Food Control 79 (2017) 227–233

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Development of a rapid magnetic bead-based immunoassay for sensitive detection of zearalenone

Fengchun Zhao, Qiang Shen, Huimin Wang, Xiao Han, Zhengyou Yang*

Department of Microbiology, College of Life Science, Key Laboratory for Agriculture Microbiology, Shandong Agricultural University, Taian 271018, China

ARTICLE INFO

Article history:
Received 18 January 2017
Received in revised form
19 March 2017
Accepted 31 March 2017
Available online 3 April 2017

Keywords:
Zearalenone
Monoclonal antibody
Magnetic beads
ELISA
Rapid

ABSTRACT

Here we demonstrate a novel magnetic bead-based enzyme-linked immunosorbent assay (MB-ELISA) for zearalenone (ZEN) detection. Firstly, an anti-ZEN monoclonal antibody (mAb) was prepared by hybridoma technique, and immobilized on carboxyl modified MBs to obtain mAb-MBs. In addition, the biotinylated ZEN-BSA was labelled by streptavidin-HRP for use as competitor. Based on the mAb-MBs and streptavidin-HRP labelled ZEN-BSA, a MB-ELISA which contains only one 20 min antigen-antibody reaction step and takes no more than 45 min for dozens of samples analysis was developed. The half maximal inhibitory concentration (IC_{50}) and limit of detection (LOD) of the MB-ELISA are 1.78 ng/mL and 0.13 ng/mL, respectively. And the MB-ELISA working range for corn samples analysis is from 5.0 μg/kg to 255.2 μg/kg. The recoveries for ZEN spiked corn samples ranged from 82.3 to 110.5% with coefficient of variation (CV) under 8.9%. For natural corn samples analysis, the results of MB-ELISA showed good agreement with the results of conventional direct competitive ELISA ($R^2 = 0.9742$).

1. Introduction

Mycotoxins are secondary metabolites produced by various fungi species. Mycotoxins contaminative agricultural products constitute a major health and economical problem (Pereira, Fernandes, & Cunha, 2014). Zearalenone (ZEN) is one of the most widely distributed mycotoxins produced by several *Fusarium* species, in particular *Fusarium graminearum* and *Fusarium culmorum* (Zinedine, Soriano, Molto, & Mañes, 2007). ZEN is highly resistant to temperature, and it has been found in many cereal products such as corn, flour, malt, soybeans and rice (Kwaśniewska, Gadzała-Kopciuch, & Cendrowski, 2015). EFSA (2011) reported that the frequency of occurrence of ZEN in corn (369 in 838 samples, 44%) was significantly higher. Some studies have evidenced that products contaminated by ZEN could cause various toxic effects, including teratogenesis, carcinogenicity, neurotoxicity and estrogenic effect (Benzoni et al., 2007; Collins et al., 2006; Heneweer et al., 2007). Considering the risk of ZEN contamination, 60 μg/kg ZEN in wheat and corn has been set as the maximum residue levels (MRLs) in China (Zhang et al., 2011), while the European Union (EU) legal limit of ZEN is 350 μg/kg in corn and 100 μg/kg in other cereals (Wang et al., 2016).

A number of methods have been reported for mycotoxins analysis. Classic chromatographic methods with high accuracy including gas chromatography (GC) (Qian et al., 2015), gas chromatography–mass spectrometry (GC–MS) (Ferreira, Fernandes, & Cunha, 2012; Pereira, Fernandes, & Cunha, 2015), high performance liquid chromatography (HPLC) (Giovannoli, Passini, Di Nardo, Anfossi, & Baggiani, 2014; Ndube, van der Westhuizen, Green, & Shephard, 2011) and HPLC coupled with tandem mass spectrometry (HPLC-MS/MS) (Arroyo-Manzanares, Huertas-Perez, Gamiz-Gracia, & Garcia-Campana, 2013). However, the instrumental methods usually require extensive sample preparation and expensive instruments, which limit their application in routine monitoring of mycotoxins. To avoid this limitation, the easy-to-use antibody-based immunoassays have been widely developed for mycotoxins determination (Lee, Wang, Allan, & Kennedy, 2004; Ling et al., 2014; Salem and Ahmad, 2010).

Since the first radioimmunoassay for ZEN reported by Thouvenot and Morfin (1983), a series of immunoassays for ZEN monitoring have been developed, such as ELISA (Liu, Nie, Zhao, Meng, & Wu, 2015; Pei et al., 2013), chemiluminescence immunoassay (Wang et al., 2013), fluorescence immunoassay (Zhan, Huang, Chen, Li, & Xiong, 2016), fluorescence polarization immunoassay (Zhang et al., 2017a), lateral flow immunoassay (Sun et al., 2014), and dot-immunoassay (He, Xu, Zhang, Li, & Huang, 2014). Among

* Corresponding author.
E-mail address: zhyouyang@sdau.edu.cn (Z. Yang).

http://dx.doi.org/10.1016/j.foodcont.2017.03.051
0956-7135/

these methods, ELISA is the most popular one due to its low-cost, high sensitivity and high throughput. But microplate-based ELISAs, including preceding coating, blocking, prolonged incubations and multiple washing steps, are always time-consuming (Urusov, Petrakova, Vozniak, Zherdev, & Dzantiev, 2014). As an alternative, magnetic beads (MBs) are always selected as an immobile phase to avoid preceding coating, blocking and reduce the incubation time (Radoi, Targa, Prieto-Simon, & Marty, 2008; Speroni, Elviri, Careri, & Mangia, 2010; Wang et al., 2014; Yu et al., 2016; Zhang et al., 2017b). Therefore, magnetic bead-based ELISA (MB-ELISA) that combined magnetic bead technology with ELISA not only has the advantage of high throughput, but also reduced the detection time.

Recently, a MBs-based immunoassay for screening ZEN in cereal and feed has been reported (Zhang et al., 2015). However, this immunoassay was an indirect competitive ELISA and needs as long as 90 min for incubation steps. Hence, the procedure showed less superiority over the conventional ELISA. In this study, a new MB-ELISA strategy was established to detect ZEN in corn samples. The developed MB-ELISA contains only one 20 min antigen-antibody incubation step and takes no more than 45 min for dozens of samples detection. This analytical method is rapid, simple, cheap, and quite suitable for routine monitoring of low molecular weight compound in food samples.

2. Materials and methods

2.1. Materials and reagents

ZEN (purity $\geq$ 99%), T-2 toxin (purity $\geq$ 98%), deoxynivalenol (purity $\geq$ 98%), aflatoxin B 1 (purity $\geq$ 98%), ochratoxin A (purity $\geq$ 98%), streptavidin-horseradish peroxidase (SA-HRP), bovine serum albumin (BSA), ovalbumin (OVA), complete Freund's adjuvants, incomplete Freund's adjuvants and TMB were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Carboxyl modified polystyrene magnetic microspheres (diameter 0.5–1 μm) were purchased from BaseLine Chromtech Research Center (Tianjin, China). Protein G resin was purchased from GenScript Biotechnology Co., Ltd (Nanjing, Jiangsu, China). *O*-(Carboxymethyl)hydroxylamine hemihydrochloride, D-Biotin *N*-Succinimidyl Ester, *N*-Hydroxysulfosuccinimide sodium salt (sulfo-NHS), *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl) and other chemical reagents were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China).

2.2. Preparation of monoclonal antibody

The hapten of ZEN (zearalenone-6′-carboxymethyloxime) was synthesized using the method described by Thouvenot & Morfin (Thouvenot & Morfin, 1983). Briefly, 20 mg ZEN was added to 5 mL pyridine contained 40 mg *O*-(carboxymethyl)hydroxylamine hemihydrochloride and stirred for 24 h at room temperature. The solvent was removed under vacuum. The residue was transferred to a separatory funnel with 20 mL of 1% $NaHCO_3$ (pH 8.0) and washed three times with ethyl acetate. Then, the aqueous phase was adjusted to pH 3.0 by 6 M HCl, and extracted three times with 20 mL of ethyl acetate. The extracts were dried over anhydrous sodium sulfate and concentrated under vacuum to obtain the hapten of ZEN. Active ester method was used to prepare hapten-protein conjugates (McAdam, Hill, Beasley, & Skerritt, 1992). Molar ratio of the hapten to carrier protein was 30:1. The conjugates (ZEN-BSA and ZEN-OVA) were dialyzed against PBS (10 mM, pH 7.4) for three days to remove residual ester.

ZEN-BSA was used as immunogen, and five BALB/C mice (6–8 weeks old, supplied by Animal Experiment Center of Shandong University) were immunized intraperitoneally for four times. One week after the last booster injection, serum was collected from the caudal vein of each mouse and tested by a noncompetitive indirect ELISA (ZEN-OVA used as coating antigen). The mouse which showed the highest titer was euthanized for monoclonal antibody (mAb) production. The spleen cells were mixed with mouse myeloma cells (P3-X63-Ag8.653) at a 10:1 ratio in the presence of 50% (w/v) PEG 4000 for cell fusion. The cultivation procedure of hybridoma cells was the same as previous study (Wang et al., 2011). An indirect competitive ELISA (icELISA) was carried out for screening the hybridoma cells using ZEN-OVA as coating antigen and ZEN as competitor. The selected hybridoma cell line was subcloned by the limited dilution technique and used to produce mAb.

2.3. Immobilization of mAb on carboxyl modified magnetic beads

Firstly, 2 mL of 5 mg/mL carboxyl modified magnetic beads (MBs) in stock solution was transferred into a 10-mL tube and washed three times with 5 mL of 50 mM MES buffer (pH 6.0). The tube was placed in a magnetic field to separate the MBs and remove the supernatant. Then, 2 mL of MES buffer containing 40 mg EDC·HCl and 60 mg sulfo-NHS was added to suspend the MBs. The mixture was shaken gently for 30 min at room temperature. After that, the activated MBs were washed three times with PBS (10 mM, pH 7.4) and resuspended in 1 mL PBS. Subsequently, 1 mL of mAb (0.5 mg/mL) in PBS was mixed with the activated MBs and shaken gently for 4 h at room temperature. Finally, the mixture was washed four times with PBS (10 mM, pH 7.4) to obtain mAb-MBs. The products were suspended in 2 mL PBS (contains 2% skim milk and 50% glycerol (v/v)) and stored at −20 °C for further use.

2.4. Preparation of HRP-labelled ZEN-BSA

ZEN-BSA was labelled with SA-HRP for use as a competitor of immunoassays. Firstly, ZEN-BSA was biotinylated by D-Biotin *N*-Succinimidyl Ester. The ester was added to the ZEN-BSA solution (molar ratio was 30:1) and stirred at room temperature for 4 h. Then, the mixture was dialyzed against PBS (10 mM, pH 7.4) for three days to remove residual ester. After centrifugation at 12,000 rpm for 10 min, the biotinylated ZEN-BSA in the supernatant was obtained. The biotinylated ZEN-BSA was then mixed with SA-HRP, and the SA-HRP-labelled antigen was obtained based on the high affinity of biotin and streptavidin. The SA-HRP-labelled biotinylated ZEN-BSA (ZEN-BSA-biotin-SA-HRP) was referred to as ZEN-BSH. The ratio of biotinylated ZEN-BSA to SA-HRP was optimized by MB-ELISA. Finally, equal volume proportion of glycerol was added into the ZEN-BSH solution and stored at −20 °C for further use.

2.5. Development of MB-ELISA

A direct competitive MB-ELISA was developed as below. Firstly, 50 μL per well of ZEN standards or samples in 17.5% methanol-water were added to 96-well plate (Costar, Corning, NY, USA). Subsequently, 25 μL per well of ZEN-BSH (1/10000) in MPBS (10% skim milk in 100 mM PBS, pH 7.4) was added. Thirdly, 25 μL per well of mAb-MBs (1/100) in PBS (100 mM, pH 7.4) was added. The plate was shaken well and incubated at 37 °C for 20 min in wet box. After the incubation, the plate was placed on a magnetic base for 2 min to precipitate the MBs. Then, the liquids were discarded, and the plate was patted to dry with a paper towel. The plate was washed two times with 150 μL PBST (10 mM PBS with 0.025% (v/v) Tween-20, pH 7.4). After washing, TMB substrate (100 μL per well) was added and incubated for 15 min at 37 °C. Finally, 50 μL per well of 2 M H_2SO_4 was added to stop the reaction and the absorbance was