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Use of Cloneable Peptide−MBP Fusion Protein as a Mimetic Coating Antigen in the Standardized Immunoassay for Mycotoxin Ochratoxin A

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Supporting Information

ABSTRACT: The quality of mycotoxin conjugates is essential to the development of reliability of immunoassays for mycotoxins. However, conventional mycotoxin conjugates are usually synthesized by chemical methods, which are harmful to the environment and yield unwanted cross-reactions. In this study, using ochratoxin A (OTA) as a model system, a selected OTA mimotope (phage-displayed peptide) that specifically binds to anti-OTA antibody was expressed as soluble and monovalent fusions to maltose binding protein (MBP). These prepared fusion proteins can serve as a mimetic coating antigen in both a quantitative chemiluminescent enzyme-linked immunoassay (CLEIA) and a qualitative dot immunoassay for OTA. One of the prepared mimetic coating antigen (L12-206-MBP)-based CLEIAs exhibited a half-inhibition concentration (IC_{50}) of 0.82 ng/mL and a working range of 0.30−2.17 ng/mL, which resemble those of the conventional OTA−OVA conjugate-based immunoassay. The dot immunoassay developed with both the OTA−OVA conjugate and the mimetics showed identical visual cutoff values of 5 ng/mL. The mimetic coating antigen proposed here is an OTA-free product and can be prepared reproducibly as a homogeneous product and facilitates standardization of immunoassays for the mycotoxin OTA.

KEYWORDS: *mycotoxin, ochratoxin A, immunoassay, mimotope, peptide*

INTRODUCTION

Mycotoxins are secondary metabolites that have been associated with severe toxic effects to humans and animals produced by several species of fungi including *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria*.[1] The frequently occurring mycotoxins, such as aflatoxins, ochratoxins, citrinin, patulin, and fusarium toxins, can contaminate a wide variety of foods as a result of fungal infection in crops, in the field during growth, at harvest, or in storage.[2,3] With regard to the negative effects and wide distribution of mycotoxins, risk assessment or legal limits have been conducted or set by many countries.[4−6] To minimize the risk of mycotoxins, many studies have been focused on analytical methods for mycotoxins including high-performance liquid chromatography, liquid chromatography−tandem mass spectrometry,[7,8] and gas chromatography−tandem mass spectrometry.[9] Besides chromatography methods, the development of immunoassays such as enzyme-linked immunosorbent assay, dipstick immunoassay, and gel-based immunoassay, as well as lateral flow assay, has also opened up widespread use for prevention of mycotoxin risk because of sensitivity, rapidity, high-throughput, and, in some cases, capability of working on site.[10−13]

As a rule, immunoassays can be categorized into competitive and noncompetitive formats. To date, in regard to the detection of small molecules, such as mycotoxins, the competitive immunoassay format has been used almost exclusively.[14] Hence, preparing mycotoxin conjugates (coating or competing antigens) is a necessary and key step. However, the coventional chemical synthetic method for mycotoxin conjugates yields heterogeneous products that are composed of various molecules conjugated at different molar ratios, which hampers the ability to standardize immunoassay systems and harms the environment and human health.[15,16] Therefore, it is significant to discover mycotoxin conjugates mimetics with security and stability and apply them to immunoassays for mycotoxins.

In recent years, several research works have been conducted to develop alternative forms of mycotoxin conjugates. One of those depends on anti-idiotypic antibody technology. Varieties of anti-idiotypic antibodies have been developed as surrogate competing antigens for the detection of aflatoxin B_1, fumonisin B_1, deoxynivalenol, and trichothecene mycotoxin T-2.[17−20] Although the preparation of anti-idiotypic antibody is time-consuming and costly, more works need to be done to promote its widespread application.[21] Another antigen surrogate are mimotopes obtained by phage-displayed peptide technology. Mimotopes (phage-displayed peptides) known to be able to mimic the interaction between epitopes in antigens and their corresponding antibodies have been applied to immunoassays for small molecule contaminants including mycotoxins, antibiotics, and drug residues.[22−24] For example, using antizearalenone (ZEN) monoclonal antibody as a target, two mimotopes of ZEN were selected from a heptapeptide random phage-displayed library and applied to analyze ZEN in cereals.[25] Recently, a phage-displayed peptide-based immunoassay for aflatoxins was also established, and the IC_{50} value was 0.29 ng/

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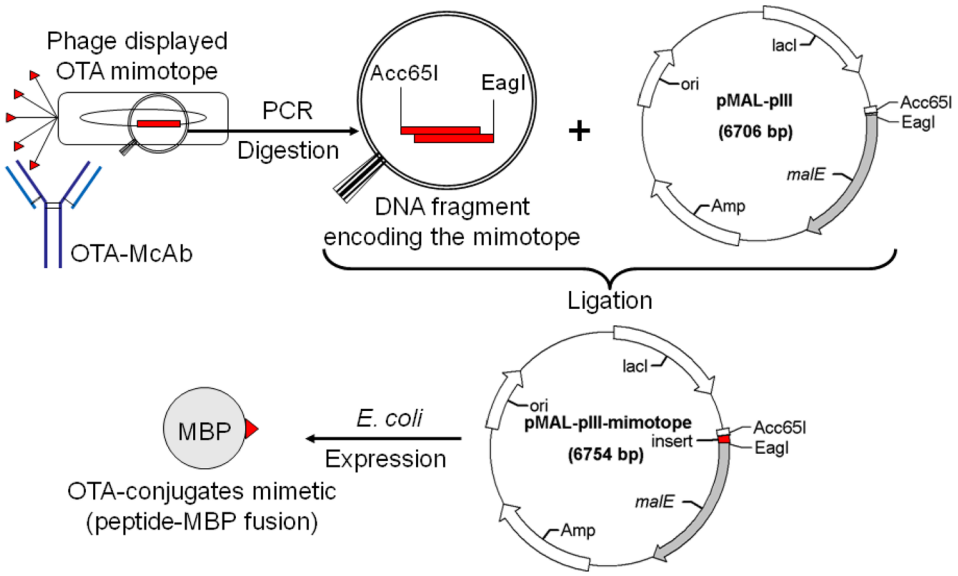

Figure 1. Schematic presentation of the construction of expression plasmid for mimetic OTA conjugate.

mL.[26] Nevertheless, current research concerning the mimotope is usually based on the phage serving as a competitive antigen, and there are few reports of mimotopes used as a coating antigen in quantitative immunoassay.

In addition to using mimotopes in the form of phage-displayed peptides, the chemosynthetic peptide has also been employed as a substitute for mycotoxin conjugate in immunoassays.[27−29] The chemosynthetic peptide allows precise control of peptide concentration and conformation, with a simple molecular weight and structure, and thus is conducive to analyzing the peptide structure−function relationship. However, the problem of environmental pollution and high cost during the chemosynthesis of peptide conjugate chemosynthesis is still inevitable.

We have previously reported ochratoxin A (OTA) mimotopes selected from a second-generation peptide library.[30] Here, using OTA as a model analyte, substitutes for OTA conjugates were produced by genetically fusing OTA mimotopes to a maltose binding protein (MBP) and expressed in *Escherichia coli* cells. The resulting peptide−MBP fusion proteins (mimetic OTA conjugate), which are "cloneable" homogeneous and OTA-free products, worked successfully in quantitative and qualitative immunoassays for measuring OTA in cereals and would facilitate standardization of immunoassays for mycotoxin OTA.

MATERIALS AND METHODS

Chemicals and Reagents. Mycotoxin ochratoxin A, aflatoxin B_1 (AFB_1), zearalenone (ZEN), citrinin (CIT), and horseradish peroxidase (HRP)-conjugated goat anti-mouse antibody were obtained from Sigma (St. Louis, MO, USA). Phage particles displaying OTA mimotopes (named L12-204, L12-206, L12-214, L12-311, L12-314, L12-317, and L12-330), anti-OTA monoclonal antibody (OTA-McAb), and OTA conjugated with ovalbumin (OTA−OVA) were previously prepared in our laboratory.[30,31] Anti-MBP Tag mouse monoclonal antibody was purchased from CWBIO (Beijing, China). 3,3′-Diaminobenzidine (DAB) substrate was purchased from Bio Basic Inc. (Toronto, ON, Canada). SuperSignal ELISA Pico chemiluminescent substrate was obtained from Thermo Scientific (Waltham, MA, USA). Polyvinylidene fluoride (PVDF) membrane was purchased from Millipore Co. (Bedford, MA, USA). The *p*MAL-pIII vector, restriction enzymes *Acc*65I and *Eag*I, and amylose resin were purchased from New England Biolabs, Inc. (Beverly, MA, USA). Taq polymerase, minibest DNA fragment purification kit, and T4 DNA ligase were purchased from TaKaRa (Dalian, China). Commercial ELISA kit for OTA was purchased from Defeng (Taiyuan, China). All inorganic chemicals and organic solvents were of reagent grade.

Construction of Expression Plasmid for Mimetic OTA Conjugate. An expression plasmid was constructed aiming to reproducibly prepare mimetic OTA conjugate (peptide−MBP fusion protein). The schematic diagram of constructing expression plasmid is presented in Figure 1. First, phage particles displaying OTA mimotopes were used for single-stranded phage DNA isolation and purification as described by He et al.[25] Then phage DNA fragment encoding the selected OTA mimotope was amplified by polymerase chain reaction (PCR) using the forward primer (5′-CAT GCC CGG GTA CCT TTC TAT TCT C-3′) and reverse primer (5′-CCC TCA TAG TTA GCG TAA CG-3′). The PCR was performed in a total volume of 100 μL (containing 200 μM each dNTP, 100 nM each forward and reverse primer, 1 U TaKaRa Taq, and 1 μL of single-stranded phage DNA; 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, cycled 30 times). PCR product was purified using a DNA fragment purification kit (Tiangen, Beijing, China) and then digested with *Acc*65I and *Eag*I restriction enzymes (37 °C for 5 h). After the digested DNA fragment was purified by 8% polyacrylamide gel electrophoresis (PAGE), it was ligated into an *Acc*65I and *Eag*I-digested vector pMAL-pIII and transformed into *E. coli* TB1. The constructed expression plasmid was extracted from the transformants and sequenced as described previously.[32]

Preparation and Characterization of Mimetic OTA Conjugate. After the construction of expression plasmid, the mimetic OTA conjugate (peptide−MBP fusion protein) was prepared through the technique of protein engineering. Initially, expression plasmid was transformed to *E. coli* TB1 cells and cultured in LB medium (containing 0.2% glucose and 100 μg/mL ampicillin) at 37 °C by shaking (250 rpm) to OD_{600} ~0.5. The culture was then induced with 0.4 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and shaken at 25 °C for 16 h. After that, the periplasmic fusion proteins were extracted by osmotic shock as described previously.[33] Following the preparation of the cold osmotic shock fluid, the OTA conjugates mimetics were purified by amylose affinity chromatography.[33] Afterward, a Western blot analysis was carried out to validate the mimetic OTA conjugates according to the protocol described previously.[32]

Chemiluminescent Immunoassay Established with Mimetic OTA Conjugate. A quantitative chemiluminescent enzyme-linked immunoassay (CLEIA) for OTA was developed with mimetic OTA conjugate. Briefly, purified mimetics (diluted in PBS, pH 7.4) was

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coated in microplate wells for 2 h at 37 °C and blocked for 1 h at 37 °C with 3% skim milk in PBS. The plate was washed three times with PBST. Afterward, 50 μL of anti-OTA monoclonal antibody (OTA-McAb) (diluted in PBS, pH 7.4) was added in the coated wells in the presence of 50 μL of sample extracted solution or OTA standard solution at 37 °C for 30 min. The plate was then washed three times, and 100 μL of a 1:2000 dilution of HRP-conjugated goat anti-mouse antibody was added to the wells for 30 min at 37 °C. The peroxidase activity was finally developed with 100 μL of chemiluminescent substrate, and chemiluminescent intensity was measured by a luminescence reader (Thermo Scientific). To measure the optimized dilution of the immunoassay reagents, a checkerboard assay was conducted by using different dilutions of mimetics and anti-OTA-McAb in advance. To compare the performance as coating antigen in immunoassay, chemosynthetic OTA−OVA conjugates and phage particles (OTA mimotope displays on phage) were also coated in microplate wells, respectively, and tested as above-described in CLEIA procedures.

Qualitative Immunoassay Based on Mimetic OTA Conjugate. A qualitative dot immunoassay was also developed using mimetic OTA conjugate. The procedure was performed as follows: A 0.45 μm PVDF membrane marked with 7 mm × 7 mm squares was soaked with methanol at room temperature for 10 s and placed on a drenched filter paper as previously described.[31] Then 3 μL of serial dilutions of mimetic OTA conjugate in PBS was spotted onto the squares and incubated at 37 °C for 2 h. After washing with PBST, the membrane was blocked by soaking in 3% skimmed milk in PBS for 30 min. After rinsing in PBST, 7 mm squares were cut, dried, and stored at 4 °C until used.

After the preparation of mimetics-spotted membrane, the assay was performed as follows: Membrane strips were soaked in 10% (v/v) methanol−PBS spiked with sample extracts or various concentrations of OTA and containing the appropriate dilution of OTA-McAb, incubated for 15 min at room temperature. After washing with PBST, the strips were incubated for 15 min in a dilution solution of HRP-conjugated goat anti-mouse antibody, washed, and incubated with TMB substrate. The membrane was rinsed with tap water, and the color intensity of the OTA-positive control and sample test zones was visually compared to that of the OTA-negative control (which has the most intense color).

Validation of Mimetic OTA Conjugate-Based Immunoassay. Validation of the mimetic OTA conjugate-based immunoassay was performed by analyzing the reproducibility and by determining the OTA concentrations in spiked cereal samples and incurred samples.

For the determination of reproducibility, CLEIAs were performed using the mimetic OTA conjugate produced within one batch (intra-assay) and three batches (interassay), respectively. Then the coefficients of variation (CVs) of the IC_{50} of the calibration curves for intra- and interassays were calculated.

To measure the recovery of OTA, OTA-negative samples (corn, rice, and wheat samples) confirmed by HPLC (<0.6 μg/kg) were spiked with OTA at various concentrations, respectively. Both OTA-negative samples and spiked samples were extracted as described below: 5 g of finely ground samples mixed with 10 mL of methanol/water (50:50, v/v) was ultrasonically extracted for 20 min; after centrifuging, the supernatant was diluted 5-fold with PBS for the dot immunoassay analysis or diluted 5-fold with PBS and subsequently diluted 4-fold with PBS containing 10% methanol for the CLEIA analysis. As to the analysis of incurred samples, cereals and feedstuffs were prepared as above.

RESULTS AND DISCUSSION

Production and Characterization of Mimetic OTA Conjugate. Phage single-stranded DNA fragment isolated from seven selected OTA mimotopes (L12-204, L12-206, L12-214, L12-311, L12-314, L12-317, and L12-330) were PCR amplified, respectively (Figure 2A), and digested by *Eag*I and *Acc*65I (Figure 2B). As to the vector of *p*MAL-pIII, it was digested with the same enzymes and isolated by 8% agarose gel

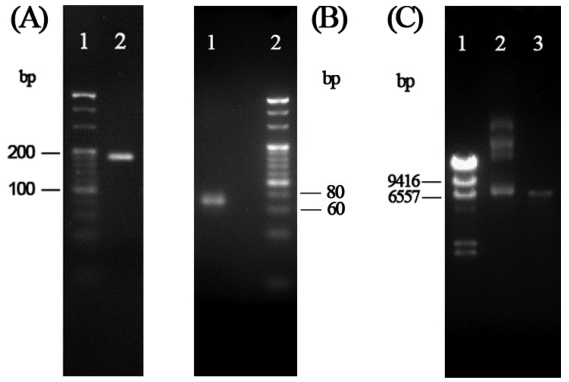

Figure 2. Agarose gel electrophoresis of the results for PCR and digestion: (A) PCR product of DNA fragment (182 bp) that encodes the OTA mimotope (L12-206, others not shown;. (B) DNA fragment (68 bp) digested with restriction enzymes *Acc*65I and *Eag*I; (C) vector pMAL-pIII (6.7 kb) before (lane 2) and after (lane 3) digestion with the same restriction enzymes.

electrophoresis (6.7 kb) (Figure 2C). Seven expression plasmids of mimetic OTA conjugate were constructed and confirmed by sequencing, respectively (data of DNA sequencing is available in the Supporting Information Figure S1).

The constructed expression plasmids were transformed into *E. coli* TB1 cells, and peptide−MBP fusion proteins (mimetic OTA conjugate) were expressed by IPTG inducing. An orthogonal experiment was implemented to analyze the effect of IPTG concentration, induction temperature, and time, which showed that the optimal fusion protein expression was induced with 0.4 mM IPTG at 25 °C for 16 h. As shown in the results of SDS-PAGE and Western blot, the purified mimetic OTA conjugates (named L12-204−MBP, L12-206−MBP, L12-214−MBP, L12-311−MBP, L12-314−MBP, L12-317−MBP, and L12-330−MBP, respectively) all ran as a single band at the predicted molecular mass (calculated as 44 kDa for the 1:1 fusion of peptide and MBP) and specifically bind to the anti-MBP antibody (Figure 3).

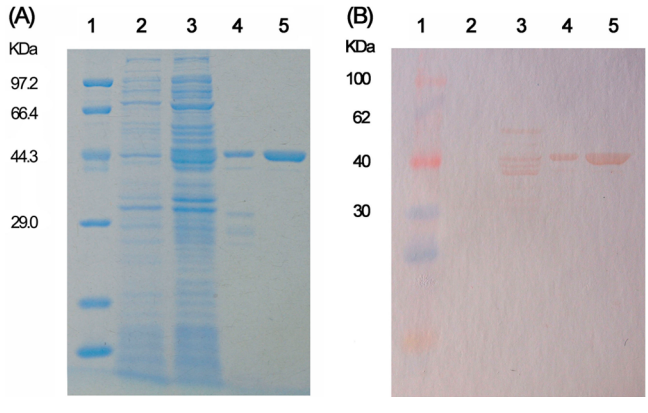

Figure 3. (A) SDS-PAGE and (B) Western blot of expression of mimetic OTA conjugate (L12-206−MBP, others not shown). Blots were stained with Coomassie brilliant blue or probed with anti-MBP mouse monoclonal antibody. Lanes: 2, whole-cell extract harvested immediately prior to IPTG induction or, 3, 16 h after IPTG induction; 4, osmotic shock supernatant; 5, purified L12-206−MBP by amylose affinity chromatography.

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Chemiluminescent Immunoassay for OTA Using Mimetic OTA Conjugate. Competitive inhibition curves of chemiluminescent immunoassay based on mimetic OTA conjugate (L12-204−MBP, L12-206−MBP, L12-214−MBP, L12-311−MBP, L12-314−MBP, L12-317−MBP,and L12-330−MBP) were established, respectively, and the sensitivity of immunoassay was determined. Among the seven mimetic-based inhibition curves, the IC_{50} values were 1.23 ± 0.11, 0.88 ± 0.05, 1.62 ± 0.12, 1.92 ± 0.17, 1.30 ± 0.06, 1.54 ± 0.12, and 1.30 ± 0.10 ng/mL, respectively (Figure 4A, the value is the average of five replicates ± standard deviation). According to the cross-reactivity test, no reaction with mycotoxin AFB_1, ZEN, or CIT was found in mimetic-based chemiluminescent immunoassay (Figure 4B). Among the seven curves, inhibition curves established with L12-206−MBP revealed the highest sensitivity; the IC_{50} was 0.82 ng/mL, and linearity ranged (20−80% inhibition of the maximal chemiluminescent intensity) from 0.31 to 2.17 ng/mL (Figure 4C, the value is the average of three replicates). For comparison, the value of IC_{50} and working range for conventional OTA−OVA cojugate -based immunoassay were 0.90 and 0.21−3.88 ng/mL; the competitive inhibition curves between the OTA−OVA conjugate and mimetic OTA conjugate had no significant difference. However, the phage-displayed OTA mimotope-based immunoassay did not exhibit a typical sigmoid curve; with the addition of OTA standard, the chemiluminescent intensity did not decrease for the phage-displayed peptide-coated microwells (Figure 4C), which indicated that phage-displayed mimotopes of OTA are not adaptable for polystyrene microwells directly serving as coating antigens in quantitative immunoassays.

The influences of buffer ionic strength and pH were investigated to evaluate the effect on mimetic-based immunoassay for OTA. The appropriate buffer ionic strength was tested with 5, 10, 25, and 50 mM methanol−PBS (Figure 5A). According to the result, ionic strength between 10 and 50 mM has little significant influence on the assay. Taking both IC_{50} and RLU_{max}/IC_{50} into consideration, optimized performance was achieved at 10 mM. At the optimal ionic strength, the pH effect was investigated between 6.0 and 9.0 (Figure 5B). The result suggests the best performance at pH 7.4 when the RLU_{max}/IC_{50} was the highest. In addition, methanol that affects antigen−antibody interaction is generally utilized as OTA solvent and extraction agent; the proper content of methanol was estimated using OTA prepared in various concentrations of (5, 10, 20, and 40%, v/v). The relative luminescent intensity declined and IC_{50} increased significantly when methanol concentration was >10%. The highest RLU_{max}/IC_{50} and the lowest IC_{50} were observed at 10% methanol−PBS (Figure 5C).

Qualitative Immunoassay Based on the Mimetic OTA Conjugate. After being employed in the quantitative immunoassay, the mimetic OTA conjugate (L12-206−MBP) was also utilized to develop a qualitative dot immunoassay to visually detect OTA without instrumentation. A checkerboard assay was performed by using different dilutions of immunoreagents (mimetic OTA conjugate and OTA-McAb). The selected mimetic OTA conjugate diluted to 10, 25, 50, and 100 μg/mL and OTA-McAb diluted to 1:500, 1:1000, 1:2000, and 1:4000 were tested, respectively. The assay incubated with 100 μg/mL mimetic OTA conjugate and 1:2000 dilution of OTA-McAb for 15 min was chosen to be the minimum concentration of immunoreagents for a clear and sufficient intensity of spot (Figure 6A). Increasing the incubation time beyond 15 min did not increase the spot color intensity (data not shown).

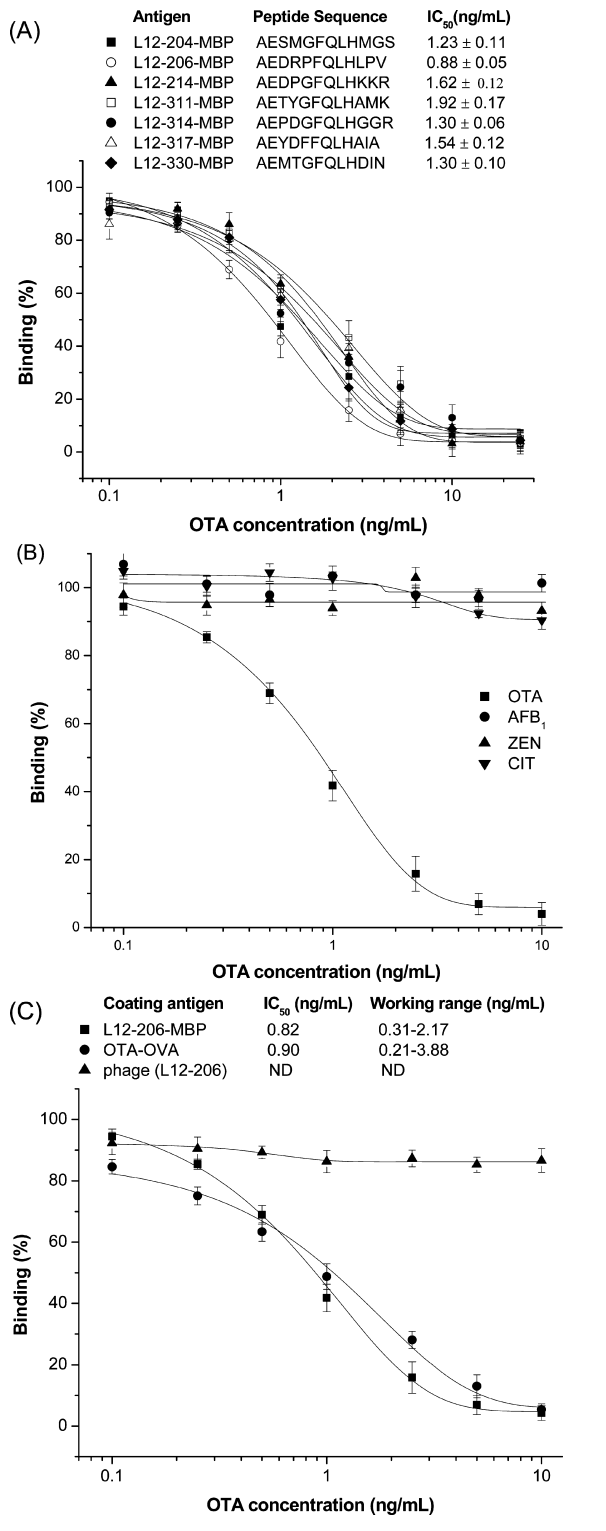

Figure 4. (A) Dose−response curves of the CLEIAs for OTA established with seven mimetic OTA conjugates (L12-204−MBP, L12-206−MBP, L12-214−MBP, L21-311−MBP, L12-314−MBP, L12-317−MBP, and L12-330−MBP. The value is the average of five replicates ± standard deviation. (B) Cross-reactivity of the mimetic OTA conjugate (L12-206−MBP)-based CLEIA toward mycotoxin OTA, AFB_1, ZEN, and CIT. (C) Dose−response curves of the CLEIAs using the mimetic OTA conjugate (L12-206−MBP, 5 μg/mL), OTA−OVA conjugate (2 μg/mL) and phage (L12-206, 2.5 × 10^8 pfu/mL) as coating antigens, respectively. The vertical bars indicate the standard deviation (n = 3).

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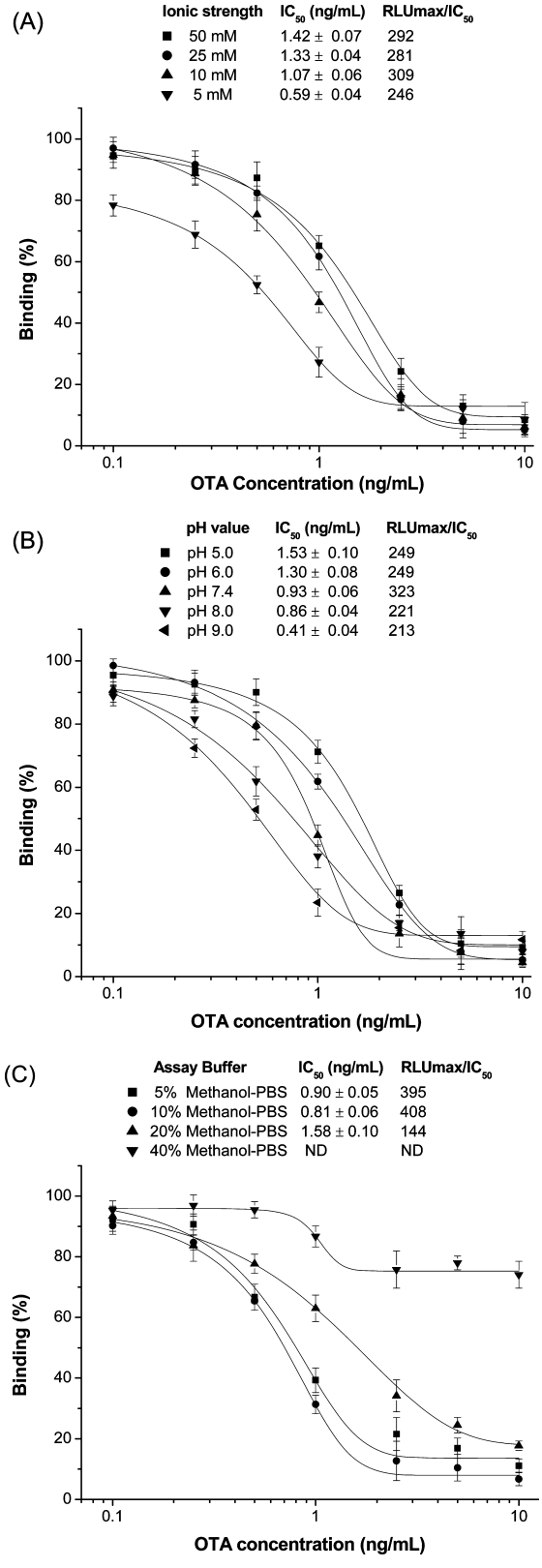

Figure 5. (A) Ionic strength, (B) pH, and (C) methanol effects on the performance of the mimetic OTA conjugate-based chemiluminescent ELISA. Each experiment was implemented in triplicate, and the means of the triplicates are plotted (error bars represent the standard deviation).

To visually evaluate the cutoff levels (the lowest concentration of OTA able to achieve 100% inhibition and therefore

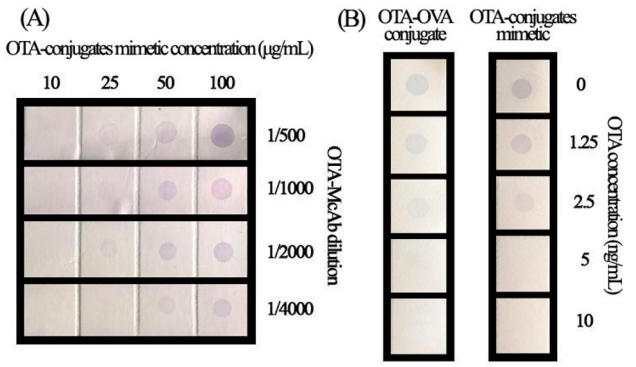

Figure 6. OTA–OVA conjugates and mimetic OTAconjugate-based dot immunoassays: (A) mimetic OTA conjugate (ranging from 10 to 100 μg/mL) spotted onto PVDF membranes incubated with different dilutions of OTA–McAb (ranging from 1/500 to 1/4000); (B) evaluation of the cutoff level (smallest amount of OTA able to achieve 100% inhibition and therefore no spot color development) of the dot immunoassay assessed visually. Mimetic OTA conjugate and conventional OTA–OVA conjugates were applied to the assay as coating antigen, and OTA levels (0, 1.25, 2.5, 5.0,10 ng/mL) were analyzed, respectively.

no color development) of the mimetic OTA conjugate-based dot immunoassay, OTA was analyzed at different concentrations (0, 1.25, 2.5, 5.0, 10 ng/mL). As presented in Figure 6B, the intensity of the spot decreased with increasing OTA concentration, and no spot color development on the membrane was obtained when the OTA standard concentration rose beyond 5.0 ng/mL. The cutoff level of the mimetic OTA conjugate-based dot immunoassay was 5.0 ng/mL, which was equal to that of the OTA–OVA conjugate-based dot immunoassay (thecutoff level was 5.0 ng/mL, Figure 6B).

Validation Studies. The validation of the CLEIA based on the mimetic OTA conjugate (L12-206–MBP) was carried out by measuring the OTA recovery and reproducibility. Table 1 shows the recoveries of OTA added to corn, rice, and wheat samples analyzed by three methods. In the first two assays (CLEIAs set up with both the mimetic OTA conjugate and OTA–OVA), the spiked and detected OTA contents were in accord with each other. The recovery of cereals spiked with 10–100 μg/kg OTA ranged from 82.6 to 134.0% determined by the mimetic OTA conjugate-based CLEIA and from 90.2 to 132.0% by conventional OTA–OVA-based assay. In the case of the dot immunoassay, a clear dot was observed for adding cereals OTA <50 μg/kg, but no dot appeared for OTA ≥50 μg/kg.

As for the reproducibility, it is evaluated by determining the CVs of the IC_{50} of the calibration curves. The CV was 5.3% for intra-assay and 10.4% for interassay, respectively. Furthermore, the mimetic OTA conjugate kept at −20 °C until use for 1 year remained effective, indicating the stable performance of the mimetic OTA conjugate-based immunoassay (data not shown).

Twenty samples of cereal and feedstuffs purchased from the Chinese market were analyzed using mimetic OTA conjugate-based CLEIA, dot immunoassay, and commercial ELISA kit for OTA, respectively. Among the 20 samples, 5 were positively detected by CLEIA and commercial ELISA kit (their limits of detection were both 5.0 μg/kg). The results obtained from CLEIA and commercial ELISA kits were in agreement with each other. However, no positive sample was detected by dot immunoassay because of its cutoff level (50 μg/kg), higher than

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Table 1. Recoveries of OTA Added to Corn, Rice, and Wheat Samples in Determinations Performed by CLEIAs and Dot Immunoassay Based on OTA−OVA Conjugate and Mimetic OTA Conjugate

		mimetic OTA conjugate-based CLEIA (n = 3)			OTA−OVA conjugate-based CLEIA (n = 3)			
matrix	spiked (μg/kg)	detected (μg/kg)	recovery (%)	RSD (%)	detected (μg/kg)	recovery (%)	RSD (%)	mimetic OTA conjugate-based dot immunoassay (n = 3)
corn	10	10.2 ± 0.7	102.0 ± 7.0	6.9	9.8 ± 0.6	98.0 ± 6.0	6.1	−[a] − −
	20	26.7 ± 1.8	133.5 ± 9.0	6.7	22.7 ± 1.0	113.5 ± 5.0	4.4	− − −
	50	41.3 ± 3.4	82.6 ± 6.8	8.2	46.0 ± 3.6	92.0 ± 7.2	7.8	+[b] + −
	100	92.3 ± 5.2	92.3 ± 5.2	5.6	98.2 ± 6.3	98.2 ± 6.3	6.4	+ + +
rice	10	13.4 ± 0.8	134.0 ± 8.0	6.0	12.7 ± 0.7	127.0 ± 7.0	5.5	− − −
	20	19.2 ± 1.8	96.0 ± 9.0	9.4	23.0 ± 1.1	115.0 ± 5.5	4.8	− − −
	50	51.1 ± 3.4	102.2 ± 6.8	6.7	48.2 ± 2.9	96.4 ± 5.8	6.0	+ + ±[c]
	100	89.3 ± 6.5	89.3 ± 6.5	7.3	90.2 ± 6.0	90.2 ± 6.0	6.7	+ + +
wheat	10	12.5 ± 0.9	125.0 ± 9.0	7.2	13.2 ± 1.2	132.0 ± 12.0	9.1	− − −
	20	23.3 ± 1.9	116.5 ± 9.5	8.2	20.6 ± 2.1	103.0 ± 10.5	10.2	− − −
	50	51.2 ± 2.4	102.4 ± 4.8	4.7	54.5 ± 1.3	109.0 ± 2.6	2.4	+ + +
	100	97.0 ± 4.5	97.0 ± 4.5	4.6	93.2 ± 6.2	93.2 ± 6.2	6.7	+ + +

[a]Negative, an obvious dot was observed. [b]Positive, no dot was observed. [c]Negative/positive, an unclear dot was observed.

the OTA concentration in contaminated samples (data are available in the Supporting Information, Table S1).

In conclusion, we described a new approach for developing coating antigen mimetics in immunoassay. The conventional antigen, OTA conjugates, was replaced with mimetic OTA conjugate (peptide−MBP fusion protein). This fusion protein mimics the reaction between the OTA and antibody and serves as a substitute for the OTA conjugates in immunoassays. These peptide−MBP fusion proteins (mimetic OTA conjugate) are cloneable and homogeneous products expressed from constructed express plasmids, which can be prepared reproducibly in large scale and with low cost. The peptide−MBP fusion protein provides practical sensitivity, specificity, and reliability in the standard qualitative and quantitative immunoassays.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figure S1 and Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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