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Research Article

Soy 11S Globulin Acid Subunits as the Novel Food Polymer Carrier

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Aflatoxins were conjugated with soy 11S globulins acid subunits and the hapten-specific monoclonal antibodies (McAbs) cross-reactive with four major aflatoxins were achieved using indirect competitive ELISA screening procedure. The two antibodies (clones 1B2 and 2D3) had similar reaction efficiency with aflatoxins B1, B2, and G1 but showed a weak cross-reaction to G2. The clone 4C5 exhibited the highest sensitivity for all four aflatoxins. The concentrations of aflatoxins B1, B2, G1, and G2 at 50% inhibition for 4C5 were 1.1, 1.2, 2.1, and 17.6 pg mL^{-1}. The results indicated that soy 11S globulin acid subunits were suitable novel carriers for aflatoxin antigen in immunization experiments and clone 4C5 could be used for simultaneous analysis of total aflatoxins.

1. Introduction

Aflatoxins are highly toxic and carcinogenic compounds, which are a group of structurally related toxic metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* [1]. The kinds of aflatoxins include aflatoxins B1, B2, G1, G2, and M1. Aflatoxins frequently contaminate a wide range of foods and animal feedstuffs. Aflatoxin B1 (AFB1) is the most toxic. These aflatoxins are found consistently contaminating feed and food supplies in many areas [2, 3]. A number of well-established methods have been reported for analyzing aflatoxins in various food systems, such as near infrared spectroscopy [4], high-performance liquid, thin liquid chromatography [5], and immunoaffinity chromatography-performance liquid chromatography [6]. As immunoassay methods, enzyme-linked immunosorbent assay (ELISA) is well suited for the rapid, routine diagnostic application of aflatoxin detection [7]. These methods are advantageous in the simple operation, portability of the equipment, and handholding validation and reliable for the analysis of a large number of samples.

Currently, the conventional immunogenic carriers used in ELISA are bovine serum albumin (BSA), human serum albumin (HSA), and keyhole limpet hemocyanin (KLH). However, when these proteins are used as the immunogen carrier, the affinity of the corresponding antibodies is relatively weak [8]. Additionally, KLH and HSA are also relatively expensive and not easily obtainable. The purpose of this study was to find a suitable protein instead of BSA, KLH, and the like. The ideal carrier should have a comparatively stable structure and strong water-solubility, regardless of being under any organic solvent circumstances. Soy 11S globulin is an inhomogeneous protein whose molecular weight ranges from 340 to 375 kDa [9] which is made up of six acid subunits (A1, A2, A3, A4, A5, and A6) and six alkaline subunits (B1, B2, B3, B4, B5, and B6). In this paper, we report a method in which aflatoxins were conjugated with soy 11S globulins acid subunits initially and the production of generic monoclonal antibodies against major aflatoxins using a two-step screening procedure. Details for the production of desired hybridoma clones are described in this paper.

2. Materials and Methods

2.1. Chemicals and Instruments. Bovine serum albumin (BSA), ovalbumin (OVA), standards of aflatoxins (AFs) B1, B2, G1, G2, and M1, N-hydroxysuccinimide (NHS), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimidehydrochloride

(EDC), tetramethylbenzidine (TMB), goat anti-mouse immunoglobulin horseradish peroxidase (IgG-HRP), and RPMI-1640 medium with l-glutamine and HEPES (free acid, 283.3 g/L) were obtained from HyClone; hypoxanthine, aminopterin, and thymidine (HAT), poly(ethylene glycol) (PEG) 1500, and complete and incomplete Freund's adjuvant were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Mouse monoclonal antibody isotyping kit was obtained from Roche Diagnostics Corporation (Indianapolis, USA). SP2/0 myeloma cells were purchased from China Center for Type Culture Collection (CCTCC). 96-well microtiter plates (Corning-Costar, 3590) and cell culture plates (6, 24, and 96 wells) were from Iwaki, Japan. Polystyrene 96-well microtiter plates were from Costar (Corning, Massachusetts, USA). Female Balb/c mice were purchased from Centers for Disease Control and Prevention of Jilin province.

The absorbance at 450 nm was detected by using a Spectra Max microplate reader (Molecular Devices, USA) ELISA plates were washed with well wash Plus and well absorbances were measured with a microtiter plate reader, which was controlled by a personal computer containing the standard software package Easy Software. They were both from Thermo Electron Co. (MA, USA). Fluorescence measurements were performed on an F-4500 spectrophotometer (HITACHI, Japan) equipped with a 150 W xenon lamp and 5 nm slit width and 1.00 cm quartz cell at a scanning speed of 1200 nm min^{-1}; the temperature was controlled by digital aqueous thermostat.

2.2. Preparation of Soy 11S Globulin and Soy 7S Globulin. Soy 11S globulin and soy 7S globulin were isolated according to a new method [10], which is a modification of the procedure of Deak et al.

2.3. Isolation and Purification of Soy 11S Globulin Acid Subunits. 1.5425 g DTT was added to a solution of 500 mL soy 11S globulin (11 mg/mL, PH 8.0) and shaken for about 1 h; then the protein solution was heated for 30 min and centrifugal separation 3 times (10000 r·min^{-1}, 20 min) at 4°C [11]. The freeze-dried precipitation was basic subunits (BS), and the freeze-dried supernatant was acid subunits (AS). The acid subunits were identified by SDS-PAGE patterns.

2.4. Preparation and Characterization of Aflatoxin B1-AS Conjugates. Aflatoxin B1 was first converted to AFB1-O-carboxymethyloxime (AFB1-oxime) as previously described [12]. Structure characterization of AFB1-oxime was performed by NSI/MS (spray voltage: 2.8 kV, capillary Temp: 270°C, capillary voltage: 36 V, and lens voltages: 115 V). AFB1-AS conjugates were prepared by the EDC-ester method, avoiding unfavorable coupling of activating reagent (DCC) with AS [13]. Briefly, 2 mL of a solution of 4.5 mg soy 11S globulin acid subunits and 50 mg EDC were added to a solution of 2.0 mg AFB1-oxime in 2 mL of dry ethanol and the resulting solution was shaken for 48 h in a closed vial at room temperature. 80 microliters of the AFB1-oxime solution was added to an ice-cold solution of 2.0 mg AS in 230 μL of coupling buffer and shaken for 6 h. The protein conjugates (AFB1-AS) were subsequently purified on a Sephadex G-25 column using PBS as mobile phase [14]. AFB1-AS conjugate was characterized using UV-vis absorption spectrophotometer (UV-2300, Techcomp).

2.5. Preparation of Coating Antigen (AFB1-OVA). AFB1-oxime was conjugated with OVA and the coating antigen (AFB1-OVA) was prepared. Equal volume of glycerol was added to the AFB1-OVA solution and then stored at −30°C after dialyzing.

2.6. Preparation and Characterization of Monoclonal Antibodies

2.6.1. Immunization. Six five-week-old female Balb/c mice were subcutaneously immunized with AFB1-AS conjugates in the initial immunization. The initial dose consisted of 50 μg of AFB1-AS conjugate intraperitoneally injected using Freund's complete adjuvant and the remaining two subsequent injections were given with Freund's incomplete adjuvant at 2-week intervals. After each injection, Antisera were collected from the caudal vein of each mouse and assayed for anti-aflatoxin B1 antibodies [15] by indirect competitive ELISAs (ciELISAs) with aflatoxins B1, B2, G1, and G2 as the competitors. An intraperitoneal booster was given to the mouse whose antiserum exhibited better cross-reactivity (CR) and higher sensitivity with aflatoxins 3 days prior to cell fusion.

2.6.2. Cell Fusion and Screening. At the beginning, SP 2/0 murine myeloma cells were cultured in RPMI-1640 media supplemented with 20% fetal bovine serum. Cell fusion was carried out as described by Devi et al. [16]. The myeloma cells were mixed with mice splenocytes and centrifuged at the ratio of 5–10 : 1. One milliliter of PEG 1500 was dropped into the cell pellet about 1 min at 37°C. After an addition of 30 mL of RPMI-1640 about 5 min, the cells were left aside for 5–10 min. And then the fused cells were mixed with selective semisolid media (RPMI 1640 medium supplemented with 20% (v/v) foetal bovine serum (FBS), 100 μg/mL streptomycin, 100 U/mL penicillin, 1% (w/v) methyl cellulose, 1% (v/v) HEPES, HAT, and 2% (v/v) HFCS) and plated to 6-well plate (1.5 mL/well). The plates were examined for the presence of hybridomas; then culture supernatants were assayed from the wells which contained hybridomas, after 12 days later. Only those hybridomas which maintained absorption values were chosen for further selection, because they had good cross-reactivity with aflatoxins B1, B2, G1, and G2 and had no cross-reactivity with OVA. According to standard methods with some modifications, cells from each interested well were subcloned by limiting dilution to ensure monoclonality [15]. Monoclonal cell strains which secreted desired antibodies were screened out by 2-3 limiting dilutions. According to the freezing protocol, 30 min at 4°C, the resulting hybridoma clones were cryopreserved and propagated in freezing solution, in gas of liquid nitrogen jar overnight, and then stored in liquid nitrogen [17].

2.6.3. Indirect Competitive ELISA Screening. Indirect competitive ELISAs were applied in the two-step screening procedure. To eliminate antibodies and screen positive wells that react with OVA, beside AFB1-OVA (diluted 1 : 250 in coating buffer), OVA alone (twofold concentration of AFB1-OVA) intervals with AFB1-OVA at one row were also coated to plates (100 μL/well). The indirect competitive ELISA was carried out to screen clones that had good cross-reactivity and high sensitivity to four aflatoxins from positive wells [18, 19]. To obtain high affinity antibodies, gradiently decreased concentrations of four aflatoxins (added concentrations were 100, 50, 20, and 10 ng mL^{-1} and the corresponding final concentrations were 50, 25, 10, and 5 ng mL^{-1} resp.) were used for the first screening and the following two or three screenings after subclone.

2.6.4. Titer Assessment and Isotype Determination. Each strain of hybridoma cell was injected into three Balb/c mice. The ascites fluid collected from these three mice was pooled and either used directly in the immunoassays [20] on protein A-sepharose columns by affinity chromatography. The antibody titer was defined as the reciprocal of the highest ascites dilution which gave an absorbance greater than 2.0-fold the background absorbance of the negative serum in the first dilution [21, 22]. The Mabs antibodies isotypes were performed with a commercially available ISO2-1 kit from Sigma in direct ELISAs.

2.6.5. Evaluation of Antibody Cross-Reactivity and Sensitivity. The indirect competitive ELISA format (AFB1-OVA was diluted 1 : 1000 in coating buffer) was used to evaluate the monoclonal antibody sensitivity. Cross-reactivity with aflatoxin M1 was also performed, beside the major aflatoxins. Required as working concentration, the optimum dilution of antibody was defined as the dilution which gave an absorbance most close to 1.0. These data were converted to antibody inhibition, expressed as % $B/B0$, where $B0$ was the absorbance in the absence of analyte and B was the absorbance at each analyte concentration [23]. CR was determined by comparing the IC50 values (concentration resulting in half-maximum inhibition) of analytes for different aflatoxins and calculated as CR (%) = (IC50 AFB1/IC50 analyte) × 100 [24].

3. Results and Discussion

3.1. SDS-PAGE Patterns Identification of Soy 11S Globulin Acid Subunits. They include M. Prestained protein molecular marker 1, soy 11S globulin, marker 2, soy 7S globulin, marker 3, soy 11S globulin acid subunits, and, marker 4, soy 11S globulin basic subunits. Soy 11S globulin basic subunits SDS-PAGE pattern was shown in Figure 1. This result suggests that soy 11S globulin acid subunits were prepared successfully.

3.2. Electrospray Mass Spectrometry Identification of AFB1-Oxime. NSI/MS pattern was shown in Figure 2. The relative molecular mass of AFB1 was 312.3, the peak of m/z 408.06 was quasi-molecular ion peaks of AFB1 carboxymethyl activation-[AFB1-O+Na]+, consistent with the molecular mass of the deserved product C17H12O6. The peak of the m/z 422.06 may be the molecular ion peak of nitrogen conjugate of AFB1 sodium salt-[AFB1-O-N+Na]+; the peak of m/z 312.1 was AFB1 quasi-ion peaks. The result can be preliminarily concluded that the activation product is AFB1 carboxyl activation-AFB1-oxime.

3.3. UV-Vis Absorption Spectrogram Characterization. Ultraviolet absorption spectrogram of AFB1-AS and soy 11S globulin acid subunits was shown in Figure 3. The maximum absorption of AFB1-AS is consistent with AS at 290 nm. This result suggested that AFB1-AS were prepared successfully.

3.4. Resonance Light Scattering Spectra Analysis. Fluorescence spectra analysis of AFB1-AS was shown in Figure 4 at different coupling ratios. The characteristic fluorescence peak of acidic subunits occurs at 350 nm. The conjugation of AFB1 to acidic subunits not only resulted in the decrease of fluorescence intensity of acidic subunits, but also led to the blue shift of maximum emission wavelength from approximately 350 to 320 nm as the coupling ratio increased.

3.5. Monitoring Antiserum Titers and Isotype Determination. AFB1-AS conjugate induced all of the six Balb/c mice to produce hapten-specific antibodies 12 days after the initial immunization. On day 105, 4 of the six mice tested gave high antibody titers more than 8,000 but presented different degrees of reaction capability and sensitivity. Number 1, number 2, and number 4 mice were successively chosen for B-lymphocytes donors and for the subsequent fusion experiments with higher sensitivity and better cross-reactivity. Then we injected three stable hybridoma lines into Balb/c F1 hybrid mice and 8–15 mL ascitic fluid was collected from each mouse. All the last three class-specific McAbs 1B2, 2D3 and 4C5 were IgG class and had no cross-reactivity with BSA and OVA. The results of isotype determination and titration are shown in Table 1.

3.6. Evaluation of Antibody Cross-Reactivity and Sensitivity. The cross-reactivity, sensitivity, and the minimal inhibition values for three monoclonal antibodies are presented in Table 2. As shown in Figure 5, graphs are plotted as percentage inhibition ($B/B0$) against mass of toxin (pg mL^{-1}). In view of different McAbs and different aflatoxins, experiments were performed with different toxins concentration gradient. The antibodies showed good cross-reactivity. The variation coefficients were between 0.2% and 8.8% and the absolute absorbances ($B0$) were between 0.8 and 1.2 units. The two monoclonal antibodies, 1B2 and 2D3, which had similar reaction efficiency with B1, B2, and G1, however, showed a weak cross-reaction with G2. The clone 4C5 had the highest affinity to four aflatoxins and then was grouped in category 3.

The cross-reactivity of three McAbs with aflatoxin M1 was also performed. As shown in Figure 6, graphs plotted as percentage inhibition ($B/B0$) against AFM1 (pg mL^{-1}), experiments were performed with different concentration gradient of aflatoxin M1, aiming at different McAbs. The antibodies

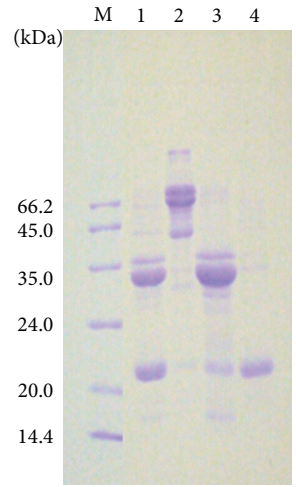

Figure 1: SDS-PAGE patterns of soy 11S globulin acid subunits.

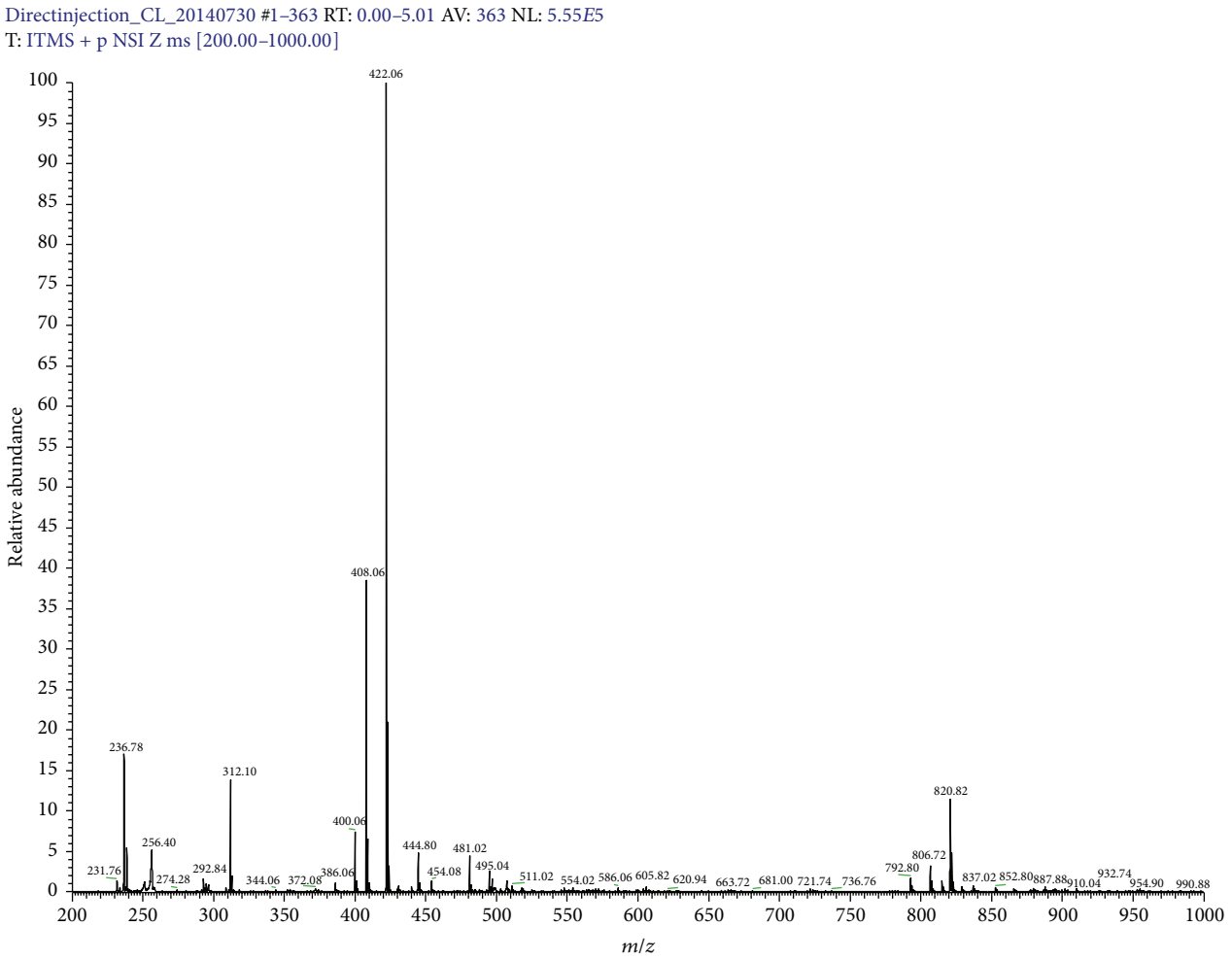

Figure 2: Electrospray mass spectrometry of AFB1-oxime.

Table 1: Results of titers and isotypes of ascites antibodies.

Clones	Isotype	Titer of crude ascites ($\times 10^4$)	OD450 values			
			Negative control serum	Blank control	1% AS	1% OVA
1B2	IgG1	69.0	0.102	0.06	0.07	0.07
2D3	IgG2a	87.5	0.087	0.05	0.06	0.06
4C5	IgG2a	432.0	0.082	0.04	0.05	0.05

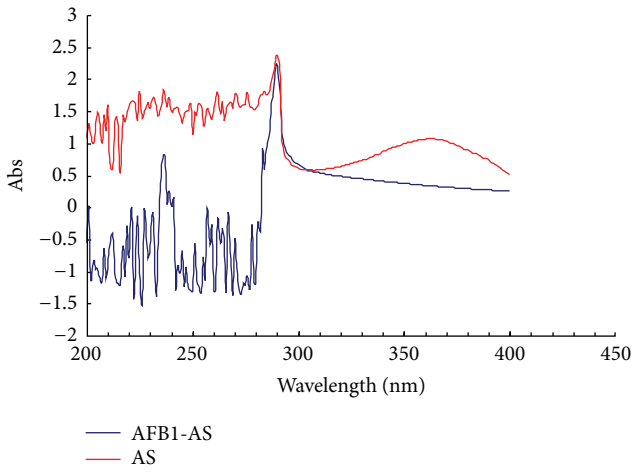

Figure 3: UV-vis absorption spectra of AFB1-AS and AS.

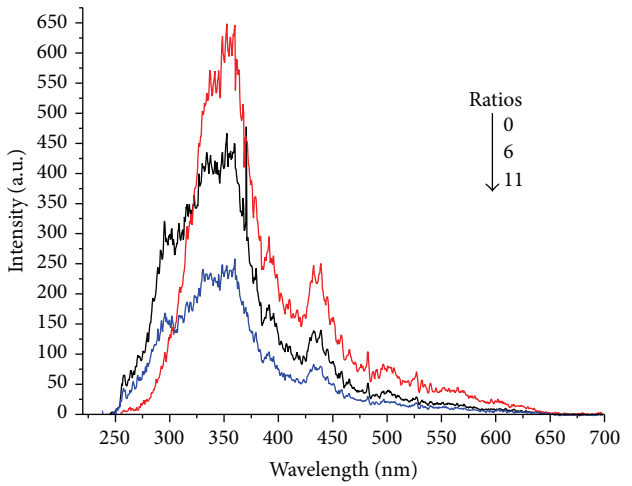

Figure 4: The effect of combination ratios on the resonance light scattering spectra of acidic subunit.

showed good cross-reactivity with aflatoxin M1. The variation coefficients were between 0.5% and 8.6% and the absolute absorbances ($B0$) were between 0.8 and 1.2 units. The cross-reactivity, sensitivity, and the minimal inhibition values for three monoclonal antibodies are presented in Table 3. The clone 4C5 showed the lowest cross-reactivity but exhibited the uppermost sensitivity; next came 1B2 and 2D3. The results of comparison showed that clone 4C5 was the best generic monoclonal antibody against the major aflatoxins.

Table 2: Results of sensitivity (expressed as pg mL^{-1}), cross-reactivity (CR%), and the minimal inhibition values of five monoclonal antibodies with four major aflatoxins.

Aflatoxins	Clones		
	1B2	2D3	4C5
Sensitivity (pg mL^{-1})			
B1	86.0	378.2	1.1
B2	95.5	759.3	1.2
G1	100.5	374.8	2.1
G2	405.6	2237.6	17.6
Cross-reactivity (%)			
B1	100.0	100.0	100.0
B2	92.1	58.5	94.6
G1	83.6	113.7	60.8
G2	18.7	15.9	5.9
Minimal inhibition (pg mL^{-1})			
B1	—*	105.7	—*
B2	0.7	39.8	0.02
G1	0.2	46.6	0.03
G2	1.6	25.9	—*

*Results were out of the measuring range.

Table 3: Results of sensitivity (expressed as pg mL^{-1}) and cross-reactivity (CR%) and the minimal inhibition values of five monoclonal antibodies with aflatoxin M1.

Aflatoxins M	Clones		
	1B2	2D3	4C5
Sensitivity (pg mL^{-1})	201.0	1147.2	13.2
Cross-reactivity (%)	42.8	35.7	9.0
Minimal inhibition (pg mL^{-1})	0.8	26.8	—*

*Refers to that the result was out of the measuring range.

3.7. Discussion. Though procedures have been established for the production of aflatoxins-BSA/OVA conjugates and antibodies to AFB1-BSA/OVA, this study was the first report of aflatoxins being conjugated with other carriers (such as soy 11S globulins acid subunits) and the evaluation of antibody sensitivity and cross-reactivity. The result stated that the AFB1-AS conjugate was prepared successfully. Soy 11S globulin acid subunits have stable structure and strong water-solubility, which are capable of conjugate with aflatoxin under organic solvent circumstances. AFB1-AS conjugate could induce Balb/c mice to produce hapten-specific antibodies, which exhibited the highest sensitivity for all four

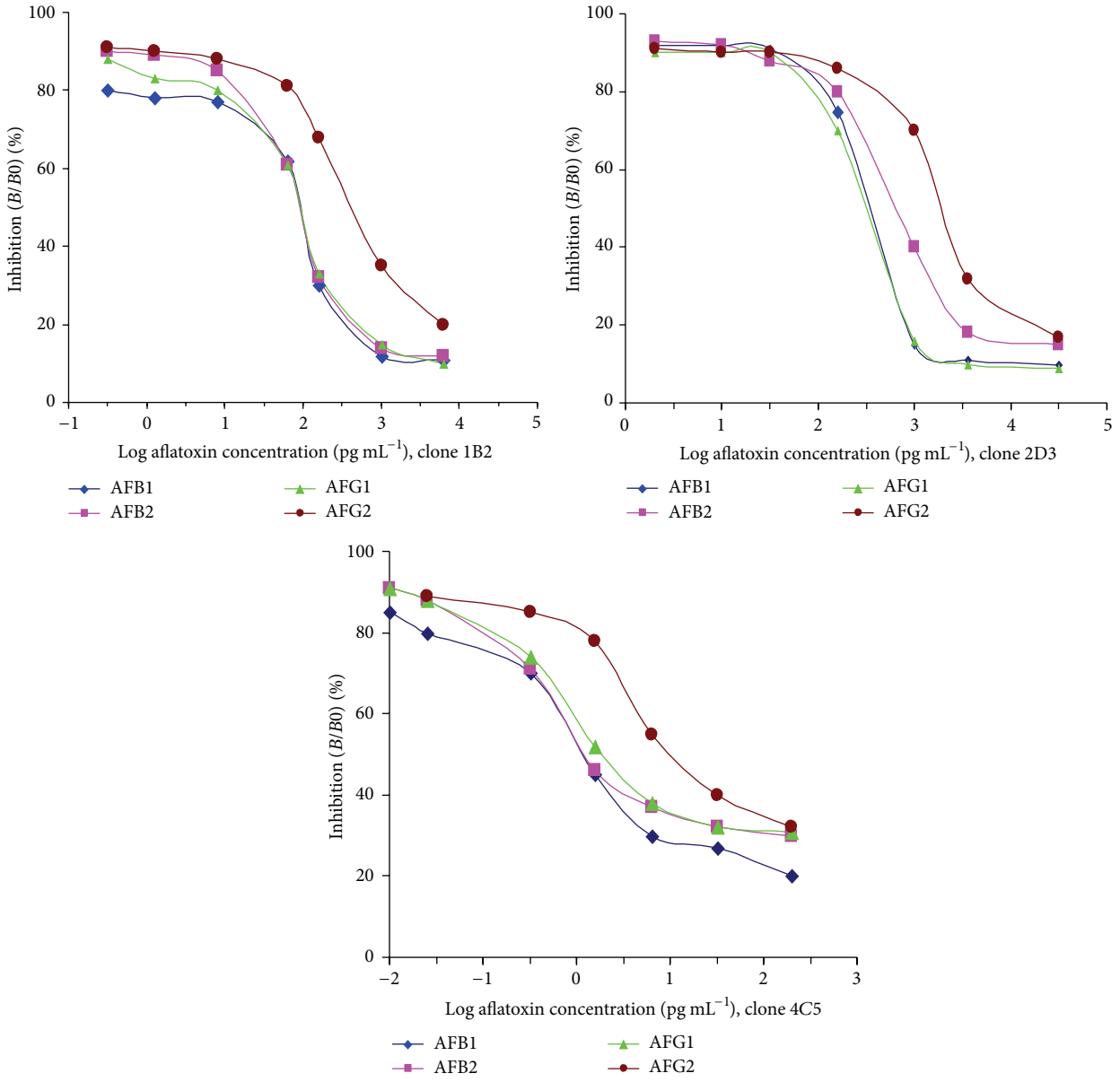

Figure 5: Cross-reactivity of three clones of monoclonal antibodies to four major aflatoxins.

aflatoxins. Nowadays, soybean seeds are cheap in China and soy 11S globulin acid subunits could be isolated from defatted soybean seeds easily. Our research proved that soy 11S globulin acid subunits are a suitable carrier for AFB1-oxime in immunization experiments and could be represented as a cheaper alternative to conventional immunogenic carriers such as BSA or OVA.

4. Conclusions

The novel AFB1-AS conjugates were prepared successfully and the three generic monoclonal antibodies were established by a modified two-step screening procedure for the determination of total aflatoxins. For clone 4C5, which exhibited the highest sensitivity for all four aflatoxins, the concentrations of aflatoxins B1, B2, G1, and G2 at 50% inhibition were 1.1, 1.2, 2.1, and 17.6 pg mL^{-1}, respectively. The results indicated that soy 11S globulin acid subunits are suitable novel carriers for aflatoxin antigen in immunization experiments and the two-step screening procedure (indirect competitive ELISA) was proved to be superior especially for the generation of antihapten McAbs.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

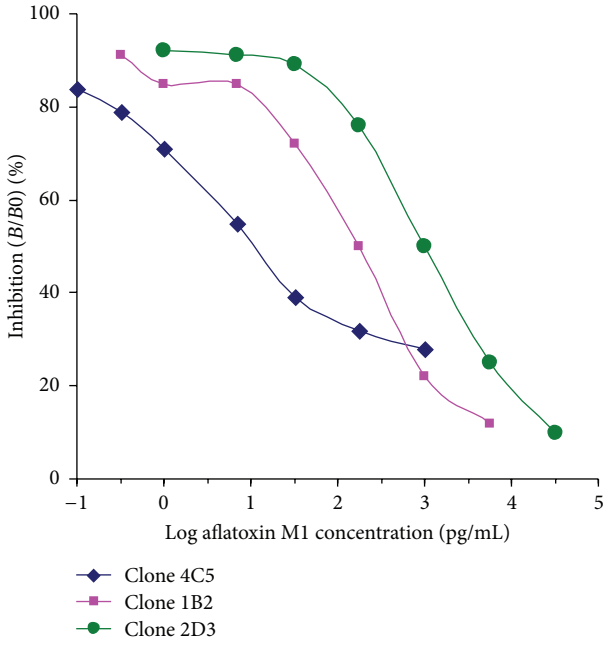

Figure 6: Cross-reactivity of three McAbs to aflatoxin M1.

Acknowledgments

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References

[1] E. E. Creppy, "Update of survey, regulation and toxic effects of mycotoxins in Europe," *Toxicology Letters*, vol. 127, no. 1–3, pp. 19–28, 2002.

[2] M. T. Nguyen, M. Tozlovanu, T. L. Tran, and A. Pfohl-Leszkowicz, "Occurrence of aflatoxin B1, citrinin and ochratoxin A in rice in five provinces of the central region of Vietnam," *Food Chemistry*, vol. 105, no. 1, pp. 42–47, 2007.

[3] A. P. Magnoli, L. Tallone, C. A. R. Rosa, A. M. Dalcero, S. M. Chiacchiera, and R. M. T. Sanchez, "Commercial bentonites as detoxifier of broiler feed contaminated with aflatoxin," *Applied Clay Science*, vol. 40, no. 1–4, pp. 63–71, 2008.

[4] J. M. Hernández-Hierro, R. J. García-Villanova, and I. González-Martín, "Potential of near infrared spectroscopy for the analysis of mycotoxins applied to naturally contaminated red paprika found in the Spanish market," *Analytica Chimica Acta*, vol. 622, no. 1-2, pp. 189–194, 2008.

[5] I. Var, B. Kabak, and F. Gök, "Survey of aflatoxin B_1 in helva, a traditional Turkish food, by TLC," *Food Control*, vol. 18, no. 1, pp. 59–62, 2007.

[6] A. C. Manetta, L. Di Giuseppe, M. Giammarco et al., "High-performance liquid chromatography with post-column derivatisation and fluorescence detection for sensitive determination of aflatoxin M_1 in milk and cheese," *Journal of Chromatography A*, vol. 1083, no. 1-2, pp. 219–222, 2005.

[7] E. Calleri, G. Marrubini, G. Brusotti, G. Massolini, and G. Caccialanza, "Development and integration of an immunoaffinity monolithic disk for the on-line solid-phase extraction and HPLC determination with fluorescence detection of aflatoxin B1 in aqueous solutions," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 44, no. 2, pp. 396–403, 2007.

[8] W. Haasnoot, G. Cazemier, J. du Pré, A. Kemmers-Voncken, M. Bienenmann-Ploum, and R. Verheijen, "Sulphonamide antibodies: from specific polyclonals to generic monoclonals," *Food and Agricultural Immunology*, vol. 12, no. 1, pp. 15–30, 2000.

[9] S. Arjunaraja, P. Massari, L. M. Wetzler, A. Lees, J. Colino, and C. M. Snapper, "The nature of an in vivo anti-capsular polysaccharide response is markedly influenced by the composition and/or architecture of the bacterial subcapsular domain," *The Journal of Immunology*, vol. 188, no. 2, pp. 569–577, 2012.

[10] N. A. Deak, P. A. Murphy, and L. A. Johnson, "Characterization of fractionated soy proteins produced by a new simplified procedure," *Journal of the American Oil Chemists' Society*, vol. 84, no. 2, pp. 137–149, 2007.

[11] L. Chen, P. V. Vadlani, and R. L. Madl, "High-efficiency removal of phytic acid in soy meal using two-stage temperature-induced *Aspergillus oryzae* solid-state fermentation," *Journal of the Science of Food and Agriculture*, vol. 94, no. 1, pp. 113–118, 2014.

[12] K. Daigo, S. Sugita, Y. Mochizuki et al., "A simple hybridoma screening method for high-affinity monoclonal antibodies using the signal ratio obtained from time-resolved fluorescence assay," *Analytical Biochemistry*, vol. 351, no. 2, pp. 219–228, 2006.

[13] C. Cervino, D. Knopp, M. G. Weller, and R. Niessner, "Novel aflatoxin derivatives and protein conjugates," *Molecules*, vol. 12, no. 3, pp. 641–653, 2007.

[14] L. Chen, R. L. Madl, and P. V. Vadlani, "Nutritional enhancement of soy meal via *Aspergillus oryzae* solid-state fermentation," *Cereal Chemistry*, vol. 90, no. 6, pp. 529–534, 2013.

[15] H. Aycicek, A. Aksoy, and S. Saygi, "Determination of aflatoxin levels in some dairy and food products which consumed in Ankara, Turkey," *Food Control*, vol. 16, no. 3, pp. 263–266, 2005.

[16] K. T. Devi, M. A. Mayo, K. L. N. Reddy et al., "Production and characterization of monoclonal antibodies for aflatoxin B1," *Letters in Applied Microbiology*, vol. 29, no. 5, pp. 284–288, 1999.

[17] J. K. Gathumbi, E. Usleber, and E. MaErtlbauer, "Production of ultrasensitive antibodies against aflatoxin B1," *Letters in Applied Microbiology*, vol. 32, no. 5, pp. 349–351, 2001.

[18] D. Zhang, P. Li, Q. Zhang et al., "Production of ultrasensitive generic monoclonal antibodies against major aflatoxins using a modified two-step screening procedure," *Analytica Chimica Acta*, vol. 636, no. 1, pp. 63–69, 2009.

[19] A. H. Heussner, I. Moeller, B. W. Day, D. R. Dietrich, and E. O'Brien, "Production and characterization of monoclonal antibodies against ochratoxin B," *Food and Chemical Toxicology*, vol. 45, no. 5, pp. 827–833, 2007.

[20] A. Y. Kolosova, W.-B. Shim, Z.-Y. Yang, S. A. Eremin, and D.-H. Chung, "Direct competitive ELISA based on a monoclonal antibody for detection of aflatoxin B1. Stabilization of ELISA kit components and application to grain samples," *Analytical and Bioanalytical Chemistry*, vol. 384, no. 1, pp. 286–294, 2006.

[21] J. D. Beatty, B. G. Beatty, and W. G. Vlahos, "Measurement of monoclonal antibody affinity by non-competitive enzyme immunoassay," *Journal of Immunological Methods*, vol. 100, no. 1-2, pp. 173–179, 1987.

[22] G. A. Canziani, S. Klakamp, and D. G. Myszka, "Kinetic screening of antibodies from crude hybridoma samples using

Biacore," *Analytical Biochemistry*, vol. 325, no. 2, pp. 301–307, 2004.

[23] J. J. Manclús, A. Abad, M. Y. Lebedev et al., "Development of a monoclonal immunoassay selective for chlorinated cyclodiene insecticides," *Journal of Agricultural and Food Chemistry*, vol. 52, no. 10, pp. 2776–2784, 2004.

[24] K. E. Sapsford, C. R. Taitt, S. Fertig et al., "Indirect competitive immunoassay for detection of aflatoxin B1 in corn and nut products using the array biosensor," *Biosensors and Bioelectronics*, vol. 21, no. 12, pp. 2298–2305, 2006.