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An ultrasensitive electrochemical sensing platform for Hg^{2+} based on a density controllable metal-organic hybrid microarray

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ABSTRACT

A novel electrochemical Hg^{2+} biosensor was developed on the basis of a metal-organic hybrid microarray, in which the nicking endonuclease (NE) assisted target-triggered strand release strategy was realized *via* the DNA cyclic amplification technique. The metal-organic hybrid microarray was fabricated using the SAM of 1, 4-benzenendithiol as soft template, and the density of the microarray could be adjusted by controlling the surface coverage of 1,4-benzenendithiol molecules. In the presence of Hg^{2+}, capture DNA (cDNA) with an indicator at one end could hybridize with the reporter DNA (rDNA) through the stable T–Hg^{2+}–T linkage, forming the nicking recognition site. After the nicking reaction, the electrochemical indicator dissociated from the electrode surface. The released rDNA and Hg^{2+} could be reused in the sensing system and initiate the next cycle, and more electroactive indicator dissociated from the electrode surface, resulting in a significant signal decrease. The constructed DNA biosensor could detect Hg^{2+} in a wide linear range from 15 pM to 500 nM, with an ultrasensitive detection limit of 5 pM (S/N=3). Furthermore, the biosensor exhibited excellent stability, good reproducibility and high selectivity towards other divalent ions. The proposed sensing system also showed a promising potential for the application in real aquatic product sample analysis.

1. Introduction

Mercuric ions (Hg^{2+}), the ionic style of mercury, are very toxic environmental pollutants, which would affect the immune and nervous systems, alter genetic expression, and cause serious damage to mammals' health even at low concentrations (Morel et al., 1998; Nolan and Lippard, 2008). The upper limit of Hg^{2+} mandated by United States Environmental Protection Agency (EPA) is 2 ppb (10 nM) in drinking water. Therefore, the development of highly sensitive and selective assay methods for determination of Hg^{2+} is of significant importance (Clevenger et al., 1997; Morita et al., 1998; Mor-Piperberg et al., 2010; Liu et al., 2009). Classical methods for Hg^{2+} detection, such as atomic absorption/emission spectroscopy (ABS/AES) (Butler et al., 2006), inductively coupled plasma mass spectrometry (ICP-MS) (Wang et al., 2007), and cold vapor atomic absorption spectroscopy (Bibby and Mercier, 2002) are widely used. However, they generally require expensive and sophisticated instrumentations and thus limit their applications in the routine and effective monitoring of Hg^{2+}. Because of their advantages of simplicity, low cost, portability, and especially feasibility of miniaturization, electrochemical methods, such as differential pulse stripping analysis (Nolan and Kounaves, 1999; Bonfil et al., 2000), chronopotentiometric stripping analysis (Augelli et al., 2005) and quartz crystal microbalance analysis (Ruys et al., 2000), have been developed. However, few methods can meet the requirement of selectivity and sensitivity at the same time, because of the non-specific interaction between the electrode modifier and Hg^{2+} ions.

As the intrinsic and specific interaction between Hg^{2+} and thymine (T), the Tanaka group reported in 2006 that T–T mismatches could selectively capture Hg^{2+} to form T–Hg^{2+}–T base pairs (Tanaka et al., 2006). In contrast, other metal ions, such as Cu^{2+}, Ni^{2+}, Pd^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, Mg^{2+}, Ca^{2+}, Fe^{2+} and Ru^{2+}, do not show any notable effect on the stability of T–Hg^{2+}–T DNA duplex (Miyake et al., 2006). This strategy could provide a high selectivity toward environmental Hg^{2+} detection over other related heavy metal ions. More recently, a series of Hg^{2+} DNA biosensors based on T–Hg^{2+}–T are developed. Kong et al. (2009) fabricated an electrochemical label-free biosensor for Hg^{2+} detection with AuNPs-functionalized rDNA as a signal amplifier, which showed a detection limit of 0.5 nM. Park et al. (2012) implemented an electrochemical detection of Hg^{2+} using graphene oxide as the active indicator with a detection limit of 1 nM. In addition, Niu et al. (2011) designed an electrochemical biosensor for

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Hg^{2+} determination based on target-induced hybridization and the detection limit of 0.6 nM was observed. A most recent report by Zhuang et al. (2013) presented an Hg^{2+} electrochemical biosensor, which used DNA hairpins as recognition elements and exhibited a detection limit of 2.5 nM. It is indicated that all these electrochemical DNA (E-DNA) biosensors could meet the EPA criterion of Hg^{2+} detection in drinking water. However, the Hg^{2+} tends to bio-accumulate in the mammals' body which could gradually accumulate to higher concentrations and eventually cause serious health damage. Therefore, there is an urgent demand to design and develop novel electrochemical biosensors to achieve higher sensitivity for Hg^{2+} detection.

Considerable efforts have been made to develop novel E-DNA biosensor with high sensitivity. Two types of strategies have been investigated towards signal amplification. Firstly, by optimizing the substrate fabrication of E-DNA biosensor, the immobilized amount of cDNA probes was enhanced and the electron transfer was improved (Huang et al., 2009). Secondly, the structure of target identification unit was optimized using DNA hairpins or stem-loop molecular beacons for the detection of targets (Wang et al., 2012; Li et al., 2011; Zhuang et al., 2013), or adopting the rolling circle amplification (RCA), hybridization chain reaction (HCR), polymerase chain reaction (PCR) and ligase chain reaction (LCR) based E-DNA biosensors (Chen et al., 2010; Bi et al., 2013; Choi et al., 2012; Hashimoto et al., 2006). The use of nanomaterials, such as Au or Pt nanoparticles, quantum dots and conductive polymers, as signal amplifiers in the E-DNA biosensors has shown to significantly improve the detection sensitivity (Chen et al., 2008; Wang et al., 2003; Gerard et al., 2002). However, few reports are available about the development of metal-organic hybrid microarray based E-DNA biosensors.

Nuclease signaling amplification (NSA) has been reported as a new method for amplified DNA/target detection, which is based on DNA/target recycling amplification with the assistance of various nucleases, including endonucleases and exonucleases (Chen et al., 2010; Tong et al., 2011). Various nucleases can lead to direct recycling and reuse of DNA/target, which in turn results in substantial signal amplification for DNA/target determination. In particular, the nucleases reactions can be performed in an isothermal condition without sophisticated instrumentation and therefore have wide applications in routine bioanalysis.

Herein, we described an E-DNA biosensor for Hg^{2+} detection based on a metal-organic hybrid microarray coupling the NE assisted target-induced strand release strategy. Highly oriented hybrid microarray of $Ni(en)_3Ag_2I_4$ (where en represents diaminoethane) was fabricated by a facile and reliable method based on the 1,4-benzenendithiol modified Au substrate through the Ag–S covalent interaction. In addition, the density of the microarray could be controlled by adjusting the distribution of the 1,4-benzenendithiol layer. The 5′–SH modified stem-loop structured cDNA molecules were immobilized onto the $Ni(en)_3Ag_2I_4$ microarray surface by Ag–S bonding. The electroactive 3′-methylene blue (MB) probe approaching the electrode surface resulted in an obvious current signal. The rDNA with four T–T mismatches could not hybridize with the cDNA on the electrode surface in the absence of Hg^{2+}. However, in the presence of Hg^{2+}, they could hybridize through T–Hg^{2+}–T coordination chemistry. Following the hybridization, the nicking recognition site formed and triggered the nicking reaction. After several cycles, indicator molecules dissociated from the electrode surface and led to the decrease in the peak current of MB, which could be used for the electrochemical quantitative determination of Hg^{2+}. The detection limit of this biosensor using this method was 5 pM, which reached the EPA limit of Hg^{2+}. In addition, the proposed method was also applied to the determination in real aquatic environmental samples.

2. Experimental section

2.1. Chemicals and materials

The NE (Nb.BtsI) was obtained from New England Biolabs. All oligonucleotides were synthesized by TaKaRa biotechnology Co., Ltd. (Dalian, China), and their base sequences were as follows: cDNA: 5′-SH-$(CH_2)_6$-GGT ACG CAG **CTC TTC TC↓C ACT GC**G TAC C-3′-methylene blue (the **CTCTTCT** sequence represents the Hg^{2+} binding sites; the **CGTCAC** sequence is the recognition sequence of NE, and the arrow indicates the nicking position), rDNA: 5′-CGT GAC GTT GTG TT-3′. Ethylenediaminetetraacetic acid (EDTA), 1,4-benzenendithiol, hexaammineruthenium (III) chloride (RuHex, 98%), tris (2-carboxyethyl) phosphine hydrochloride (TCEP), potassium ferricyanide ($K_3Fe(CN)_6$) and potassium ferrocyanide ($K_4Fe(CN)_6$) were purchased from Sigma-Aldrich. $AgNO_3$, KI, N, N-dimethylformamide (DMF), 1,2-ethylenediamine, KCl, NaCl, NaH_2PO_4, Na_2HPO_4, $HgCl_2$, $CoCl_2$, $ZnCl_2$, $MgCl_2$, $CaCl_2$, $Pb(NO_3)_2$, $MnCl_2$, $CuSO_4$, $Ni(NO_3)_2$, $Ba(NO_3)_2$ and $CdCl_2$ were obtained from Sinopharm Chemical Reagent Co., Ltd. All solutions were made up using ultrapure water of resistivity ~18.2 MΩ cm (Millipore, MA). Aqueous environmental samples in Taihu Lake (Soochow) containing Hg^{2+} were obtained from Entry-exit Inspection and Quarantine Bureau, Jiang Su Province, China.

2.2. Buffers

Immobilization buffer (I-buffer): 10 mM Tris–HCl + 1 mM EDTA + 0.1 M NaCl + 10 mM TCEP + 10 mM $MgCl_2$ · (pH 7.4), Mg^{2+} was added into the I-buffer to induce the formation of the stem-loop structure of DNA probes. Hybridization buffer (H-buffer): 10 mM phosphate buffer + 0.25 M NaCl (pH 7.4). Washing buffer (W-buffer): 10 mM Tris–HCl (pH 7.4). NE buffer: 20 mM Tris-acetate + 50 mM potassium acetate + 10 mM magnesium acetate + 1 mM dithiothreitol (pH 7.9).

2.3. Formation of $Ni(en)_3Ag_2I_4$ microarray modified Au substrate

The diameter of the Au substrate was controlled by the insulating tape and determined to be 1 mm. The Au substrate was pretreated following the known procedure (Zhang et al., 2007). The freshly cleaned Au substrate was immersed in the 5 mM 1,4-benzenendithiol ethanol solution for different time from 2 to 10 h to form the SAM structure and was then sufficiently rinsed with ethanol. Hybrid mother solution of $Ni(en)_3Ag_2I_4$ was prepared as described previously (Jiang et al., 2008, Shi et al., 2013a). Here special treatment was needed: the mother solution was maintained at 80 °C for 72 h, then heated to 100 °C and kept for 24 h, and finally slowly cooled to 25 °C. The sediment formed during the treatment procedure was removed from the hybrid mother solution with a filter membrane. The $Ni(en)_3Ag_2I_4$ microarray modified Au substrate was then fabricated by vertically dipping the Au substrate into the solution to crystallize at 25 °C for 10 h. For comparison, disordered $Ni(en)_3Ag_2I_4$ hybrid film modified Au electrode was prepared using the similar method but without 1,4-benzenendithiol SAM template.

2.4. DNA immobilization, hybridization and the nicking reaction

Firstly, the $Ni(en)_3Ag_2I_4$/Au was incubated into a 2 μM cDNA solution at 37 °C for 5 h in 100% humidity. The electrode was thoroughly rinsed with W-buffer to reduce the physical adsorption of cDNA and further incubated in the hybridization solution containing 1 μM rDNA and Hg^{2+} with various concentrations for 2 h at room temperature. After hybridization, the electrode was thoroughly rinsed with W-buffer and dried under a stream of