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A highly sensitive electrochemical assay for silver ion detection based on un-labeled C-rich ssDNA probe and controlled assembly of MWCNTs

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ABSTRACT

We report here a highly sensitive electrochemical sensing platform for Ag^+ detection based on Ag^+-induced conformational change of cytosine-rich single stranded DNA C-rich ssDNA probe and the controlled assembly of MWCNTs. In the protocol, the gold electrode was first modified with a dense 16-mercaptohexadecanoic acid self-assembled monolayer (MHA/SAM). The hydrophobic MHA/SAM isolated the electrode from the electroactive indicator in the aqueous solution, which resulted in the electronic transmission blocking. It was eT OFF state. In the presence of Ag^+, C–Ag^+–C coordination induced the conformational change of C-rich ssDNA probe from random-coil structure to fold into a hairpin structure, which cannot wrap on the surface of the MWCNTs. Then the "naked" MWCNTs can be assembled on the MHA/SAM gold electrode, mediating the electron transfer between the electrode and the electroactive indicator. It generated measurable electrochemical signals (eT ON). The resulting change in electron transfer efficiency was readily measured by differential pulse voltammetry at target Ag^+ concentrations as low as 1.3 nM. The linear response range for Ag^+ detection was from 10 to 500 nM. This method dose not need of electroactive molecules labeling on the C-rich ssDNA probe. Moreover, it has good selectivity to other environmentally relevant metal ions. Therefore, the developed electrochemical assay is an ideal method for Ag^+ detection with some advantages including sensitivity, selectivity, simplicity, low-cost, and no requirement for probe label preparation. We expect that this strategy could be a generalized platform for DNA-based sensing.

1. Introduction

Silver, as one of indispensible high toxic heavy metals, has been used extensively in photography, batteries and semiconductor industry [1,2]. Thousands of tons of silver and its compounds are released into the environment from industrial wastes and emissions annually [2,3]. Silver ions (Ag^+), as one of the important existence type of silver, could produce dose-dependent cytopathogenic effects on many kinds of cell types, including human gingival fibroblasts, keratinocytes, human tissue mast cells and endothelial cells, and so on, because it could bind with various metabolites and inactivate sulfhydrylenzymes [4–6]. In addition, owing to strong oxidation, Ag^+ can easily enter into the human body, leading to internal organ edema, and even to death. Thus, it is of great importance to develop sensitive and selective methods for detection of trace amounts of Ag^+ in environmental and food related samples.

Traditional methods, such as atomic absorption spectroscopy [7,8], and inductively coupled plasma-mass spectroscopy (ICP-MS) [9,10], as well as ion-selective electrodes (ISEs) [11] had been used to detect trace levels of Ag^+ in aqueous media. Most of them were somewhat sophisticated, expensive and time consuming. Recently, a number of highly sensitive and selective methodologies for the determination of Ag^+ have been developed, based on chromophores [12], semiconductor nanocrystals [13], gold nanoparticles [14], carbon based materials [15,16], oligonucleotides [17], DNAzymes [18]. Among these methodologies, DNA–metal base pairs as the sensing elements currently attract considerable attention for sensitive and specific detection of Ag^+. Since Ono et al. found Ag^+ can selectively coordinate cytosine (C) bases to form stable C–Ag^+–C complex [19], many novel colorimetric biosensors [20,21] as well as fluorescent biosensors [22,23] have been developed to detect Ag^+ in aqueous media by taking advantage of specific C–Ag^+–C interaction and signal amplification of nanomaterials. Although these methods have their own advantages, in practical detection, they still have some limitations and disadvantages, such as high background signal, relatively low sensitivity, high cost in synthesis of dye labeled DNA sequence and so on. Electrochemical assays demonstrate its superiority in sensitivity, simple instrumentation and easy

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miniaturization, which is very important in practical application. But until now, few electrochemical assays for Ag^+ detection using the specific interaction of Ag^+ with C–C mismatches and signal amplification of nanomaterials have been developed.

Carbon nanotubes (CNTs) exhibit great chemical stability, large aspect ratio, excellent electrical conductivity, and high electrocatalytic activity [24]. In addition, CNTs can interact with biomolecules through versatile covalent and nocovalent functionalization strategies. These properties make CNTs widely used in biosensors. Among these CNTs based sensors, single-walled carbon nanotubes (SWCNTs) were mostly used [22]. As reported, MWCNTs, which has similar properties, was also confirmed to be a good material in nucleic acid detection. The process of the ssDNA–CNT association undergoes much faster on MWCNTs than that of on SWCNTs. Moreover, MWCNTs can also be well dispersed when wrapped by ssDNA [25–27].

In this paper, we demonstrate the first use of the specific interaction of Ag^+ with C–C mismatches and the controlled assembly of MWCNTs for developing a highly sensitive electrochemical sensing platform to detect Ag^+. In the protocol, one un-labeled C-rich ssDNA probe was employed. The presence of Ag^+ can induce conformational change of cytosine-rich DNA probe from random-coil structure to fold into a hairpin structure, which cannot wrap on the surface of the MWCNTs. Then the "naked" MWCNTs can be assembled onto the 16-mercaptohexadecanoic acid self-assembled monolayer (MHA/SAM) modified gold electrode, resulting in the electron transfer between the electrode and the electroactive indicator (ferrocenecarboxylic acid (FcCOOH)). It generated measurable peak current signals. As the peak current on the gold electrode depended on the assembly of MWCNTs, which was correlative with the concentration of Ag^+, the Ag^+ ions could just be indirectly detected. Here, the effect of Na^+ concentration and incubation time of MWCNTs/Ag^+/C-rich ssDNA mixture with MHA/SAM modified electrode on this assay had been investigated, respectively. Under the optimized conditions, the assay showed good electrochemical response upon the addition of Ag^+ and had good selectivity to other environmentally relevant metal ions.

2. Experimental

2.1. Materials and instruments

Multi-walled carbon nanotubes (MWCNTs) (purity >90%, diameter <10 nm, length 5–15 μm) were purchased from Shenzhen Nanotech Port Company (Shenzhen, China). The cytosine-rich single stranded DNA (C-rich ssDNA) was purchased from Sangon Inc. (Shanghai, China). The sequence of the C-rich ssDNA probe used in this work was as follows: 5′-CTCTCTTCTCTTCATTTTTCAACACAACACAC-3′. 3-(*N*-morpholino)propanesulfonic acid (MOPS), $AgNO_3$ were obtained from Dingguo bio-technology Co. Ltd. (Beijing, China). 90% 16-mercaptohexadecanoic acid (MHA) and ferrocenecarboxylic acid (FcCOOH) were purchased from Sigma Aldrich Chemical Co. All other chemicals were obtained from Reagent & Glass Apparatus Corporation of Changsha and were used without further purification. All solutions were prepared and diluted using ultrapure water (18.2 MΩ cm) from the Millipore Milli-Q system.

Electrochemical measurements were performed at room temperature using a CHI660A electrochemical workstation (Shanghai Chenhua Instrument Corporation, China). A conventional three-electrode cell was employed, which involved a gold working electrode of a diameter of 2 mm, a platinum wire counter electrode, and a saturated calomel reference electrode (SCE).

2.2. Pretreatment of MWCNTs

To lower the background and improve the utilization efficiency of C-rich DNA, the purchased MWCNTs were further purified. Briefly, 5 mL of 10 mM MOPS buffer (pH 7.0) containing 0.5 mg/mL MWCNTs was sonicated (200 W) for 2 h in ice bath using an ultrasonic crasher to obtain a black dispersed suspension. Then the resulting suspension was centrifuged at 1000 rpm for 5 min to remove large MWCNTs. The small piece of MWCNTs supernatant was collected and then for further sensing application. And the final concentration was about 0.1 mg/mL.

2.3. Preparation of MHA/SAM modified gold electrode

The insulated 16-mercaptohexadecanoic acid self-assembled monolayer modified gold electrode can be a good platform for controlled assembly of MWCNTs as signal transduction. It was prepared as follows. The gold electrode (2 mm diameter) was dipped in freshly prepared piranha solution (H_2SO_4/H_2O_2, 7:3 by volume) for 10 min and rinsed with ultrapure water thoroughly. Then the gold electrode was polished carefully with alumina powder of 0.3 μm and 0.05 μm, followed by sequentially sonicated for 5 min each in ultrapure water, ethanol, and ultrapure water. The electrode was then scanned in 0.1 M H_2SO_4 between −0.2 V and 1.55 V at 100 mV/s until a reproducible cyclic voltammogram (CV) was obtained. After being washed with ultrapure water and dried with purified nitrogen. The pretreated electrodes were immersed into an ethanol solution of MHA (20 mM) for 24 h at 25 °C to allow formation of a dense MHA self-assembled monolayer. The electrodes (denoted as MHA/SAM modified electrodes, hereafter) were then thoroughly rinsed using ethanol to remove MHA adsorbed on the electrode surface and followed by drying under mild N_2 stream.

2.4. Analytical procedure

Different concentrations of Ag^+ were incubated in MOPS buffer (100 μL, pH 7.0) containing 150 mM of $NaNO_3$ and 20 nM of C-rich ssDNA probe for 10 min at room temperature. Then 20 μL of dispersed MWCNTs (0.1 mg/mL) was added to this mixture and incubated for 15 min under vibration. The resulting solution (10 μL) was dropped on the surface of MHA/SAM modified electrodes and incubated in a humid atmosphere at room temperature for 80 min. Subsequently, the electrode was thoroughly rinsed with ethanol and ultrapure water to remove MWCNTs weakly adsorbed on the electrode surface. Then the electrodes were dried under N_2 stream before electrochemical measurements. All electrochemical measurements were conducted in 20 mM PBS buffer solution (pH 7.0) containing 5 mM FcCOOH and 0.1 M $NaClO_4$. Cyclic voltammograms (CV) measurements were recorded using a step potential of 1 mV within the potential range from −0.2 V to +0.6 V at a scan rate of 100 mV/s. Differential pulse voltammogram (DPV) has been performed within the potential range from 0 V to +0.6 V under modulation amplitude of 50 mV and sample width 16.7 ms. The reported DPV curves were baseline correction. Electrochemical impedance spectroscopy (EIS) was performed in the frequency range from 0.1 Hz to 100 kHz with 10 mV as the frequency modulation at a bias potential of 0.24 V.

3. Results and discussion

3.1. Experiment principle

The principle for electrochemical detection of Ag^+ based on MWCNTs and un-labeled silver ion specific oligonucleotide was demonstrated in Fig. 1. The clean gold electrode was first modified with a dense MHA/SAM. The hydrophobic MHA/SAM isolated