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Label free electrochemical sensor for Pb^{2+} based on graphene oxide mediated deposition of silver nanoparticles

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ABSTRACT

By the use of graphene oxide (GO) as catalytic probe and deposition substrate, a novel label free electrochemical biosensor has been fabricated for sensitive detection of Pb^{2+}. As a result of Pb^{2+} induced conformational change of aptamer, GO can be captured on electrode surface. The Ag^{+} reduction process can be accelerated by the captured GO, which resulted in the formation of silver nanoparticles (AgNPs) on GO surface. Through detecting the oxidation signal of the formed AgNPs by voltammetry, highly sensitive detection of Pb^{2+} can be realized. The detection limit of this assay for Pb^{2+} was 80 pM with a linear range from 0.1 nM to 10.0 μM. The developed sensor is simple, sensitive, selective, and do not need complex tagging process, thus holds great potential for Pb^{2+} and other molecular detection in real samples.

1. Introduction

Aptamers are artificial nucleic acid sequences. Due to their inherent selectivity, affinity, chemical stability, diversity of target molecules, simplicity in vitro screening and easy modification, aptamers are widely applied in bioassay, biological engineering and nanotechnology fields [1,2]. So far, some aptamer-based sensors have been constructed and successfully used for the detection of a variety of targets, including small molecules [3], large biological molecules [4], and even whole cells [5]. Electrochemical sensors, owing to their intrinsic remarkable advantages, such as simplicity, high sensitivity, low cost and high stability, have attracted great attention in the field of biosensing.

Lead ion (Pb^{2+}) can enter the human blood and disperse throughout the entire body tissues. It can combine with a variety of amino acids and prevent their normal physiological activities. Hematopoietic system is the most sensitive system to Pb^{2+}. The synthesis of hemoglobin can be hindered by Pb^{2+}, which cause anemia of children [6]. Due to favourable electrochemical characteristics and easy-to-made surface renewal, hanging mercury drop electrodes or mercury film modified electrodes are widely used for anodic stripping voltammetry (ASV) analysis of Pb^{2+} and other heavy metal ions [7]. However, because of the high toxicity, stability and volatility of mercury, there is highly desirable to substitute it with other less toxic materials. Bismuth film modified electrodes, as promising alternative materials, are of great interest due to the attractive properties including simple preparation, high sensitivity, well-defined and reproducible stripping signal and low toxicity [8].

So far, two kinds of functional nucleic acid molecules have been used to construct Pb^{2+} sensors. One is the RNA-cleaving DNAzyme. Some DNAzyme-based sensors have been developed for the detection of Pb^{2+} by colorimetric [9], fluorescent [10] and electrochemical [11] methods. However, these methods still have several shortcomings, such as high cost, complicated operation or using unstable molecules (such as RNA) etc., restricting their practical applications. Aptamer, as a substitute, is the other kind of function DNA molecule that has been used for Pb^{2+} detection [12,13]. Because no RNA molecules are contained in the DNA aptamer, its stability is relatively higher. The allosteric effect of the aptamer can be driven by Pb^{2+} to form the specific G-quadruplex structure. By the use of the Pb^{2+}-driven G-quadruplex, Wang et al. constructed a DNA molecular device for the detection of Pb^{2+} [14]. This label free sensor is simple, but its detection limit is only 20 nM. In addition, some aptamer based Pb^{2+} detection methods need hemin as a cofactor or fluorescent labelling [15,16], which make the detection procedure more complex. Therefore, it is still necessary to design a label free, highly sensitive, easy-operating and low-cost aptasensor for Pb^{2+} detection.

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Silver nanoparticles (AgNPs), due to its low oxidation potential and mild dissolution conditions, can be directly detected by electrochemical stripping method. Some signal enhancement methods based on the deposition of AgNPs on nanomaterials surface, have been developed to detect DNA and protein [17–20]. The detection sensitivity can be significantly elevated after silver enhancement reaction. However, in these methods, the pre-marking of nanomaterial is required for the deposition of AgNPs. Graphene oxide (GO) is a single layer of carbon atoms which are closely packed in a honeycomb-like crystal lattice. The unique properties of GO, such as large specific surface area, low production cost and catalytic properties etc., make it great potential application in the field of biosensing [21–24]. Recently, by the use of GO as deposition substrate to increase the loading amount of signal reporter, some highly sensitive electrochemical sensors have been fabricated [25,26]. However, in these sensors, the labeling of GO with recognition molecule is still unavoidable. It has been reported that ssDNA can bind to the surface of GO through non-covalent π-π interactions while dsDNA cannot [27,28]. Based on this phenomenon, several fluorescence analysis methods have been developed [29,30], but there are rare reports of electrochemical sensors.

Herein, for the first time, a novel label free electrochemical sensor has been constructed for the highly sensitive and selective detection of Pb^{2+} based on the strong binding ability of GO to ssDNA and GO mediated deposition of AgNPs. The configuration of the aptamer can be regulated by Pb^{2+} and then GO can be captured onto the electrode surface through the strong interaction between GO and ssDNA. GO is not only used as a deposition substrate to increase the loading amount of AgNPs, but also as a catalyst to accelerate the Ag^{+} reduction process. The formed AgNPs can be directly detected by electrochemical stripping method in one-step, thus simple and highly sensitive detection of Pb^{2+} can be achieved.

2. Experimental

2.1. Materials

High performance liquid chromatography (HPLC) purified DNA sequences were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). Capture probe: 5′-SH-TTTTTCACCCTCCCAC-3′; Pb^{2+} aptamer: 5′-GGGTGGGTGGGTGGG T-3′. mercaptohexanol (MCH), silver nitrate ($AgNO_3$), hydroquinone (HQ) and other reagents were of analytical grade and used without further purification. 100 μM DNA stock solutions were prepared with ultrapure water and stored in −20 °C. The DNA stock solutions were further diluted to a required concentration with 0.1 M PBS (pH = 7.0) buffer solution before use. Ultrapure water was used throughout the experiment (Millipore, ≥18.2 MΩ cm).

2.2. Instruments

All electrochemical tests were performed on a CHI 760D electrochemical workstation with a three electrodes system, comprising of a gold working electrode (2 mm in diameter), a platinum wire counter electrode and a Ag/AgCl reference electrode (3 M KCl). Circular dichroism (CD) experiments were carried out on a model 420CD spectrometer (Lake Wood, NJ, USA). Atomic force microscopy (AFM) analysis was performed with a Multimode 3D atomic force microscope (Bruker Nano Inc., USA). The UV–vis absorption spectrum was recorded on a TU-1950 instrument (Beijing, China). Fourier transform infrared spectrum (FTIR) was recorded on a Nicolet 6700 FT-IR spectroscopy (Thermo Fisher Scientific, USA). Transmission electron microscopy (TEM) image was characterized by a high-resolution transmission electron microscopy (HRTEM) on a Philips Tecnai G2 F20 microscope (Philips, Netherlands) with an accelerating voltage of 200 kV.

2.3. Synthesis of graphene oxide (GO)

GO was synthesized from graphite powder by the modified Hummers method [31,32]. Briefly, graphite powder (12 g) was added to a 50 mL mixture of concentrated H_2SO_4, $K_2S_2O_8$ (10 g), and P_2O_5 (10 g) at 80 °C. The mixture was reacted for 6 h and then diluted with 2 L water, filtered, washed using a 0.2 μm nylon Millipore filter, and dried in air overnight. The oxidized graphite was added to concentrated H_2SO_4 (460 mL) and cooled to 0 °C using an ice bath. Then $KMnO_4$ (60 g) was slowly added with temperature controlled below 10 °C. After that the mixture was allowed to react at 35 °C for 2 h, distilled water (920 mL) was slowly added for further reaction for 2 h at a temperature below 50 °C. Water (2.8 L) and 30% H_2O_2 (50 mL) were finally added to produce a brilliant yellow color along with bubbling. The mixture was suspended in water and subjected to dialysis for one week to remove the residual salts. After drying at 50 °C overnight, the obtained graphite oxide was exfoliated into GO by ultrasonication at 0.05 wt% aqueous dispersion for 30 min. The unexfoliated graphite oxide was removed by ultrafiltration at 2000 rpm for 5 min.

2.4. Pretreatment of gold electrode

First, gold electrode was soaked in piranha solution (V_{H2O2}: V_{H2SO4} = 3:7) for 5 min. After washing with distilled water, it was polished on 1.0 μm, 0.3 μm and 0.05 μm alumina slurry, respectively, followed by successively ultrasonic cleaning in ethanol and distilled water to remove the residual aluminum powder. Next, the gold electrode was electrochemically cleaned by cyclic voltammetry (CV) scanning at a potential range of 0 ∼+ 1.6 V in 0.5 M sulfuric acid solution, until the steady CV curve was obtained. Finally, gold electrode was taken out, rinsed with distilled water, and dried with nitrogen for later modification.

2.5. Construction of the label free electrochemical Pb^{2+} sensor

Firstly, the capture probe was treated with TCEP (2.0 μM, 0.1 M PBS) at 37 °C for 30 min to reduce the possible existence of disulfide bond. Then, 5 μL of the treated capture probe (1.0 μM) was dripping on the prepared gold electrode surface, and incubated under humid environment overnight. During the reaction process, capture probe will be immobilized on the gold electrode surface through Au-S bond. After washing to remove the non-immobilized capture probe, the modified electrode was then blocked with 1.0 mM MCH solution for 1.5 h to prevent the non-specific adsorption. At the same time, the captured MCH will help the vertical distribution of capture probe on the gold electrode surface, which is benefit for the following hybridization process. Next, 5 μL of 1.0 μM aptamer was dropped onto the electrode surface and hybridized with the capture probe at 37 °C for 1.5 h to form double stranded DNA. Then, 5 μL different concentrations of Pb^{2+} solutions (25 mM Tris-HAc, pH = 7.2, containing 300 mM $NaNO_3$) were added and incubated at 37 °C for 1.5 h. After washing with the buffer solution (0.1 M Tris-HNO_3, pH = 7.2, containing 0.05% Tween 20) and drying with nitrogen, 5 μL 0.06 mg mL^{-1} GO solution was dropped onto the electrode surface and incubated at 25 °C for 1 h. After washing to remove the non-captured GO, the electrode was then soaked into the silver enhancement solution (containing 0.25 mM $AgNO_3$ and 0.25 mM HQ) for 5 min under dark. Finally, the electrode was removed and rinsed for electrochemical detection (Noting, before the next modification step, the electrode surface should be carefully rinsed with buffer to remove non-specific adsorption substrate).