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Analytical Methods

PVP-coated gold nanoparticles for the selective determination of ochratoxin A via quenching fluorescence of the free aptamer

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ABSTRACT

This paper describes an aptamer/gold nanoparticle-based assay for ochratoxin A (OTA) detection. This assay is based on the use of an aptamer labeled with carboxyfluorescein (FAM) at its 5′-end and gold nanoparticles (AuNPs) that act as quenchers of fluorescence. When OTA is absent in the system, the fluorescently labeled aptamers are adsorbed on the surface of AuNPs. The fluorescence signal of the fluorescein-labeled OTA aptamer generated is quenched by the fluorescence resonance energy transfer effect of AuNPs. When OTA is present in the system, the fluorescently labeled aptamer binds to OTA and forms a folded structure, which can resist the adsorption of AuNPs. Thus, the fluorescent signal can be retained. The detection limit of this sensing platform is 5 nM, and the linear detection range is 10–1000 nM (R^2 = 0.994). The procedure was validated by the quantitation of OTA in spiked ginger powder samples and were found to be free of interference by the sample matrix. The recoveries and the relative standard deviation varied from 89.0% to 117.8% and from 1.9% to 6.3%, respectively.

1. Introduction

Ochratoxins are a group of mycotoxins produced by several fungal species of the genera *Aspergillus* and *Penicillium* (Wang et al., 2017). Ochratoxin A (OTA) is the most prevalent of this group and is considered as one of the most important toxic fungal secondary metabolites in food safety regulation. The contamination of OTA occurs widely in a large number of foodstuff, such as cereals, beans, coffee, pork meat, eggs, and spices, all over the world (Arroyo-Manzanares, Gamiz-Gracia, & Garcia-Campana, 2012; Belakova, Benesova, Mikulikova, & Svoboda, 2011; García-Moraleja, Font, Mañes, & Ferrer, 2015; Thirumala-Devi et al., 2001). Moreover, it is responsible for chronic diseases in humans and animals. Numerous studies have shown that OTA not only presents liver and kidney toxicity but also exerts carcinogenic, teratogenic, mutagenic, and immunosuppressive effects (Zhu, Ren, Nie, & Xu, 2016). The United Nations Cancer Research Organization also lists OTA as Class 2B carcinogen (Fernandez-Baldo, Bertolino, Messina, Sanz, & Raba, 2010). Thus, OTA has been categorized as a potential carcinogen, and countries have established limits to OTA levels in food and beverages (Dou, Chu, Kong, Luo, & Yang, 2016; Gu, Long, Zhou, & Shi, 2016). Thus, the establishment of an OTA detection method with high sensitivity, high selectivity, and easy operation is imminent.

At present, the detection of OTA technology mainly involves traditional detection methods, such as chromatography, high-performance liquid chromatography (HPLC) coupling with fluorescent detection or mass spectrometry (MS), gas chromatography (GC), and GC coupled with MS (Ahmed, Farag, Soliman, Abdel-Samed, & Naguib, 2007; Flajs, Domijan, Ivic, Cvjetkovic, & Peraica, 2009). However, these traditional methods tend to require complex methodology, long detection period, cumbersome sample pretreatment processes, long processing times, and professional operators, among others. These disadvantages limited OTA detection in practical applications.

In recent years, a vast number of nanomaterials, such as nanoparticles (Karabchevsky, Mosayyebi, & Kavokin, 2016; Liu et al., 2015; Su, Goldberg, & Stoltz, 2016), nanotubes (Xin & Li, 2014; Yue, Han, Zhu, Wang, & Zhang, 2016), nanosheets (Gao et al., 2016; Wang et al., 2010), nanoislands (Jung, Park, Kang, & Jeong, 2016; Liu, Cai, Chen, Zhang, & Kong, 2016), and nanowires (Akihama & Hane, 2012; Moon, Zhang, Myung, & Haberer, 2014; Riedel et al., 2017), have been utilized to establish a new style of analytical tools. Among these nanomaterials, AuNPs have been widely utilized to establish biosensors because of their unique characteristics, including chemical stability, ease of

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synthesis, excellent compatibility with biomolecules, and unique optical, thermal, and electronic (Jin, Wu, Li, Mirkin, & Schatz, 2003b; Su, Ke, Cai, & Yao, 2012; Sun et al., 2013).

Aptamers are a class of single-stranded oligonucleotide sequences that can bind with high specificity and affinity to a wide range of targets (Wang, Fan, Liu, & Dong, 2017), such as cells (Wang, He, Zhai, He, & Yu, 2017), proteins (Li, Du, Yang, & Tang, 2013; Pu et al., 2011), toxins (Sharma et al., 2016) and other inorganic or organic molecules (Choi, Yoon, Baeg, & Kim, 2009; Zheng, Zou, & Lou, 2012); thus, these sequences can be sensitive for target molecule detection. The aptamer of OTA was first obtained by Cruz-Aguado in 2008 (Cruz-Aguado & Penner, 2008). Subsequently, a series of aptasensor for OTA detection have been established (Wang et al., 2016; Yang, Wang, Marty, & Yang, 2011; Zhang et al., 2015; Zhu et al., 2015). However, these OTA aptasensors are limited by the procedures and the complex modification steps; hence, the sensitivities of these methods still need to be improved. Therefore, we established a simple FRET system for OTA detection by using AuNPs and 6-carboxy fluorescein (FAM) labeled OTA aptamer.

In the present study, in combination with AuNPs, a FAM-aptamer specific for OTA was utilized; when OTA is absent in the system, the fluorescently labeled aptamer is adsorbed on AuNPs surface. Then, the fluorescence signal is quenched by FRET effect. When OTA is present in the system, the fluorescently labeled aptamer binds to the OTA and forms a folded structure, which can resist the adsorption of AuNPs; thus, the fluorescent signal is retained. Therefore, OTA can be quantitatively detected by determining the intensity change of the fluorescence signal of the AuNPs interacting with the fluorescently labeled aptamer. To eliminate the non-specific adsorption of OTA onto AuNPs, we used polymer materials (poly(vinylpyrrolidone), PVP) as AuNPs coating to prevent non-specific target adsorption.

2. Materials and methods

2.1. Materials and reagents

The FAM modified OTA aptamer with the sequence of 5′-FAM -GATCGGGTG TGGGTGGCGTAAAGGGAGCATCGGACA-3′ (Guo, Ren, Wang, & Wang, 2011) was synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China). DNA stock solution was prepared by dissolving oligonucleotides in 10 mM Tris buffer (pH 8.0) containing 120 mM NaCl, 20 mM $CaCl_2$ and 5 mM KCl and was stored at −20 °C before use. OTA, Ochratoxin B (OTB), Ochratoxin C (OTC), Aflatoxin B_1 (AFB_1), Zearalenone toxin (ZEN), *N*-acetyl-L-phenylalanine (NAP), Warfarin, trisodium citrate and poly (vinyl pyrrolidone) (PVP) were purchased from Sigma Aldrich (St. Louis, MO, USA). Trimethylolaminomethane (Tris) ($C_4H_{11}NO_3$) and gold chloride ($HAuCl_4{\cdot}4H_2O$) were obtained from Beijing Chemical Reagent Company (Beijing, China). The ochratoxin was dissolved in absolute ethanol to prepare OTA stock solution (1 mM) and the stock solution was stored at −20 °C. The purified water was prepared using a Milli-Q ultra-high purity water system (Millipore, Bedford, MA, USA). All chemicals were of analytical grade and used as received.

2.2. Apparatus

A UV-2600 spectrophotometer (Shimadzu, Japan) was employed to characterize the UV vis-spectroscopy of AuNPs. A JASCO J-810 circular dichroism (CD) spectropolarimeter (Tokyo, Japan) was employed to confirm the aptamer conformation. A Hitachi 600 transmission electron microscope (Hitachi, Japan) was used to obtain the high resolution transmission electron microscopy (HRTEM) image. A Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc. France) was used to record the fluorescence spectra. Meanwhile, emission spectra were recorded under excitation of 492 nm and an emission range from 500 nm to 630 nm. The excitation and emission slit widths were both set at 10 nm.

2.3. Analysis of aptamer conformation with CD

The CD spectra were recorded using an optical chamber with a path length of 1 cm and an instrument scanning speed of 200 nm/min with a time constant of 1 s. CD spectra was the accumulation of three scans from 240 to 340 nm.

2.4. Synthesis of AuNPs

All glassware were thoroughly soaked in freshly prepared nitrohydrochloric acid solution, rinsed with triple distilled water, and fully dried prior to use. AuNPs were prepared by the reduction of chloroauric acid by the classical sodium citrate approach (Frens, 1973; Grabar, Freeman, Hommer, & Natan, 1995). In brief, 5 mL of 1% trisodium citrate solution was rapidly injected into 50 mL of 1% $HAuCl_4{\cdot}4H_2O$ solution under stirring and boiling conditions. After boiling for 15 min, heat was removed to allow the reaction solution to cool to room temperature. Then, the solution was centrifuged at 10,000 rpm for 20 min, the supernatant was discarded, and the precipitation was resuspended in ultrapure water and stored at 4 °C prior to use.

2.5. Characterization of AuNPs

The formation of AuNPs was monitored using a UV–vis spectrophotometer in a range of 350–800 nm with a resolution of 1 nm. The morphology, size, and shape of AuNPs were analyzed by HRTEM. The HRTEM image was obtained using a conventional transmission mode at an accelerating voltage of 200 kV.

2.6. Fluorescent detection of OTA

A routine for OTA quantitative measurement is as follows: 200 μL of 200 nM FAM-modified aptamer solutions and 400 μL of OTA solution of different concentrations were mixed and incubated for 30 min. Subsequently, 200 μL of Au@PVP was introduced to obtain final volumes of 800 μL and incubated for 2 h at room temperature. Then, the fluorescence spectra was measured.

2.7. Application

Ginger, which is easily contaminated by OTA (Thirumala-Devi et al., 2001), was selected for actual sample testing. Aliquots (1 g) of non-contaminated ginger powder bought from a supermarket were spiked with different concentrations of OTA and mixed in a vortex mixer. After 10 mL of extraction solvent [methanol:water, 6:4 (v/v)] was added, the samples were mixed with an orbital shaker for 30 min at room temperature and then centrifuged at 6000*g* for 10 min. The supernatant was passed through a 0.45 mm syringe filter and diluted with Tris buffer (1:10, v/v) for recovery studies. For statistical analyses, four separate samples with different concentrations of OTA (10, 50, 100, and 500 nM) were prepared and each concentration level was analyzed in three replicates.

3. Results and discussion

3.1. Assay strategy

This method is based on the interaction of OTA aptamer and AuNPs. As shown in Scheme 1, in the absence of OTA, the FAM-modified OTA aptamer exists in a random coil and is easily adsorbed on the surface of the AuNPs. Then, FAM fluorescence was quenched by the FRET effect from dye to AuNPs. When OTA is present in the system, the specific binding between FAM-modified aptamer and OTA lead to the formation of the antiparallel G-quadruplex structure, which can resist the adsorption of AuNPs; thus, the fluorescent signal remained. Based on this principle, we can quantitatively detect OTA.