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A reusable electrochemical biosensor for highly sensitive detection of mercury ions with an anionic intercalator supported on ordered mesoporous carbon/self-doped polyaniline nanofibers platform

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ABSTRACT

In this paper, a reusable electrochemical biosensor was developed for highly sensitive detection of mercury ions (Hg^{2+}) using an anionic intercalator, which was based on Hg^{2+}-induced conformational change of a thymine-rich, single-stranded DNA (ssDNA) supported on the platform of ordered mesoporous carbon (OMC) and self-doped polyaniline (SPAN) nanofibers. In the presence of Hg^{2+}, the mercury-specific oligonucleotides were induced and folded into hairpin structure through mismatched thymine-Hg^{2+}-thymine (T-Hg^{2+}-T) base pairs from random coils. Then, the indicators intercalated into the hairpin structure and increased electric signal. OMC and SPAN nanofibers possessed excellent electrical conductivity which synergistically accelerated electron transfer and greatly improved the efficiency of electrochemical reaction at the electrode interface. Meanwhile, SPAN nanofibers strongly adhered to the electrode surface and attached rod-like OMC homogeneously and firmly, which provided a stable platform for DNA immobilization. Under the optimal conditions, the detection limit (LOD) for Hg^{2+} was 0.6 fM (S/N = 3). Furthermore, the biosensor could be easily regenerated by cysteine for cyclic utilization. Some environmental samples including lake sediment pore water and tap water were analyzed by the developed biosensor, the relatively satisfactory results indicated that it provided a green and promising strategy for detection of trace Hg^{2+} in the practical application.

1. Introduction

In recent decades, heavy metal pollution has been one of the most serious concerns because of their bioaccumulation and biotoxicity to human health and the ecological system. Particularly mercury, one of the most dangerous and highly toxic heavy metal pollutants, is widely distributed in the environment [1]. Mercury exists in various forms, such as divalent mercuric ion (Hg^{2+}), mercury sulfide (HgS), methylmercury (CH_3Hg^+) [2], of which the water-soluble mercuric ion is one of the most common and stable forms [3]. Long-term exposure to mercury in the environment and mercury accumulation in living organisms would cause a series of serious health problems such as kidney and liver damage, central nervous system defects, cardiomyopathy and arrhythmia, respiratory failure, endocrine disorders [4]. The world health organization (WHO) regulated that the total Hg^{2+} concentration in drinking water should be less than 0.001 mg/L [5]. Besides, pore water analyses could be used for toxicity identification and sediment quality assessment [6]. As a consequence, it is very important and significant to develop a simple, green, sensitive and low-cost sensor for quantitative determination of Hg^{2+} in the environment, especially in the aqueous media.

Many efforts were paid to develop various analytical methods for detection of Hg^{2+}. Traditional detection techniques include atomic absorption-emission spectroscopy [7], inductively coupled plasma mass spectrometry (ICP-MS) [8], cold vapor atomic fluorescence spectroscopy (CVAFS) [9], etc. While these methods have high sensitivity and accuracy, they generally require complicated and time-consuming sample preparation processes, professional operators, expensive and cumbersome machines and a large number of samples, which make them unsuitable for real-time detection to

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a large extent. In recent years, electrochemical sensors have been widely applied for detection of heavy metal ions, organic pollutants and biomolecules due to many advantages such as simplicity, portability, low cost and high sensitivity [10–13].

Since A. Ono and co-workers found that some base pairs could specifically bind to metal ions and form stable metal-mediated DNA duplexes, many sensors based on the metal-mediated base pairs were developed to detect metal ions [14,15]. It has been proved that Hg^{2+} can selectively coordinate with thymine (T) bases to form mismatched thymine-Hg^{2+}-thymine (T-Hg^{2+}-T) base pairs which are more stable than Watson-Crick A (adenine)-T pairs due to that the melting temperature (T_m) of the DNA duplexes containing T-T mispair in the presence of Hg^{2+} is higher than that of natural pair [16]. Recently, various approaches based on these unique properties have been developed to construct sensor platforms for targets detection by using advanced materials, such as quantum dots [17], metal nanoparticles [18], graphene [19], and carbon nanotubes [20]. Ordered mesoporous carbon (OMC) has attracted much attention since the first synthesis in 1999 [21]. Due to its well-ordered pore structure, high specific surface area, tunable pore diameters in the mesopore range as well as high thermal stability, flexible framework composition, electron transfer resistance and chemical inertness, OMC has been applied in catalysis [22], sensing [23,24], bioreactor construction [25], energy storage [26], etc. As one of the most promising conducting polymers, polyanilines (PAN) are also extensively applied in electrochemical sensors due to its specific properties, such as high electrical conductivity, good environmental stability and biocompatibility, facile synthesis, lower cost, homogeneity, special redox properties, and strong adherence to the electrode surface [27–30]. However, the conductivity of PAN will be significantly reduced when the pH of the solution is higher than 4 [31], which largely limits its application in biosensors. In order to overcome this drawback, self-doped polyanilines (SPAN), a type of polyaniline derivatives, have been widely prepared by chemical modification of polyanilines itself or chemical polymerization of appropriate monomers or electrochemical synthesis [32]. Compared with the parent PAN, SPAN with functional groups such as sulfonic and carboxylic acids, have their own unique properties. Especially, they can show good electric conductivity and electrochemical activity in an extended pH range. Therefore, SPAN can be widely applied to construct some biosensors that usually require neutral, slightly acidic or alkaline solutions due to the specificity of biomolecules such as DNA, RNA, enzyme, protein.

On the other hand, it is very important to choose a suitable signal indicator for improving the detection performance of electrochemical sensors. Of which, the label-free signal indicators have been widely applied in various sensors, especially DNA sensors, due to their good electrochemical performance and flexible, simple and stable features. In addition, cationic indicators such as methylene blue (MB) have a clear advantage with regards to intercalation rate, but would lead to a high background signal because of nonspecific adsorption in contrast with anionic indicators [33,34]. Therefore, the biosensors using anionic indicators could obtain better detection effects and lower detection limit due to the obviation of nonspecific adsorption. Disodium-anthraquinone-2, 6-disulfonate (AQDS), a kind of typical anionic indicators, was used for the proposed electrochemical biosensor.

In this paper, a kind of SPAN nanofibers was synthesized and applied to construct a reusable and sensitive electrochemical biosensor combining with OMC and using AQDS as the electric signal indicator for highly sensitive detection of Hg^{2+} based on the Hg^{2+}-induced conformational change of a thymine-rich, single-stranded DNA (ssDNA). SPAN nanofibers and OMC modified glassy carbon electrode (GCE) showed a remarkable capability of faster electron transfer and excellent stability. AQDS was selected as the electroactive signal indicator owing to its unique properties and good electrochemical performance. Gold nanoparticles (AuNPs) were electrodeposited on the SPAN/OMC modified electrode surface to immobilize mercury-specific oligonucleotides. Meanwhile, cysteine was used as a regenerant of the biosensor for cyclic utilization. Furthermore, in order to investigate the practical application ability, the constructed sensor was applied for detection of Hg^{2+} in lake sediment pore water and tap water.

2. Materials and methods

2.1. Chemicals and apparatus

OMC was synthesized as described previously in our laboratory [35–37]. Disodium-anthraquinone-2,6-disulfonate (AQDS) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Hydrochloroauric acid ($HAuCl_4{\cdot}4H_20$) and aniline (AN) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Tris(2-carboxyethyl)phosphine (TCEP), 6-Mercaptohexanol (MCH), tris(hydroxymethyl) aminomethane (Tris) and *N,N*-dimethylformamide (DMF) were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-cysteine and all the other chemicals were of analytical grade and used as received. The preparation process of SPAN nanofibers was described in Section 1 of the Supplementary information (SI). The following buffer solutions were used in this study: DNA stock solution: 1 × TE buffer solution (pH 8.0); hybridization buffer solution: 10 mM Tris-HCl buffer solution (pH 7.0) containing 0.2 M NaCl; detection buffer solution: 50 mM phosphate buffer solutions (pH 7.0) containing 0.3 M NaCl (PBS); washing buffer solution: 10 mM Tris-HCl buffer solution (pH 7.0) containing 0.2 M NaCl (TBS). All solutions were prepared with ultrapure water (18 MΩ cm, Milli–Q, Millipore). The oligonucleotides were synthesized by Sangon (Shanghai, China) and purified by high-performance liquid chromatography, and the sequence of capture DNA probe was as follows: 5'-SH-$(CH_2)_6$-TTC TTT CTT CCCC TTG TTT GTT-3'.

Cyclic voltammetry (CV) measurements, differential pulse voltammetry (DPV) measurements and electrochemical impedance spectroscopy (EIS) measurements were carried on CHI760D electrochemical workstation (Chenhua Instrument, Shanghai, China). The conventional three-electrode system used in this work was made up of a modified glassy carbon electrode (GCE, 3 mm diameter) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a Pt foil auxiliary electrode. Scanning electron microscopy (SEM) images of the materials were obtained by an S-4800 scanning electron microscope (Hitachi Ltd, Japan). High resolution transmission electron microscopy (HRTEM) images were obtained by a Tecnai G2 F20 S-TWIX electron microscope (FEI, Holland). An AFS-9700 atomic fluorescence spectrophotometer (Kechuang Haiguang Instrument, Beijing, China) was used to analyze environment samples for comparison with the proposed biosensor. A model PHSJ-3F laboratory pH meter (Leici Instrument, Shanghai, China) was employed for pH measurements of buffer solutions.

2.2. Preparation of the electrochemical Hg^{2+} biosensor

To construct the electrochemical Hg^{2+} biosensor, the bare GCE was carefully polished in alumina slurry at first. After rinsing with ultrapure water, the electrode was sonicated in acetone, ethanol and water successively. Then, the electrode was electrochemically treated in 0.5 M H_2SO_4 by cyclic voltammetry between −0.5 V and 1.5 V at 50 mV/s until a steady state redox curve was observed.

The self-assembled film was attached onto the GCE surface by layer-by-layer means. First, 5 μL SPAN nanofibers suspension (dispersed in DMF and ultrasonic processing before each use for