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Multiplex electrochemical aptasensor for detecting multiple antibiotics residues based on carbon fiber and mesoporous carbon-gold nanoparticles

Falan Li [a], Xiangyou Wang [a,b,*], Xia Sun [b,**], Yemin Guo [b]

[a] School of Engineering, Northeast Agricultural University, No. 59 Mucai Street Xiangfang District, Harbin 150000, Heilongjiang Province, PR China
[b] School of Agriculture and Food Engineering, Shandong University of Technology, No. 12 Zhangzhou Road, Zibo 255049, Shandong Province, PR China

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ABSTRACT

We proposed a multiplex electrochemical aptasensor based on a screen-printed carbon electrode (SPCE), which was modified by carbon nanofibers (CNFs) and mesoporous carbon-gold nanoparticles (OMC-AuNPs) for the ultrasensitive detection of kanamycin (KAN) and streptomycin (STR). The designed aptasensor had some characteristics of a high electrochemical conductivity and a high specific surface area because of rod-like OMC-AuNPs and CNFs. Moreover, CNFs and OMC-AuNPs could be homogeneously and firmly adhered to the surface of SPCE, and complimentary strands of aptamers could also be well immobilized on the surface of the electrode modified with CNFs and OMC-AuNPs. In the absence of KAN and STR, the aptamers bound to their complimentary strands. Upon addition of KAN and STR, the aptamers bound to their targets, which led to the complementary strands released from the aptamers and more changes of current peaks because of the aptamers labelled with CdS and PbS. Under the optimal conditions, this aptasensor showed a high stability and a selectivity toward KAN and STR with limits of detection (LODs) as low as 87.3 and 45.0 pM, respectively. The applicability of the developed aptasensor was successfully assessed by detection of KAN and STR in a spiked milk sample without any interference from the sample matrix. It is expected that the proposed aptasensor can be easily detect residues of other antibiotics in milk by replacing different aptamers.

1. Introduction

Aminoglycoside antibiotics (AAs) are the oldest classes of essential antibiotic agents to treat bacterial infections by killing or slowing the growth of bacteria [1]. As commonly used AAs, Kanamycin (KAN) and streptomycin (STR) are produced by the fermentation of *Streptomyces kanamyceticus* [2,3]. The residual amount of KAN and STR in foods may lead to serious toxic side effects such as hearing losing, liver and kidney damages, and allergic reactions to the drugs [4,5]. Therefore, it is extremely important to control and monitor the residues of KAN and STR in foods and it is urgent to avoid drug abuse.

The European Union (EU) has established maximum residue limits for KAN (150 μg/kg) and STR (200 μg/kg) in milk to protect food safety and public health [6]. Therefore, it is necessary to sensitively detect the residues of KAN and STR. Various analytical methods including enzyme-linked immunosorbent assay (ELISA) [7], immunoassays [8], gas chromatography-mass spectrometry [3], colorimetric methods [9,10], and thin-layer chromatography [11] have been employed to detect KAN and STR in animal-derived foods and biological samples. Recently, various analytical methods such as liquid chromatography (LC) [12,13], ELISA [14], surface plasmon resonance [15,16], LC-tandem mass spectrometry (LC–MS/MS) [17], and capillary electrophoresis [18] have been used to detect multiple antibiotic residues. Although these traditional methods are stable and reliable, they are limited by their disadvantages such as large amounts of reagents, tedious sample pre-treatment, sophisticated steps, and costly instrument [19,20]. Therefore, it is necessary to develop an easy and sensitive multiplex assay to detect multiple antibiotic residues. We have developed an aptasensor that is used to simultaneously detect multiple antibiotic residues with KAN and STR used as a model in this study.

Because of good specificity, usability, and simplicity, the aptasensor has attracted considerable attention [21–23]. Aptamers

* Corresponding author at: School of Agriculture and Food Engineering, Shandong University of Technology, No. 12 Zhangzhou Road, Zibo 255049, Shandong Province, PR China.
** Corresponding author.
E-mail addresses: wxy@sdut.edu.cn (X. Wang), sunxia2151@sina.com (X. Sun).

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are short single-stranded DNA (ssDNA) or RNA sequences prepared by an in-vitro selection technique called systematic evolution of ligands by exponential enrichment [24,25], which can selectively and sensitively bind to their pre-selected targets including metal ions [26,27], small organic molecules [28,29], proteins [30,31], and even whole cells or micro-organisms [32–34]. Used as recognition probes in bioassays, aptamers have many advantages over antibodies, for example, low batch–batch variability, availability, long-term stability, resistance to denaturation and degradation, amenability for automated synthesis, and inherent selectivity [35–37]. Therefore, aptamers are promising as molecular recognition elements in biosensing applications. To date, a variety of aptamers with high specificity and affinity against KAN and STR have been generated [38,39]. Colorimetric [40–42] and electrochemical [43,44] aptasensors have also been developed on the basis of these aptamers. Recently, aptamer-based assay has been used for simultaneously electrochemical detection of multiple analytes [22,45]. In simultaneous detection of multiple targets, it is important to fabricate sensitivity and distinguishability signal labels. Cd, Pb and other metal nano-ions are easily detected simultaneously using differential pulse voltammetry (DPV) at different electrochemical redox potentials [46,47]. Different targets can be simultaneously detected because different aptamers are labelled with Cd and Pb ions, respectively. In this study, the aptamers of KAN and STR were labelled with CdS and PbS, respectively. Aptamers were hybridized with their complimentary strands. The targets could be combined with aptamers so that the complementary strands were released from the aptamers, causing the change in the corresponding signals for quantitative detection of the targets.

A key factor of aptasensor construction is the signal amplification. As a result, various nanomaterials have been used to develop sensors [48–51]. Ordered mesoporous carbon (OMC) is a carbon material, and can be used as an effective nanomaterial in construction of electrochemical biosensors because of its ordered mesostructure, high stability, high conductivity, high specific surface area, and good biocompatibility [52,53]. In addition, OMC is liable to adsorb metal molecule and biomolecule because of its high pore volume [53]. Gold nanoparticles (AuNPs) as nano-sized precious metal particles, have been manufactured and applied in electrochemical analysis because of their unique characteristics such as easy synthesis, chemical stability, and large effective surface area [54–58]. The introduction of AuNPs in OMC improves electron transfer performance, biocompatibility of nanomaterials, and binding capability to DNA. Carbon fibers (CNFs) have become attractive materials in electrochemistry [59]. CNFs are manufactured from polymeric precursor fibers through carbonization. The high carbon content (>95%) offers CNFs the characteristics such as corrosion resistance, large surface area, and high electrical conductivity [60,61].

In this paper, we focused on developing a disposable and portable aptasensor to satisfy the requirements of on-site monitoring of KAN and STR in real milk samples. A novel electrochemical aptasensor based on a screen-printed carbon electrode (SPCE) modified by CNFs and OMC-AuNPs was developed for ultrasensitive detection of trace KAN and STR in complex matrices. CNFs promoted the electron transfer of the aptasensor owing to their good conductivity. OMC-AuNPs could improve the efficiency of electron transfer because of their abundant mesoporous structures and excellent electron transfer activities. A large number of complimentary strands of aptamers were adsorbed on the surface of OMC-AuNPs that have a large specific surface area. In this work, a high affinity ssDNA KAN aptamer (KAP) [62] and a high affinity ssDNA STR aptamer (STP) [3] were used as sensing agents. In the absence of KAN and STR, aptamers bound to their complimentary strands. When the targets (KAN and STR) were presented, the aptamers were released from their complementary strands, with more significant change of CdS and PbS corresponding to the current peaks. As far as we know, such an aptasensor has not been reported. This aptasensor, for the first time to be reported, showed high sensitivity, high selectivity, and extremely broad dynamic ranges of KAN and STR. And it was successfully demonstrated for detection of KAN and STR in spiked milk samples.

2. Experimental

2.1. Reagents

KAN, STR, oxytetracycline, tobramycin, and other antibiotics were purchased from Sinopharm Chemical Reagent Co. OMC and CNFs were purchased from Yoshikura Nanotechnology Co. (Nanjing, China) and DeKe-DaoJin Co. (Beijing, China), respectively. Bovine serum albumin (BSA) was obtained from BioDev-Tech. Co. (Beijing, China). All aptamers and other oligonucleotides were purchased from Sangon Biotechnology (Shanghai, China), and sequences were listed as follows: KAN aptamer (KAP), 5′-NH_2-AGATGGGGGTTGAGGCTAAGCCGA-3′; STR aptamer (STP), 5′-NH_2-GGGGTCTGGTGTTCTGCTTTGTTCTGTCGGGTCGT-3′; complementary single strand of KAP (cKAP), 3′-NH_2-TCTACCCCCAACTCCGATTCGGCT-5′; complementary single strand of STP (cSTP), 3′-NH_2CCCCAGACCACAAGACGAAACAAGACAGCCCAGCA-5′. The buffer solution uesd in this study was phosphate buffered solution (PBS, 0.2 M NaH_2PO_4, 0.2 M Na_2HPO_4). Most of the solutions in the experiment were prepared with ultrapure water.

2.2. Apparatus

All electrochemical assay measurements were carried out with an electrochemical workstation (CHI660D, Shanghai CH Instruments Co., China). SPCE, consisting of conventional three-electrode configuration (working electrode, auxiliary electrode and reference electrode) was fabricated (Zensor R&D Co., China). Scanning electron micrographs were taken using a scanning electron microscope (SEM, FEI Sirion 200, FEI, USA). Transmission electron microscopic (TEM) images were acquired with a FEI Tecnai G2F20S-TWIN at 200 kV (FEI, USA). UV–vis spectra were obtained using a Shimadzu UV-2550 spectro-scopy (Shimadzu, Japan). Fourier transformation infrared (FT-IR) spectra were recorded using a Nicolet 380 FT-IR spectrometer (Thermo Fisher Scientific Co., USA).

2.3. Preparation of OMC-AuNPs

The glassware was thoroughly cleaned in freshly prepared aqua regia (HCl:HNO_3, 3:1) and ultrapure water, and then dried. AuNPs precursor was prepared by 10 mL 1% $HAuCl_4$ aqueous solution and 3.95 mL 1% trisodium citrate. The color of the solution changed from yellow to wine red, which indicated the formation of AuNPs. 10 mg OMC was ultrasonically dissolved in 0.3 mL 5% nafion/ethanol solution to obtain a black homogeneous suspension. Then OMC homogeneous suspension and AuNPs were mixed with assistance of magnetic stirring. The OMC-AuNPs solution was stored in a brown bottle at 4 °C.

2.4. Preparation of distinguishable signal probes

CdS/KAP and PbS/STP nanochains were prepared as the distinguishable signal probes. According to the reported method [63,64], CdS/KAP and PbS/STP nanochains were prepared through *in situ* growth of CdS or PbS on DNA chains. In other words, the desired CdS or PbS selective growth was given on a DNA template. The KAP solution was added to $Cd(NO_3)_2$ (0.2 mmol/L,