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Practical and regenerable electrochemical aptasensor based on nanoporous gold and thymine-Hg^{2+}-thymine base pairs for Hg^{2+} detection

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ABSTRACT

A simple, practical and reusable electrochemical aptasensor, based on thymine-Hg^{2+}-thymine (T-Hg^{2+}-T) coordination chemistry and nanoporous gold (NPG) for signal amplification, was designed for sensitive and selective detection of mercury ions (Hg^{2+}). The thiol modified T-rich hairpin capture probe was self-assembled onto the surface of the NPG modified electrode for hybridizing with ferrocene-labeled T-rich probe in the presence of Hg^{2+} via T-Hg^{2+}-T coordination chemistry. As a result, the hairpin capture probe was opened, and the ferrocene tags were close to the NPG modified electrode. Taking advantage of the amplification effect of NPG electrode for increasing the reaction sites of thiol modified capture probe, the proposed electrochemical aptasensor could detect Hg^{2+} quantitatively in the range of 0.01–5000 nM, with a detection limit as low as 0.0036 nM which is much lower than the maximum contamination level for Hg^{2+} in drinking water defined by the U.S. Environmental Protection Agency. Moreover, the proposed electrochemical aptasensor can be regenerated by adding cysteine and Mg^{2+}. The aptasensor was also used to detect Hg^{2+} from real water samples, and the results showed excellent agreement with the values determined by atomic fluorescence spectrometer. This aptasensor showed a promising potential for on-site detecting Hg^{2+} in drinking water.

1. Introduction

Mercury pollution is a global environmental problem and has a series risk to endanger public health. Inorganic mercury, elemental mercury and Hg^{2+}, is released into the environment through a variety of natural (e.g., volcanic, oceanic emissions, and forest fires) and anthropogenic (e.g., fossil fuel combustion, solid waste incineration, coal and gold mining, wood pulping, and chemical manufacturing) sources (Huang et al., 2013a; Li et al., 2009; Nolan and Lippard, 2008; Zeng et al., 2013a, 2013b). Emitted elementary mercury vapors are easily transported in the atmosphere and eventually oxidized to Hg^{2+}. Exposure to trace amount of Hg^{2+} can cause serious human health such as erethism, arrhythmia, cardiomyopathy, kidney damage, and central nervous system defects (Ekino et al., 2004; Lee et al., 2007). The maximum contamination level (MCL) for Hg^{2+} in drinking water is defined by the U.S. Environmental Protection Agency (EPA) to be 10 nM (Nolan and Lippard, 2008). Hence, it is essential to monitor Hg^{2+} levels with sensitivity and selectivity in aqueous systems.

Aptamers are oligonucleotide or peptide molecules that bind to a specific target molecule. Scientific studies have found that oligonucleotides can interact with metal ions with high specificity (Zhou et al., 2016). It has been previously demonstrated that Hg^{2+} can selectively bind between two DNA thymine (T) bases and promote these T–T mismatches to form stable T–Hg^{2+}–T base pairs (Miyake et al., 2006; Zhang et al., 2015). The Hg^{2+}-mediated T-Hg^{2+}-T pairs are more stable (the binding constant is about 4.14×10^6 L mol^{-1}, higher than that of the Watson-Crick A-T pairs) (Li et al., 2006). Moreover, Hg^{2+} can be incorporated into the DNA duplex without largely altering the double helical structure because the van der Waals radius of mercury (≈1.44 Å) is smaller than the base pair spacing of the DNA duplex (≈3.4 Å) (Miyake et al., 2006). As a consequence, DNA-based aptasensors have been developed for highly sensitive and selective detection of Hg^{2+}(Huang et al., 2013a; Kim et al., 2012; Zhang et al., 2015). Wu and co-workers have developed a “turn off” electrochemical sensor for Hg^{2+} based on target-induced structure-switching DNA, enabling the detection limit to be 0.06 nM (Wu et al., 2010). Our team developed an electrochemical sensor based on electrodeposited graphene-Au modified electrode and nanoAu carrier amplified signal strategy for

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attomolar mercury detection, with a detection range of 1.0 aM–100 nM and detection limit as low as 0.001 aM (Zhang et al., 2015a). Xiong and associates developed a ratiometric electrochemical aptasensor for sensitive detection of Hg^{2+} based on thymine (T)-rich stem-loop (hairpin) DNA probe, and the detection limit of 0.08 nM is much lower than the EPA limit of Hg^{2+} in drinking water (Xiong et al., 2015). Moreover, aptamer based fluorescent, colorimetric and quartz crystal microbalance sensors have been proposed. Aptamer functionalized gold nanoparticles based fluorescent probe for the detection of Hg^{2+} ion in aqueous solution has been proposed and Hg^{2+} can be detected in a range of 20–1000 nM (Tan et al., 2013). Colorimetric aptasensor was proposed with sensitivity of 5 nM by naked-eye, and detection limit of approximately 0.15 nM by absorption spectroscopy (Zhu et al., 2015). Quartz crystal microbalance aptasensor for detection of Hg^{2+} based on signal amplification with gold nanoparticles has been reported with a detection limit of 0.24 nM (Dong and Zhao, 2012). These protocols are highly sensitive and selective but complex and they require multiple assembly steps, which may affect the stability and reproducibility of the aptasensors. Comparison of these detection methods, electrochemical aptasensors have advantages in detection limit and detection range. Meanwhile, traditional methods [e.g., atomic absorption/emission spectroscopy, ultraviolet-visible (UV–vis) spectrophotometry, and cold vapor atomic absorption spectrometry (CV-AAS)] for Hg^{2+} detection in aqueous systems are very sensitive, selective, accurate and can be used to detect different kinds of metal ions. However, these methods need expensive and complex equipment, materials and include time consuming extraction steps to eliminate the excipients, contaminants and interfering ions (Huang et al., 2013b; Lai et al., 2016; Qiu et al., 2016). Moreover, the analysis must be performed in a specialized laboratory by skilled personnel. Comprehensive consideration these factors, electrochemistry-based detection schemes have attracted considerable interest due to their remarkable sensitivity, inherent simplicity, low cost, portability, and are suitable for automated detection and sensor miniaturization (Tang et al., 2008; Zhang et al., 2007).

In developing highly sensitive electrochemical aptasensors, amplified detection strategy is the central research topic (Wu et al., 2014). Utilization of nanomaterials (e.g., nanoporous materials, metal nanoparticles, carbon-based nanomaterials, magnetic nanoparticles, polymeric NPs, quantum dots, etc.) as electrode materials to construct sensing platforms and carriers for increasing the upload of electrochemical tags has been studied extensively (Chen and Chatterjee, 2013; Fan et al., 2008; Hu et al., 2011; Zhang et al., 2016b, 2014a; Zhou et al., 2014a, 2014b, 2016). These nanomaterials possess large surface area, abundant binding sites, and provide a synergic effect among conductivity, catalytic activity, and biocompatibility, which are beneficial to significantly increase the amount of the immobilized DNA probe and in consequence obtain the amplified electrochemical detection signals (Feng et al., 2010; Gong et al., 2009; Huang et al., 2008; Wu et al., 2014; Xu et al., 2012; Zhang et al., 2007; Zhou et al., 2014a). Nanoporous gold (NPG), distinct from inert bulk gold, has been found to possess remarkable catalytic activity towards oxidation reactions (Fujita et al., 2012), and the controllable three-dimensional nanoporous metal film has been proved to be an excellent electrode material in constructing aptasensor in our previous work (Zhang et al., 2016a, 2014b). Compared with bulk gold electrode, NPG electrode can enlarge the electrochemical detection signals (e.g., the current of differential pulse voltammetry, the charge of chronocoulometry, the electron-transfer resistance of electrochemical impedance spectra etc.) and lower the detection limits by the enhancement of electron transfer on the electrode surface and the enlargement of the surface area of the substrate electrode (Ding and Erlebacher, 2003; Xiong et al., 2015; Zhang et al., 2016a).

Here we propose a simpler electrochemical approach based on the NPG electrode and highly specific and stable structure of T-Hg^{2+}-T for Hg^{2+} detection in aqueous solution. Compared with the common Hg^{2+} aptasensors, the proposed method exhibits higher sensitivity and selectivity toward Hg^{2+} even in the presence of other competitive heavy metal ions. Moreover, Hg^{2+} detections in river water, tap water, and landfill leachate samples are performed to demonstrate the practical use of this aptasensor. The developed aptasensor is simple, practical, and regenerable and it may be an alternative method for Hg^{2+} detection in environmental and other applications.

2. Materials and methods

2.1. Materials

All oligonucleotides used in the present study were synthesized and high-performance liquid chromatography (HPLC)-purified by Sangon Biotech. Co., Ltd. (Shanghai, China). Their base sequences are as follows: 5′-SH-$(CH_2)_6$-GGCGACGTTTTGTCGCC-3′ (P1, capture probe) and 5′-GACTTTTCGTGCGG-$(CH_2)_6$-Fc-3′ (P2, ferrocene-labeled T-rich DNA probe) (Xiong et al., 2015). Au/Ag alloy was kindly provided by Prof. Ding, Y., Shandong University, Jinan 250100, P. R. China. NPG was prepared according to our previous method (Zhang et al., 2016a). Au/Ag alloy leaf was sandwiched between two supporting papers and cut into small pieces of any size. Then float onto concentrated nitric acid for dealloying (1 h) or water for rinsing (3 times). Tris (2-carboxyethyl) phosphinehydrochloride (TCEP), 6-mercaptohexanol (MCH), and cysteine were purchased from Sigma-Aldrich Chemical Co. All other chemicals were of analytical grade and were used without further purification. Ultrapure water (18.2 MΩ cm) was used throughout the experiments. The buffers involved in this work are as follows: Tris–EDTA buffer (TE, 10 mM Tris–HCl and 1 mM EDTA, pH 8.0), Tris–HCl buffer (100 mM, pH 7.4) and phosphate-buffered saline buffer (PBS, 10 mM, pH 7.4).

2.2. Apparatus

Electrochemical measurements were carried out on a CHI760D electrochemistry system (Chenhua Instrument, China). The three-electrode system used in this work consisted of a glass carbon electrode (GCE, 3 mm in diameter) as working electrode, a saturated calomel electrode (SCE) as reference electrode and a Pt foil auxiliary electrode. The microstructures of Au-Ag alloy and dealloyed NPG were observed using a JSM-6700F field emission scanning electron microscope (JEOL Ltd., Japan).

The Brunauer-Emmett-Teller (BET) specific surface area and pore volumes were measured by ASAP 2020 Accelerated Surface Area and Porosimetry System (Micromeritics Instrument Corporation, USA). All works were performed at room temperature (25 °C) unless otherwise mentioned.

2.3. Aptasensor fabrication

The GCE was polished in the aqueous slurry of alumina and rinsed with deionized water. Residual alumina particles were thoroughly removed by sonicating GCE in ethanol and deionized water for 5 min, respectively. Then the GCE was sonicated in “piranha solution” (H_2SO_4: 30% H_2O_2 =3:1, V/V), and rinsed with ultrapure water. After being dried with nitrogen gas, prepared NPG foil was coated onto a pretreated GCE via physical adsorption (Zhang et al., 2016a). Before self-assembly of the thiol modified oligonucleotide capture probe P1 on NPG, P1 was dissolved in 100 mM Tris–HCl buffer (containing 1.0 M NaCl, 2 mM $MgCl_2$, pH 7.4) and incubated in dark for 2 h to reduce disulfide bonded oligomers (Yang et al., 2010). Then 10 μL of 1 μM P1 solution was placed onto the freshly prepared NPG electrode for 10 h to obtain the GCE/NPG/P1 electrode. The P1 modified NPG electrode was then passivated with 2 mM MCH for 1 h followed by washing with 10 mM PBS buffer (pH 7.4) to reduce nonspecific adsorption of P1 and to obtain a well aligned DNA monolayer (Levicky et al., 1998).