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Fabricated aptamer-based electrochemical "signal-off" sensor of ochratoxin A

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ABSTRACT

An ultrasensitive and rapid electrochemical platform for the specific detection of ochratoxin A (OTA) was developed. In this method, three single-stranded DNA molecules, including the aptamer, were immobilized on the surface of an electrode. Binding of the OTA target analyte to the aptamer changed the redox current of methylene blue (MB), which was used as the electrochemical probe, in a manner that was dependent on OTA concentration. With signal enhancement from gold nanoparticle-functionalized DNA, the sensitivity of this method for OTA was as low as 30 pg/mL, and the effective sensing range was from 0.1 to 20 ng/mL. To investigate the sensing process, the conformational switch of the aptamer was studied by circular dichroism (CD), which confirmed the recognition of the aptamer by the target OTA. Given its sensitivity and rapid detection, we believe this approach has the potential to be a main technology for the detection of toxins in the field of food safety, and in other areas.

1. Introduction

Recent reports have described food poisoning events associated with microcystins from polluted water, aflatoxins from tainted corn, and ochratoxins from contaminated food. Ochratoxins are dangerous by-products of several fungal species, mainly in the *Aspergillus* and *Penicillium* genera, which can contaminate foods and beverages including cereals, beans, nuts, spices, dried fruits, coffee, cocoa, beer, and wine (Cruz-Aguado and Penner, 2008; Clarke et al., 1993; Breitholtz et al., 1991; Xu et al., 2009). Ochratoxin A (OTA) is the most toxic and known to be hepatotoxic, nephrotoxic, teratogenic and mutagenic to a wide variety of mammalian species. OTA may also exist in human blood and breast milk (Barna-Vetro et al., 1996). Thus, a method for the detection of OTA is urgently needed, to help prevent the risk of human OTA consumption. Conventional instrumental analytical methods such as liquid chromatography (LC), high performance liquid chromatography (HPLC), and thin layer chromatography (TCL), are acceptable, but high cost, long processing times, and the need for specially trained personnel have hindered their wide application (Nesheim et al., 1973; Ahmed et al., 2007; Hunt et al., 1979). Immunoassays such as the enzyme-linked immunosorbant assay (ELISA) do not have the disadvantages of the other methods, and may even have several advantages, including simplicity, reliability and a low requirement for technical skills (Barna-Vetro et al., 1996). We have developed several immunoassay methods for the detection of toxins, chemicals and pesticide residues (Chen et al., 2009a,b; Hao et al., 2009). However, the preparation of antibodies, including hapten, immunogen synthesis, and the rabbits immunity, is time consuming. Antibodies may also be susceptible to problems with stability or modification. These may be overcome by the use of aptamers, which are artificial nucleic acids or peptides, first described in 1990, that exhibit affinity and selectivity for a target molecule (Tuerk and Gold, 1990; Ellington and Szostak, 1990; Breaker, 1997; Cox et al., 1998; Zheng et al., 2009). The easy production and stability over long-term storage (O'Sullivan, 2002) have led to the development of aptamers against targets that include metal ions, organic molecules, peptides, proteins and whole cells (Smironov and Shafer, 2000; Stojanovic and Landry, 2002; Wilson and Szostak, 1999; Huang et al., 2005; Lin et al., 2006; Ho and Leclerc, 2004; Bang et al., 2005; Kong et al., 2009). Aptamers are currently used as alternatives to antibodies for recognizing the different biodetection targets.

Electrochemical biosensors have been widely reported because of their simple instrumentation, easy operation, low cost, high sensitivity and rapid sensing time. Our group has developed an electrochemical sensor for the detection of protein and toxin (Shim et al., 2008; Wang et al., 2009a,b). Other groups have also reported developing electrochemical sensors for DNA and other small molecules (Wu et al., 2007; Zhou et al., 2007; Huang et al., 2009; Xiao et al., 2005; Lai et al., 2006; Zuo et al., 2007; Ferapontova et al., 2008).

Here we describe the development of an aptamer-based electrochemical sensor for the detection of OTA and the real application in practical samples. The aptamer was immobilized on the surface

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of the electrode through base-pairing with a linker DNA (DNA 1). A gold nanoparticle (Au NP)-functionalized DNA 3, complementary to the aptamer (DNA 2), was used to amplify the sensing signal. In the sensing system, the target OTA competed with both DNA 1 and DNA 3 to combine with the aptamer, influencing the amount of DNA on the surface of the electrode. Methylene blue (MB), used as the electrochemical probe, was proportional to the amount of the DNA, so the redox currents of the electrochemical probe MB were proportional to the amount of OTA in the solution. The developed method was also used to detect OTA in field samples, with sensitivity in the range of pg/mL.

2. Materials and methods

2.1. Equipments

Electrochemical measurements used a CHI 660A electrochemical workstation (Chenhua Instrument Company, Shanghai, China) based on a conventional three-electrode system with the modified glassy carbon electrode (GCE) as the working electrode, the platinum wire as the auxiliary electrode, and a saturated calomel electrode as the reference electrode. The surface of electrode was characterized with the JSM 6380LV scanning electron microscope. The spatial conformation was studied based on the Jasco J-810 circular dichroism spectrum.

2.2. Reagents

DNA 1: 5′-CCA CACCCG ATC-NH_2-3′; DNA 2 (aptamer): 5′-GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA-3′; DNA 3: 5′-HS-TGT CCG ATG CTC-3′. Oligonucleotides were synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequence of the aptamer (DNA 2) was designed according to the reported reference (Jorge and Gregory, 2008a,b). The following reagents were used without further purification: hydrogen tetrachloroaurate(III) ($HAuCl_4$), methylene blue, p-aminobenzene sulfonic acid, were from Sigma–Aldrich and trisodium citrate was from Fluka.

2.3. Preparation of Au nanoparticle (NP)-functionalized DNA 3 probe

Gold NPs were synthesized using the classic Frens's method with slight modification (Frens, 1973). Briefly, 1 mM $HAuCl_4$ (100 mL) was added to a round-bottom flask and heated to boiling with continuous stirring, before rapidly adding 10 mL 38.8 mM sodium citrate under stirring. The mixture was refluxed for another 15 min before cooling to room temperature with continuous stirring. A 1 mL aliquot of the gold nanoparticle solution was centrifugated at 10,000 r/min for 15 min and the precipitate redispersed in 50 μL pure water.

The conjugation of DNA 3 to the Au NPs was carried out according to the literature. Briefly, a thiol-mediated DNA 3 solution was added to 10 μL of concentrated Au NPs solution and incubated overnight before incubating with 1 μL 2 mol/L NaCl and DNA 3 mixture for 24 h. After centrifugation at 16,000 r/min for 15 min, as much supernatant as possible was adsorbed with a pipet to remove unbound DNA 3. The precipitate was rinsed and centrifuged twice, and redispersed in 50 μL 0.05 M Tris–HCl buffer containing 0.2 M NaCl (pH 7.4). Immobilization of DNA 3 on the surface of the Au NPs was characterized by agarose gel electrophoresis.

2.4. Preparation of the modified electrode

A glassy carbon electrode (GCE) was polished sequentially by 1.0, 0.5 and 0.01 μm alumina slurry, followed by ultrasonic cleaning in ethanol and ultrapure water for 5 min each. The treated electrode was immersed in 20 mM p-aminobenzene sulfonic acid and scanned at 0.1 V/s between −0.5 and 0.5 V for 30 min. After this, the electrode was functionalized with the sulfonic groups for the following DNA immobilization. Then the electrode was washed twice with ultrapure water and immersed in 40 mM PCl_5 solution for 30 min to activate the sulfonic groups on the surface. Finally, the modified electrode was washed three times and dried under nitrogen gas. Amino-modified single-stranded DNA 1 was immobilized to the treated electrode by adding 6 μL of 5 μM DNA 1 solution (10 mM Tris–HCl buffer, 1 M NaCl, pH 7.4) to the surface of electrode and peptide bond formation allowed to occur for 2 h. The DNA 1 modified electrode was rinsed thoroughly three times with ultrapure water and dried under nitrogen gas.

The electrode was placed in aptamer solution (DNA 2, 5 μM) for 4 h. After rinsing and drying three times as above, 20 μL of DNA 3–Au NPs was placed on the surface to obtain the electrode/DNA 1/aptamer/DNA 3 structure shown in the scheme. After careful rinsing and drying treatment, the functionalized electrode was used as the work electrode in the three-electrode system. Immobilization of the DNA 3-Au NPs was characterized by scanning electron microscope (SEM). A system without aptamer was used as the control (Scheme 1).

Prior to electrochemical detection of OTA, the electrode was put in a series of OTA concentrations for 15 min, followed by three rapid rinses with buffer solution. Subsequently, the electrode was put into 8×10^{-5} M methylene blue (MB) Tris–HCl for 10 min followed by three rinses. Cyclic voltammograms (CVs) were performed in 5 mL 100 mM degassed Tris–HCl solution (pH 7.4) at a scan rate of 100 mV/s.

2.5. Sample preparation

The red wine samples were all purchased from the supermarket. Samples made in China were used in this study. The red wine samples were treated with the solid phase extracted column according to the reported method. The spiked samples were prepared by adding different volumes of OTA standard solution previously to the wine samples which were lacking OTA.

3. Results and discussion

As shown in the scheme, detection was based on specific recognition of the aptamer by OTA, which induced a current change in the redox reaction. The specific sequence of the aptamer to OTA was selected by the classic systematic evolution of ligands by exponential enrichment (SELEX) technology. The selected aptamer used in our research had the strong and specific affinity to the target OTA (Cruz-Aguado and Penner, 2008). The surface of a naked glass carbon electrode was carboxylated with the bifunctional molecule p-aminobenzene sulfonic acid. By forming peptide bonds between amino and carboxyls group, amino-modified DNA 1 was immobilized to the electrode surface as a linker. DNA 2, the aptamer against OTA was a bridge DNA that was linked to the electrode through partial hybridization with DNA 1. Au NP-functionalized DNA 3 was also conjugated to the electrode through hybridization to the DNA 2 aptamer. After the DNAs were immobilized to the electrode, the target molecule solution was added to the detection system at different concentrations, and competed with DNA 1 and DNA 3 to combine with the DNA 2 aptamer. The more target molecules in the detection system, the more aptamer (DNA 2) was combined with the target and released into solution. According to the previous reported literature (Wang et al., 2009b), the MB could specifically interact with guanine (G). So, most of the MB molecules were adsorbed by the aptamer (DNA 2) while DNA 1 and DNA 3 also