Food Chemistry 268 (2018) 242–248

Contents lists available at ScienceDirect

Food Chemistry

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

Analytical Methods

A peptide/maltose-binding protein fusion protein used to replace the traditional antigen for immunological detection of deoxynivalenol in food and feed

Yang Xu[a,b], Hongwei Yang[a,b], Zhibing Huang[a,b,*], Yanping Li[a,b], Qinghua He[a,b], Zhui Tu[a], Yanwei Ji[a,b], Wenjie Ren[a,b]

[a] State Key Laboratory of Food Science and Technology, Nanchang University, No. 235 Nanjing East Road, Nanchang 330047, China
[b] Sino-German Joint Research Institute, Nanchang University, No. 235 Nanjing East Road, Nanchang 330047, China

ARTICLE INFO

Keywords:
Deoxynivalenol
Mimic epitope peptide
Enzyme-linked immunosorbent assay
Immunochromatographic assay

ABSTRACT

A deoxynivalenol (DON) epitope clone (D_8) was obtained by phage display technology using anti-DON monoclonal antibodies as a target molecule. Subsequently, a DON antigen mimic (D_8-maltose-binding protein [MBP]) was synthesized by fusing the mimic epitope peptide with MBP. An enzyme-linked immunosorbent assay (ELISA) and urchin-like gold nanoparticle immunochromatographic assay was developed based on D_8-MBP for detection of DON in maize and wheat. The half-maximal inhibitory concentration, lower detection limit, and linear range of the D_8-MBP ELISA were 57.98 ± 0.97, 9.83, and 11.32–286.77 ng/mL, respectively. The sensitivity of the D_8-MBP ELISA was nearly 2.5 times higher than that of traditional ELISA using DON-bovine serum albumin (BSA). The detection threshold of the colloidal gold immunochromatographic assay for D_8-MBP was 25 ng/mL. Thus, D_8-MBP could be used to replace the traditional DON-BSA antigen for the immunological detection of DON, permitting low cost, rapid detection of DON.

1. Introduction

Deoxynivalenol (DON) is a well-known mycotoxin and secondary metabolite produced by *Fusarium* fungi having a poor nutritional status (Tralamazza, Bemvenuti, Zorzete, Garcia, & Corrêa, 2016; Palacios et al., 2017). DON is toxic in humans and animals and can cause acute toxicity, reproductive toxicity, cytotoxicity, and immune toxicity, among other toxic effects (Pestka, 2010). Worldwide, corn, wheat, barley, other grains, and grain products can be polluted by DON; severe DON pollution has been reported in the United States of America, Canada, and China, among other countries (Tanaka et al., 1988). Accordingly, rapid detection and screening for DON in food and feeds are needed for identification of DON contamination, and the development of novel highly sensitive detection methods for DON is essential.

To date, many methods, including thin layer chromatography (TLC) (Trucksess, Flood, Mossoba, & Page, 1987), high-performance liquid chromatography (HPLC) (Suman, Bergamini, Catellani, & Manzitti, 2013; Zhang, Wong, Krynitsky, & Trucksess, 2014), gas chromatography (GC) (Cunha & Fernandes, 2012), near-infrared spectroscopy (NIRS) (Peiris et al., 2010), enzyme-linked immunosorbent assays (ELISAs) (Li et al., 2015), and colloidal gold immunochromatographic assays (GICAs) (Foubert, Beloglazova, & Saeger, 2017; Huang, Xu, Li, Zhang, & He, 2012; Xu et al., 2010), have been developed for detection of DON. Due to the low sensitivity and complicated sample pretreatment required for TLC, this method is not suitable for rapid screening and detection of DON in food and feed. Others methods have high detection sensitivity and accuracy; however, these methods require large, expensive equipment and highly skilled technicians and are therefore not suitable for the rapid detection of DON (Xu et al., 2010).

ELISA and GICA are two immunoassay technologies based on the specific binding reaction of the antibody and antigen for detecting various substances (Zangheri et al., 2015). ELISA and GICA have various advantages, such as short detection time, no need for large instruments, and suitability for rapid screening of large numbers of samples (Zhou et al., 2015; Meng et al., 2014). For detection of DON, competitive immunoassays are often carried out, and DON antigens, such as DON-bovine serum albumin (BSA) and DON-ovalbumin (OVA), need to be synthesized (Qiu et al., 2015). Conventional DON antigens are typically synthesized by chemical methods, which require toxic reagents; thus, this synthesis method may have serious adverse effects on the health of technicians and the environment (Zhang et al., 2015).

In recent years, researchers have focused on the development of

* Corresponding author at: State Key Laboratory of Food Science and Technology, Nanchang University, No. 235 Nanjing East Road, Nanchang 330047, China.
E-mail address: hzbchem@ncu.edu.cn (Z. Huang).

https://doi.org/10.1016/j.foodchem.2018.06.096
Received 4 August 2017; Received in revised form 13 November 2017; Accepted 19 June 2018
Available online 20 June 2018
0308-8146/

antigen substitutes for immunoassays. The three-dimensional structure of the DON antigen can be simulated by anti-idiotype antibodies, which have been used to replace the traditional DON antigen for DON immunological analysis (Qiu et al., 2015). However, two monoclonal antibodies (MAbs) are needed for preparation of anti-idiotype antibodies by hybridoma technology, and the preparation process is long and complex (Lode et al., 2013). Another method for preparation of anti-idiotype antibodies is based on phage display technology; however, this method also has several disadvantages, such as the need for several immune reactions to build an antibody library, and the process is also complex, with antibodies often expressed in inclusion bodies (Nur, Shoenfeld, & Blank, 2013). In addition, phage display involves the use of organisms, and accurate quantification cannot be carried out easily because of the complex structures of phages (Wang et al., 2013).

Alternatively, phage display peptide technology may have some advantages, including large library capacity, simple screening procedures, and the lack of a need for helper phage rescue. Additionally, analog epitope peptides are easily synthesized by biological or chemical methods, and the structures of peptides are simple. The structures of peptides can also be easily modified to improve the detection sensitivity in immunoassays (Ivarsson et al., 2014). In our previous work, fumonisin B_1 (FB_1) and ochratoxin A (OTA) mimotope phage clones were successfully obtained with their MAbs as target molecules from phage random 12 peptide library biopanning (Xu et al., 2014; He et al., 2013). In this study, DON mimotope phage clones were screened out using phage display peptide technology with anti-DON-MAbs as a target molecule, and the mimic epitope peptide and maltose-binding protein (MBP) were fused and expressed further using molecular biology techniques. The clones were then used to replace the traditional DON capturing antigen for immunological detection of DON, permitting nontoxic and rapid detection of DON in immunoassays.

2. Materials and methods

2.1. Materials and reagents

Standards for mycotoxin, such as aflatoxin B_1 (AFB_1), deoxynivalenol (DON), FB_1, zearalenone (ZEN), OTA, and gold chloride acid, as well as hydroquinone, sodium citrate, PEG 8000, and Tween-20 were obtained from Sigma-Aldrich (St. Louis, MO, USA). Horseradish peroxidase (HRP)-labeled anti-M13 antibodies were purchased from GE Healthcare Inc. (Piscataway, NJ, USA). The Ph.D.-12 phage display peptide library kit, *Eag*I, restriction endonuclease *Acc*65I, amylose resin, and pMAL-pIII vector were obtained from New England Biolabs (Beverly, MA, USA). The Taq polymerase, nucleic acid and protein marker, minibest DNA fragment purification kit, and T4 DNA ligase were obtained from TaKaRa (Dalian, China). *Escherichia coli* ER2738, *E. coli* TB1, *E. coli* DH5, and pMAL-P III plasmid were preserved in our laboratory. Isopropylthio-β-d-galactoside (IPTG) and 3,3,5,5-tetramethylbenzidine (TMB) were purchased from Bio Basic Inc. (Markham, ON, Canada). Yeast extract and tryptone were from Oxoid (Cambridge, UK). BSA and OVA were obtained from Sangon Biotech (Shanghai, China). Sartorius CN 140 nitrocellulose (NC) membranes (CN140) were purchased from Sartorius (Göttingen, Germany). The plastic packing, probe pad, sample pad, absorbent pad, and goat anti-mouse IgG antibodies were obtained from JieYI Biotechnology Co., Ltd. (Shanghai, China). Purified water was obtained using a Millipore Milli-Q water system (Millipore, Bedford, MA, USA). Anti-DON-MAbs and traditional DON-BSA conjugate antigen were prepared in our laboratory (Xu et al., 2010). A PriboFastDON ELISA kit was purchased from Pribolab Pte. Ltd. (Singapore). The M13KE insert extension primer (5′-^{HO}CATGCCCGGG TAC-CTTTCTATTCTC-3′) and the −96 g gIII sequencing primer (5′-CCCTCATAGTTAGCG-TAACG-3′) were synthesized by Invitrogen (China). The colony polymerase chain reaction (PCR) primers M13R (5′-^{HO}AGCGGATAACAATTTCACACAGGA-3′) and MalE (5′-^{HO}TTTACC TTCTTCGATTTTCATATCGG-3′) were also synthesized by Invitrogen. All the other chemicals were of analytical grade.

2.2. Apparatus

Immunoassay absorbance was obtained using a Thermo Multiskan MK3 Spectrum (Thermo Scientific, USA). Maxisorp polystyrene 96-well plates were obtained from Nunc (Roskilde, Denmark). PCR amplification was carried out using a TCA0096 PCR instrument (Thermo Scientific). Ultraviolet (UV)-visible spectra were obtained using an Ultrospec 4300 Pro UV–Visible Spectrophotometer (Amersham, NJ, USA). Dispersion of the urchin-like gold nanoparticles (UGNs) was observed using a JEM-2100 transmission electron microscope (TEM; Pulstec, Japan). The BioDot XYZ platform used for solution spraying and the CM 4000 guillotine cutter were purchased from BioDot (Irvine, CA, USA).

2.3. Biopanning and identification of DON mimotopes

The DON mimotopes were biopanned from a 12-mer phage-displayed peptide library using anti-DON-MAbs as a target molecule. During the first round of panning, anti-DON-MAbs were diluted to 100 μg/mL and coated onto 96-well plates (100 μL/well). The plates were then incubated at 37 °C for 60 min, washed three times with PBST (0.01 M phosphate-buffered saline [PBS], pH 7.4, plus 0.1% [v/v] Tween-20), and blocked with 3% BSA (300 μL/well) at 37 °C for 60 min. After washing three times with PBST, 100 μL of the 12-mer phage-displayed peptide library (1.0×10^{11} pfu) was added to the plates, and the plates were incubated at 37 °C for 60 min. After washing three times with PBST, 100 μL elution buffer containing 0.2 M glycine-HCl (pH 2.2) and BSA (1 mg/mL) was added. The elution mixture was then rocked gently for 20 min at 25 °C, and the eluate was pipetted into a microcentrifuge tube and neutralized with 15 μL Tris-HCl (1 M). One microliter of the eluate was removed for determination of the phage titer, and the remaining eluate was used for PCR amplification. In the second panning round, the plates were coated with 75 μg/mL anti-DON-MAbs (100 μL/well). The concentration of Tween-20 was increased to 0.25%, and 3% OVA was substituted for 3% BSA. After incubation at 37 °C for 30 min, 100 μL DON (10 μg/mL) was added for elution of specific phages. During the third round of selection, the concentration of the anti-DON-MAbs used for coating was decreased to 50 μg/mL. The concentration of Tween-20 was increased to 0.5%, and 3% BSA was included in the PBST elution. After incubation at 37 °C for 30 min, specific phages were eluted with 100 μL DON (10 μg/mL). Individual phage isolates from the elution were evaluated for anti-DON-MAb binding by phage ELISA and DNA sequencing, as described our previous study (Qiu et al., 2015; Xu et al., 2014). The sequencing of positive clones was carried out using −96 gIII as primers.

2.4. Biosynthesis and characterization of D_8-MBP

D_8-MBP was biosynthesized as described previously (Xu et al., 2014; He et al., 2013). Briefly, single-stranded phage DNA was extracted from the phage particle. Next, the DON mimotope was displayed and amplified by PCR using M13EP and the −96 gIII as the insert extension primer and sequencing primer, respectively. The amplified DON mimotope genes were digested using the restriction endonuclease Acc651 and the Eagl enzyme at 37 °C for 5 h; the gene was then inserted into the pMAL-pIII plasmid to construct the epitope fusion peptide and MBP expression vector. The expression vector was then transformed into *E. coli* DH5 using M13R and MalE as primers, and the insertion was verified by colony PCR. The expression vectors were extracted from validated positive clones and transformed into *E. coli* TB1 cells; expression was induced by 0.4 M IPTG at 25 °C for 16 h. The fusion proteins were extracted using an osmotic shock procedure and purified by amylose resin. The expression and purification of the proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)