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A sensitive electrochemical aptasensor for multiplex antibiotics detection based on high-capacity magnetic hollow porous nanotracers coupling exonuclease-assisted cascade target recycling

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ABSTRACT

A multiplex electrochemical aptasensor was developed for simultaneous detection of two antibiotics such as chloramphenicol (CAP) and oxytetracycline (OTC), and high-capacity magnetic hollow porous nanotracers coupling exonuclease-assisted target recycling was used to improve sensitivity. The cascade amplification process consists of the exonuclease-assisted target recycling amplification and metal ions encoded magnetic hollow porous nanoparticles (MHPs) to produce voltammetry signals. Upon the specific recognition of aptamers to targets (CAP and OTC), exonuclease I (Exo I) selectively digested the aptamers which were bound with CAP and OTC, then the released CAP and OTC participated new cycling to produce more single DNA, which can act as trigger strands to hybrid with nanotracers to generate further signal amplification. MHPs were used as carriers to load more amounts of metal ions and coupling with Exo I assisted cascade target recycling can amplify the signal for about 12 folds compared with silica based nanotracers. Owing to the dual signal amplification, the linear range between signals and the concentrations of CAP and OTC were obtained in the range of 0.0005–50 ng mL^{-1}. The detection limits of CAP and OTC were 0.15 and 0.10 ng mL^{-1} (S/N=3) which is more than 2 orders lower than commercial enzyme-linked immunosorbent immunoassay (ELISA) method, respectively. The proposed method was successfully applied to simultaneously detection of CAP and OTC in milk samples. Besides, this aptasensor can be applied to other antibiotics detection by changing the corresponding aptamer. The whole scheme is facile, selective and sensitive enough for antibiotics screening in food safety.

1. Introduction

Antibiotics are large and natural group of pharmaceuticals used in animals for the treatment of diseases and also as animal growth promoters. However, the use of antibiotics may lead to drug residues and accumulation of antibiotics. The accumulation of antibiotics in food-producing animals has become a concern, because of their potential to cause serious threats to public health. Thus, it was highly desirable to develop a facile and sensitive multiplex assay and sensor for their screening.

Various analytical methods and strategies have been developed for simultaneous detection of multiple antibiotic residues, including enzyme-linked immunosorbent assay (ELISA), liquid chromatography (LC) (Posyniak et al., 2003; Kim et al., 2014), liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Gantverg et al., 2003; Castellari et al., 2009), and surface plasmon resonance biosensor (Karaseva and Ermolaeva 2012). It's well known that these traditional methods are reliable, sensitive and stable, but some of the problems limit their application, such as expensive apparatus, high cost and lengthy preparation sample time. Electrochemical aptasensor has attracted considerable attention for its good portability, ease to use, simplicity and low-cost (Liu and Ma, 2014; Cui et al., 2014; Jampasa et al., 2014). Aptamers have highly affinity toward their targets and have attracted substantial attention as recognition elements in electrochemical detection especially in multiplex detection (Wu et al., 2012; Jiang et al., 2013). Therefore, we hope to fabricate an electrochemical aptasensor for simultaneous detection of multiple antibiotic residues, and chloramphenicol (CAP) and oxytetracycline (OTC) were used as models in the manuscript.

To fulfill the purpose of simultaneous detection, it's important to fabricate the sensitive and distinguishable signal tags. Some metal ions based nanotracers, such as Cd, Pb ions, have attracted much attention for signal source, because it can be easily and

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simultaneously detected by stripping voltammetry at different electrochemical oxidation–reduction potential (Feng et al., 2012; Xu et al., 2014). Therefore, different nanotracers encoded with Cd, Pb ions can be employed for simultaneous detection respectively. In order to achieve a higher sensitivity, it is important to find a carrier with high specific surface area and space to encapsulate more metal ions inside it for making the nanotracers. Up to now, various nanoparticles, such as mesoporous silica (Tao et al., 2015; Zhang et al., 2013; Li et al., 2015b), carbon nanotube (Anirudhan et al., 2014) have been successfully synthesized with apparently increased loading capacity. Among them, magnetic mesoporous silica (MMS) has attracted much attention due to their superior properties, such as high specific surface area, excellent biological compatibility, and easily for separation. If MMS was modified with amino groups, it can absorb a lot of Cd and Pb ions (Yi et al., 2006; Feng et al., 2012; Xu et al., 2014). In order to further enlarge the encapsulation capacity of metal ions in MMS's inner surface, we hope to enlarge the pore size and load space of MMS. Recently, the hollow porous nanoparticles (HPs) have increasingly attracted more interests, especially when mesoporous silica spheres were used as sacrificial support. HPs have larger specific area at the internal and external surface, which are suitable for metal ions encapsulation (Li et al., 2013; Yang et al., 2012). Therefore, amino functioned magnetic hollow porous nanoparticles (MHPs) can be acted as ideal supports for metal ions. What is more, the larger hollow space and high internal specific surface area greatly enhanced the signal loading capacity and amplified the signals; magnetic led to a simplified separation and washing steps, the metal ion probe could be detected directly without acid dissolution.

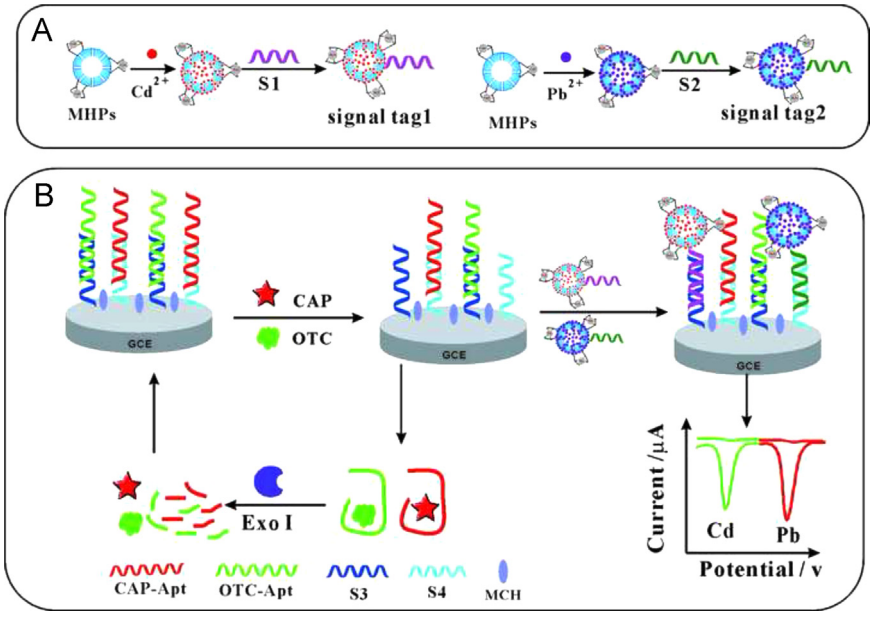
In order to further amplify the signal, nucleases were employed in the study for recycling amplification. Nucleases are specific enzymes that can cleave the phosphodiester bonds in nucleic acids either from the interior or the ends. Among them, exonuclease I (Exo I) has attracted increasing interests, due to the sensitive structure digestion for the single strand DNA in the direction of 3′ to 5′ (Wu et al., 2009). Some reports about the use of Exo I have been described. For example, Wei et al. designed a label-free aptasensor assay employing Exo I as the digesting nuclease for the sensitive and selective detection of ATP (Wei et al., 2015). Wang et al. developed an insertion approach electrochemical aptasensor for mucin 1 detection based on exonuclease-assisted target recycling (Wen et al., 2015). Jiang's group proposed a sensitive protein detection method by protect the trigger DNA from exonuclease I digestion (Li et al., 2015a). Therefore, the Exo I assisted target recycling is convenient, which did not need more immobilization step, just inject the Exo I into the solution. Thus Exo I-assisted cascade strategy was employed in the manuscript coupled with MHPs based nanotracer for dual signal amplification.

Thus, in this study, a novel electrochemical aptasensor was developed for simultaneous detection of antibiotics, chloramphenicol (CAP) and oxytetracycline (OTC) as models, based on metal ions encoded MHPs and Exo I-assisted cascade multiple amplification strategy. In this assay, MHPs were used as soluble carriers, which can not only increase the immobilization amount of metal ions for signal enhancement but also simplify the separation and detection steps. The presence of targets CAP and OTC result in the release of aptamers from the double strand DNA. Based on the selectivity digestion of aptamer-CAP (or aptamer-OTC) complex from the 3′-termini to 5′-end of aptamers, the targets were released for another round recycling, leading to an increase of the single strand DNA and thus improved the signals (Scheme 1B). Therefore, the present aptasensor based on MHPs and Exo I-assisted cascade multiple amplification strategy provided a simple method to improve the sensitivity and selectivity for the detection of CAP and OTC.

2. Experiment

2.1. Reagents and apparatus

Tetraethyl orthosilicate (TEOS) (99.99%, Aldrich), (3-aminopropyl)-triethoxysilane (APTES), glutaraldehyde (25% aqueous solution), tris (2-carboxyethyl) phosphine hydrochloride (TCEP), CAP and OTC were purchased from Sigma-Aldrich Co., Ltd (St Louis, MO, USA). 6-mercapto-1-hexanol (MCH) and $HAuCl_4$ were purchased from Aladin Co., Ltd (Shanghai, China). $Cd(NO_3)_2$, $Pb(NO_3)_2$, Tris, sodium citrate, and all other chemicals were of analytical reagent grade, and doubly distilled water was used in all the



Scheme 1. Schematic representation of simultaneous electrochemical detection of chloramphenicol (CAP) and oxytetracycline (OTC) based on MHPs cascade multiple amplification strategy.