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Analytical Methods

Development and optimization of a multiplex lateral flow immunoassay for the simultaneous determination of three mycotoxins in corn, rice and peanut

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ABSTRACT

A multiplex lateral flow immunoassay (LFA) is developed for the simultaneous on-site determination of three mycotoxins (aflatoxin B_1, zearalenone and ochratoxin A) in corn, rice and peanut. By systematically optimizing the preparation of antibody-gold nanoparticle conjugates, the size of gold nanoparticle and the position of capture antigen, the developed LFA can obtain a visual detection limit of 10 μg/kg for aflatoxin B_1, 50 μg/kg for zearalenone and 15 μg/kg for ochratoxin A. For quantitative analysis, the limits of detection were 0.10–0.13 μg/kg for aflatoxin B_1, 0.42–0.46 μg/kg for zearalenone, and 0.19–0.24 μg/kg for ochratoxin A, which were far below the regulatory limits set by the European Commission. At the spiked concentrations of 0.5–10.0 μg/kg, the mean recoveries of the three mycotoxins ranged from 86.2 to 114.5% with coefficients of variation less than 16.7%. These results demonstrated that the developed immunoassay can be used for routine monitoring of mycotoxin contamination.

1. Introduction

Mycotoxin is a kind of toxic metabolites produced by fungi and it extensively exists in agricultural product (Anater et al., 2016; Brase, Encinas, Keck, & Nising, 2009). Because of the potential toxic effects such as carcinogenicity, mutagenicity, neurotoxicity, immunosuppressive and estrogenic effects (Brase et al., 2009; Rocha, Freire, Erlan, Izabel, & Rondina, 2014), mycotoxin can pose serious hazard to human and animal health. Surveillance survey indicated that mycotoxin contamination has been a global problem. It is estimated that approximately 25% of the world's crop production is contaminated with mycotoxin (Choudhary & Kumari, 2010), which can result in the reduction of livestock production, the increase of health care and regulatory costs, and thus will lead to tremendous economic losses. For protecting the health of consumers, maximum tolerable and guideline levels of mycotoxin have been established by many countries and areas (European Commission, 2006; Food and Drug Administration, US, 2011; Ministry of Health, PRC, 2011).

The analytical approaches for mycotoxin detection can be broadly divided into two categories: instrumental analysis and bioanalytical method (Anfossi, Baggiani, Giovannoli, D'Arco, & Giraudi, 2013; Turner, Subrahmanyam, & Piletsky, 2015). Instrumental methods mainly comprise liquid chromatography (Wang et al., 2008) and LC coupled with mass spectrometry (Hickert et al., 2015; Liao et al., 2015). These methods have high accuracy and precision, and are usually used for quantitative and qualitative analysis. However, they need expensive equipment and skilled personnel, and also require sophisticated sample preparation which often consumes hours and even days (Maragos & Busman, 2010). Thus, instrumental method is not suitable for the application in normal laboratory and field environment. The requirement for timely monitoring mycotoxin contamination in food industry has demanded more rapid and cost-effective methods, therefore, bioanalytical method especiallyimmunoassay has been developed for mycotoxin detection (Beloglazova et al., 2014; Li et al., 2013; Soleri et al., 2015; Song et al., 2014).

Lateral flow immunoassay (LFA) is one of the most widely used immunoassay because of its low-cost, easy-to-use and rapidity

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(Anfossi et al., 2013). However, routine LFA can only detect one target molecule at one time. By comparison, the multiplexing format of LFA can simultaneously detect several target chemicals, thus it can further reduce operating cost and improve detection efficiency (Li et al., 2013; Song et al., 2014). As aflatoxin B_1 (AFB_1), zearalenone (ZEA) and ochratoxin A (OTA) frequently occurred in foodstuff (Bennett & Klich, 2003), they were selected as target chemicals in the development of a multiplex LFA in this study. In previous report, Li et al. (2013) have developed a multi-component immunochromatographic assay for qualitative analysis of the three mycotoxins in agro-food. However, only qualitative analysis was achieved and their assay sensitivities needed further improvement. In this study, we presented both qualitative and quantitative analysis of the three mycotoxins in corn, rice and peanut with higher assay sensitivities.

For the development of a LFA method, the major challenge is to achieve high assay sensitivity while maintaining its rapidity. Hence, for acquiring satisfactory assay sensitivity, the optimization of immune-parameters is extremely important. Among the immune-parameters, the preparation of antibody-gold nanoparticle (Ab-GNP) conjugate is the most important factor since it directly relates with assay sensitivity. Flocculation test has been widely used to optimize the preparation of Ab-GNPs (Paek, Lee, Cho, & Kim, 2000; Xie et al., 2003; Ye et al., 2010), whereas it was originally designed to stabilize Ab-GNPs conjugate (Horrisberger, Rosset, & Bauer, 1975), not to pursue high assay sensitivity. Thus, if the flocculation test is suitable for optimizing assay sensitivity of competitive LFA still remained questionable. In addition, the effects of the size of gold nanoparticle and the test line position also remained unexplored. Therefore, we systematically investigated the effects of these three important immune-parameters on assay sensitivity of the developed LFA in this study.

2. Material and methods

2.1. Reagents and equipments

Chemical standards of AFB_1, ZEA and OTA, bovine serum albumin (BSA), and chloroauric acid were purchased from Sigma-Aldrich (St. Louis, MO). Goat anti-mouse IgG, Tween 20 and Triton X-100 were obtained from Thermo Fisher Scientific (West Palm Beach, FL). Purified water was prepared using a Milli-Q water purification system (Millipore, Bedford, MA). Other reagents were purchased from Beijing Regent Corporation (Beijing, China). Nitrocellulose (NC) membrane HF 180 was purchased from Millipore Company (Millipore, Bedford, MA). Glass fiber membrane SB 08, absorbent pad CH 37, adhesive backing card were purchased from ShangHai GoldBio Co. Ltd. (Shanghai, China). The synthesis of AFB_1-BSA, ZEA-BSA and OTA-BSA conjugates and the preparation of respective anti-mycotoxin monoclonal antibodies (mAbs) were briefly described in Supplementary material. Strip reader (i-Check I type) was provided by Clover Technology Group (Beijing).

HM 3030 Dispensing Platform and ZQ 2000 Guillotine Cutting Module used for strip assembly were purchased from ShangHai GoldBio Co. Ltd. (Shanghai, China).

2.2. Synthesis of gold nanoparticles (GNPs)

GNPs were prepared as described by Frens (1973) with some modifications. Briefly, 100 mL of 0.01% (m/v) chloroauric acid in de-ionized water was heated to boiling, then an aliquot (1.0, 1.6 or 2.5 mL) of 1.0% tri-sodium citrate (w/v) was added under constant stirring. After reaction for 15 min, the solution was left to cool, and de-ionized water was complemented to the initial volume. Supplemented with 0.05% sodium azide, the obtained GNPs is stable at 4 °C for several months. Transmission electron microscopy (TEM, JEOL USA Inc., Peabody, MA) was used to determine the particle sizes of GNPs.

2.3. Flocculation test

To determine the optimal pH value and the minimum antibody amount for stabilizing Ab-GNPs conjugate, flocculation test was performed according to literature (Paek et al., 2000; Xie et al., 2003; Ye et al., 2010) with some modifications. First, the optimal pH value was measured. Briefly, the pH values of GNPs (32 nm) were adjusted to 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 8.5 and 9.0 respectively by adding different amount of 0.1 M K_2CO_3, then each aliquot (1.0 mL) of these GNPs solution was mixed with 20 μg of respective anti-mycotoxin mAbs and incubated for 30 min. Subsequently, 100 μL of 10% NaCl (w/v) was added and mixed. After incubating for another 30 min, the absorbance of the solution was determined at 520 nm and 580 nm by UV–vis spectrophotometer (Varian Cary 50, Agilent Technologies Inc., Palo Alto, CA). Then the minimum antibody amounts were tested. The GNPs solutions were adjusted to the optimal pH values as determined above, then 0.16, 0.31, 0.63, 1.3, 2.5, 5.0, 10, 20, 40 and 80 μg of respective anti-mycotoxin mAbs in 100 μL of water were separately added into 1.0 mL of the GNPs solution and incubated for 30 min. Afterwards, 100 μL of 10% NaCl (w/v) was added and mixed. Following incubation for another 30 min, the absorbance of the solution was determined at 520 nm and 580 nm by UV–vis spectrophotometer. The differential absorbance ($A_{520\ nm}-A_{580\ nm}$) was used to evaluate the flocculation level. The optimal pH value and antibody amount to stabilize Ab-GNPs was the smallest pH value and antibody amount at which flocculation did not occur (Paek et al., 2000).

2.4. Checkerboard titration test

To measure the optimal pH value and antibody amount that can result in the best assay sensitivity, checkerboard titration test was performed. Briefly, aliquots (1.0 mL for each aliquot) of 32 nm GNPs was adjusted to pH 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 respectively by adding 0.1 M K_2CO_3, then 1.3, 2.5, 5.0, 10, 20 and 40 μg of respective anti-mycotoxin mAbs in 100 μL of water were separately added into GNPs with different pH values in the form of checkerboard, and the mixtures were incubated for 30 min. Then 100 μL of 1.0% BSA in 10 mM citrate buffer (pH 6.5) was added. After three centrifugation at 5000*g* for 10 min and re-suspended with 0.1% BSA in 10 mM citrate buffer (pH 6.5), the prepared Ab-GNPs conjugates were then applied to conjugate pad and subjected to LFA as described below. By adjusting dilution times with 0.1% BSA aqueous solution, the different Ab-GNPs conjugates were made to result in identical test line intensities. Then the cut-off value, which was defined as the lowest concentration to make test line invisible, was determined for each Ab-GNPs conjugate.

2.5. Preparation of detection reagents

The purified anti-AFB_1, anti-ZEA and anti-OTA mAbs were dialyzed against phosphate buffer saline (PBS, 10 mM, containing 0.15 M sodium chloride, pH 7.4) at 4 °C for 24 h with constant stirring. Before conjugation between antibody and GNPs, the optimal pH value and antibody concentration were determined by checkerboard titration test as described above. With gentle stirring, 10.0 mL of GNPs solution was adjusted to the optimal pH values (pH 6.5 for AFB_1, pH 7.5 for ZEA, pH 6.5 for OTA) with 0.1 M K_2CO_3, then 1.0 mL of purified mAb (0.025 mg/mLanti-AFB_1, 0.050 mg/mL anti-ZEA, and 0.025 mg/mL anti-OTA) was added. After incubation at room temperature for 60 min, 1.0 mL of 1.0%