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Electronic Supporting Information

A "green" method to detect aflatoxin B_1 residue in edible oil based on colloidal gold immunochromatographic assay

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Experimental

Reagents

All inorganic chemicals and organic solvents were of reagent grade quality. Carboxymethyloxime (CMO), bovine serum albumin (BSA), Freund's complete adjuvant, Freund's incomplete adjuvant, N, N'-dimethylformamide(DMF), were products of Sigma-Aldrich (St. Louis, MO, USA), 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride(EDC), N-hydroxysuccinimide (NHS), pyridine, were obtained from J&K Scientific LTD. (Beijing, China). HAT, Cell-culture media (DMEM), fetal bovine serum (FBS) and supplements were obtained from Gibco Life Technologies Corporation (Carlsbad, CA, USA). 96-well microtiter plates was purchased from Corning-Costar (Tewksbury, MA, USA).

Preparation of immunogen

For preparation of AFB_1-BSA, AFB_1 (1.5 mg) was reacted with CMO (3 mg) in methanol/pyridine (2 mL, 1:1) at 70 °C for 5 h. After drying, residues were dissolved in distilled water. pH of the solution was adjusted to 3.0 with HCl (0.1 M), and ethyl acetate (3 mL) were used to extract for three times. The product of AFB_1-oxime was dissolved in DMF (0.5 mL) after ethyl acetate was volatilized. AFB_1-oxime (0.45 mL) was transferred to an amber glass bottle, and then EDC (30 mg) and NHS (8.64 mg) were added. The volume was adjusted to 1.5 mL with DMF and the mixture was stirred overnight at 30 °C. Then the mixture were reacted with BSA (4.2 mg/mL, 3 mL) which was dissolved in 0.1 M phosphate-buffered saline (PBS, pH=7.4) at room temperature for 2 h. The supernatant after centrifugation (4 °C , 3000 rpm, 10 min) was dialyzed against PBS (10 mM, pH=7.4). PBS was changed three times a day, and dialysis was performed for 3 days. The final solution was stored at 4 °C until use.

Preparation of antibody

AFB_1–BSA (30 μg) in 0.1 mL sterilized PBS was emulsified with an equal volume of Freund's complete adjuvant. This was injected intraperitoneally into each of several 7-week-old female BALB/c mice. Ten days after injection, serum was collected from the caudal vein of each mouse and titers of antisera were determined by indirect ELISA (iELISA). The spleen was removed aseptically and splenocytes were fused with cells of the Sp2/0 murine myeloma cell line at a ratio of 5:1 in the presense of polyethylene glycol 4000 (PEG 4000). After fusion, cells were suspended in DMEM HAT medium containing 16% FBS and added to 96-well micro plates. After 7 days, culture supernatants from each well were assayed using indirect competitive ELISA. ELISA-positive hybridoma cells were cloned by the limiting dilution method. Cloned hybridoma cells (1.0×10^7 in PBS) were intraperitoneally injected into BALB/c mice, pre-treated with intraperitoneal injection

of 0.2 mL pristane, and grown as ascite tumours. The immunoglobulin fraction was prepared from the ascitic fluids. Antibodies were purified by protein-A-agarose affinity chromatography.

Results and discussion

Determination of antigen

Bruker autoflex MALDI-TOF-MS system (Bruker, Germany) was used for the identification of antigen. The molecular weight difference between BSA and AFB_1-BSA shows clearly in the MALDI-TOF-MS spectra (Fig. S1). A significant shift of the peak maximum of $\Delta m/z$=5140. The ratio of AFB_1: BSA was 14:1 calculated from the $\Delta m/z$.

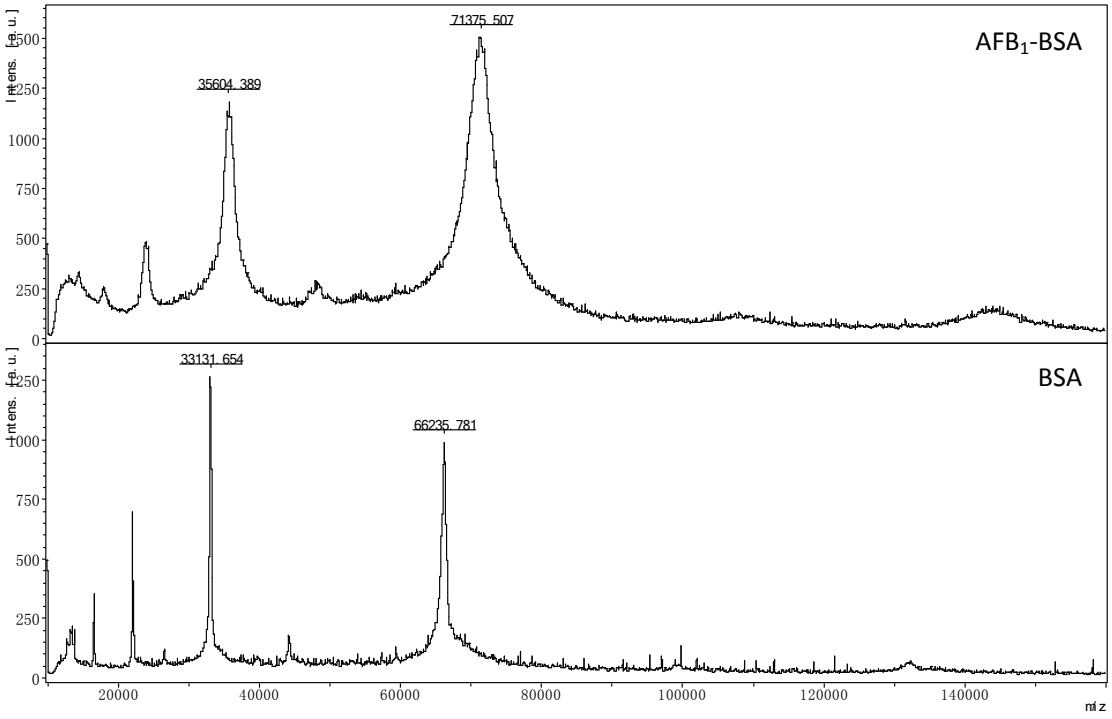

Fig. S1 MOLDI-TOF/MS spectra of AFB_1-BSA and BSA

Characterization of monoclonal antibody

Many monoclonal antibodies showed positive results against AFB_1 (data were not shown). Antibody 4H12 had the highest affinity among them. IC_{50} of 4H12 determined by the indirect ELISA methods were 0.36 ng/mL. The isotype of antibody 4H12 was determined as IgG_1, the light chain was Lambda and the affinity constant was reach to 7.9×10^9 L / mol。

Characterizations of colloidal gold and antibody-colloidal gold conjugate

Characterizations of colloidal gold and antibody-colloidal gold conjugate were performed according to the previous reports.[1-3] The formation of the colloidal gold was confirmed and characterized by Transmission Electron Microscopy (JEM 2010, Japan). UV spectra of the colloidal gold solution, the antibody-colloidal gold conjugate solution were obtained by Unico spectrophotometry (UV2802, China).

Fig. S2 shows the TEM images of the gold particles, and the diameters of the colloidal gold obtained were about 30 nm. As it was shown in Fig. S3, the maximum absorbance of the colloidal gold was 529 nm. For the antibody-colloidal gold conjugate after centrifugation, the peak shifted from 529 nm to 532 nm, similar as what was reported previously.[1-3]

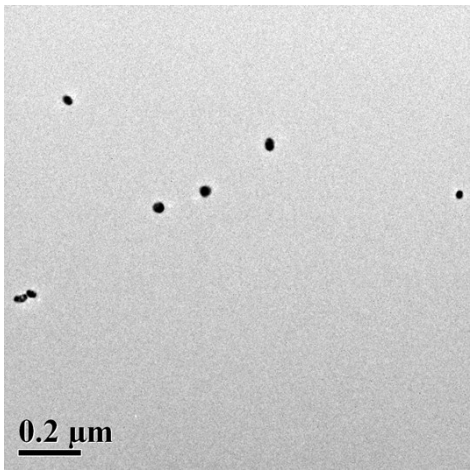

Fig. S2 TEM image of colloidal gold particles.

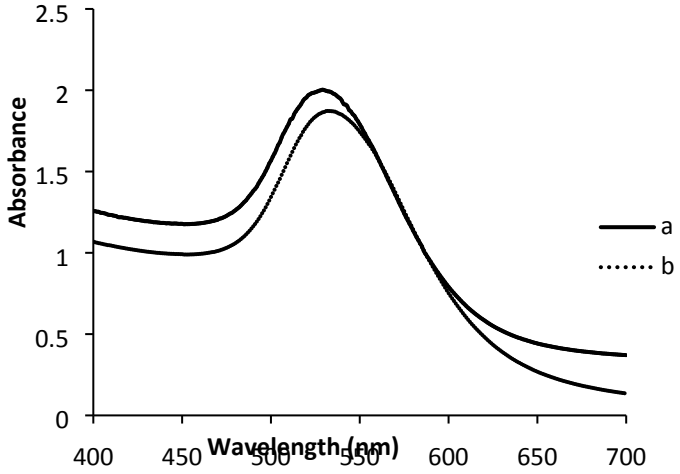

Fig. S3 UV spectra of the colloidal gold solution and antibody-colloidal gold conjugate solution. Curve a: colloidal gold solution; Curve b: antibody-colloidal gold conjugate solution, after centrifugation and resuspension, diluted 10 times with water.

Reference:

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3. X. Li, P. Li, Q. Zhang, R. Li, W. Zhang, Z. Zhang, X. Ding and X. Tang, *Biosens Bioelectron*, 2013, **49**, 426-432.