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Highly sensitive homogeneous electrochemical aptasensor for antibiotic residues detection based on dual recycling amplification strategy

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ABSTRACT

The ubiquitous presence of antibiotic residues in foodstuff have serious health consequences for consumers from allergic reactions to the evolution of antibiotic-resistant bacteria. To address this problem, a novel homogeneous electrochemical aptasensor with high sensitivity and specificity is designed for antibiotic residues detection based on target-induced and T7 exonuclease-assisted dual recycling signal amplification strategy. It was realized by the remarkable diffusivity difference between hairpin probe and the mononucleotides towards the negatively charged indium tin oxide electrode. For the proof-of-concept experiment, ampicillin, was employed as a model analyte to examine the desirable properties of this assay. A low detection limit of 4.0 pM toward ampicillin with an excellent selectivity could be achieved, which has been successfully applied to assay antibiotic in milk. What's more, compared with the immobilization-based electrochemical means, the proposed sensing system avoids the tedious and time-consuming steps of electrode modification, making the experimental processes much simpler and more convenient. With the advantages of high sensitivity, excellent selectivity and simple operation, it is believed that this strategy possesses great potential for the simple, easy and convenient detection of antibiotic residues in food safety field.

1. Introduction

Antibiotics are generally used for treating infections in modern healthcare. However, improper use of antibiotics in food industry may lead to undesired levels of residues in food supplies (Ang et al., 1997; Wang et al., 2010; Zhang et al., 2013a), and the development of strains of antibiotic resistant bacteria, thus causing significant risks to consumers and raising the cost to patient treatments (Dasenbrock and LaCourse, 1998; Zhang et al., 2010). Ampicillin (AMP), a broad-spectrum β-lactam antibiotic, is widely used in veterinary medicine for the treatment and prevention of primary respiratory, gastrointestinal, urogenital and skin bacterial infections in food-producing animals (Bogialli et al., 2004). Therefore, the detection of AMP residues in foodstuffs is very important for human health protection. In the past decades, many efforts have been made to develop efficient methods to detect the AMP residues in agricultural products and waters, including immunoassay (Kloth et al., 2009), high-performance liquid chromatography-mass spectrometry (Chen et al., 2015; Cheng et al., 2015; Kipper et al., 2011; Meng et al., 2015; Straub and Voyksner, 1993; Tylova et al., 2013), nuclear magnetic resonance (Reinscheid, 2006), colorimetry (Khan et al., 2015; Xu et al., 2004), fluorescence detection (Fernández-González et al., 2003; Pena et al., 2010), Raman spectroscopy (Andreou et al., 2015), electrochemiluminescence (Li et al., 2011) and dual fluorescence–colorimetric biosensor (Song et al., 2012a). For example, Ban's group developed an aptasensor to detect AMP using its single-stranded DNA aptamer and gold nanoparticles-based dual fluorescence–colorimetric method (Song et al., 2012a). However, these methods suffered from the drawbacks of long analysis time, high cost, the need for skilled manpower, sophisticated instruments or unsatisfactory sensitivity, which limited them as the on-site assays in practical applications. Hence, it is urgent to develop new sensing strategies which provide simple, high sensitivity and convenient detection of AMP residue in food safety field.

Aptamers are single stranded deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) oligonucleotides isolated through Systematic Evolution of Ligands by EXponential enrichment (SELEX), which can specifically bind to their targets, such as small molecules, drugs, virus, bacteria, proteins and even intact tumor cells with high affinity and selectivity (Mascini et al., 2012; Tang et al.,

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2008; Wu et al., 2013). Compared to other molecular recognition systems, aptamers exhibit many advantages, including simple synthesis, easy labeling, good stability and wide applicability (Pavlov et al., 2004; Tolle et al., 2015). Up to now, numerous aptasensors have been developed based on chemiluminescence (Wang et al., 2013; Zhang et al., 2014), fluorescence (Li et al., 2014; Wang et al., 2015b; Zhang and Zhang, 2012), colorimetry (Li et al., 2012; Tang et al., 2012), electrochemistry (Wang et al., 2014b; Zhou et al., 2007), and so on. Among them, electrochemical aptasensors show significant advantages, such as rapid, high sensitivity, low cost and no need of sophisticated instruments (Liu et al., 2014, 2013b). However, most of the reported electrochemical aptasensors in the heterogeneous assaying processes require immobilizing the recognition elements or capture probes on the electrode surface before collecting electrochemical signal, which are generally laborious and time-consuming (Wang et al., 2014b; Zhou et al., 2007). Also, the spatial hindrance effect of the electrode surface and the loss of configuration freedom caused by binding targets on the solution-electrode interface usually make these heterogeneous assays suffer from relatively low hybridization efficiency and enzyme kinetics compared to homogeneous assays (Liu et al., 2012). Therefore, faster and easier-to-use homogeneous electrochemical strategies have been developed for the detection of various targets (Xuan et al., 2012, 2013; Zhang et al., 2013b). Additionally, the substrate electrode is very important for the design and manufacture of homogeneous electrochemical sensor. Recently, the application of indium tin oxide (ITO) electrodes has attracted increasing interest due to its prominent characteristics, such as high electrical conductivity, wide electrochemical working window, excellent substrate adhesion, stable electrochemical and physical properties and low cost. For example, Hsing and colleagues have demonstrated solution-phase electrochemical strategies for sensitive detection of DNA and mercury ion (Xuan et al., 2012, 2013). Very recently, our group also has developed highly sensitive homogeneous electrochemical biosensors for the detection of methyltransferase (Li et al., 2015b) and human telomerase activity (Liu et al., 2015).

In order to obtain high sensitivity, many nucleic acid amplification-based techniques have been paid more and more attention, such as rolling circle amplification (Liu et al., 2013a), strand displacement amplification (Feng et al., 2011), hybridization chain reaction amplification (Li et al., 2015a; Niu et al., 2010; Ye et al., 2015), nicking enzyme-assisted amplification (Li et al., 2014) and exonuclease-assisted amplification (Li et al., 2015b; Wang et al., 2014a; Zhou et al., 2014). Notably, T7 exonuclease-assisted signal amplification strategies are more attractive in constructing versatile biosensing platform, since no specific recognition sequences are required, making T7 more suitable for constructing versatile biosensing platforms (Zhang et al., 2015).

Inspired by the aforementioned developments, herein, we developed a homogeneous electrochemical aptasensor with high sensitivity and specificity for antibiotic detection using target-induced and T7 exonuclease-assisted dual recycling signal amplification strategy. For the proof-of-concept experiment, AMP, was employed as a model analyte to examine the desirable properties of this assay. To the best of our knowledge, few studies have been previously reported to detect antibiotics by this method (Wang et al., 2016, 2015a). It has been applied to detect AMP residues in milk samples. Therefore, it is believed that this strategy possesses great potential for the simple, easy and convenient detection of antibiotic residue in food safety field.

2. Experimental

2.1. Reagents and materials

Klenow Fragment polymerase (5000 U mL^{-1}) and T7 Exonuclease (10,000 U mL^{-1}) were purchased from New England Biolabs (Ipswich, MA, USA) and used without further purification. Deoxyribonucleoside triphosphates (dNTPs) were purchased from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). Ampillin, amoxicillin, penicillin, benzylpenicillin, lincomycin hydrochloride were obtained from Aladdin (Shanghai, China). Tris(hydroxymethyl)aminomethane (Tris), hydrochloric acid, sodium chloride and magnesium chloride were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals are of analytical grade and used without further purification. Ultrapure water (18.2 MΩ cm at 25 °C) obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA) was used throughout the experiments. All oligonucleotides used in this work were synthesized and HPLC-purified by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China), and the sequences of the DNA probes are listed in Table 1. All oligonucleotides were used as provided and diluted in 50 mM Tris-HCl buffer solution (pH 7.5, containing 50 mM NaCl, 10 mM $MgCl_2$) to give the stock solutions of 10 μM.

2.2. Electrode pretreatment and electrochemical measurement

The ITO electrode was pretreated by ultrasonic washing in an Alconox solution (8 g of Alconox per liter of water), isopropanol and ultrapure water for 10 min each, respectively. Then the electrode was immersed in 1 mM NaOH solution for 5 h and then ultrasonic washing in ultrapure water for 10 min at room temperature. After that, a negatively charged ITO working electrode surface with an active surface area of ca. 0.12 cm^2 was obtained for use. All electrochemical measurements were carried out on an Autolab (PGSTAT 302N) electrochemical analyzer (Metrohm Autolab B.V., Netherlands) using a conventional three-electrode system at room temperature, with an ITO electrode as the working electrode, a platinum wire as the auxiliary electrode, and an Ag/AgCl/saturated KCl electrode as the reference electrode. Differential pulse voltammetric (DPV) measurements were carried out in 50 mM Tris-HCl (pH 7.5, containing 50 mM NaCl, 10 mM $MgCl_2$) with the potential window ranging from −0.20 to −0.40 V.

2.3. Electrochemical assay for AMP

In this experiment, the hairpin probe (HP) DNA probe solution was firstly heated to and kept at 90 °C for 5 min and gradually cooled to room temperature to form stem-loop structure. Then the target-induced conformational change of the HP resulting in the exposure of the primer recognition fragment and the subsequent KF polymerization reactions were performed in 40 μL Tris-HCl

Table 1
Sequences of the oligonucleotides used in the experiments[a].

Name	Sequence (from 5′ to 3′)
HP	Methylene blue–**GCGGGC**GGTTGTATAGCGG*AAAAAAAAA*CCGCTATACAACC AAAAAA
Primer	AAAATGTATAGCGG

[a] The boldface letters in HP indicate the sequences of AMP aptamer, and the letters in italic represent the sequences of the spacer DNA between the AMP aptamer and the primer recognition fragment. The underlined letters in HP represent the sequences complementary to each other to form a hairpin structure. The red letters in HP and Primer represent the sequences complementary to each other, and the blue letters in HP represent the sequences extend-DNA.