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An aptamer based lateral flow strip for on-site rapid detection of ochratoxin A in *Astragalus membranaceus*

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ABSTRACT

An aptamer based lateral flow strip based on competitive format was developed for on-site rapid detection of ochratoxin A (OTA) in *Astragalus membranaceus*. Some crucial parameters that might influence the sensitive detection, such as the characterization of the colloidal gold, size and shape of gold nanoparticles (AuNPs), amount of AuNPs-aptamer conjugate, migration rate and the addition amount of methanol, were investigated to provide the optimum assay performance. To perform the test, 1 g sample was extracted with 2.5 mL of methanol-water (80:20, *v/v*) and diluted by 4-fold running buffer to eliminate the matrix and methanol interferences. Under optimized conditions, the aptamer-based assay showed a visual limit of detection (LOD) of 1 ng mL^{-1}, and with no significant cross-reactivity with several homologous toxins. The whole detection could be completed within 15 min without special equipment because of available visual results. One out of nine *A. membranaceus* samples was found to be positive of OTA, which was in a good agreement with those obtained from LC–MS/MS analysis. The results demonstrated that the aptamer-based lateral flow assay could be used as a rapid, reliable, cost-effective and robust on-site screening technique for mycotoxins at trace level in complex matrices without special instrumentation.

1. Introduction

Astragalus membranaceus (Huangqi in Chinese) as a well-known traditional Chinese medicine (TCM) and dietary supplement has been widely used in clinic for promoting health over 2000 years [1]. However, the safety related to its quality has settled off alarms since contaminated studies have been frequently reported on this TCM [2–4]. Similar with other herbs, *A. membranaceus* is susceptible to contaminants such as mycotoxins during the processes of planting, harvesting, processing, transportation and storage posing potential risks to human health [5]. Among the mycotoxins, ochratoxins (OTA) [6], which is derived from the species of fungi including *Penicillium verrucosum*, *Aspergillus ochraceus*, *Aspergillus carbonarius* and *Aspergillus niger* [7], presents nephrotoxic, hepatotoxic, teratogenic and immunotoxic properties as to be classified as possibly carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer [8]. Owing to its chemical stability to heat treatments and hydrolysis during food processing [9], OTA may occur in a large variety of foods, spices, and TCMs because of a carry-over effect [10]. Since the common occurrence and extreme toxicity, most countries and organizations have set guidelines and recommendations for the maximum residue levels (MRLs) of 5 μg kg^{-1} acceptable in cereals for OTA by the World Health Organization and the European Union [11], and 10 μg kg^{-1} by China [12]. The determination of mycotoxins in TCMs has been reported frequently, such as rapid analysis and identification of multi-class mycotoxins in *Morinda officinalis* [13] and detection of OTA in ginger [14]. But, until now, the detection of OTA in *A. membranaceus* has not been reported. Taking the above-mentioned facts into consideration, developing a reliable method for sensitive and accurate detection of OTA in *A. membranaceus* is in urgent demand.

The instrument-based methods such as high performance liquid chromatography-fluorescence detector (HPLC-FLD) and liquid chromatography tandem mass spectrometry (LC–MS/MS) are widely accepted as reference methods with good accuracy and reproducibility [15,16]. However, these instrumental methods require expensive equipments, trained personnel as well as costly and time-consuming pretreatments, which are unsuitable for

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getting high throughput on-site detection. Alternatively, screening approaches such as enzyme-linked immunosorbent assay (ELISA), immunochromatographic assay (ICGA) and electrochemical biosensors [17] are in increasing application owing to their rapidity and simplicity [18–20]. However, it should be noted that these screening methods heavily relies on antibody, which may encounter some drawbacks due to the difficulty of production, limited stability, and the complexity of modification of suitable antibody. So, searching for succedaneum for antibody is imperative.

Aptamer has been provided as a good alternative to antibody because of its high specificity, easy and reproducible production and liable modification. A couple of aptamer based assays, such as electrochemical method [21,22], enzyme-linked aptamer assays [23] and structure-switch signaling aptamer [24], have been developed as a promising tool for OTA detection. However, these methods usually take long time owing to multiple incubation and complicated separation steps, which limits their widespread application. Lateral flow strip assay regarded as the one-step approach has attracted much attention in on-site detection of multiple analytes. Recently, most lateral-flow immunoassay methods have been reported in corn or feedstuff samples [25], and only an aptamer-based chromatographic strip assay was reported for the detection of OTA in red wine [26]. In view of the fact of high occurrence of OTA in TCMs and few references regarding lateral flow strip, it is necessary to develop an aptamer based lateral flow strip for on-site rapid detection of OTA in real TCM samples.

In the present work, a specific gold nanoparticles (AuNPs) and aptamer detecting-probe for the lateral flow strip were constructed. Owing to the advantages derived from AuNPs and aptamers, a visible, rapid and one-step lateral flow assay was established for screening of OTA in real *A. membranaceus* from different sources in China. The detection performances were validated to be accurate enough to allow the trace determination of OTA. The positive results were further confirmed by LC–MS/MS. The results have indicated the aptamer based lateral flow assay as a reliable, on-site and rapid tool for OTA screening in real complex TCM samples.

2. Experimental

2.1. Materials and reagents

Ochratoxin A (OTA), aflatoxin B_1 (AFB_1), zearalenone (ZEN), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), T-2, nivalenol (NIV) and deoxynivalenol (DON) were purchased from SUPELCO (Bellafonte, PA, USA). $HAuCl_4{\cdot}3H_2O$, Tris (2-carboxyethyl) phosphine (TCEP), bovine serum albumin (BSA), ovalbumin (OVA), DTT and streptavidin were provided by Sigma-Aldrich (St. Louis, MO, USA). Sodium hydrogen phosphate (Na_2HPO_4), monobasic potassium (KH_2PO_4), sodium chloride (NaCl), potassium chloride (KCl) and other reagents were from Beijing Chemical works (Beijing, China). Ethyl acetate (HPLC grade) was purchased from Fisher Scientific (Fisher Scientific, Fair Lawn, NJ, USA). Water was obtained from a Milli Q purification system (Millipore). All other inorganic chemicals and organic solvents were of analytical grade or better. Cellulose fibre, glass fibre, nitrocellulose (NC) membranes (Sartorius CN140, Sartorius CN95, Millipore135, Vivid 90, and Vivid 170, 20×2.5 cm), absorbent paper, Polyvinyl chloride (PVC) sheets were purchased from Jieyi Biotech Shanghai, Co., Ltd. (Shanghai, China).

Thiol-modified aptamer (5′-GAT CGG GTG TGG GTG GCC TAA AGG GAG CAT CGG ACA TTT TTT TTT TTT TTT TTT TT-SH-3′) and two complementary biotin-modified DNA probes (No. 1, 5′-CTC CCT TTA CGC CAC CCA CAC CCG ATC-Biotin-3′; No. 2, 5′-AAA AAA AAA AAA AAA AAAAA-Biotin-3′) were manufactured from Sangon Biotech Co., Ltd. (Shanghai, China).

2.2. Preparation of gold nanoparticles

AuNPs were obtained with an average particle diameter of 10 nm according to the previous report [27] with some modifications. Briefly, 2.5 mL of 1% aqueous sodium citrate solution was mixed with 100 mL of boiling water. After constant stirring, 1 mL of 1% $HAuCl_4{\cdot}3H_2O$ was added rapidly into the mixture followed. The color of the solution changed from gray to purple and finally turned to wine-red, then the solution was kept boiling for another 12 min, and gradually cooled to room temperature. The prepared AuNPs were protected from light and stored at 4 °C, and the morphology was characterized by using a UV–vis spectrometer

2.3. Preparation of AuNPs-aptamer conjugate

The AuNPs-aptamer conjugate was accomplished via gold-sulfur coordination reaction between AuNPs and the thiolated DNA strands. Briefly, prior to the conjugation, the thiolated aptamer was activated by adding 3 μL of 5 mg mL^{-1} TCEP into 3 μL of 100 μM SH-aptamer, the mixture was incubated for 2 h at 4 °C. 5 mL AuNPs solution was adjusted to pH 8.5 with 0.2 M K_2CO_3 and concentrated by centrifugation to 1 mL. Subsequently, the concentrated AuNPs solution was mixed with the activated aptamer. The freshly prepared mixture was left at room temperature overnight, and aged by adding salt solution, and the salt-aging process was repeated until a final concentration of 160 mM NaCl was reached. To remove excess SH-aptamer and AuNPs, ultra-centrifugal filters were used and the precipitate was re-suspended in 0.01 M PBS (pH 7.4) containing 0.5% PEG, 2% sucrose, 0.1% Tween 20, 0.02% $MgSO_4$, 0.05% $(NH_4)_2SO_4$, and 1% OVA. To determine the number of aptamer loaded on the gold nanoparticles, the aptamer was chemically displaced from the nanoparticle surface using DTT, and the number of aptamer per particle for each aliquot was calculated through dividing the concentration of fluorescent aptamer by the concentration of nanoparticles [28]. The stability of AuNPs-aptamer conjugate was compared with the unlabelled AuNPs to prevent the aggregation induced by salt ions from 170 mM NaCl [29].

2.4. Preparation of test and control zones

To immobilize capture and control probes on a NC membrane, streptavidin was used as an intermediate to react with the biotinylated DNA probe 1 and probe 2 to form the streptavidin-biotin DNA conjugate [30]. Primarily, for the test zone, 35 μL of 10 μM DNA probe1 was mixed with 35 μL of 1 mg mL^{-1} streptavidin and incubated for 2 h at 4 °C, then 30 μL of PBS was added into the mixture. The control zone was similarly constructed as the above-described procedure except for DNA probe2 instead of DNA probe1. The prepared capture and control probes were immobilized on the test zone and control zone for the following analysis, respectively.

2.5. Assembly of the aptamer-based strip

Commonly, the aptamer-based strip is comprised of a sample pad (cellulose fibre), a conjugate pad (glass fibre), NC membrane, and an absorbent pad on a backing layer. The sample pad was previously saturated with PBS solution containing 1% OVA and dried at 37 °C and kept under dry conditions. The conjugate pad was pretreated with PBS solution containing 1% OVA, 0.25% Tween 20, 2% sucrose, and 0.02% sodium azide and dried at 65 °C, and was cut into sections (4×0.5 cm), followed by the AuNPs-aptamer conjugate pipette and dried at 37 °C, and stored under dry conditions. The NC membrane was attached with capture and control probes on the test and control zones, respectively, and dried for 2 h at 37 °C. The distance between the two lines was settled at 5 mm. Finally, followed by fabricating the NC membrane on a plastic adhesive