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A highly sensitive aptasensor for OTA detection based on hybridization chain reaction and fluorescent perylene probe

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ABSTRACT

An optical aptasensor was developed for ultrasensitive detection of ochratoxin A (OTA) based on hybridization chain reaction (HCR) amplification strategy and fluorescent perylene probe (PAPDI)/DNA composites. Dendritic DNA concatamers were synthesized by HCR strategy and modified on magnetic nanoparticles through aptamer as medium. A large amount of PAPDI probe aggregated under the induction of DNA concatamers and caused fluorescence quenching. In the presence of OTA, the PAPDI/DNA composites were released from magnetic nanoparticles due to the strong affinity between aptamer and OTA. In ethanol, PAPDI monomers disaggregated and produced strong fluorescence. The present method displays excellent sensitivity and selectivity towards OTA.

1. Introduction

Ochratoxin A (OTA) has been reported as one of the most toxic and cancerigenic substances to a wide variety of mammalian species (Duarte et al., 2010a). However, it was found that a series of food products (including cereals, wheat, barley, beans, corn, coffee and wine) were susceptible to OTA contamination (Duarte et al., 2010b). OTA contamination in food samples would not cause any observable physical damages (decay, mildew etc.) at the start of the infection. Thus it was hard to diagnose OTA at the initial stage of infection to avoid further damage (McKeague et al., 2014). It is essential to develop a sensitive method for low level OTA detection.

Recently aptamer-based detection methods have been rapidly developed due to their noticeable merits such as high binding affinity, ease labeling, remarkable target diversity, convenient automated-synthesis and high stability (Jiang et al., 2014). A variety of colorimetric, electrochemical, fluorescent, and surface plasmon resonance biosensors with high sensitivity (Lee et al., 2014; Rivas et al., 2015; Wang et al., 2015b; Zhu et al., 2015) have been developed for the detection of low concentration of OTA.

Furthermore, a series of signal amplification strategies have been developed to realize ultrasensitive and trace determination of OTA in foods and drinks (Hun et al., 2013; Jiang et al. 2013). The most common strategy to amplify the signals in mycotoxin detection is using catalysts (such as enzyme). For example, rolling-circle amplification (RCA) is an isothermal nucleic acid amplification process that synthesized a long single-stranded nucleic acid product based on DNA polymerase and a circular, single-stranded nucleic acid template (Huang et al., 2013). The electrochemical aptasensor based on RCA displayed super high sensitivity with the limit of detection of 0.065 pg/mL for OTA detection (Huang et al., 2013); Another amplification technique depending on exonucleases catalysed the stepwise removal of mononucleotides from the 3′-end or 5′-end of single-stranded or double-stranded DNA. Yang et al. (2014) constructed OTA aptasensor based on exonuclease-catalysed target recycling amplification. OTA could be sensitively quantified by the recovery of the quantum dot electrochemiluminescence (ECL) with a detection limit of 0.64 pg/mL by this method; Nicking endonuclease, which is an enzyme cutting one strand of a dsDNA at a restriction site, could also be utilized in OTA detection. The OTA aptasensor based on target-induced strand release coupling cleavage of nicking endonuclease was developed by Hun et al. (2013). In this design, DNA polymerase and nicking endonuclease were utilized to extend and cleave DNA strand and produced an amount of targeted ssDNA; Additionally, loop-mediated isothermal amplification (LAMP), as an important nucleic acid amplification technique, has been designed for electrochemically aptasensing of OTA too (Xie et al., 2014). In their design, aptamers were utilized as the forward outer primer to trigger the LAMP reaction, and DNA polymerase was used to catalyse the extension

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of DNA and strand displacement reaction. This strategy displayed excellent sensitivity and selectivity with a detection limit of 0.3 pM.

In comparison with above amplification strategies, Hybridization Chain Reaction (HCR) method is the most attractive among them due to its enzyme-free process and kinetics-controlled reaction (Zhao et al., 2015). In HCR, long double-strand DNA structure was constructed by hybridization of different oligonucleotides. HCR amplification strategy has been widely used in the detection of DNA, proteins, biothiols, metal ions, virus, cancer cells, and endonuclease activities (Yang et al., 2015; Ge et al., 2014). However, OTA detection using HCR amplification strategy has not been fully investigated (Wang et al., 2015a) yet. Further optimization and improvement are still required for low level OTA detection.

Perylene tetracarboxylic acid diimide (PDI) derivatives have been reported as fluorescence probe due to their high fluorescence quantum yield and photostability (Würthner, 2004; Wang and Yu, 2010). It has been proved that PDI derivatives were remarkable fluorescent probe for optical sensor (Wang et al., 2014; Li et al., 2015; Wang et al., 2011). In this work, a highly sensitive fluorescent aptasensor for OTA determination was designed based on branching-growth of HCR strategy and the fluorescent perylene probe. In this design, positively charged perylene derivative, [(N,N′-bis(propylenetrimethylammonium)-3,4,9,10-perylenediimide), PAPDI] was synthesized and served as fluorescent probe, while dendritic DNA concatamers synthesized by HCR strategy were utilized to induce the aggregation of PDI probe and further amplify the detection signal. OTA could be detected when it was bound with aptamer and released the DNA/PAPDI composite to produce strong fluorescent signals. This strategy was optimized to achieve highly sensitive detection of OTA and verified in the corn sample for its effectiveness for low level OTA detection in practical applications.

2. Experimental

2.1. Chemicals and apparatus

Oligonucleotides used in this study were synthesized and purified with ultra PAGE by Sangon Biotechnology Co., Ltd. (Shanghai, China). The oligonucleotides stock solution was prepared by dissolving the oligonucleotide in 10 mM Tris–HCl buffer (pH 8.4, 200 mM NaCl) and was stored at 4 °C before use.

Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), aflatoxin A1 (AFB1), and ochratoxin A (OTA) were purchased from Sigma-Aldrich Chemical Co. (USA). Ochratoxin B (OTB), Zearalenone, and Fumidil were purchased from Leon Technology Co. Ltd. (Beijing). All these mycotoxins including OTA, OTB, AFB1, Zearalenone, and Fumidil were dissolved in 80% methanol for further use. Amino-modified magnetic nanoparticles (AMNPs) were purchased from the Aladdin Reagent Co., Ltd. (Shanghai, China). 3,4,9,10-Perylenetetracarboxylic dianhydride (PDI), N,N′-dimethyl-1,3-propanediamine, methyl iodide and tri (hydroxymethyl) amino methane hydrochloride (Tris–HCl) were obtained from Shanghai Chemical Reagent Co.

Perylene probe [(N,N′-bis(propylenetrimethylammonium)-3,4,9,10-perylenediimide), PAPDI] was synthesized according to our previous report (Wang and Yu, 2010). All other chemicals were of analytical grade. Deionized water (18.2 MΩ cm at 25 °C) was used throughout the experiments.

2.2. Apparatus

All fluorescence measurements were performed on a Shimadzu RF-5301PC spectro-fluorometer equipped with a 150 W xenon lamp as the excitation source (Ushio Inc., Japan). The Zeta potential measurement was carried out at 25 °C (Zetasizer Nano 90, Malvern), and the surface charge of nanoparticles was determined in 100 mM Tris–HCl (pH 7.4, containing 200 mM NaCl). Scanning electron microscopy (SEM) images were taken by JSM-7800F (Japan).

2.3. Preparation of aptamer modified magnetic nanoparticles

The AMNPs were functionalized with the OTA aptamer according to literature (Hua et al., 2013). Briefly, 10 nmol carboxyl-modified OTA aptamer was dissolved in 100 μL of MES buffer solution (10 mM, pH 5.2). Then, 50 μL of 2 mM EDC and 50 μL of 2 mM NHS in pH 5.2 MES buffer were mixed with the above aptamer solution and incubated for 30 min to activate the carboxyl group. After that, the mixture was injected into 500 μL of 5 mg/mL AMNPs (100 mM Tris–HCl, pH 8.0) and incubated overnight at room temperature with gentle shaking. Then, the aptamer-modified AMNPs were washed three times with TT buffer (100 mM Tris–HCl, Tween-20 0.01%, pH 7.4). The obtained product was dissolved in 1 mL of Tris–HCl (100 mM, pH 7.4) and stored at 4 °C before use.

2.4. Fluorescence detection of OTA

Typically, 10 μL of the as-prepared aptamer-AMNPs suspension was mixed with 100 μL of TS buffer (containing 100 mM, pH 7.4, Tris–HCl and 200 mM NaCl). Then, 2 μL of 10 μM DNA1, DNA2, DNA3, DNA4, DNA5, and DNA6 were added consecutively. The final mixture was incubated at 85 °C for 10 min, naturally cooled to room temperature, and incubated at room temperature for another 30 min. Subsequently, the obtained mixture was magnetically separated and washed with 100 μL of TS buffer solution for three times, and dispersed into 100 μL of TS buffer solution. PAPDI (2 μL, 100 μM) was mixed with the above suspension, followed by the magnetic separation and washed for 3 times using the TS buffer solution. The obtained deposit was dissolved in 100 μL of TS buffer. 10 μL prepared PAPDI/DNA/ AMNPs suspension was taken and mixed with 20 μL Tris–HCl (400 mM, pH 7.4) buffer solution, 10 μL 1 M NaCl solution, and 10 μL OTA with different concentrations (the final concentration of methanol was 16%). After incubation at room temperature for 1 h, the dispersion was magnetically separated. Finally, the supernatant was diluted with 400 μL of ethanol. The fluorescence spectra were recorded on a fluorescence spectrometer and standard curve was calculated based on detection results. The same procedure was performed to estimate the selectivity of the method by replacing OTA with other mycotoxins.

2.5. Assay of gel electrophoresis

In order to verify the hybridization of DNA on AMNPs, polyacrylamide gel electrophoresis (PAGE) was performed. Firstly, different concentrations of OTA (0, 20 pM, and 1.0 nM) were incubated with DNA/aptamer/AMNPs nanocomposites for 2 h at room temperature. The released DNA concatamers were used for Gel Electrophoresis analysis. A 12.0% native polyacrylamide gel was prepared by using 1 × TBE buffer (100 mM Tris–HCl, 83 mM boric acid, 1 mM EDTA, pH 8.0). The gel was run at 100 V for 60 min with the loading of 5 μL of each sample into the lanes at room temperature. After staining with Goldview Nucleic Acid Gel Stain (Biotium, USA) for 30 min, the gels were scanned using a Gel Imaging System (Bio-Rad, USA).