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Ultrasensitive and eco-friendly immunoassays based monoclonal antibody for detection of deoxynivalenol in cereal and feed samples

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ABSTRACT

Ultrasensitive immunoassays, including an indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) and a lateral-flow immunochromatographic assay (ICA), were developed based on a monoclonal antibody for the analysis of deoxynivalenol in food and feed samples. With 0.01 M PBS, 20% ethanol–PBS, and 60% ethanol–PBS extraction, which are environmentally safe, the 50% inhibitory concentration (IC_{50}) and limit of detection (LOD) values were 1.83–4.68 μg/kg and 0.241–0.664 μg/kg, respectively, with recovery rates of 87.7%–137% and coefficient variation values of 3.99–9.88% (intra-assay) and 4.17–9.81% (inter-assay) for the ic-ELISA relative to the results obtained by liquid chromatography–tandem mass spectrometry (LC–MS). For the ICA strip, the visual LODs were 10–150 μg/kg, the cut-off values were 50–750 μg/kg, and the calculated LODs were 1.97–46.8 μg/kg, with different sample extraction solutions, and the recovery rates were 66.7%–127%. These methods are sensitive, simple and safe, providing an auxiliary analytical tool for screening the massive samples in markets.

1. Introduction

Trichothecenes are a large class of mycotoxins produced by *Fusarium* fungi, which fall into four main types. The type A and B trichothecenes are the main natural contaminants, of which the type B trichothecenes are the most harmful. Deoxynivalenol (DON) is the main representative of the type B trichothecenes, which also include 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), nivalenol (NIV), and c X (FX). DON occurs in almost all kinds of cereals and is highly toxic to humans and animals, inhibiting the synthesis of DNA, RNA, and proteins, and causing gastrointestinal symptoms and vomiting, with neurotoxic and immunotoxic effects (Gerez, Desto, & Bracarense, 2017; Peng, Chen, Nussler, Liu, & Yang, 2017; Wentzel, Lombard, Du Plessis, & Zandberg, 2017; Yu et al., 2017). DON is also classified as a group 3 carcinogen by the International Agency for Research on Cancer (Kim et al., 2014). To protect human health from exposure to DON, the European Union sets different limits of DON in foodstuffs, with minimum limit as 200 μg/kg in processed cereal-based foods and maximum limit as 1750 μg/kg in unprocessed maize, durum wheat and oats. China also set the limit at 1000 μg/kg in foodstuffs.

Common methods for the detection of DON include liquid chromatography–tandem mass spectrometry (Al-Taher et al., 2017), gas chromatography–mass spectrometry (Rodriguez-Carrasco, Molto, Manes, & Berrada, 2017), and liquid chromatography (Dall'Asta, Sforza, Galaverna, Dossena, & Marchelli, 2004). Although these chromatographic methods are sensitive and can be used for the analysis of multiple mycotoxins (Wang et al., 2017), they are not suitable for screening mass samples. Furthermore, the complex sample pretreatment and highly skilled personnel required limit the widespread use of these methods. Newly developed sensor methods for the detection of DON, such as Raman (Yuan et al., 2017), chemiluminescent detection (Li, Xia, Zhao, Wang, & Jiang, 2017), and the microfluidic method (Soares et al., 2017), are also not suitable for screening real mass samples. Immunoassays are another important method, and include chemiluminescence enzyme immunoassays (Zhang, Zhou, & Zhou, 2015), electrochemical immunosensors (Lu, Seenivasan, Wang, Yu, & Gunasekaran, 2016), enzyme-linked immunosorbent assays (ELISAs), and lateral-flow devices (LFDs). Zhang developed a competitive direct ELISA with a limit of detection (LOD) of 0.15–0.48 mg/kg in real samples (Zhang et al., 2017). Fang Ji developed ELISA method for DON determination in grains with linear detection range between 0.01 and

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100 μg/mL (Ji, Li, Xu, & Shi, 2007). Yu developed a lateral-flow immunoassay for the simultaneous detection of fumonisin B1 and DON, with cut-off values in buffer of 2.0 and 40 ng/mL, respectively (Yu et al., 2015). J. Liu developed lateral-flow immunoassay for DON with a limit of detection about 0.30 mg/kg (Liu, Zanardi, Powers, & Sumanb, 2012). Kyeong-Yeol also prepared a lateral-flow strip for the simultaneous detection of DON and zearalenone, with cut-off values of 500 and 10 ng/g, respectively, in spiked rice and corn (Kim et al., 2014). Now, the extensive monitoring programs require to develop convenient, efficient, and inexpensive techniques. Additionally, to better fit the market test demand, safe and clear sample pretreatment methods for the extraction of different mycotoxins are also important. In this study, we prepared a more-sensitive monoclonal antibody for the development of an indirect competitive ELISA (ic-ELISA) and lateral-flow immunochromatographic assay (ICA). Both of these methods were optimized for different extraction solutions, to allow the safe and clear - detection and lay a foundation for simultaneous detection of various mycotoxins by ic-ELISA and lateral-flow ICA strip methods on uniform extraction methods.

2. Materials and methods

2.1. Reagents and materials

Standards (purity ≥ 98%) including DON, 3-AcDON, 15-AcDON, NIV, FX, T-2 toxin, HT-2 toxin, and diacetoxyscirpenol (DAS) were obtained from J&K Scientific Ltd (Shanghai, China). Butaneboronic acid (BBA, 98%), succinic anhydride (98%), 4-dimethylamiopyridine (DMAP, 98%), bovine serum albumin (BSA, 96%), keyhole limpet hemocyanin (KLH, 98%), 1-ethyl-carbodiimidehydrochloride (EDC, 98%), *N*-hydroxysuccinimide (NHS, 98%), and carbodiimide (CDI, 98%) were obtained from Sigma (St. Louis, MO, USA). A goat anti-mouse immunoglobulin (IgG) antibody was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). Other reagents are analytically pure and were obtained from the National Pharmaceutical Group Chemical Reagent Co., Ltd (Shanghai, China). Cereal and feed samples were obtained from the Jiangsu Entry–Exit Inspection and Quarantine Bureau (Nanjing, China) and confirmed by LC-MS.

Nitrocellulose (NC) high-flow plus membrane (PuraBind™ RP) was obtained from Whatman-Xinhua Filter Paper Co. (Hangzhou, China). The sample pad (CB-SB08), polyvinylchloride (PVC) backing card, and absorption pad (SX18) were supplied by Goldbio Tech Co. (Shanghai, China).

All buffer solutions were prepared with ultrapure water (Milli-Q Purification System, Millipore Co., Bedford, MA, USA). AirJet Quanti™ 3000 and BioJet Quanti™ 3000 were the aerosol dispensers used (XinqidianGene-Technology Co. Ltd, Beijing, China), and a CM 4000 strip-cutting instrument was used (Gene, Shanghai, China). The strip scan reader was provided by Huaan Magnech Bio-Tech Co., Ltd (Beijing, China).

The design of molecular structure is based on the ChemBioDraw Ultra 14.0. The date processing is based on Origin 8.5.

2.2. Preparation of antigens

Because DON contains three free hydroxyl groups, the antigens were prepared in two different ways. The protein was first diluted in sodium carbonate–bicarbonate buffer (CB, pH 9.6) at 5 mg/mL.

The procedure used to prepare the first antigen (A) is shown in Fig. S1. DON (1 mg) was diluted in 0.5 mL of pyridine and added to 5 mg of BBA (forming DON–BBA) for blocking. The mixture was reacted in the dark overnight, 3.2 mg of DMAP and 7 mg of succinic anhydride were added and the mixture was reacted in 50 °C for 4 h with stirring. The reaction was stopped by the addition of 100 μL of ultrapure water, and then dried under nitrogen (forming 3-HS-DON-BBA). The residue was diluted in water and extracted three times with trichloromethane. The trichloromethane was merged and dried under nitrogen. The residue was then diluted in methanol, and shaken overnight for deblocking (forming 3-HS-DON). The hapten 3-HS-DON was identified by mass spectrum. 3-HS-DON (1 mg) was mixed with 1.2 mg of NHS and 2.5 mg of EDC in 400 μL of dimethylformamide, and reacted at room temperature for 4 h with stirring. This solution was then added dropwise into 5 mg of protein solution and reacted at room temperature overnight with stirring. The resulting conjugates were dialyzed against 0.01 M phosphate buffer solution (PBS) for 3 days in the dark (Li et al., 2012).

To prepare the second antigen (B), DON (1 mg) was diluted in 0.5 mL of dimethyl sulfoxide with 8 mg of CDI and reacted at room temperature for 30 min with stirring. This reaction was stopped with 50 μL of ultrapure water, and then added dropwise into 5 mg of protein solution and reacted at room temperature for 4 h with stirring. The resulting conjugates were dialyzed against 0.01 M PBS for 3 days in the dark (Maragos & McCormick, 2000).

Because DON has no characteristic absorption peak within the ultraviolet–visible light region, the antigens were characterized with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Because the molecular weight of the KLH protein is high, only the DON–BSA antigen was characterized.

2.3. Immunization and preparation of antibody

DON conjugates with KLH (3-HS-DON-KLH-A1 and DON-KLH-B1) were used as the immunogens and its conjugates with BSA (3-HS-DON-BSA-A2 and DON-BSA-B2) were used as the coating antigens. Female BALB/c mice (8–10 weeks of age) were injected with two immunogens and their sera were tested with the ic-ELISA using the two coating antigens. After six rounds of immunization, the serum antibody titers and inhibitory effects of mice were stable. The mouse with the highest serum antibody titer and lowest 50% inhibitory concentration (IC_{50}) was then selected for cell fusion. The cell fusion and screening procedures were performed as previously reported (Wang et al., 2009). The hybridoma cell lines were obtained with three rounds of screening and subcloning. The hybridoma cells (1×10^7) were then injected into the peritoneal cavities of mice to induce ascites production and monoclonal antibody (mAb) were purified from the ascites with the caprylic acid–ammonium sulfate precipitation method (Kuang et al., 2013).

2.4. Characterization of the ic-Elisa

2.4.1. Development of the ic-ELISA

The ic-ELISA was performed as previously reported (Kong et al., 2015). The coating antigen was diluted in coating buffer (0.05 M CB, pH 9.6) and added to a 96-well plate (100 μL/well). The plate was incubated at 37 °C for 2 h, washed three times, and blocked with 200 μL/well blocking buffer (2% gelatin in 0.05 M CB, pH 9.6) at 37 °C for 2 h. After three washes, 50 μL of mAb and 50 μL of standard solution were added to each well and incubated at 37 °C for 30 min. After three washes, 100 μL of horseradish-peroxidase-labeled goat anti-mouse IgG antibody was added to each well and incubated at 37 °C for 30 min. After four washes, 100 μL of enzyme substrate was added and incubated at 37 °C for 15 min in the dark. The reaction was stopped with 2 M sulfuric acid (50 μL/well). The absorbance was measured at 450 nm in a microplate reader.

2.4.2. Optimization of the ic-ELISA

Different parameters were evaluated to optimize the ic-ELISA, including the ionic strength (NaCl content: 0.2%, 0.4%, 0.8%, 1.6%, or 3.2%, [w/v]), pH (5.0, 6.0, 7.4, 8.6, or 9.6), and ethanol content (0%, 5%, 10%, 20%, or 40%) of the dilution buffer (Yan, Liu, Xu, Kuang, & Xu, 2015).