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A novel sandwich-type electrochemical aptasensor based on GR-3D Au and aptamer-AuNPs-HRP for sensitive detection of oxytetracycline

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ABSTRACT

In this paper, a novel sandwich-type electrochemical aptasensor has been fabricated and applied for sensitive and selective detection of antibiotic oxytetracycline (OTC). This sensor was based on graphene-three dimensional nanostructure gold nanocomposite (GR-3D Au) and aptamer-AuNPs-horseradish peroxidase (aptamer-AuNPs-HRP) nanoprobes as signal amplification. Firstly, GR-3D Au film was modified on glassy carbon electrode only by one-step electrochemical coreduction with graphite oxide (GO) and $HAuCl_4$ at cathodic potentials, which enhanced the electron transfer and loading capacity of biomolecules. Then the aptamer and HRP modified Au nanoparticles provide high affinity and ultrasensitive electrochemical probe with excellent specificity for OTC. Under the optimized conditions, the peak current was linearly proportional to the concentration of OTC in the range of $5 \times 10^{-10} - 2 \times 10^{-3}$ g L^{-1}, with a detection limit of 4.98×10^{-10} g L^{-1}. Additionally, this aptasensor had the advantages in high sensitivity, superb specificity and showed good recovery in synthetic samples. Hence, the developed sandwich-type electrochemical aptasensor might provide a useful and practical tool for OTC determination and related food safety analysis and clinical diagnosis.

1. Introduction

Oxytetracycline (OTC) is a broad-spectrum antimicrobial drug as a tetracycline derivative obtained from Streptomyces rimosus (Leung et al., 2013), widely used in human medicine, eterinary medicine, fruits and vegetables to prevent bacterial diseases (Zengin et al., 2014). However, the misuse of these medicines may have some adverse affects, such as allergic reactions and antibiotic resistance and drug residues. Drug residues in the food chain may endanger human health (Zhu et al., 2012), WHO has announced a maximum allowable level of 0.1 mg L^{-1} in drugs and human food (Yu et al., 2013). Therefore, sensitive and selective methods for the determination of OTC in biological fluids are highly advisable.

Many methods for the determination of OTCs have been reported, such as high performance liquid chromatography (HPLC) (Zhao et al., 2011), enzyme-linked immunosorbent assay (ELISA) (Zhang et al., 2013), capillary electrophoresis (CE) (El-Attug et al., 2011), immunoassays (Frasconi et al., 2010) and electrochemical methods (Li et al., 2012). Some of these methods have been found to be cumbersome, possessing poor precision and specificity and uneconomical (Li et al., 2014; Kim et al., 2014; Karaseva et al., 2012). However, electrochemical method was an alternative approach owing to its simplicity, rapidness, and low cost detection. Electrochemical aptasensor has attracted considerable attention for its good portability, ease to use, simplicity and low-cost (Liu et al., 2014; Cui et al., 2014; Jampasa et al., 2014). Aptamers are artificially selected nucleic acid sequences with high selectivity, structural stability and flexibility, easy regeneration capabilities and high resistance against denaturation (Zhuo et al., 2014; Batra and Pundir, 2013; Meng et al., 2012). And they have highly affinity toward their targets and have attracted substantial attention as recognition elements in electrochemical detection (Wu et al., 2012; Jiang et al., 2013). Therefore, we hope to fabricate an electrochemical aptasensor for detection of antibiotic residues, and oxytetracycline (OTC) were used as model in the manuscript. Recently, graphene (GR) which is a new class of two-dimensional sheet of sp^2 bonded carbon atoms has been received more attention as modified materials of electrode because of its large specific surface area, excellent electronic transport property (Wu et al., 2010). As a general rule, graphene is prepared by chemical or thermal reduction of graphite oxide (GO) (Yan et al., 2010; Li et al.,

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2013; Li et al., 2013; Xu et al., 2012). However, these methods require highly toxic reducing agents (such as hydrazine hydrate, and hydroquinone (HQ)) and high temperatures in the chemical synthesis processes (Coskun et al., 2012). Exfoliated GO has been reported to be electrochemically reduced into GR at cathodic potentials (potential: −1.5 V vs. Ag/AgCl) (Sheng et al., 2011). At such negative potentials, $HAuCl_4$ can also be reduced to form three-dimensional (3D) gold nanostructures on a planar electrode (Zhang et al., 2009). In view of this method, Liu et al. (2011) and Zhong et al. (2014) prepared graphene-three dimensional nanostructure gold nanocomposite (GR-3D Au) by a one-step electrochemical coreduction. The conductivity and surface area of the GR-3D Au are significant improvement than pure graphene film because of regularly spaced GR layers around Au nanoparticles (Pruneanu et al., 2011).

In this work, a novel sandwich-type electrochemical aptasensor based on GR-3D Au and aptamer-AuNPs-horseradish peroxidase (aptamer-AuNPs-HRP) nanprobes was prepared and utilized for the detection of OTC. GR-3D Au can not only enhance the electron transfer ability but also provide abundant recognition sites for the conjugation of OTC antibody (Ab). Besides, the utilization of aptamer-AuNPs-HRP nanoparticles offered an enzymatically amplified current signal and increased the affinity between nanoprobes and OTC. Under optimal experimental conditions, the experimental observations showed excellent sensitivity and specificity, and maybe provide a new hope for detecting OTC in related food safety analysis and clinical diagnosis.

2. Materials and methods

2.1. Materials and reagents

Oligonucleotides were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The OTC aptamer, which is purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China), had the sequence: 5′-SH-CGA CGC ACA GTC GCT GGT GCG TAC CTG GTT GCC GTT GTG T. The OTC antibody (Ab) and horseradish peroxidase (HRP) were obtained from Dingguo Biotechnology Co. Ltd. (Beijing, China). Bovine serum albumin (BSA) was obtained from Sigma Aldrich (St. Louis, MO, USA). Gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$) was obtained from Aladdin Chemistry Co., Ltd. (Shanghai, China). All other chemicals were of analytical grade and obtained from Sinopharm Chemical Reagent Co. Ltd (Beijing, China). All solutions were prepared using ultrapure water, which was obtained through a Millipore Milli-Q water purification system (Billerica, MA, USA) and had an electric resistance >18.25 MΩ.

2.2. Preparation of aptamer-AuNPs-HRP nanprobes

Gold nanoparticles (AuNPs) were synthesized by using citrate sodium as the reducing reagent under vigorous stirring. In brief, 200 mL sample of aqueous $HAuCl_4$ 0.01% (w/v) solution was boiled with vigorous stirring, and 3 mL of 1% (w/v) trisodium citrate solution was quickly added to the boiling solution. Then, the solution turned from pale yellow to wine red in a few minutes, indicating the formation of AuNPs. The solution was boiled for another 15 min and cooled down to room temperature (RT) under stirring. The resulting solution was stored at 4 °C for further use.

Before conjugation, the prepared AuNPs was concentrated as followed. Firstly, 1 mL of AuNPs solution was centrifuged at 10,000 rpm for 10 min. Then 300 μL of ultrapure water was added after the supernatant was removed. The mixture was adjusted with 0.1 M NaOH solution until pH about 8. Secondly, 3 μL of HRP (5 μM) was added to the above solution. This solution was stirred at RT for 10 min, ensuring the ratio of molar concentration of HRP and AuNPs was 50:1. The mixture was stored at 4 °C for 2 h. Thirdly, 60 μL SH-DNA (10 μM) was added to the mixture with vigorous stirring for 10 min at RT. And the ratio of molar concentration of AuNPs and HS-DNA was 1:2000. The solution was kept for 24 h at 4 °C. Fourthly, 40.33 μL of phosphate buffer (PB) (100 mM, pH 7.4) was added to the solution with vigorous stirring for 10 min. Subsequently, 13.90 μL of phosphate buffer saline (PBS) buffer (10 mM, pH 7.4, containing 3.0 M NaCl) was added for 30 min with stirring. The resulting solution was kept for 24 h at 4 °C. Finally, the mixture was centrifugated at 10,000 rpm for 15 min the supernatant was discarded. 300 μL of 10 mM PBS buffer was added to the red precipitate. The resulting aptamer-AuNPs-HRP conjugates were obtained and stored at 4 °C until use. AuNPs and aptamer-AuNPs-HRP nanoprobes were analysised by UV–vis spectroscopy, respectively.

2.3. Preparation of GR-3D Au/GCE

Graphite oxide(GO) was briefly prepared according to our previous works (Yu et al., 2013; Wu et al., 2010). Then exfoliated GO was obtained by ultrasound of GO (1.0 mg mL^{-1}) dispersion using a sonifier. The obtained brown dispersion was then centrifuged at 3000 rpm for 5 min to remove precipitate. GR-3D Au was prepared as the reported paper (Liu et al., 2013). Bare glassy carbon electrode (GCE) was first polished with 0.3 and 0.05 μM alumina slurry to a mirror-like surface and washed with ultrapure water. 10 μL of the exfoliated GO suspension was dropped onto the surface of freshly polished GCE and dried at RT. The prepared electrode was immersed in the solution containing 2.8 mM $HAuCl_4$ and 0.1 M H_2SO_4. The one-step electrochemical co-reduction was performed by cyclic voltammetry (CV) in a potential range from 0.0 to −1.5 V with the scan rate of 50 mV s^{-1}. The resultant GR-3D Au/GCE was washed in deionized water and dried at RT for further used.

2.4. Fabrication of the modified electrode

10 μL 100 μg mL^{-1} Ab dissolved in PBS buffer was dropped onto the GR-3D Au/GCE and kept overnight at RT. Then the unbound Ab was removed by ultrapure water and PBS. 10 μL BSA (0.5%) in PBS was employed to block non-specific binding sites for 2 h. After being rinsed with PBS in agitation condition, the electrodes were soaked in different concentration of OTC and washed again with PBS buffer. The OTC coated GCE was incubated with 10 μL aptamer-AuNPs-HRP nanprobes for 1 h at RT through the strong discrimination with the target. The sandwich-type aptasensor was fabricated successfully.

2.5. Electrochemical measurements

All electrochemical measurements including differential pulse voltammetry (DPV), CVs, and electrochemical impedance spectroscopy (EIS) were performed at RT using a three-electrode system consisting of a Ag/AgCl reference electrode, a platinum wire as auxiliary electrode, and modified glassy carbon electrode (GCE) as the working electrode. DPV, CV and EIS were carried out using a CHI 660D electrochemical workstation (Shanghai CH Instruments, China). All electrochemical data were recorded in 0.01 M PBS (pH 7.4) containing 0.25 mM KCl, 1 mM HQ and 10 mM H_2O_2 at RT. DPV was recorded within the potential range from −0.2 to 0.6 V at a pulse amplitude of 0.05 V and a pulse width scan of 0.06 s. CV was performed within pH 7.4 PBS containing 0.25 mM KCl and 5.0 mM $K_3[Fe(CN)_6]$ at scan rate of 50 mV s^{-1}. EIS was performed in 5.0 mM $K_3Fe(CN)_6$ working solution in the frequency range of 0.1–10^5 Hz with amplitude of 0.005 V.

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