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Development of an aptasensor for electrochemical detection of tetracycline

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ABSTRACT

Developing the rapid, simple and sensitive biosensor system for tetracycline detection is very important in food safety. In this paper, we developed a label-free aptasensor for electrochemical detection of tetracycline. The reorganization of tetracycline binding aptamer was confirmed by Isothermal Titration Calorimetry, Kd = 5.18×10^{-5} mol L^{-1}. According to the electrochemical impendence spectroscopy (EIS) analysis, there was a linear relationship between the log concentration of tetracycline and the charge transfer resistance (ΔRet) from 5.0 to 5.0×10^{3} ng mL^{-1} of the tetracycline conc. The detection limit was 1.0 ng mL^{-1} within a detection time of 15 min. The average of assemble rate *Q* was at 82.4% with a differential batches' RSD of 4.6%. The current change of this aptasensor lies within at 8.5% after a storage of 15 days under 4 °C. The result aptasensor had shown a good reproducibility with an acceptable stability in tetracycline detection. The recoveries of TET in spiked milk samples were in the range of 90.0 −95.7%.

1. Introduction

Tetracyclines (TCs) are a group of broad-spectrum antibiotics containing four condensed aromatic rings. According to the difference of sources, TCs can be divided into two types: natural tetracycline (tetracycline, oxytetracycline, chlortetracycline and demethylation aureomycin) and semisynthetic tetracycline (methacycline, doxycycline and minocycline) (Dai, 2003). Most commonly used TCs have oxytetracycline (OTC), doxycycline (DOX) and tetracycline (TET). They have been widely used in animal breeding industry as antibiotics and growth promoters (Wang, Zhao, Quan, & Chen, 2011). However, their widespread use has led to TC residues in animal foods, which becomes one of the most noticeable problems for food safety. Therefore, it is very important to develop a rapid, simple, sensitive and specific detection method to detect TCs in food products.

The common techniques used to detect TCs include microbiological assay (Nagel, Molina, & Althaus, 2011), enzyme-linked immunoassay (ELISA) (Chafer-Pericas et al., 2010), high-performance thin-layer chromatography (HPTLC) (Meisen et al., 2010), capillary electrophoresis (CE) (Ibarra, Rodriguez, Miranda, Vega, & Barrado, 2011), electrochemical (Kim, Kim, Niazi, & Gu, 2010), liquid chromatography-mass spectrometry (LC/MS) (Bousova, Senyuva, & Mittendorf, 2013), high performance liquid chromatography (HPLC) (Yang, Yang, & Yan, 2013). Microbiological assay is simple with low cost, but time-consuming, and lack of sensitivity and specificity. ELISA has high specificity, but is time-consuming (multiple incubations and washing steps) with high background absorption and susceptible to being influenced by sample matrix. CE has the advantages of rapid separation, analysis, and low cost, but not suitable for small molecular detection. LC/MS, HPTLC and HPLC need expensive and large instrument. The electrochemical technologies have gained a great deal of attention, mainly because of their ease in operation, high specificity and sensitivity, easy to miniaturization and amenability to automation.

Wang et al. (2011) developed a tetracycline sensor using Molecularly Imprinted Polymer Modified Carbon Nanotube-Gold Nanoparticles Electrode which has high specificity, but the linear range is narrow and the sensitivity can not meet the requirements. The linear range is from 0.1 to 40.0 μg mL^{-1}, and the detection limit was 40.0 ng mL^{-1}. Que et al. (2013) developed a sensitive electrochemical immunoassay of tetracycline residues by using platinum-catalyzed hydrogen evolution reaction (HER) on an anti-TC antibody modified immunosensor, which has high sensitivity and specificity. The detection limit is 6 pg mL^{-1} However, this

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immunosensor is complex and has some difficulty for the real samples analysis in the complex system by using platinum nanoparticle based HER (platinum nanoparticle is relatively active in the aqueous solution, and the catalytic property is easily affected by the external conditions (Que et al., 2013)).

Aptamers are single-stranded RNA or DNA oligonucleotides screened from synthetic DNA/RNA libraries which rely on hydrogen bonding, electrostatic and hydrophobic interactions for high affinity, specific recognition to their target. The selection of the aptamers for the specific target is based on the SELEX procedure (systematic evolution of ligands by exponential enrichment) (Ellinglon & Szostak, 1990; Tuerk & Gold, 1990). Comparing to traditional immunological and chemical recognition molecules, aptamers have better target versatility, stronger affinity, and higher specificity. In addition, aptamers are more stable because of their tolerance with temperature and other physical conditions (Citartan, Gopinath, Tominaga, Tan, & Tang, 2012; Shangguan et al., 2006). Aptamers are easy to produce through chemical synthesis. And the modification process is simple and the molecule is resistant to denaturation and degradation (Kirby et al., 2004). Moreover, aptamers are taking advantages in small molecular weights, non-toxicity, immunogenicity, and good osmosis in tissue (White, Sullenger, & Rusconi, 2000). So far, aptamers have been widely used in analytical chemistry, biochemistry, molecular biology, medicine, food, environment and other fields, which provides a new, efficient and fast identification platform for basic research, drug analysis, medical inspection, environmental monitoring and food safety (Fischer, Tarasow, & Tok, 2007; Lares, Rossi, & Ouellet, 2010; Tombelli, Minunni, & Mascini, 2005).

Kim et al. (2010) developed a label-free electrochemical aptasensor on a screen printed gold electrode for the detection of TET using square-wave voltammetry (SWV) technique. The detection limit is 10.0 nmol L^{-1} (4.4 ng mL^{-1}), but the stability and reproducibility of the method were not discussed. Zhou, Li, Gai, Wang, and Li (2012) developed an electrochemical TET aptasensor with multi-walled carbon nanotubes (MWCNTs) modification, monitored by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The detection limit is 5.0 nmol L^{-1} (2.2 ng mL^{-1}). However, these aptasensor is relatively complex and needs 30 min for reaction. At present, more attentions have focused on the research of TET aptasensor.

In this work, we developed a simple label-free electrochemical aptasensor for the specific detection of TET using TET-binding aptamer, which was screened by Isothermal Titration Calorimetry, as bio-recognizer. The interaction between TET and aptamer was investigated by the electrochemical probe of ferricyanide and monitored by electrochemical impedance spectroscopy (EIS).

2. Materials and methods

2.1. Reagents and apparatus

Tetracycline (purity: 98%) was purchased from Sigma Company (St. Louis, MO, USA). Single-strand DNA library of random sequence synthesized by Sangon Biotech Company (Shanghai, China). All other chemicals were of analytical grade, purchased from Guangzhou Chemical Reagent Company (Guangdong, China). All solutions were prepared with ultrapure water (resistivity: 18.2 MΩ/cm).

The ITC experiment was carried out on a MicroCal™ Auto iTC_{200} system (GE Corporation, USA). AFM was performed using Multimode AFM (Nanoscope III, Thermo Co., USA). Electrochemical analysis was performed at room temperature using an electrochemical analyzer Epsilon Autolab (BAS Corporation, USA) for CV and DPV analysis, CHI660C electrochemical workstation (Shanghai Chenhua Instrument Corporation, China) for EIS analysis. All experiments were carried out by using a conventional three-electrode system which was consisted of an aptamer modified gold electrode or gold electrode (2 mm) as the working electrode, a platinum wire as the auxiliary electrode, and a Ag/AgCl reference electrode. The impedance measurements were performed in the presence of a $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1, 5 mmol L^{-1}) mixture, in a solution that contained KCl (0.1 mol L^{-1}). They were recorded by applying a potential equivalent to that of the open circuit (OCP), 0.22 V (vs. Ag/AgCl) over the frequency range of 0.1 Hz–10^5 Hz. The DPV measurements were performed in 80 mmol L^{-1} Na_2HPO_4–$C_6H_{10}O_8$ (citric acid) buffer, pH 7.0, containing 50 mmol L^{-1} NaCl and 2 mmol L^{-1} $K_4[Fe(CN)_6]$ in the potential range from +0.3 V to +0.7 V with a pulse amplitude of 50 mV.

2.2. Preparation of aptamer

The 13 bp single-strand DNA with random sequence were synthesized, respectively. After dissolved this single-strand DNA into an 8 mmol L^{-1} Na_2HPO_4–$C_6H_{10}O_8$ (citric acid) buffer (pH 5.0), respectively, which contained 1 molL^{-1} NaCl, a 0.2 mmolL^{-1} single-strand DNA solution was obtained as titrant solution. With the same buffer, the TET solution of 5.0×10^{-3} mmol L^{-1} was prepared as ligand solution. Prior to use, the buffer, titrant solution and ligand solution were degassed for 30 min. Afterwards, MicroCal™ Auto iTC200 was used to analyze the affinity between single-strand DNA and TET. According to the affinity analysis, a single-strand DNA, which has a high affinity to TET was screened and named AP. Finally, a fixed groups (5′-NH_2–$(CH_2)_6$–) was introduced into the 5′ end of AP, which was used as bio-recognizer to develop aptasensor. The Auto iTC_{200} running parameters: cell temperature 25 °C, number of injections 20, reference power 5 μCal/s, the volume (μL) of titrant 2, stirring speed 750 rpm, duration time 4 s (the time that the instrument should take to inject), injection spacing time 180 s, filter period 5 s.

2.3. Fabrication of aptamer-based electrochemical sensor

The gold electrode was sequentially polished using 1.0 μm, 0.3 μm, 0.05 μm alunima slurry, and then sonicated in ultrapure water for 5 min. After that, the gold electrode was immersed in Piranha solution [30% H_2O_2/98% H_2SO_4 (3:7, v/v)] for 10 min and washed twice with ultrapure water and ethanol for 5 min, respectively. After washing, EIS responses of the gold electrode were recorded.

After the analysis of EIS, the active gold electrode was rinsed with ultrapure water and immersed into 2 μmol L^{-1} AP solution containing 1 mol L^{-1} NaCl to self-assemble for 24 h at 4 °C. At this point, the AP modified gold electrode was obtained. To remove the non-fixed AP, the modified electrode was rinsed with ultrapure water after incubated in AP solution. After washing, EIS responses of the modified electrode were recorded.

2.4. Electrochemical measurements

The prepared aptasensor was incubated in 1.0 ng mL^{-1} concentration of TET solution at room temperature for 15 min. After washing with ultrapure water to remove the nonspecific combining of TET, EIS responses of the aptasensor were recorded.

In the same way, the prepared aptasensor was sequentially incubated in 5.0 ng mL^{-1}, 10.0 ng mL^{-1}, 50.0 ng mL^{-1}, 1.0×10^2 ng mL^{-1}, 5.0×10^2 ng mL^{-1}, 1.0×10^3 ng mL^{-1} and 5.0×10^3 ng mL^{-1} concentration of TET solution at room temperature for 15 min. After washing with ultrapure water to remove the nonspecific combining of TET, EIS responses of the aptasensor were