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Production of a Monoclonal Antibody against Ochratoxin A and Its Application to Immunochromatographic Assay

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A monoclonal antibody (Mab) against ochratoxin A (OTA) was produced from the hybridoma cell line C7G25, which was established by the fusion of Sp2/0-Ag14 myeloma cells with spleen cells isolated from a BALB/c mouse immunized with the OTA−bovine serum albumin conjugate. This Mab belongs to the IgG_{2a} heavy-chain subclass with a κ-type light chain. The level of 50% inhibition concentration was 1.20 ng/mL in a competitive direct enzyme-linked immunosorbent assay (cdELISA), and the detection limit was 0.12 ng/mL. This antibody is specific for OTA but also shows cross-reactivity with ochratoxin B (31.7%) in a cdELISA. On the basis of the sandwich format using the produced Mab against OTA, a rapid immunochromatographic assay was developed to efficiently detect OTA. This method was able to detect up to 500 ng/mL of OTA in <10 min.

KEYWORDS: Ochratoxin A; monoclonal antibody; enzyme-linked immunosorbent assay (ELISA); colloidal gold; immunochromatographic assay

INTRODUCTION

Ochratoxin A (OTA; **Figure 1**) as secondary metabolite is a mycotoxin produced by fungi growing on agricultural commodities in the field or during storage. OTA is mainly produced by *Aspergillus* and *Penicillium* species in starch-abundant cereals and causes nephrotoxicity, hepatotoxicity, and carcinogenicity in animal (*1*, *2*). With the amounts of imported or exported agricultural commodities increasing in recent years, monitoring of OTA is essential for food safety in the worldwide market. Many research groups have been studying the monitoring methods of naturally occurring OTA in a number of agricultural commodities (*3*, *4*). Like many other mycotoxins, OTA is generally detected or quantified by using the principle of thin-layer chromatography, high-performance liquid chromatography with fluorescence detection, or gas chromatography (*5*−*7*). However, in cases when numerous analyses are to be carried out, the references or the official methods chosen by a public authority might be considered as time-consuming or laborious for the analyst. The methods above are also either lacking in specificity or limited by sensitivity. Therefore, attempts were made to develop an immunochemical method for the detection of OTA. An immunoassay-based method is usually simple and rapid compared to chromatographic techniques. It has further benefited from the application of monoclonal antibody (Mab) technology (*8*). When a suitable antibody is chosen, it can then be used in a rapid immunological method such as an enzyme-linked, radioimmunoassay, or immunoaffinity chromatography system. The enzyme-linked immunosorbent assay (ELISA) method has been established and applied to the determination of OTA in various samples such as maize, peanuts, milk, and barley (*9*−*11*).

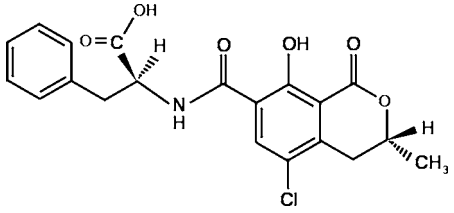

Figure 1. Structure of ochratoxin A.

An immunochromatographic assay, also simply called a strip assay, has been developed recently. This technique is based on the principle of the highly specific immunological reactions between antigens and antibodies that are used for the rapid detection of specific compounds in biological fluids. There are several advantages such as its user-friendly format, the very short time needed to obtain results, its low cost, field evaluation, and its long-term stability. Recently, the immunochromatographic assays using a Mab were reported for the detection of ricin, a plant toxin, and botulinum neurotoxin type B (*12*, *13*). However, a rapid strip test format was not established for the detection of mycotoxins including OTA, even though many immunochromatographic strip test kits for drugs and toxins have

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been already commercialized. In this study, a Mab against OTA was produced, and a rapid and sensitive immunochromatographic assay for OTA detection was developed.

MATERIALS AND METHODS

Supplies. Ochratoxin A, ochratoxin B (OTB), aflatoxin B_1 (AFB_1), fumonisin B_1 (FB_1), bovine serum albumin (BSA), hypoxanthine, thymidine, aminopterin, L-glutamine, penicillin, streptomycin, fetal bovine serum (FBS), OTA−BSA conjugate, horseradish peroxidase (HRP), Freund's complete adjuvant (FCA), Freund's incomplete adjuvant (FIA), poly(ethylene glycol) (PEG 1500), 3,3′,5,5′-tetramethylbenzidine (TMB) dihydrochloride, 2,6,10,14-tetramethylpentadecane (pristane), and myeloma cells (Sp2/0-Ag14) were from Sigma Chemical Co. (St. Louis, MO). Purified Recomb Protein A/G, Slide-A-Lyzer dialysis cassettes, the rabbit anti-mouse IgG Fc specific antibody, goat anti-mouse IgG-HRP, and T-Gel purification kit were purchased from Pierce (Rockford, IL). A Mouse Immunoglobulin Isotyping ELISA Kit was obtained from BD PharMingen (San Diego, CA). Eight-week-old female BALB/c mice were purchased from Biogenomics (Washington, DC). Colloidal gold (40 nm in diameter) was obtained from BBInternational (Cardiff, U.K.). A Linomat IV HiFlow Plus Assembly Kit was purchased from Millipore (Bedford, MA).

OTA−HRP conjugates were prepared following the method as described by Chu et al. (*14*). Phosphate-buffered saline (PBS) solution was made as 0.138 M NaCl and 2.7 mM KCl in 0.01 M phosphate buffer, pH 7.4. To prepare PBS with 0.05% (v/v) Tween 20 (PBST), Tween 20 was added to PBS before the pH was adjusted. Serum-free DMEM consisted of Dulbecco's Modified Eagle Medium (Gibco-BRL, Gaithersburg, MD) with added L-glutamine (2 mM), penicillin (100 units/mL), and streptomycin (100 μg/mL). Complete DMEM was serum-free DMEM plus 10% (v/v) heat-inactivated FBS. Heat inactivation of FBS was achieved at 56 °C for 30 min. HT medium consisted of complete DMEM with 10% FBS, sodium hypoxanthine (100 μM), and thymidine (16 μM). HAT consisted of HT medium plus aminopterin (0.4 μM). All other chemicals used were of analytical reagent grade.

Production of Mab against OTA (Anti-OTA Mab). Mab was produced according to the standard method of Kohler and Milstein (*8*) with some modifications. Four 8-week-old BALB/c female mice were each immunized intraperitoneally (ip) with the OTA−BSA conjugate (0.25 mg/mL) emulsified with an equal volume of FCA as an immunogen. Subcutaneous injections of the immunogen (0.25 mg/mL for each mouse) mixed with FIA were carried out in the interval of 2 weeks until a strong immune response to the antigen was recognized. The mice were finally boosted intravenously (iv) with the OTA−BSA conjugate (0.5 mg/mL for each mouse) dissolved in PBS after 1 week for generating high-affinity antibodies. To check the immune response, test sera from mice were periodically collected to determine the titer of the sera. For hybridoma production, the lymphocytes (1×10^8 cells) collected from the spleen of the immunized mouse showing the best antibody titer were fused with the Sp2/0-Ag14 myeloma cell line using PEG fusion medium, and then the hybrid cells were resuspended on 96-well plates in HAT medium with feeder layers of mouse peritoneal macrophage (*15*). The cells were cultured in a CO_2 incubator at 37 °C. The supernatants of cell cultures were screened by modified competitive direct ELISA (cdELISA) for the presence of antibodies binding to OTA. The hybridomas of interest were cloned by limiting dilution and extended. Cells exhibiting inhibition by OTA in fusion wells were transferred to 24-well plates. Positive hybridomas were subsequently grown in HT medium. Selection was made on the basis of antibody production, affinity, and sensitivity. The cloning procedure was repeated three times for the selected clone.

Mature female BALB/c mice were injected (ip) with 0.5 mL of 2,6,-10,14-tetramethylpentadecane (pristane) 7 days before receiving ip injection of the hybridoma cell line [$(1-10) \times 10^6$ cells] suspended in DMEM. Ascites fluid was developed for 2 weeks after the injection and was collected every other day for ~7 days. The ascites fluid was centrifuged at 6000*g* for 5 min to remove cell debris and then frozen at −20 °C. Purification of Mabs was achieved using a T-Gel purification kit according to the manufacturer's instructions. The purified anti-OTA Mabs were used for subsequent experiments.

ELISA. Screening of positive hybridoma cells was carried out using direct enzyme immunoassay with a modified double-antibody solid-phase cdELISA performed according to the method of Hack et al. (*16*). The 96-well plate was coated with rabbit anti-mouse IgG Fc specific antibody (0.1 mL/well with 2 μg/mL) in 0.05 M carbonate−bicarbonate buffer for 1 h at 37 °C and washed three times with fresh PBST. Diluted culture supernatant fluid (100 μL) was transferred from the tissue culture plates to 96-well plates and then incubated for 1 h. After a washing step with fresh PBST, the competition step was performed by the addition of 50 μL of OTA (0−2 μg/mL) and 50 μL of diluted OTA−HRP (1:1000 in PBST) followed by 1 h of incubation. Following an additional washing step, 100 μL of fresh substrate solution (TMB/H_2O_2) was added. After the reaction had been stopped by the addition of 50 μL of 2 M H_2SO_4, the color development was quantitatively measured at 450 nm using a microplate reader (ThermoMax, Molecular Device, Sunnyvale, CA). The cdELISA was performed to determine the sensitivity and specificity of the Mab secreted from the selected cell line. Mabs against OTA (anti-OTA Mab, 0.1 mL/well with 2 μg/mL) were coated on the 96-well plate overnight at 4 °C and washed with fresh PBST. Fifty microliters of OTA standard (0−2 μg/mL) and 50 μL of diluted OTA−HRP (1:1000 in PBST) were added consecutively to each well. For evaluation of the cross-reactivity of anti-OTA Mab, 50 μL of some mycotoxins (OTB, AFB_1, and FB_1, 0−2 μg/mL) or BSA (0−2 μg/mL) were used instead of OTA standard. After 1 h of incubation at 37 °C, the plate was washed and the bound peroxidase was determined by the color development at 450 nm using the microplate reader.

Determination of Isotypes of Anti-OTA Mab. The isotypes of anti-OTA Mab were determined by the Mouse Immunoglobulin Isotyping ELISA Kit using class and subclass specific anti-mouse immunoglobulin (IgG). The class of heavy-chain and light-chain types of the Mab could be determined by an indirect ELISA system. Briefly, antibodies that are specific for the individual isotypes were coated into the 96-well plate for overnight at 4 °C. After coating, 50 μL of supernatant from cloned hybridomas was added and washed three times with fresh PBST. Diluted anti-mouse IgG−HRP (1:100 in PBS containing 1% BSA) was added for binding to the anti-OTA Mab. Following an additional washing step, 100 μL of fresh substrate solution (TMB/H_2O_2) was added. Peroxidase reaction was saturated and stop solution (2 M H_2SO_4) was added into the plate, and color development was quantitatively measured at 450 nm using the microplate reader.

Measurement of Cross-Reactivity. To determine the specificity of the Mab, cross-reactivity of anti-OTA Mab with an ochratoxin analogue (OTB), some mycotoxins (AFB_1 and FB_1), and BSA was evaluated through cdELISA. Cross-reactivity was determined as

$$\text{cross-reactivity (\%)} = \frac{\text{concn of standard OTA inhibiting 50\% of antibody binding}}{\text{concn of other compounds inhibiting 50\% of antibody binding}} \times 100$$

Preparation of Colloidal Gold Probes. Purified anti-OTA Mabs in PBS were dialyzed against distilled water for 6 h at 4 °C and filtered to determine the optimum conditions of pH and antibody concentration for conjugation. Colloidal gold in 1 mL of 2 mM borax buffer was conjugated with the purified anti-OTA Mab. The pH values tested for colloidal gold ranged from 5.0 to 9.5 and the amount of anti-OTA Mab added from 0 to 32 μg. After conjugation, 10% NaCl was added, and the conjugate solutions were read at 520 nm with a spectrophotometer. On the addition of ionic substances (NaCl) to the colloidal gold solution, the attracting force becomes greater than electrostatic repulsion between gold particles, which leads to an aggregation accompanying a color change from red to blue (*17*).

Colloidal gold (40 nm in diameter) was used for the conjugation of anti-OTA Mab. One milliliter of colloidal gold was mixed by adding 120 μL of 20 mM borax buffer (pH 7.0), and then anti-OTA Mab solution (125 μg/mL) was added. The mixture was gently mixed for 30 min at room temperature and blocked by 10% (w/v) filtered BSA for 30 min. This mixture was centrifuged at 7000*g* for 15 min at 4 °C, and then the supernatant was discarded. Borax buffer (2 mM; pH 7.0) was added to the mixture. After centrifugation for washing and

discarding the supernatant, the gold pellets were resuspended by adding 20 mM Tris-buffered saline (pH 8.0) with 1% BSA and 0.1% sodium azide. These anti-OTA Mab coated colloidal gold probes were stored at 4 °C until use.

Preparation of Immunochromatographic Test Strip. An immunochromatographic test system was prepared as follows: OTA−BSA and protein A/G were diluted with 0.2% (w/v) BSA and 3% (v/v) methanol in 10 mM PBS (pH 7.4), respectively. By using Linomat IV, 3 μL of OTA−BSA (0.2 mg/mL) and 3 μL of protein A/G (0.5 mg/mL) were sprayed separately near one end (top) of the strip of high-flow nitrocellulose membrane (FF85; capillary rise, 4 cm/75−100 s; W × L, 0.5 mm × 25 mm; Scheicher & Schuell BioScience, Keene, NH) and dried for 1 h at room temperature. Even though nitrocellulose membrane has been used as a protein-binding membrane, the exact binding mechanism remains unknown (*18*, *19*). The purpose of drying the membrane at room temperature is to fix and stabilize protein or protein conjugate to the nitrocellulose. The protein A/G and the OTA−BSA were immobilized on the control and test line, respectively. The anti-OTA Mab−colloidal gold conjugate (50 μL/strip) was added near the other end (bottom) and dried again. After drying, the coated membrane was attached on the plastic backing, and then an absorbent pad was also attached on the end of the nitrocellulose membrane. The nitrocellulose membrane was cut to the appropriate size (4 mm width) for making the immunochromatographic strip.

Assay of OTA on Test Strip. The assay was carried out by adding various concentrations of the standard OTA solution (0−10 μg/mL) into the microplate wells. Subsequently, the test strips were dipped into the wells vertically. The combined solution of the test OTA and detection reagent rose up the membrane.

RESULTS AND DISCUSSION

Screening of Hybridoma Cells. The molecular mass of OTA is 403.8 Da, which is too small to be immunogenic. To overcome the limit of OTA recognition in an immune response, the OTA−BSA conjugate was constructed as the immunogen instead of OTA itself. However, immunization of the OTA−BSA conjugate might induce many kinds of antibodies that recognize not only OTA but also BSA (*20*). A new method for the screening of monoclonal antibodies to mycotoxins was developed using a double-antibody solid phase in a direct enzyme immunoassay (*19*). In general, indirect enzyme immunoassays are used for the screening procedures of these antibodies, in which the immunoplates are coated with the respective toxin−protein conjugates. These conjugates have to be different from the conjugates used for immunization to avoid cross-reactions between the antibody and the carrier. Thus, modified cdELISA was performed using the anti-mouse IgG Fc specific antibody as solid phase for trapping the Mab and the OTA−HRP conjugate for the detection of the specificity of the bound antibody. In addition, competitive indirect ELISA (ciELISA) was implemented simultaneously in the screening steps. Of the 288 wells tested, 9 gave a positive signal at the first screening. The cells of supernatants exhibiting inhibition by OTA were transferred from the fusion plates into 24-well plates. Cultures showing the highest percentage of inhibition with OTA as competitor were cloned by limiting dilution. For the cloning procedure, the cells were plated in HT medium on a layer of macrophage feeder cells and the final 257 clones obtained from the wells that had been seeded with 1 cell/well. After the limiting dilution, nine clones showing a positive signal by the modified cdELISA were selected at the second screening. Finally, a stable hybridoma cell line, C7G25, producing antibodies against OTA was established and used for subsequent tests.

Cross-Reactivity and Classification of Anti-OTA Mab. The type, form, specificity, and sensitivity of immunoassay developed for the detection of various mycotoxins are dependent on the Mab characteristics. The Mabs against mycotoxins have been characterized in terms of affinity, cross-reactivity to closely related metabolites, assay performance, sensitivity, immunoglobulin class and subclass, stability, and ability to determine mycotoxins from contaminated samples (*21*). Therefore, cdELISA was performed to determine the sensitivity of the purified anti-OTA Mab from the stable hybridoma cell line C7G25. From the standard curve of cdELISA using the anti-OTA Mab and OTA−HRP conjugate, a detection range of OTA was between 0.12 and 100 ng/mL, and the 50% inhibition concentration (IC_{50}) was 1.2 ng/mL (**Figure 2**). OTA and OTB are structurally related mycotoxins on human health. The chemical structures of OTA and OTB are very similar. The latter compound lacks only the chlorine atom in the isocoumarin ring (*22*). Therefore, the cross-reactivity of anti-OTA Mab with OTB, AFB_1, FB_1, and BSA was also examined using the cdELISA. The antibody was specific to OTA, whereas it had a weak cross-reaction with OTB (31.7%). There was no reactivity with AFB_1, FB_1, and BSA.

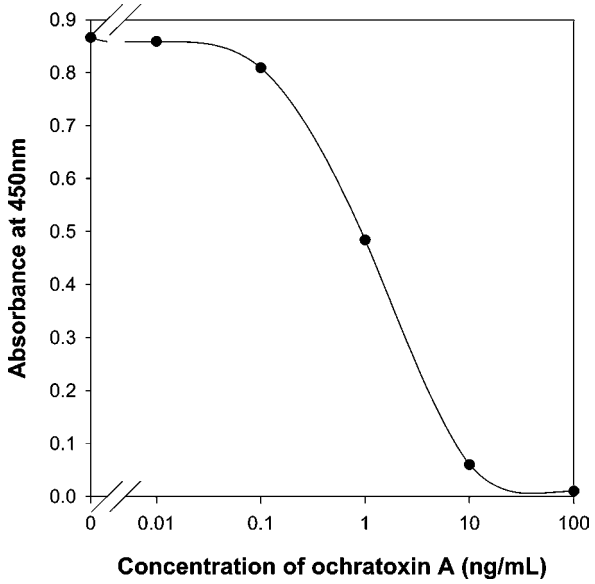

Figure 2. Standard curve of anti-OTA Mab from selected hybridoma cell line C7G25 in competitive direct ELISA assay.

Isotype determinations of Mabs are essential to give information on the chemical properties of the antibody in relation to isolation, conjugation procedures to enzyme, and assay sensitivities (*21*). The analysis of the isotype of the anti-OTA Mab showed that the anti-OTA Mab produced from the C7G25 cell line was the IgG_{2a} subclass with a κ-type light chain by the indirect ELISA. These anti-OTA Mabs were tested for suitability as a strip assay kit for OTA detection.

Conjugation of Anti-OTA Mab with Colloidal Gold. Colloidal gold has been generally used as an immunospecific probe for electron microscopy and immunoblotting (*23*, *24*). Recently, it was also used in an antibody capture assay (*25*). In this study, anti-OTA Mabs were conjugated to 40 nm gold particles offering maximum visibility due to the least steric hindrance in the case of IgG conjugation (*26*). During the conjugation procedure, proteins are adsorbed onto the surface of the gold particle within a matter of seconds (*27*). There is a minimal antibody concentration that can stabilize the gold particles. Therefore, optimum conditions of pH and antibody concentration for conjugation with the colloidal gold can be determined by measuring the differential absorbance ($A_{520} - A_{580}$) (*28*). As a result of preliminary titration, the optimum pH of 2 mM borax buffer in the colloidal gold particles was 7.0

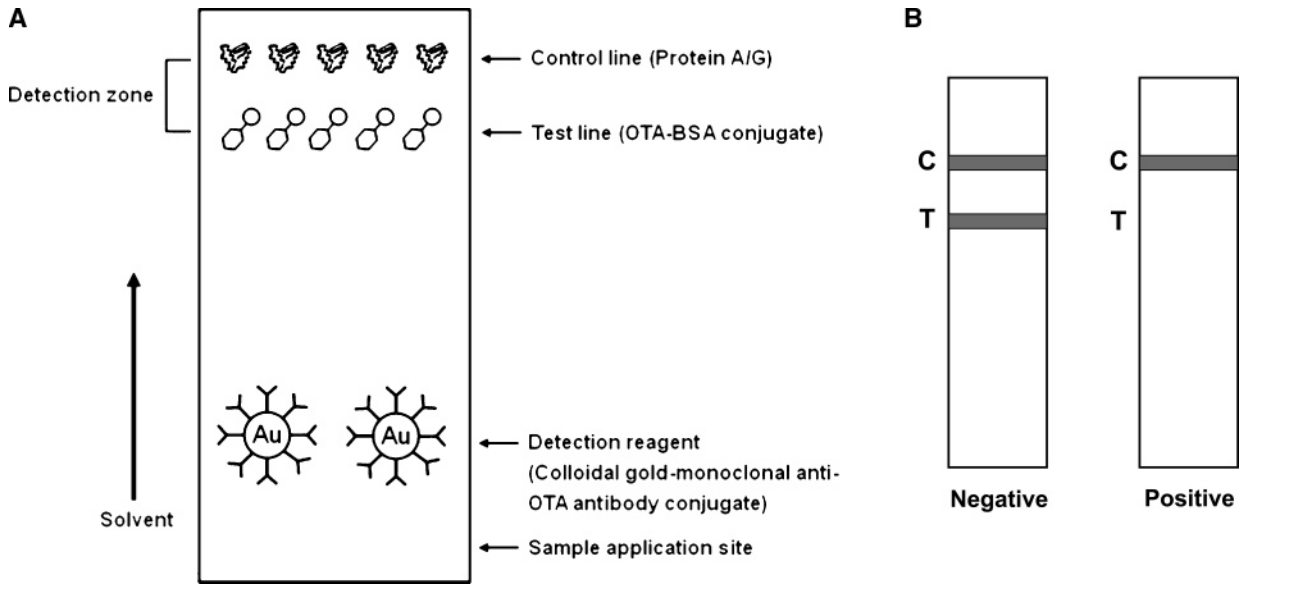

Figure 3. (**A**) Schematic description of immunochromatographic test strip. (**B**) Illustrations of immunochromatographic test results. C and T stand for control line and test line, respectively.

and a minimum amount of anti-OTA Mabs was 10 μg to stabilize the gold particles for conjugation with 1 mL of colloidal gold.

Determination of Strip Test Results. The principle of this immunochromatographic test is described as one-step immunoassay in which the OTA−BSA conjugate competes with OTA, probably due to the fact that these may be present in a sample for limited antibody binding sites. A schematic description of the immunochromatographic test format is shown in **Figure 3A**. In the absence of OTA in the sample, the solution of red antibody−colloidal gold conjugate and sample moves upward chromatographically by the capillary action across the membrane. This solution then migrates to the immobilized OTA−BSA conjugate zone on the test region. The red antibody−colloidal gold conjugate then was trapped to the OTA−BSA to form a visible line as the antibody complexes with the OTA−BSA conjugate. Therefore, the formation of a visible precipitant in the test zone occurs when the test sample is negative for OTA (**Figure 3B**). When OTA is present in a sample, the OTA competes with the OTA−BSA conjugate on the test zone for the limited antibody binding sites. When a sufficient concentration of OTA was present, it would occupy the limited antibody binding sites, preventing attachment of the colored antibody−colloidal gold conjugate to the OTA−BSA conjugate zone on the test region. Therefore, the absence of the color band on the test region indicates a positive result (**Figure 3B**). Protein A/G that has a different interaction is added to the nitrocellulose membrane in the control region to verify that the assay has performed properly. This control line should always show a red color regardless of OTA. Protein A/G was used for binding an excess detector reagent (anti-OTA Mab−gold conjugate) because of its Fc-binding properties (*27*). A negative sample without OTA produced two red bands, whereas a positive sample with OTA generated only one band in red, indicating the strip test was done as expected.

Detection Limit of OTA Test Strip. Samples at various concentrations of OTA (0−10^4 μg/mL) were applicably assayed by the OTA test strips. Analysis was completed in <10 min, and the detection limit for the strip test using OTA was 500 ng/mL, which can form one band on the control line (**Figure 4**). At 500 ng/mL, OTA could bind whole anti-OTA Mabs on the surface of the gold particles, so OTA prevented binding

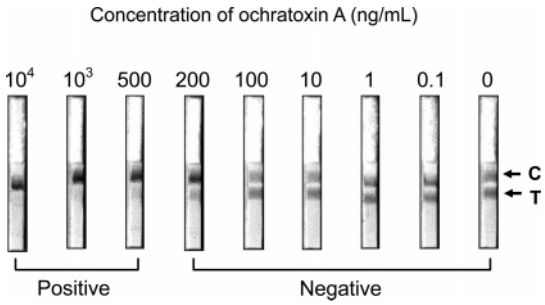

Figure 4. Immunochromatographic detection of ochratoxin A using applicable test strip. A series of dilutions (0.1–10000 ng/mL) of OTA was made in PBS. A negative sample without OTA produces two bands, whereas a positive sample with OTA shows only one band.

between the antibody−gold conjugate and the OTA−BSA on the nitrocellulose membrane. Therefore, >500 ng/mL of OTA could be detected by forming a single band on the control line. The monoclonal-based OTA test strip showed good specificity. There was only a weak cross-reaction with OTB (data not shown). The developed OTA immunochromatographic assay using the colored colloidal gold nanoparticles as a tracer provides visual evidence of the presence of OTA in a sample within 10 min.

However, the developed OTA strip assay showed low sensitivity to support in vivo OTA distribution analysis and in field OTA contamination detection. That might be due to the membrane strips constructed. The colloidal gold coated anti-OTA Mabs might be irreversibly immobilized to the membrane surface after they were dried. Therefore, the mobility of nanoparticles of colloidal gold might be problematic in the porous nitrocellulose membrane, and the colloidal gold−anti-OTA Mab conjugate might have undesirable nonspecific binding on the surface of membrane prior to the binding reaction with the immobilized OTA−BSA conjugate when it migrates through the membrane pores. The immunochromatographic assay using colloidal gold has several advantages. One is that the enhancement procedure provides an opportunity to improve assay sensitivity. The sensitivity of immunogold detection could be increased ~1000-fold with the silver enhancement reagent (*13*).

In conclusion, the OTA strip assay described in this study might provide potential applications to a general assay method

for the detection of various toxins in biological fluids that requires no separation steps. Moreover, the assay is better than other immunoassays, such as radioimmunoassay and ELISA, with respect to its overall speed and simplicity for the detection of various toxins. These characteristics make strip assay a potential candidate for the development of rapid toxin detection kits.

ABBREVIATIONS USED

OTA, ochratoxin A; ELISA, enzyme-linked immunosorbent assay; BSA, bovine serum albumin; FBS, fetal bovine serum; OTA−BSA, ochratoxin A−BSA conjugate; HRP, horseradish peroxidase; FCA, Freund's complete adjuvant; FIA, Freund's incomplete adjuvant; PEG, poly(ethylene glycol); TMB, 3,3′,5,5′-tetramethylbenzidine; PBS, phosphate-buffered saline; PBST, PBS with 0.05% (v/v) Tween 20; DMEM, Dulbecco's Modified Eagle's Medium; cdELISA, competitive direct ELISA; anti-OTA Mab, monoclonal antibody against OTA; IgG, immunoglobulin G; ciELISA, competitive indirect ELISA; IC_{50}, 50% inhibition concentration; OTB, ochratoxin B; AFB_1, aflatoxin B_1; FB_1, fumonisin B_1.

LITERATURE CITED

(1) Delacruz, L.; Bach, H. The role of ochratoxin A metabolism and biochemistry in animal and human nephrotoxicity. *J. Biopharm. Sci.* **1990**, *1*, 277−304.

(2) Kuiper-Goodman, T.; Scott, P. M. Risk assessment of the mycotoxin ochratoxin A. *Biomed. Environ. Sci.* **1989**, *2*, 179−248.

(3) Milanez, T. V.; Leitao, M. F. F.; The effect of cooking on ochratoxin A content of beans, variety 'Carioca'. *Food Addit. Contam.* **1996**, *13*, 89−93.

(4) Patel, S.; Hazel, C. M.; Winterton, A. G. M.; Gleadle, A. E. Survey of ochratoxin A in UK retail coffees. *Food Addit. Contam.* **1997**, *14*, 217−222.

(5) Pittet, A.; Royer, D. Rapid, low cost thin-layer chromatographic screening method for the detection of ochratoxin A in green coffee at a control level of 10 μg/kg. *J. Agric. Food Chem.* **2002**, *50*, 243−247.

(6) Medina, A.; Jimenez, M.; Gimeno-Adelantado, J. V.; Valle-Algarra, F. M.; Mateo, R. Determination of ochratoxin A in beer marketed in Spain by liquid chromatography with fluorescence detection using lead hydroxyacetate as a clean-up agent. *J. Chromatogr. A* **2005**, *1083*, 7−13.

(7) Soleas, G. J.; Yan, J.; Goldberg, D. M. Assay of ochratoxin A in wine and beer by high-pressure liquid chromatography photodiode array and gas chromatography mass selective detection. *J. Agric. Food Chem.* **2001**, *49*, 2733−2740.

(8) Kohler, G.; Milstein, C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **1975**, *256*, 495−497.

(9) Kwak, B. Y.; Shon, D. H. Detection of ochratoxin A in agricultural commodities using enzyme-linked immunosorbent assay. *Food Sci. Biotechnol.* **2000**, *3*, 168−173.

(10) Kawamura, O.; Sato, O.; Kajii, H.; Nagayama, S.; Ohtani, K.; Chiba, J.; Ueno, Y. A sensitive enzyme linked immunosorbent assay of ochratoxin A based on monoclonal antibodies. *Toxicon* **1989**, *27*, 887−897.

(11) Morgan, M. R.; McNervey, R.; Chan, H. W. Enzyme-linked immunosorbent assay of ochratoxin A in barley. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 1481−1484.

(12) Shyu, R. H.; Shyu, H. F.; Liu, H. W.; Tang, S. S. Colloidal gold-based immunochromatographic assay for detection of ricin. *Toxicon* **2002**, *40*, 255−258.

(13) Chiao, D. J.; Shyu, H. F.; Hu, C. S.; Chiang, H. Y.; Tang, S. S. Colloidal gold-based immunochromatographic assay for detection of botulinum neurotoxin type B. *J. Chromatogr. B* **2004**, *809*, 37−41.

(14) Chu, F. S.; Lau, H. P.; Fan, T. S.; Zhang, G. S. Etheylenediamine modified bovine serum albumin as protein carrier in the production of antibody against mycotoxins. *J. Immunol. Methods* **1982**. *55*, 73−78.

(15) Shulman, M.; Wilde, C. D.; Kohler, G. A better cell line for making hybridomas secreting specific antibodies. *Nature* **1978**, *276*, 269−270.

(16) Hack, R.; Martlbauer, E.; Terplan, G. A monoclonal antibody to the trichothecene T-2 toxin: Screening for the antibody by a direct enzyme immunoassay. *J. Vet. Med. B* **1987**, *34*, 538−544.

(17) Hayat, M. A., Ed. Chapter 1. Molecular interactions between colloidal gold, proteins, and living cells. In *Colloidal Gold: Principles, Methods, and Applications*; Academic Press: San Diego, CA, 1989; Vol. 2, pp 1−17.

(18) Harlow, E., Lane, D., Eds. Chapter 12. Immunoblotting. In *Antibodies: A Laboratory Manual*; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1988; pp 471−511.

(19) Jones, K. D. Troubleshooting protein binding nitrocellulose membranes, Part 1: Principles. *IVD Technol.* **1999**, *5* (2), 32−41.

(20) Chu, F. S.; Chang, C. F.; Hinsdill, R. D. Production of antibody against ochartoxin A. *Appl. Environ. Microbiol.* **1976**, *31*, 831−835.

(21) Candlish, A. A. G.; Smith, J. E.; Stimson, W. H. Monoclonal antibody technology for mycotoxins. *Biotechnol. Adv.* **1989**, *7*, 401−418.

(22) Knasmuller, S.; Cavin, C.; Chakraborty, A.; Darroudi, F.; Majer, B. J.; Huber, W. W.; Ehrlich, V. A. Structurally related mycotoxins ochratoxin A, ochratoxin B, and citrinin differ in their genotoxic activities and in their mode of action in human-derived liver (HepG2) cells: implications for risk assessment. *Nutr. Cancer* **2004**, *50*, 190−197.

(23) Faulk, W. P.; Taylor, G. M. An immunocolloidal method for electron microscope. *Immunocytochemistry* **1971**, *8*, 1081−1083.

(24) Moeremans, M.; Daneels, G.; Dijek, A. V.; Langanger, G.; Mey, J. D. Sensitive visualization of antigen−antibody reactions in dot and blot immune overlay assays with immunogold and immunogold/silver staining. *J. Immunol. Methods* **1984**, *74*, 353−360.

(25) Gupta, R.; Talwar, G. P.; Gupta, S. k. Rapid antibody capture assay for detection of group-A *Streptococci* using monoclonal antibody and colloidal gold-monospecific polyvalent antibody conjugate. *J. Immunoassay* **1992**, *13*, 441−455.

(26) Chandler, J.; Gurmin, T.; Robinson, N. The place of gold in rapid tests. *IVD Technol.* **2000**, *6* (2), 37−49.

(27) Bullock, G. R., Petrusz, P., Eds. Chapter 10. The colloidal gold marker system for light and electron microscopic cytochemistry. In *Techniques in Immunocytochemistry*; Academic Press: London, U.K., 1983; Vol. 2, pp 217−284.

(28) Paek, S. H.; Lee, S. H.; Cho, J. H.; Kim, Y. S. Development of rapid one-step immunochromatographic assay. *Methods* **2000**, *22*, 53−60.

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