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Research paper

Ultrasensitive electrochemical biosensor for silver ion based on magnetic nanoparticles labeling with hybridization chain reaction amplification strategy

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ABSTRACT

Silver ion (Ag^+) is a highly toxic heavy metal ion to aquatic organisms and accumulates in the human body via the food chain. Therefore, fast and accurate detection of Ag^+ in water and food resources has become a critical issue within the scope of human health. Herein, we developed an ultrasensitive electrochemical biosensor for detection of Ag^+ based on magnetic Fe_3O_4@gold core-shell nanoparticles (Fe_3O_4@Au NPs) labeling with hybridization chain reaction (HCR) amplification strategy. In this sensing strategy, the magnetic Fe_3O_4@Au NPs were selected for labeling with HCR product and enrichment on the surface of magnetic gold electrode. Thiolated-oligonucleotide (S1) was firstly immobilized on the surface of Fe_3O_4@Au NPs through Au–S chemical bond. In the presence of Ag^+, cytosine-rich DNA oligonucleotide S2 hybridized with S1 to form an intramolecular duplex, in which Ag^+ can selectively bind to cytosine–cytosine mismatches forming C–Ag^+–C complex. The exposed stem of the C–Ag^+–C complex opened two alternating ferrocene-labeled DNA hairpins (H_1 and H_2) in turn and triggered HCR to form a supersandwich DNA structure on the surface of Fe_3O_4@Au NPs. The HCR products modified Fe_3O_4@Au NPs were brought to the surface of magnetic gold electrode for direct electrochemical measurements. The proposed strategy led to a low detection limit of 0.5 fM and a wide dynamic range of 1 fM–100 pM for target Ag^+. The developed biosensor was highly selective and its practical applicability in tap water and lake water samples was also investigated with a satisfactory result.

1. Introduction

Most of the heavy metal ions are hazardous carcinogens and pollutants, which lead to serious health and environment concerns in recent decades [1,2]. Silver, as a rare but naturally occurring metal, is widely employed in industries such as electronics, photography and pharmacy and is always unrestrictedly released into the environment from industrial wastes. Silver ion (Ag^+) is a common form of silver. As a highly bioactive cation, Ag^+ exhibits strong antimicrobial activity at low concentrations and exerts serious biological effects on human health at high levels [3,4]. Therefore, much attention is currently devoted to the development of highly sensitive methods for the accurate detection of trace amounts of Ag^+. Comparison with traditional techniques (e.g., AAS and ICP-MS) [5–10], efforts have been made to develop more advanced methods, such as colorimetric [11,12], fluorescent [12–14], and chemical and electrochemical sensors [15–21], for detection of Ag^+.

As an important magnetic nanoparticles (MNPs), Fe_3O_4 nanoparticles have attracted an increasing attention due to their potential applications in biomedicine and biosensors owing to their good stability and biocompatibility, high surface area, low toxicity, and easy preparation and separation [22–24]. Important progress has been made in surface functionalization of Fe_3O_4 MNPs via the loading of other nanomaterials in chemical synthesis process. Gold coating over Fe_3O_4 magnetic nanoparticles is an even more attractive composite system [25–27]. With Au coating, Fe_3O_4 magnetic nanoparticles can be stabilized more efficiently in corrosive biological conditions and readily functionalized through the Au-S chemistry. The coating also renders the magnetic nanoparticles with plasmonic properties. This makes the Fe_3O_4@Au core/shell

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Table 1
Probes and sequences of the oligonucleotides used in this work.

Name	Sequence (5′–3′)
S1	SH–CAC TTC TCT CTT CTC TTC CCT CTC
S2	AGG AGT AGA CTA GAT CGG ACA CAC ACC CAA CAC AAC ACA CAA CTC–NH_2
H_1	TGT CCG ATC TAG TCT ACT CCT ACT GTG AGG AGT AGA CAT GAT–NH_2
H_2	AGG AGT AGA CTA GAT CGG ACA ATC TAG TCT ACT CCT CAC AGT–NH_2

composite nanoparticles extremely interesting for magnetic, optical, and biosensing applications [28].

Hybridization chain reaction (HCR) is a novel amplification technique via triggered cascade of DNA polymerization by initiator or target molecules under mild conditions without enzymes [29,30]. HCR is a kinetics-controlled reaction in which a cascade of hybridization events between two species of metastable DNA hairpin probes is triggered to form a long dsDNA structure [31–38]. HCR signal amplification strategy shows high sensitivity and selectivity toward targets. Recently, Li et al. reported a novel enzyme-free fluorescent biosensor for the detection of DNA based on hybridization chain reaction amplification and the separation via core–shell Fe_3O_4 polydopamine nanoparticles [39]. Inspired by the aforementioned work, herein, we report the chemical synthesis and functionalization of magnetic and gold-coated magnetic Fe_3O_4 nanoparticles and the immobilization of single-stranded mercapto oligonucleotides onto Fe_3O_4@Au nanoparticles. Upon introduction of silver ion (Ag^+), Ag^+ was intercalated into the duplex DNA chain based on cytosine–Ag^+–cytosine coordination chemistry [40,41]. With the trigger probe, and then another exposed part of it opened two ferrocene-labeled DNA hairpins (H_1 and H_2), which directly induced the happen of hybridization chain reaction and the formation of extended dsDNA polymers. This is an enzyme-free homogeneous electrochemical biosensing strategy for ultra-sensitive detection of Ag^+ based on Fe_3O_4@Au core-shell magnetic nanoparticles and HCR signal amplification.

2. Experimental

2.1. Materials and reagents

Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), (3-aminopropyl)triethoxysilane (APTES), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and $HAuCl_4{\cdot}3H_2O$ were purchased from Sigma-Aldrich (USA). *N*-hydroxy-succinimide (NHS), 1-ethyl-3-(3-dimet-hylaminopropyl) carbodiimide (EDC), ferrocenecarboxylic acid, ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), $AgNO_3$ and sodium citrate were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). All other chemicals were of analytical grade and were used as received. All aqueous solutions were prepared using sterilized water and ultra-pure water (>18 MΩ).

All the oligonucleotides used in this study were synthesized and purified by Sangon Biotechnology Co. Ltd. (Shanghai, China), and the oligonucleotide sequences are listed in Table 1. A 0.1 M PBS (pH 7.4) buffer containing 0.2 M NaCl was used as the immobilization buffer. A 20 mM HEPES buffer was used as the hybridization buffer. A 0.1 M PBS (pH 7.4) buffer containing 0.2 M NaCl and 0.1 M $NaClO_4$ was employed as HCR buffer and washing buffer. A 5 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ solution containing 0.1 M KCl and 0.1 M $KClO_4$ was used as the electrochemical impedance spectroscopy (EIS) supporting electrolyte solution. A 0.1 M PBS (pH 7.4) buffer containing 0.2 M NaCl and 0.1 M $NaClO_4$ was employed as the differential pulse voltammetry (DPV) buffer.

2.2. Apparatus

Electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV) measurements were carried out on a CHI630E electrochemical workstation (CHI Instrument Company, Shanghai, China). Ultraviolet-visible spectra were obtained on Agilent 8453 spectrophotometer. The transmission electron micrograph (TEM) and high-resolution transmission electron microscopy (HRTEM) were performed using a JEM-2100 microscope (Jeol, Japan) equipped with an energy dispersive X-ray (EDS) analysis system from Oxford Instruments. A standard three electrode cell was used for all electrochemical experiments with magnetic gold electrode (d = 4 mm) as working electrode, a platinum (Pt) sheet as an auxiliary electrode, and a saturated calomel electrode (SCE) as a reference electrode, respectively. Hybridization process was carried out on a 4 D rotation mixing equipment (Kylin-Bell, China). The crystalline structures of samples were characterized by a D8 Advance X-ray diffraction (XRD) (Bruker Germany). The gel electrophoresis was performed on the DYCP-31BN Electrophoresis Analyser (Liuyi Instrument Company, China) and imaged on the Bio-rad ChemDoc XRS (Bio-Rad, USA) to study the HCR products.

2.3. Preparation of Fe_3O_4 and Fe_3O_4@Au nanoparticles

Fe_3O_4 nanoparticles were prepared with a solvothermal reduction method according to the literature with a minor modification [42]. In brief, 0.6 g of $FeCl_3{\cdot}6H_2O$ was dissolved in 20 mL of ethylene glycol to form a clear solution, followed by the addition of 1.5 g NaAc and 1.0 g polyethylene glycol. The mixture was stirred vigorously for 30 min and then was sealed in a teflon-lined stainless-steel autoclave (100 mL capacity). The autoclave was heated to and maintained at 200 °C for 8 h. The black products were washed several times with ethanol and dried at 60 °C for 4 h in a vacuum oven. The core/shell Fe_3O_4@Au NPs were synthesized according to previous reported methods with minor modification [25]. First, the surface of Fe_3O_4 nanoparticles was functionalized with amine group via silanization with APTES. Then, the synthesis of core-shell Fe_3O_4@Au NPs was accomplished by reducing $HAuCl_4$ with sodium citrate. Finally, the obtained Fe_3O_4@Au NPs were magnetically separated, washed, and re-dispersed in water for the further use.

2.4. Measurement procedure for target Ag^+ detection

Scheme 1 represents the procedure including capture probe immobilization, DNA hybridization, HCR and electrochemical detection of Ag^+. The obtained Fe_3O_4@Au NPs solution (1 mg mL^{-1}) was mixed with 0.2 μM 5′-thiolated-oligonucleotide (S1) and incubated overnight at room temperature (before immobilization, S1 was activated using TCEP at room temperature for 1 h). After incubation, the mixed solution was salted to 0.2 M NaCl and allowed to stand for 4 h. To remove excess reagents, the synthesized Fe_3O_4@Au/S1 NPs were magnetically separated, washed, and re-dispersed in 0.1 M pH 7.4 PBS (containing 0.5 M NaCl) for the further use. The ferrocene-labeled oligonucleotides modified with $-NH_2$ at the 3′ end (S2, H_1 and H_2) were synthesized by the EDC-NHS method [43]. Briefly, 10 μM of oligonucleotides was incubated for 2 h at 37 °C in 2.5 mL of 10 mM pH 7.4 PBS containing 5 mM of ferrocenecarboxylic acid, 2 mM EDC, and 10 mg NHS, and 0.5 M NaCl.

The Fe_3O_4@Au/S1 NPs was incubated with 1 μM C-rich Fc-S2 and different concentration of target Ag^+ in 20 mM HEPES buffer for 4 h at 37 °C. After binding of Ag^+ with C-Ag^+-C interaction, Fe_3O_4@Au/S1-Ag^+-S2 NPs was rinsed with 0.1 M pH 7.4 PBS (containing 0.2 M NaCl and 0.1 M $NaClO_4$). Then, HCR happened by hybridizing the Fe_3O_4@Au/S1-Ag^+-S2 with the mixture containing 1 μM Fc-H_1 and Fc-H_2 (1:1 ratio) in 0.1 M pH 7.4 PBS (containing 0.2 M NaCl and 0.1 M $NaClO_4$) for 2 h at 37 °C. Subsequently,