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Label-free aptasensor for ochratoxin A detection using SYBR Gold as a probe

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ABSTRACT

A label-free sensing strategy employing aptamers, SYBR Gold, and exonuclease I (Exo I) for ochratoxin A (OTA) detection was designed. In the presence of target molecules (OTA), the conformation of the aptamer specific for OTA is switched from a random coil to an antiparallel G-quadruplex. Subsequently, Exo I is added into the mixture to digest the unfolded aptamers selectively, which are the preferred substrates of Exo I. Following the addition of SYBR Gold as probe, a strong fluorescence intensity is obtained. This aptasensor shows high selectivity toward OTA with a low limit of detection (16.5 nM). The validity of the procedure and applicability of the aptasensor are successfully assessed through the detection of OTA in spiked red wine and beer without interference from the sample matrix. Utilization of the proposed biosensor for quantitative determination of mycotoxins in food samples indicates its usefulness as a tool for verifying the effectiveness of mycotoxin control strategies.

1. Introduction

Mycotoxins are toxic metabolites produced by fungi, mostly by saprophytic molds growing on various foodstuffs, including that of animal feeds, and by many plant pathogens [1]. Ochratoxin A (OTA) is one of the mycotoxins (a natural, toxic secondary metabolite) produced by several species of the fungi *Penicillium* and *Aspergillus*. OTA has been widely detected in cereal-derived products, dried fruits, spices, beer, and wine. OTA is potentially carcinogenic to human beings. This mycotoxin is also weakly mutagenic and can cause immunosuppression and immunotoxicity [2]. To avoid the risk of OTA consumption, the detection and quantification of OTA level in contaminated raw materials are considerably useful. Various analytical methods have been established for the determination of OTA. OTA analysis is typically performed via conventional chromatographic methods, such as thin layer chromatography, high-performance liquid chromatography (HPLC), or gas chromatography coupled to ultraviolet visible, fluorescence, or mass spectrometry [3–8]. However, these methods generally require multiple steps, including extraction, extensive sample cleaning, preconcentration, and analyte derivatization, prior to detection. Such sample treatment not only makes the analysis time consuming and costly but also requires trained personnel. Immunoassays provide a simple and economical alternative to instrumental methods for OTA analysis. A common alternative for OTA detection is the immunoassay based on antigen–antibody interactions, such as enzyme-linked immunosorbent assay (ELISA). Several groups have previously established immunoassays for OTA [9–13]. Nevertheless, ELISA is a heterogeneous method and involves multiple washing steps. In addition, the storage and application conditions of the antibody, such as temperature, pH, and ionic strength, are strictly defined. All these conditions have limited the ability of the existing antibody-based methods to satisfy the current detection requirements.

Aptamers are single-stranded DNA or RNA molecules that can bind specifically to their targets and selected via an in vitro process called systematic evolution of ligands by exponential enrichment [14]. Aptamers have been considered a good candidate alternatives to replace antibodies. Furthermore, aptamers can be selected for a broad range of targets, including small molecules, proteins, nucleic acids, cells, tissues, and organisms. The binding ability of aptamers is as good as that of antibodies, but their synthesis, maintenance, and delivery are easier. Thus, aptamers are promising molecular receptors for bioanalytical applications [15,16].

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Recently, a series of assays using aptamers against OTA have been developed. These assays mainly involved fluorescence [17,18], electrochemical [19,20], and colorimetric transducers [21,22], etc. Among these assays, electrode processing is troublesome for the electrochemical method, and the sample color may interfere with the test results for the colorimetric method. Fluorescence methods have received increasing attention because of their distinct advantages, such as high sensitivity, rapid analysis, and little damage to sample [23–27]. Biosensing via fluorescence is one of the well known methods for the design of aptamer-based assays, largely because of the ease of detection, good sensitivity, and potential for high-throughput analysis. However, frequently in these assays, the aptamer needs to be labeled with fluorophores. The process of DNA modification is laborious and expensive. [28–30]. Moreover, these fluorescent and/or quenching molecules may even alter the binding properties of aptamers [31–33]. In addition, the majority of current labeled dyes, particularly near-infrared dyes, suffer from poor photostability and low emission intensities, ultimately limiting the further improvement of fluorescence methods [34,35]. Therefore, the development of label-free methods for fluorescent detection is highly desirable.

A few label-free fluorescent aptasensors for OTA detection have been reported. For example, Zhang et al. have reported a fluorescent aptasensor based on Tb^{3+} and structure-switching aptamer for label-free detection of ochratoxin A [36]. However, the Tb^{3+} is poisonous, and the performance is tedious. Lv et al. have reported a simple and sensitive label-free fluorescent aptasensor for ochratoxin A detection [37]. However, the sensing systems are in turn-off mode, and such turn-off assays might compromise specificity because other quenchers or environmental stimuli might lead to fluorescence quenching, thereby leading to "false positive" results. Considering these problems, we designed a label-free turn-on fluorescent aptasensor for OTA detection based on exonuclease I (Exo I) enzyme and SYBR Gold dye. SYBR Gold was applied as fluorescent probe because of its high sensitivity and specificity for nucleic acids, as well as its low toxicity and good stability [38]. The aptamer specific for OTA can fold to form antiparallel G-quadruplex structure upon exposure to OTA [39]. The formation of antiparallel G-quadruplex structure is resistant to Exo I digestion. The amount of aptamer left after nuclease reaction is proportional to OTA concentrations, thereby making it proportional to the fluorescence intensities from SYBR Gold that can only stain nucleic acids but not their digestion products, nucleoside monophosphates. The results of our experiment also confirmed that our design strategy was very successful. The major advantage of combining Exo I enzyme and SYBR Gold with aptamer is the elimination of the need for DNA modification, thereby significantly reducing the cost. This fluorometric method is low cost, highly sensitivity, and involves a simple performance. The elements used in the assays are cheap and commercially available. Furthermore, the method is a nearly universal one because of the exceptional structure selectivity of Exo I. The principle can be extended to the detection of other targets, such as ions, small molecules, and proteins.

2. Materials and methods

2.1. Materials

The OTA aptamers (5′-GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA-3′) [40] were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The fluorescent dye SYBR Gold (10 000× concentrated) was purchased from Invitrogen (CA, USA). Exo I was bought from TaKaRa Biotechnology Inc. (Dalian, China). OTA, ochratoxin B (OTB), ochratoxin C (OTC), aflatoxin B1 (AFB1), and zearalenone (ZEN) were purchased from Fermentek (Jerusalem, Israel). Poly (allylamine) hydrochloride (PAH), poly (diallyldimethylammonium chloride) (PDDA) were purchased from Sigma Aldrich (Shanghai, China). Binding buffer (10 mM Tris, pH 8.5, 120 mM NaCl, 5 mM KCl, 10 mM $MgCl_2$, and 20 mM $CaCl_2$) was used for the binding reaction between the aptamer and OTA. The OTA stock solution (1 mM) was prepared by dissolving ochratoxin in absolute ethanol and stored at −20 °C. Changyu Rose Red Wine was produced by YanTai ChangYu Pioneer Wine Company Limited (Yantai, China). Tsingtao beer was produced by Tsingtao beer Company Limited (Tsingtao, China). All other chemicals were of analytical grade and used as received without further purification. Ultrapure water with an electrical resistivity of 18.2 MΩ cm was obtained from a Milli-Q ultra-high-purity water system (Millipore, Bedford, MA, USA).

2.2. Instrumentation

Cary 500 Scan UV–vis Spectrophotometer (Varian, USA) was used to quantify the oligonucleotides. An Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a fluorescence detector was used to record HPLC chromatograms. A JASCO J-810 spectropolarimeter (Tokyo, Japan) was used to collect circular dichroism (CD) spectra in 10 mM Tris buffer (pH 8.5). A RF-5301PC fluorescence spectrophotometer (Shimadzu, Tokyo, Japan) was used to record the fluorescence spectra with a 150 W Xenon lamp as the excitation source. Meanwhile, emission spectra were recorded within the wavelength range of 480–620 nm upon excitation at 495 nm. Slit widths for excitation and emission were set at 3 nm. All measurements were performed at room temperature unless stated otherwise.

2.3. Analysis of aptamer conformation with CD

CD spectropolarimeter using solutions of DNA aptamer (1 μM) in an optical chamber (1 cm path length, 1 mL volume), which was deoxygenated with dry purified nitrogen (99.99%) before use and kept in nitrogen atmosphere during experiments. Each CD spectrum was the accumulation of three scans at 200 nm/min with a 1 nm band width and a time constant of 1 s. The data were collected within 230–340 nm at 0.1 nm intervals. The background of the buffer solution was subtracted from CD data.

2.4. Optimizing Exo I concentration

Increasing concentrations of Exo I (0–10 units) were added to a constant concentration of ssDNA aptamer (100 nM) in binding buffer (final volume, 400 μL). After 30 min of incubation at 37 °C, 100 μL of 10 × SYBR Gold was mixed with the samples. After equilibrating the solution in the dark at room temperature for another 15 min, fluorescence intensity was measured.

2.5. Optimization of incubation time of Exo I with ssDNA aptamer on fluorescence intensities

5 units of Exo I were added to 100 nM ssDNA aptamer in binding buffer (final volume, 400 μL). The mixture was incubated at 37 °C for 0–40 min. Subsequently, 100 μL of 10 × SYBR Gold was added to the samples, and fluorescence intensities were recorded.

2.6. Fluorescent detection of OTA

For quantitative measurement of OTA, 200 μL of solution containing 250 nM of aptamer was mixed with buffer solution of different OTA concentrations and allowed to settle for 15 min. Subsequently, 5 units of Exo I were added, and the total final volume