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Fabrication of magnetically assembled aptasensing device for label-free determination of aflatoxin B1 based on EIS

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ABSTRACT

Aflatoxin B1 (AFB1), one of the most common mycotoxins in food matrixes, has been identified as the most toxic contaminant with mutagenic, teratogenic, immunosuppressive, and carcinogenic effects. In this work, a magnetically assembled aptasensing device has been designed for label-free determination of AFB1 by employing a disposable screen-printed carbon electrode (SPCE) covered with a designed polydimethylsiloxane (PDMS) film as the micro electrolytic cell. The magnetically controlled bio-probes were firstly prepared by immobilization of the thiolated aptamers on the Fe_3O_4@Au magnetic beads, which was rapidly assembled on the working electrode of SPCE within 10 s, by using a magnet placed at the opposite side. The PDMS film with a centered hole was covered on the SPCE surface to achieve a more practicable and flexible electrochemical measurement. In this effort, a label-free aptasensor for the sensitive and selective determination of AFB1 has been developed using electrochemical impedance spectroscopy upon the biorecognition between aptamers and the targets. The developed method had a wide linear range of 20 pg mL^{-1}–50 ng mL^{-1} with a detection limit of 15 pg mL^{-1} (S/N = 3) and succeeded in spiked samples of peanuts. The developed aptasensing device shows fantastic application prospect with simple design, easy operation, low cost, and high sensitivity and selectivity characteristics. This sensing strategy represents a promising path toward routine quality control of food safety and creates the opportunity to develop facile aptasensing device for other targets.

1. Introduction

To a certain degree, about 25% of the world's agricultural commodities are contaminated by mycotoxins during crop growth, harvest, storage, or processing (Al-Taher et al., 2013). Mycotoxins are secondary metabolites produced by filamentous fungi and the most predominant mycotoxins are deoxynivalenol, zearalenone, nivalenol, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol, ochratoxin A (OTA), aflatoxin B1 (AFB1), and fumonisin B1 (FB1) (Pereira et al., 2014; Li et al., 2009). Among them, AFB1 is specified as the most toxic mycotoxin due to their capacity to bind with the DNA of cell and increasing the risk of liver cancer in human beings (Chen et al., 2017b; Groopman et al., 2008; Scholl and Groopman, 2008). Considering its mutagenic, teratogenic, immunosuppressive, and carcinogenic effects, AFB1 has been listed as group I carcinogens by the International Agency for Research on Cancer (IARC, 2002) (Shim et al., 2014). Many countries have established the maximum tolerant level of AFB1 between 0.05 and 20 ng mL^{-1} in all cereals and cereal-derived products (Babu and Muriana, 2011). The U.S. Food and Drug Administration (FDA) has set the limited level of AFB1 in corn and peanut feeds for finishing beef cattle at 300 ng mL^{-1} (Hu et al., 2015). Therefore, monitoring of AFB1 in human foods and animal feeds is significant to ensure food safety. Traditional methods such as high-performance liquid chromatography and liquid chromatography combined with mass spectrometry have been used for AFB1 determination (Herzallah, 2009; Yazdanpanah et al., 2013; Abia et al., 2013; Warth et al., 2013; Tokusoglu et al., 2005). Although such techniques provide sufficient sensitivity and accuracy, they require expensive instruments, well-trained professionals, and tedious procedures (Shim et al., 2007). Therefore, great efforts should be devoted to developing facile methods for monitoring trace levels of AFB1 in foods and feeds (Arora et al., 2011).

Aptamer (Apt), a single-stranded DNA or RNA oligonucleotides, can bind to a specific target with high affinity and specificity and has being explored as highly promising alternatives of antibodies (Chen et al.,

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2017a). Owing to its low-cost, facile synthesis, ease of modification, and high stability under non-physiological conditions (Jayasena, 1999; Barthelmebs et al., 2011; Zhang et al., 2012), biosensors based on aptamer have been developed for the detection of several mycotoxins, such as OTA and FB1 (Bonel et al., 2011; Soh et al., 2015; Shi et al., 2015; Zhao et al., 2014; Chen et al., 2015). Ever since the emerging of aptamer for the specific binding of mycotoxin AFB1 in 2012 (Patent: PCT/CA2010/001292), a series of aptasensors have come into use for AFB1 determination based on electrochemistry (Zheng et al., 2016), colorimetry (Seok et al., 2015), fluorescence (Chen et al., 2017a; Wang et al., 2016), and chemiluminescence (Shim et al., 2014) transducers. Among them, aptasensor based on electrochemistry is of particular interest in recent years thanks to its fast and reliable response, high sensitivity, low production cost, and compatibility with conventional measurement. However, most of the reported electrochemical aptasensors for AFB1 still suffer a drawback of complicated sensing procedure, specifically, requiring additional conjugating of the aptamer with enzymes or redox species.

As a typical electrochemical technique, electrochemical impedance spectroscopy (EIS) is a more effective analysis tool to probe interfacial interactions associated with biometrics events occurring at the electrode surface. Therefore, EIS based aptasensor is especially promising in convenient and truly label-free detection without the requirement of modifying the biomolecules with labels. Recently, Hianik group have proposed two novel label-free aptasensor for AFB1 while electrochemistry based on EIS was employed for acquiring the signal response by means of redox indicators (Castillo et al., 2015; Evtugyn et al., 2014). One of the typical aptasensors has been constructed through layer coating of cystamine, poly (amidoamine) dendrimers, and aptamers on gold electrode (Castillo et al., 2015). In the other work, the aptasensor was designed by covalently bounding of aptamers with polycarboxylate macrocyclic ligands and electropolymerized neutral red on glassy carbon electrode (Evtugyn et al., 2014). In both cases, however, each sample measurement takes tens hours to refresh the electrode surface, modify the electrode surface with several kinds of functional groups and/or nanoparticles, and covalently bind of aptamers on the constructed interface. These procedures are always tedious and time-consuming, which does not satisfy the rapid, simple, and high through-put detection requirements for current assays. In addition, conventional three-electrode system is involved in both designs which makes the electrochemical measurements demand large volume of electrolyte solution. This kind of aptasensors is only suitable for laboratory testing which cannot be used for on-site field testing.

In this work, a magnetically assembled aptasensing device has been fabricated for label-free determination of AFB1 by using a disposable screen-printed carbon electrode (SPCE) covered with a designed polydimethylsiloxane (PDMS) film as the micro electrolytic cell. Specifically, the magnetically controlled bio-probes were obtained by immobilization of the thiolated aptamers specific for AFB1 on the core/shell Fe_3O_4@Au magnetic beads (MBs). The aptasensor could be constructed by magnetic assembly of the bio-probes on the working electrode surface within 10 s, by using a magnet placed at the opposite side. Due to biorecognition between aptamers and targets, the introduction of AFB1 would trigger Apt/AFB1 binding, leading to the inhibition of the electron transfer between the redox probe and the electrode interface. The aptasensing device was fabricated by covering the SPCE with PDMS film having a centered hole to make the three electrodes system of SPCE flexible to approach to the electrolyte solution. The PDMS film-based micro electrolytic cell can effectively lower the solution volume down to 30 μL that offers many advantages like low measurement cost, simple operation, on-site field use, and prospects of miniaturization. On this basis, a cheap and disposable aptasensor was successfully achieved for simple, rapid, and label-free determination of AFB1 by directly measuring the increase of the electron transfer resistance (R_{et}) determined by EIS.

2. Materials and methods

2.1. Reagents and materials

Diethyleneglycol (DEG), ethyleneglycol (EG), polyethylene glycol (PEG), $HAuCl_4{\cdot}4H_2O$, $FeCl_3{\cdot}6H_2O$, and (3-aminopropyl) triethoxysilane (APTS) were purchased from Sinopharm Chemical Reagent Co. Ltd (China). Tris (hydroxymethyl) aminomethane (Tris), Tris (2-chloroethyl) phosphate (TCEP), (ethylenedinitrilo) tetra-acetic acid disodium salt (EDTA), and 6-mercapto − 1-hexanol (MCH) were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). OTA, FB1, AFB1, and aflatoxin B2 (AFB2) were obtained from Sigma-Aldrich. AFB1 aptamer: 5′-GTT GGG CAC GTG TTG TCT CTC TGT GTC TCG TGC CCT TCG CTA GGC CCA CA-SH-3′ were purchased from Sangon Biotech Co., Ltd. (China). DNA oligonucleotide stock solutions were prepared with 10 mM pH 7.4 Tris-HCl buffer containing 1.0 mM EDTA (TE buffer) and kept at 4 °C.

2.2. Instrumentations

All the electrochemical measurements were performed on a CHI660D electrochemical workstation (Chenhua Instruments Co., Shanghai, China). The SPCE containing a carbon working electrode (Φ = 3 mm), a carbon auxiliary electrode, and an Ag/AgCl reference electrode were purchased from Delta Biotech (Shanghai, China). The morphology of the samples was checked by transmission electron microscopy (TEM) technique (JEOL2100, JEOL, Japan) and scanning electron microscopy (SEM) technique (JSM-6010PLUS/LA, Japan). X-ray diffraction (XRD) spectra were carried out with a Bruker D8 ADVANCE diffractometer (Germany) with Cu Kα (λ = 1.5406 Å) radiation. X-ray photoelectron spectroscopy (XPS, ESCALAB 250 multitechnique surface analysis system, Thermo Electron Co., USA) was used to probe the binding of aptamer on Fe_3O_4@Au MBs. UV–vis absorption spectra were measured by UV-2450 spectrophotometer (Shimadzu, Japan).

2.3. Preparation of Fe_3O_4@Au MBs

The pristine Fe_3O_4 nanospheres were prepared according to our reported work.[31] Briefly, 2 mmol of $FeCl_3{\cdot}6H_2O$ was dissolved in a solution containing 10 mL of EG and 10 mL of DEG by ultrasonication for half an hour, followed by the addition of 20 mmol of sodium acetate and 2 mmol of PEG. After vigorously stirring for 30 min, the mixture was sealed in a Teflon-lined autoclave which was heated at 200 °C and maintained for 3 h. Then it was cooled to room temperature. The Fe_3O_4 nanospheres were separated by a magnet and washed three times with ethanol and water, respectively. Finally, the obtained Fe_3O_4 nanospheres was re-dispersed in 50 mL of ethanol with ultrasonication for 5 min.

The core/shell Fe_3O_4@Au MBs were prepared according to the previously reported methods with minor modification (Wang et al., 2015a, 2015b; Zhao et al., 2008; Yu et al., 2010). Typically, 40 mL of the Fe_3O_4 nanospheres suspension was mixed with 400 μL of APTS and vigorously stirred for 7 h at room temperature. After magnetic separation and washing, the APTS-coated Fe_3O_4 nanospheres was obtained and dispersed in 40 mL of water. Subsequently, 4 mL of the $HAuCl_4$ solution (1 wt%) was added dropwise into the as-synthesized APTS-coated Fe_3O_4 nanospheres solution under stirring. After refluxing at 100 °C for 30 min, 8 mL of the sodium citrate solution (1 wt%) was added dropwise into the above solution under stirring and refluxing at 100 °C for another 3 h. Finally, cooled down to room temperature and purified by magnetic separation and washing, the core/shell Fe_3O_4@Au MBs were therefore obtained and re-dispersed in 40 mL of water for further use.