeCl_3$) were supplied by sinopharm group chemical reagent Co. Ltd (Shanghai, China). Hg^{2+} stock solution (0.1 M) was prepared by dissolving $Hg(NO_3)_2$ with 1 M HNO_3 and it was diluted to desired concentration with 10 mM HEPES buffer (pH 7.4) containing 50 mM $NaNO_3$. The HEPES buffer was also used as the hybridization, washing and electrochemical test solution. All regents were used as received and all solutions were prepared with 18.2 MΩ cm ultrapure water.

2.2. Gold electrode pretreatment and oligonucleotide immobilization

Prior to modification, the gold working electrodes (of 2 mm diameter) were polished carefully with 0.05 μm alumina slurries and ultrasonically washed in ethanol and ultrapure water to remove the residues. Then the gold electrodes were electrochemically cleaned in 1 M H_2SO_4 by cyclic voltammetry from −0.4 to 1.2 V (vs. Hg/Hg_2SO_4) until a steady-state redox wave was observed.

The immobilization of the capture probe (S1) was performed by dropping 30 μL of 10 mM Tris–HCl buffer (pH 7.4) containing 1.0 μM S1 probe and 1.0 M NaCl on the cleaned gold electrode in a humidified chamber. The self-assembly was proceeded for two hours. Then the gold electrode was incubated in 1 mM MCH for 1 h to eliminate the nonspecific-bonded DNA. After being thoroughly rinsed with the washing buffer, the S1 probe modified gold electrode was stored at 4 °C in HEPES buffer for further use.



Scheme 1. Schematic representation of the enzyme-amplified electrochemical Hg^{2+} detection system.

2.3. Electrochemical detection

Electrochemical measurements were carried out on an electrochemical workstation of CHI900B (CH Instructions, Austin, TX, USA) at room temperature. A three-electrode system was used, consisting of an Ag/AgCl (3 M KCl) reference electrode, a platinum wire counter electrode, and the modified gold electrode as a working electrode.

For Hg^{2+} concentration determination, the S1 probe modified electrodes were first incubated in the hybridization buffer containing 1 μM S2 probe and varying concentrations of Hg^{2+} at 37 °C. The surface hybridization reaction of the biotinated S2 probe and the immobilized S1 led to the immobilization of biotin on the gold electrode surface. Then the biotinated gold electrodes were submerged in the HEPES buffer containing 1% BSA (w/v) and 0.1% Tween-20 (v/v) for 10 min to block the active sites of nonspecific enzyme absorption. After that, 30 μL of 4 μg mL^{-1} streptavidin-HRP solution containing 1% BSA (w/v) and 0.1% Tween-20 (v/v) was dropped on the modified electrode and allowed to interact with the immobilized biotin for 20 min. The resulting electrodes were gently washed and transferred into the test solution containing 1 mM H_2Q and 1 mM H_2O_2. Then cyclic voltammetric measurements between 0.4 and −0.4 V were performed and reduction peak currents of the enzymatic production of BQ were recorded. Electrodes modified without Hg^{2+} in the hybridization buffer were also tested and the resulting reduction peak currents were adopted as the background responses. Electrochemical detection procedures for other metal ions were the same as those for Hg^{2+} and the obtained signal responses were used to evaluate the selectivity of the sensor.

3. Results and discussion

3.1. Mercury sensor design

Scheme 1 shows the configuration of the enzyme-amplified electrochemical Hg^{2+} detection system. The sensor recognition element comprises a 15-mer capture probe (S1) with a hexanethiol at its 5′ end and a 15-mer biotinated functioned signal probe (S2). The oligonucleotides S1 and S2 include two mismatched T–T base pairs and ten complementary base pairs (underline in Table 1). The DINAMelt web server (Markham and Zuker, 2005) was used to predict the thermodynamical stability of duplexes between S1 and S2. With the duplex concentration of 1 μM in the hybridiza-