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Label free electrochemical aptasensor for ultrasensitive detection of ractopamine

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ABSTRACT

A label free electrochemical (EC) aptasensor for ultrasensitive detection of ractopamine (RAC) was developed. A special immobilization media consisting of gold nanoparticles/poly dimethyl diallyl ammonium chloride–graphene composite (AuNPs/PDDA-GN) was utilized to improve conductivity and performance of the biosensor. The RAC aptamer was attached on AuNPs of the composite membrane via Au–S bond. The fabrication process of the EC aptasensor was characterized by electrochemical impedance spectroscopy and cyclic voltammetry. The peak currents obtained by differential pulse voltammetry decreased linearly with the increasing of RAC concentrations and the sensor responds approximately logarithmically over a wide dynamic range of RAC concentration from 1.0×10^{-12} mol/L to 1.0×10^{-8} mol/L. The linear correlation coefficient of the developed aptasensor was 0.998, the limit of detection was 5.0×10^{-13} mol/L. The proposed EC aptasensor displayed good stability, reproducibility and robust operation in animal urine. Particularly, the generality of the fabrication approach of electrochemical aptasensor is highlighted with a further example for illegal drugs detection via the aptamer identification.

1. Introduction

β-Agonist are drugs that can promote bronchodilation, vasodilatation and increase heart rate, and used for the treatment of pulmonary disease and asthma (Kuiper et al., 1998). RAC is one of the typical β-agonists. It was used as feed additive in animal feeding due to its roles in decreasing adipose tissue deposition and increasing protein accretion in livestock (Xiong et al., 2006). However, excessive amount of RAC in animal meats may pose health risks to human being when the meats were consumed (Carry et al., 2009). The use of RAC in stock breeding has been banned by the majority of countries, such as China (MOA Regulation 176, 2002, PR China) and European Union (Commission decision, 1996/23/EC). Routine monitoring of RAC abuse had attracted great attention to the government regulatory agencies and the food industry.

Convention analytical methods, such as liquid chromatography (LC) (Koole et al., 1999; Blomgren et al., 2002), gas chromatography–mass spectrometry (Hernandez-Carrasquilla, 2000) and LC–tandem mass spectrometry (LC–MS/MS) (Nielen et al., 2008; Shao et al., 2009), have been developed to detect trace residue of RAC. These chromatography methods often suffer from complicated sample preparation procedures, which were time-consuming. Some rapid detection methods including enzyme immunoassay (Elliott et al., 1998), visual probes (Zhou et al., 2013), immuno chromatographic assay sensor (Zhang et al., 2009; Wang et al., 2015), surface-enhanced Raman scattering immunoassay (Zhu et al., 2011) and so on, have been developed for the determination of RAC. However, these new methods either require antibodies that were difficult to obtain or the sensitivity don not meet the MRL requirements. Electrochemical (EC) sensors show great potential due to their simple determination procedure, fast response time, high sensitivity, lower cost and portability (Chen et al., 2011). Recently, some EC and electrochemiluminescent (ECL) sensors have been developed for detection of RAC in various sample matrixes (Yang et al., 2014; Wei et al., 2015; Li et al., 2013). These EC or ECL sensors exhibited high sensitivity and fast analysis time. However, the selectivity of these EC or ECL methods depended on either monoclonal antibody for RAC or electrocatalytic

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oxidation of RAC. The sensitivity and selectivity were limited in complicated sample matrix due to the absence of specific recognition elements.

Aptamers are a kind of synthetic oligonucleotide sequence (Ellington and Szostak, 1990). They exhibit excellent biological recognition capability and can be exploited as analytical biosensors (Hermann, 2000). Aptamers have been synthetically evolved for a wide range of targets ranging from metal ions, small molecules, to proteins and even entire cells (Jo et al., 2011; Lee et al., 2010; Meyer et al., 2011). Compared with antibodies, aptamers possess some outstanding features, including high specificity, stability, easy modification with electrochemical active markers, optical markers, enzymes and other desired substances (Khezrian et al., 2013). It is facillius for the aptamer to transduce the recognition events into detectable chemical signals (Kang et al., 2008). In the case of small molecular compounds targets, the binding of aptamers induces conformational switch of the aptamer (Citartan et al., 2012). Such switch can then be transduced via colorimetric (Barthelmebs et al., 2011; Zheng et al., 2011), fluorescence (Sassolas et al., 2011; Yildirim et al., 2012), size (Alsager et al., 2014), and electrochemical responses (Lin et al.,2012). Among them, electrochemical aptasensors offer the lowest detection limit, as well as rapid, label-free quantification of analyte over a wide range of concentrations (Radi, 2011). Graphene has recently attracted tremendous interest because of its unique thermal, mechanical, and electrical properties (Novoselov et al., 2004). One of the most promising applications of graphene is electrochemical sensing (Zhou et al., 2009; Shan et al., 2009).

To our knowledge, the EC aptasensor for RAC based on the aptamer recognition has not been reported. Herein, we proposed an EC aptasensor for sensitive determination of RAC. A single strain DNA aptamer designed for recognition RAC was used for selective detection of RAC. The RAC aptamer was modified on the AuNPs/PDDA-GN composite film on a glass carbon electrode surface (GCE). The interaction between aptamer and RAC can be captured by the EC probe of ferricyanide and monitored by cyclic voltammetry (CV) and differential pulse stripping voltammetry (DPV). The proposed EC sensor showed excellent selectivity and could be used directly for the analysis of urine samples without any sample pre-treatment. Particularly, the EC aptasensor was applied to the determination of RAC in swine urine samples with satisfactory results.

2. Experimental

2.1. Chemicals and apparatus

All reagents were of analytical grade and used without further purification. Solutions were prepared using high pure water with a resistance of 18 MΩ cm. RAC, clenbuterol, salbuterol, dopamine, oxytetracycline, penicillin and Poly dimethyl diallyl ammonium chloride (PDDA, MW=100,000–200,000, 20 wt% in water) was purchased from Aldrich Company. The RAC aptamer was a single strain DNA which was provided by Dr. Yao who is from Jinan University, Guangzhou of China. The RAC aptamer contained functional sequence of AGTGCGGGC with a molecular weight of 4.06 KDa. The base pairs (bp) and dissociation equilibrium constant (K_d) for RAC of the aptamer were 13 bp and 1.66×10^{-6} mol/L. The ΔH and ΔG of aptamer binding with RAC were -0.9×10^{2} cal/mol and -7.88×10^{3} cal/mol. 5′ of RAC aptamer was modified with functional group of $-(CH_2)_3$–SH. $HAuCl_4\cdot4H_2O$ was bought from Sinopharm Chemical Reagent Co., Ltd. Graphene oxide (GO) dispersion (1 mg/mL) was bought from XF NANO, INC. Hydrazine hydrate (40 wt%) was purchased from Sinopharm Chemical Reagent Co., Ltd. Bovine serum albumin (BSA) was purchased from Beijing Cell chip Biotechnology Co., Ltd. Sodium citrate, $CaCl_2$, $MgCl_2$, and NaCl were purchased from Sinopharm Chemical Reagent Co., Ltd. Phosphate-buffered saline (PBS, 10 mM) with various pH values was prepared with stock standard solution of Na_2HPO_4 and NaH_2PO_4. The pH of the solution was measured with a PB-10 pH meter (Sartorius, Germany). Cyclic voltammetry (CV) and differential pulse stripping voltammetry (DPV) were carried out by a CHI 760 C electrochemical workstation (CHI Instrument Company, Shanghai, China). A three-electrode system was used in the measurements, composed of a GCE (2 mm in diameter) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and the Pt wire as the counter electrode.

2.2. Preparation of AuNPs

AuNPs were prepared by the reduction of $HAuCl_4$ with sodium citrate according to the conventional method (Hua et al., 2013). Briefly, 15 mL trisodium citrate (3.88×10^{-2} mol/L) was added into 150 mL of boiling solution of $HAuCl_4$ (1×10^{-3} mol/L) with magnetic stirring and the mixed solution was heated to boiling under stirring for another 30 min, producing a wine-red color solution. The solution was cooled to room temperature citrate-stabilized suspension of AuNPs was formed. The final AuNPs were characterized by using transmission electron microscope (TEM) and UV–vis (Fig. 2A and C). The size of AuNPs was about 13 nm and the concentration was 10 nmol/L. The stock solution of AuNPs was stored in a refrigerator at 4 °C for further use.

2.3. Preparation of PDDA-GNs

The preparation of PDDA-GNs was performed according to method from literature (Xue et al., 2011). Firstly, 1.5 mL GO dispersion, 1 mL PDDA (0.2 wt%), 1 mL hydrazine hydrate (4 wt%) and 4 mL H_2O was added into a flask, followed by ultra-sonication for 5 min to form a homogeneous dispersion. After stirring for 30 min at room temperature, the mixture was heated to 100 °C for 30 min. Finally, a black PDDA-GNs dispersion with a concentration of 0.25 mg mL^{-1} was obtained. Excessive PDDA and hydrazine hydrate was removed by centrifugation at 15000 rpm for 10 min. The PDDA-GNs precipitate was rinsed with water twice and then redispersed into 1 mL H_2O for the further use.

2.4. Fabrication of the sensor

GCE was polished with alumina slurry until a mirror-like surface was obtained. Then it was washed in absolute ethanol and ultra pure water for 5 min under ultrasonication. Finally, it was dried under a stream of nitrogen. 10 μL PDDA-GNs dispersion was dropped onto the surface of the GCE, dried under infrared lamp, and rinsed with water to remove loosely adsorbed graphene. The AuNPs/PDDA-GNs/GCE was prepared by immersing PDDA-GNs/GCE into AuNPs solution for 2 h and then reinsed with water and dried under a stream of nitrogen. The Citrate-capped AuNPs with negative surface charge can be adsorbed onto the PDDA-GNs with positive surface charge by electrostatic adsorption. The modified electrode (AuNPs/PDDA-GNs/GCE) was immersed in thoil modified RAC aptamer solution at 4 °C overnight. The resulted electrode was incubated in BSA solution (0.25%, w/w) for 1 h in order to block possible remaining active sites and avoid non-specific adsorption. The finished sensor (aptamer/AuNPs/PDDA-GNs/GCE) was stored at 4 °C in PBS (pH 7.0) when not use. The procedures used for construction of the sensor were shown in Fig. 1.