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Selection of Single Chain Fragment Variables with Direct Coating of Aflatoxin B_1 to Enzyme-Linked Immunosorbent Assay Plates

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Most selections of antihapten recombinant antibodies from antibody libraries were against hapten-carrier conjugates, which are different from free haptens in antigen specificity and often mislead the screening due to immunodominant epitopes in carriers. In the present study, aflatoxin B_1 (AFB_1) was directly coated to enzyme-linked immunosorbent assay (ELISA) plates and anti-AFB_1 single chain fragment variables (scFvs) were selected with the AFB_1-coated plates. Compared to the selection against AFB_1-bovine serum albumin conjugate (31 positives out of 46 random clones), the isolated scFvs against AFB_1 (44 positives out of 46 random clones) showed higher specificities for AFB_1. The clone H4 with K_D of 9.8×10^{-11} M to AFB_1−BSA and K_D of 1.2×10^{-12} to AFB1 was sequenced. In addition, polymerase chain reaction−denaturing gradient gel electrophoresis (PCR−DGGE) was applied to describe the biopanning for the first time. Our research presents a quick and robust selection technique for antihapten recombinant antibody from large naive libraries.

KEYWORDS: Aflatoxin; scFv; phage display; hapten; DGGE

INTRODUCTION

Aflatoxin B_1 (AFB_1) is an *Aspergillus flavus* metabolite that contaminates the food and feed supply, such as maize, cottonseed, peanuts, and tree nuts, during growth and storage. Dietary exposure to AFB_1 is one of the major risk factors in the multifactorial etiology of hepatocellular carcinoma (HCC), especially in populations exposed to hepatitis B virus (HBV) (*1*). To effectively monitor the occurrence of AFB_1 in food at low contamination levels, sensitive, reliable, and simple analytical methods are required. High performance liquid chromatograph (HPLC) and immunoassay are the most common methods for AFB_1 detection in food and feed. High quality antibody is very important in the competitive enzyme-linked immunosorbent assay (ELISA) (*2*). Antibody is also important in HPLC analysis, since immunoaffinity chromatograph (IAC) is often used for cleaning up the samples prior to HPLC analysis (*3*). Bypassing the immunization of animals, the recombinant antibody technology could produce low-cost antibodies with easy manipulation of affinity and specificity.

A growing number of recombinant antibodies have been used successfully in the quality control applications in food research and industry, such as the immunoassay for atrazine (*4*), wheat glutenin (*5*), and *Sclerotinia sclerotiorum* (*6*). Several in vitro methods have been developed, which greatly facilitated the construction and screening of combinatorial libraries as well as the expression of recombinant antibody fragments in bacteria (*7*).

Moghaddam et al. isolated anti-AFB_1 single chain fragment variables (scFvs) from a human lymphocyte antibody library and a semisynthetic antibody library against AFB_1−BSA (*8*). Daly et al. used AFB_1−dextran and AFB_1−BSA as antigens and panned murine anti-AFB_1 scFvs from a preimmunized phage displayed antibody library (*9*). Currently, selections of recombinant antibodies against haptens mainly choose to use hapten−carrier conjugates as antigens (*10*−*13*) for the benefits of high solubility and easy immobilization. Unfortunately, the conjugation of haptens and carriers might change the antigenicity of haptens, and some selected antibodies would only recognize the conjugates (*3*, *8*, *14*). Furthermore, phage display selection usually yielded antibody fragments against immunodominant epitopes, and antibodies against haptens were not, or only rarely, selected when the epitopes of haptens were weak (*15*).

However, when haptens are bound directly to solid supports, screening of antihapten antibody fragments against the immobilized haptens may avoid these problems. Hofstetter et al. demonstrated that it was possible to directly bind amino acids of hapten (such as *p*-aminophenylalamine) to γ-irradiated polystyrene plates (Maxisorp plates) for the detection of antibodies that stereospecifically recognized the chiral center of α-amino acid (*16*). In the present study, AFB_1 was directly coated into Maxisorp plates and we developed a new strategy that allows specific selection for single chain antibodies against AFB_1 without any carrier.

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MATERIALS AND METHODS

The Tomlinson libraries I + J, *Escherichia coli* (*E. coli*) TG1, and KM13 helper phage were purchased from Geneservice Ltd. (Cambridge, U.K.). Protein A/horseradish peroxidase conjugate (Protein A/HRP), anti-M13 monoclonal antibody/horseradish peroxidase conjugate (anti-M13/HRP), and *E. coli* HB2151 were purchased from GE Healthcare (GE Healthcare, Shanghai, China). Aflatoxins and AFB_1−BSA conjugate were Sigma products (Sigma, Shanghai, China). Maxisorp ELISA plates were from a local distributor of NUNC A/S (Recheen, Shanghai, China). Other chemicals were supplied by Shanghai Sangon Biological Engineer Technology & Service Co., Ltd. (Sangon, Shanghai, China).

Biopanning. The panning was performed as described in the manufacturer's protocol with some modifications. Briefly, Maxisorp plates were coated with 200 μL of 10 μg/mL AFB_1 or AFB_1−BSA (conjugation molar ratio 8.1) in phosphate buffered saline (PBS, pH 7.2) at 4 °C overnight, washed 3 times with PBS, and blocked with 2% Marvel milk powder in PBS (MPBS) while the MPBS-blocked blank wells were used as negative control. Subsequently, phages were added to the blocked wells (10^{12} to 10^{13} phages per well in 150 μL MPBS) and incubated at 37 °C for 2 h. The unbound and loosely bound phages were washed away with PBS containing 0.05% Tween-20 (PBST). The wash was repeated 10 times in the first round and 20 times in the second and third rounds. The bound phages were competitively eluted with 100 μL of free AFB_1 (50 μg/mL) and then treated with trypsin (1 mg/mL) at 37 °C for 15 min (*15*). The treated phages were rescued by infecting 900 μL of exponentially growing *E. coli* TG1 and selected on TYE plates (15 g of Bacto-agar, 8 g of NaCl, 10 g of tryptone, 5 g of yeast extract in 1 L) supplemented with 1% glucose, 100 μg/mL ampicillin, overnight. At the same time, the infected *E. coli* TG1 was serially diluted to titrate the eluted phages. With the helper phage KM13, phagemids were amplified and released from *E. coli* TG1 and precipitated with 20% polyethylene glycol 6000 for further rounds of selection.

Polymerase Chain Reaction−Denaturing Gradient Gel Electrophoresis (PCR−DGGE). The Vκ gene segments of scFv were amplified by PCR from the phagemids (100 ng each), recovered from each round of selection, and analyzed with DGGE. The PCR primers were GC clamp + DPK9 Fr1 (5′-CGCCC GCCGC GCCCC GCGCC CGGCC CGCCG CCCCC GCCCG CTATG CGGCC CCATT CA) (*17*) and pHEN (5′-CTATG CGGCC CCATT CA). The PCR conditions were 95 °C for 5 min, 30 cycles of 95 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, and finally 72 °C for 10 min. PCR products were separated by 8% acrylamide/bis (37.5:1) gel electrophoresis with a D-Code system (Bio-Rad, Nanjing, China). The gel contained a linear gradient of urea and formamide (35%−70%). Electrophoresis was run in 1× TAE buffer at 60 °C, 200 V for 10 min and 85 V for 14 h. Subsequently, the gel was treated in 10% ethanol containing 0.5% acetate acid for 5 min, stained with 0.5% silver nitrate for 10 min, and destained with 1.5% sodium hydroxide containing 0.3% formaldehyde and sodium borohydride.

ELISA. Maxisorp plates were coated with 25 μL of AFB_1−BSA (5 μg/mL) at 37 °C for 2 h or with 25 μL of BSA (5 μg/mL) as negative control. The coated wells were washed with PBS twice, blocked with 2% MPBS at 37 °C for 2 h, and washed with PBS. In polyclonal phage ELISA, 10 μL of phage antibodies amplified from each round of selection were mixed with 200 μL of 2% MPBS and incubated at 37 °C for 1 h. Unbound phages were removed by washing for 4 times with PBST. Anti-M13/HRP antibody in MPBS (1:5000 dilution) was added and incubated at 37 °C for 1 h. The plate was washed again 4 times with PBST. The signal was visualized with 100 μL of 3,3′,5,5′-tetramethylbenzidine (TMB, 100 μg/mL) in acetate acid buffer (pH 5.5). The reaction was stopped by adding 50 μL of 1 M H_2SO_4, and the optical densities at 450 nm (OD_{450}) and 650 nm (OD_{650}) were recorded with a MK3 microplate reader (Thermo, Shanghai, China). Individual clones from the titer plates of the third selection round were picked randomly, amplified in phage form, and used in monoclonal phage ELISA.

Soluble scFv Production. The gene of scFv was amplified by PCR reaction with primers of LMB (5′-CGA CCC GCC ACC GCC GCT G) and pHEN (5′- CTA TGC GGC CCC ATT CA) and double digested with restriction enzymes of *Nco*I and *Not*I. The enzymatic digested scFv gene fragment and pET22b(+) was linked in a ligation mixture of T4 DNA ligase at 16 °C for 16 h. The ligation mixture (10 μL) was transformed in competent *E. coli* DH5α by heating shock. The transformed cells were selected on LB agar (10 g of Bacto-agar, 5 g of NaCl, 10 g of tryptone, 5 g of yeast extract in 1 L) supplemented with ampicillin. Colonies bearing the pET−scFv construct were confirmed by restriction enzyme digestion and DNA sequencing (Sangon, Shanghai). The positive clones of pET22b(+)/H4 construct was transferred into competent *E. coli* BL21(DE3).

A single clone of BL21(DE3)/pET22b/H4 was selected and grown to midlogarithmic phase (OD_{600}=0.8) in LB broth, and IPTG was added to a final concentration of 1 mM. The cells were allowed to grow at 20 °C for another 20 h. The cells were harvested by centrifugation at 3300*g* for 15 min. The resulting pellet was resuspended in 10 mL of PBS and ultrasonicated in an ice bath. The soluble scFv was purified with a Ni-chelating affinity chromatography (GE Healthcare, Shanghai).

Competitive ELISA. Competitive ELISA was employed to evaluate the affinity and the cross-reactivity of the selected clones with AFB_1 and its closely related molecules (*9*). Maxisorp plates were coated with AFB_1−BSA and blocked as described above. AFB_1 standard solutions with a range of concentrations from 0.05 to 512 ng/mL were added to each well. Then 50 μL of soluble scFv was added to each well. The plates were incubated at 37 °C for 1 h and washed with PBST. Protein A/HRP was incubated at 37 °C for 1 h and aspirated, and then 100 μg/mL TMB was added, and OD_{450} and OD_{650} were recorded. The half maximal inhibitory concentration (IC_{50}) of free AFB_1 was determined.

Standard solutions of aflatoxin B2, G1, and G2 (0.05−250 ng/mL) were prepared, and the competitive ELISA was carried out as described above, except that aflatoxin B2, G1, or G2 was added to the plates with scFv−H4.

Sodium Dodecyl Sulfate−Polyacrylamid Gel Electrophoresis (SDS−PAGE). SDS−PAGE was performed on 15% gel under reducing conditions with a Mini-protean 3 electrophoresis system (Bio-Rad, Nanjing, China). Five microliter samples with various protein concentrations were loaded on the gel, concentrated at 80 V for 20 min, and separated at 150 V for 60 min. The gel was stained by Coomassie brilliant blue and destained with 10% acetic acid.

Surface Plasmon Resonance (SPR) Measurement. The association and dissociation kinetics of scFv (H4) for AFB_1−BSA were determined by SPR on a BIAcore 3000 instrument (GE healthcare, Shanghai, China). The AFB_1−BSA was coupled to the CM5 chip using standard carbodiimide chemistry. The scFvs were tested at 10 μL/min over the immobilized AFB_1−BSA for 1 min, followed by a 2 min delayed wash to allow the dissociation curve to be recorded. Sensograms were collected as the difference in binding to the AFB_1−BSA-coated channel against a BSA-coated channel. The sensor chip surface was regenerated using 20 μL of glycine−HCl solution after each round of binding. The BIAevaluation software 3.0 was used to analyze binding data using a simple Langmuir 1:1 model. The fit of the experimental data for models was assessed by x^2 analysis.

DNA Sequencing Assay. Positive clones determined in the competitive ELISA were sequenced by Shanghai Sangon Biological Engineer Technology & Service Co., Ltd. (Shanghai, China). Sequencing primers were LMB (CGA CCC GCC ACC GCC GCT G) and pHEN (CTA TGC GGC CCC ATT CA).

RESULTS AND DISCUSSION

To identify and clone human antibodies against AFB_1, AFB_1 and AFB_1−BSA conjugate were coated directly on Maxisorp plates and used for selection of Tomlinson libraries I + J (human scFv libraries preselected for active fold by binding to Protein L and Protein A, with a size of approximately 1.4×10^8 each). Positive phages were enriched with either free AFB_1 or AFB_1−BSA conjugate (**Table 1**). In the first round of selection, 8.0×10^2 phages were selected against AFB_1, accounting for 5% of the eluted phages (4.2×10^4) against AFB_1−BSA. In the second and third rounds of selection, the phage titers increased consistently compared to the previous rounds, which reflected an enrichment of specific clones. The titers of phage panned against MPBS-blocked wells were always at a low level of recovery in the rounds

Table 1. Comparison of Selection of Antibodies against AFB_1−BSA and AFB_1[a]

	panning against AFB_1	panning against AFB_1−BSA	blank control
titer results in the panning			
round 1	8.0×10^2	4.2×10^4	3.2×10^2
round 2	2.0×10^3	2.5×10^6	5.7×10^2
round 3	9.4×10^5	1.1×10^8	1.9×10^3
positive ratio in monoclonal ELISA after three rounds of panning	44/46	31/46	0/46
number of positive scFv recognizing free AFB_1[b]	13/14	4/8	ND[c]
unique clones	A7, C5, C11, G1, H4	D6, F8	ND

[a] The blank control followed the same selection procedure against MPBS blocked blank wells. [b] Binding to AFB_1 was scored as positive when the scFv antibodies were inhibited from binding to AFB_1−BSA coated ELISA plate wells by at least 50% using 500 ng/mL soluble AFB_1. [c] Not detected.

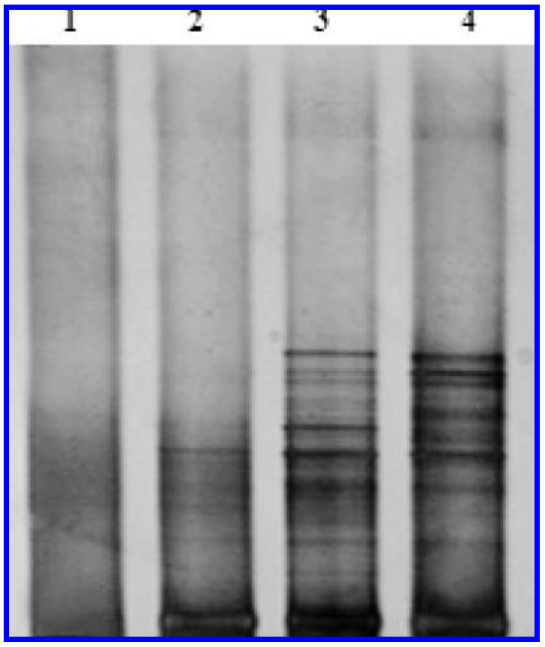

Figure 1. DGGE profiles of Vκ gene segments. Lane 1, original Tomlinson library I; lane 2, the first round of selection against AFB_1; lane 3, the second round of selection against AFB_1; lane 4, the third round of selection against AFB_1.

of selection, and no enrichment was observed. In addition, the recovery of panning against AFB_1 was less efficient than that against AFB_1−BSA, which might be due to the weaker binding of AFB_1 to the ELISA plate.

Since various scFv clones from the Tomlinson I + J libraries have an equal length in nucleotides with a high homology, PCR−DGGE was also used to confirm the enrichment of specific clones in the selection against AFB_1. In DGGE, DNA fragments of the same length, but with a different sequence, could be separated. Clones with abundance greater than 1% could be recorded as clear bands on a DGGE gel (*18*). In **Figure 1**, clear bands were detected with the increasing intensity in the three rounds of selection, indicating the enrichment of some clones. There was no clear band in the DGGE profile of the original library I (lane 1); after the first round of selection, some bands appeared (lane 2); more bands were presented in the second round of selection (lane 3) and intensified in the third round of selection (lane 4).

PCR−DGGE has been frequently used to fingerprint the natural bacterial population (*19*) and to identify mutations in short DNA fragments (*20*). Previously, the amplification of selected clones was described by the recovery increase of each round of selection. Here, we employed PCR−DGGE to evaluate the amplification of the specific clones in each round of biopanning. In addition, PCR−DGGE could provide information about the diversity and the abundance of library screening results. However, PCR−DGGE could not be used to analyze gene fragments longer than 500 bp (*21*). Since the full length of the scFv gene is more than 700 bp, PCR−DGGE cannot be applied in the study of diversity of the whole scFv. Related to the problem of resolution might be the maximum number of different DNA fragments which can be separated by DGGE. Obviously, DGGE could not separate all of the scFv gene segments in the profile of the original library with a size of 1.4×10^8 but presented them as smears. The amplification, proven by titer results and PCR−DGGE, indicated that AFB_1 could directly coat into the ELISA plates and work as an immobilized antigen in the selection.

In polyclonal ELISA, the signals of phage antibodies selected against AFB_1 increased after each round of selection, while the signals of negative controls decreased (**Figure 2A**). Stronger signals were recorded during the selection against AFB_1−BSA than that against AFB_1 (**Figure 2B**), while the signals of negative controls decreased sharply after the second round of selection. Generally, phage antibodies are scored as positive when the signals are 3 times higher than their negative controls (*8*). We noted that the phage antibodies selected against AFB_1 were positive after three rounds of selection, while the phage antibodies selected against AFB_1−BSA were positive after only two rounds of selection. When phase antibodies selected against MPBS-blocked blank wells were used in the polyclonal ELISA, the signal on the AFB_1−BSA coated wells was at a very low level and not different from the signal on BSA coated wells (**Figure 2C**). It indicated that no specific clones recognizing AFB_1−BSA or BSA was enriched.

Forty-six clones selected from each method were randomly picked out from the titer plates of the third round of selection and checked by monoclonal ELISA. Out of these 46 clones, 31 positive clones were selected against AFB_1−BSA and 44 positive clones were selected against AFB_1, while no positive clones were obtained in the selection against MPBS-blocked blank wells (**Table 1**). Seven clones with a strong signal were obtained from negative control plates (BSA coated) of AFB_1−BSA, whereas none were obtained from the negative control plates of AFB_1, suggesting that the anti-BSA phage antibodies were nonspecifically eluted in the rounds of selection against AFB_1−BSA. It appeared that selection against AFB_1 was more specific than that against AFB_1−BSA in the isolation of anti-AFB_1 recombinant antibodies. Previously, Daly et al. used AFB_1−dextran and AFB_1−BSA alternatively as antigens and selected 7 positive clones out of 96 random clones from a murine antibody library preimmunized by AFB_1−BSA (*9*). Moghaddam et al. selected 19 positive clones out of 46 random clones eluted by triethylamine (pH 11) and 24 positive clones out of 32 random clones eluted by AFB_1 (4.4 μg/mL) from a semisynthetic library (*8*). It appeared that our method was more efficient in selecting AFB_1-specific scFv than the previous methods.

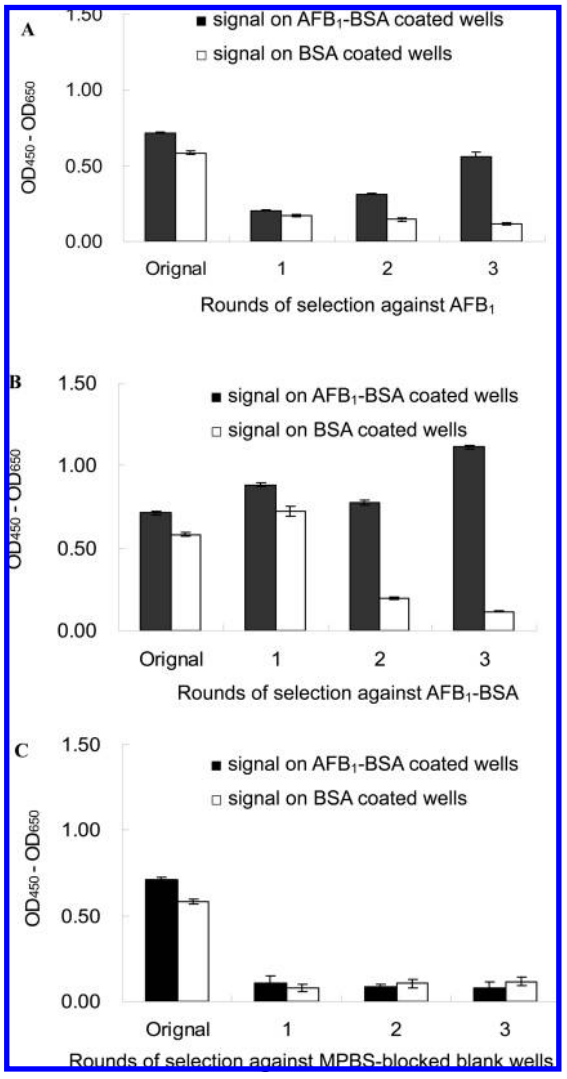

Figure 2. Binding activity of phage antibodies amplified after rounds of selection: (A) panned against AFB_1; (B) panned against AFB_1−BSA, and (C) panned against the MPBS-blocked blank wells. The data were the average of duplicates with standard deviations.

The trypsin treatment also increased the selection efficiency. After competitive elution, trypsin digestion rendered the phages without scFv noninfective (*22*) because the integrity of pIII protein was necessary for phages to recognize host bacteria. Shown in **Figure 3**, two types of pIII protein existed on M13 phages (lane 2): one was pIII−scFv fusion protein from recombinant phagemid, and the other was modified pIII from helper phage which had an additional trypsin-sensitive site between domain 2 and domain 3 (*23*). After trypsin treatment, the fusion protein was cleaved into pIII and scFv while modified pIII was cleaved into domain fractions at the additional trypsin-sensitive site (lane 3). It was reported that about 99% of eluted phages carrying no scFv became noninfective after trypsin treatment (*15*). Thus, the selection efficiency of target phages could increase dramatically.

Competitive ELISA was used to explore the binding of soluble scFv to free AFB_1. The 13 positive clones in competitive ELISA were sequenced and five unique clones were detected (**Table 1**). IC_{50} of the selected clones varied from 0.4 to 120 ng/mL (**Figure 4**). In previously reports, the IC_{50} of the selected clones were about 400 and 16 ng/mL, respectively (*8*, *9*). H4 (IC_{50}: 0.4 ng/mL) from the Tomlinson library J were chosen for further study.

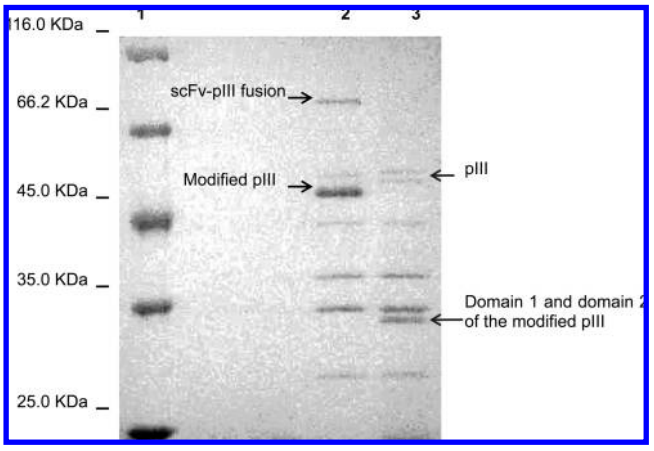

Figure 3. SDS−PAGE results of scFv−phage before and after trypsin treatment. Lane 1, protein marker; lane 2, protein profile of scFv−phage; lane 3, protein profile of scFv−phage after 1 mg/mL trypsin treatment at 37 °C for 10 min.

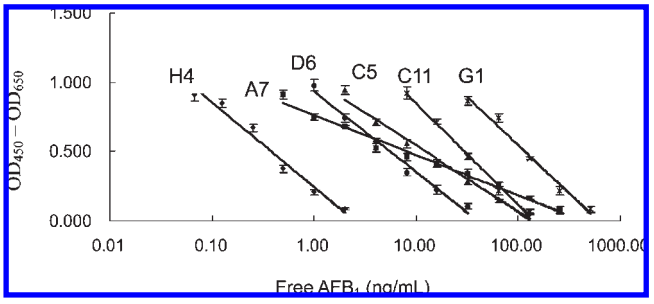

Figure 4. Binding of selected clones to free AFB_1 in competition assay. The purified scFv antibodies were titrated to determine the amount of antibody that gives an OD of 0.8−1.0. Soluble scFv was preincubated with AFB_1 standard solutions with a range of concentrations from 0.05 to 512 ng/mL. Numbers are average of duplicates with standard deviation. Name assigned for each scFv clone is shown on the top of each trend line.

Three structurally related aflatoxins (B_2, G_1, and G_2) were used to evaluate the cross reactivity of soluble scFvs of the selected clones. The IC_{50} values of AFB_2, AFG_1, and AFG_2 were determined by preparing standard curves using the competitive ELISA. The cross-reactivity values were calculated as the ratio of IC_{50} of AFB_1/IC_{50} of the similar aflatoxins. All of these selected clones showed cross-reactivity to the structurally related aflatoxins with much reduced binding efficiencies. Clone H4 had the cross reactivity of 12% with AFB_2, 42% with AFG_1, and 9% with AFG_2.

The soluble scFv was tested for their binding ability to AFB_1−BSA using SPR. A dose-dependent binding to AFB_1−BSA-coated chip was observed, and the K_D to AFB_1−BSA estimated according to binding kinetics was 9.8×10^{-11} M (**Figure 5A**). Free AFB_1 could inhibit the binding by the preincubation of the scFv with AFB_1. The scFv−H4 (0.5 μM) was incubated with increasing concentrations of AFB_1 from 0 to 2.0 ng/mL (**Figure 5B**) The competition was also dose-dependent, which indicated that scFv−H4 recognizes free AFB_1 in solution. The IC_{50} of AFB_1 obtained by SPR was similar to that obtained by ELISA. The affinity of scFv−H4 to AFB_1 was 1.2×10^{-12} M, which was estimated by the software in the competitive SPR.

Sequencing results showed that the clone C5 selected against AFB_1 and clone F8 selected against AFB_1−BSA were completely identical. It was interesting to know that a common clone could

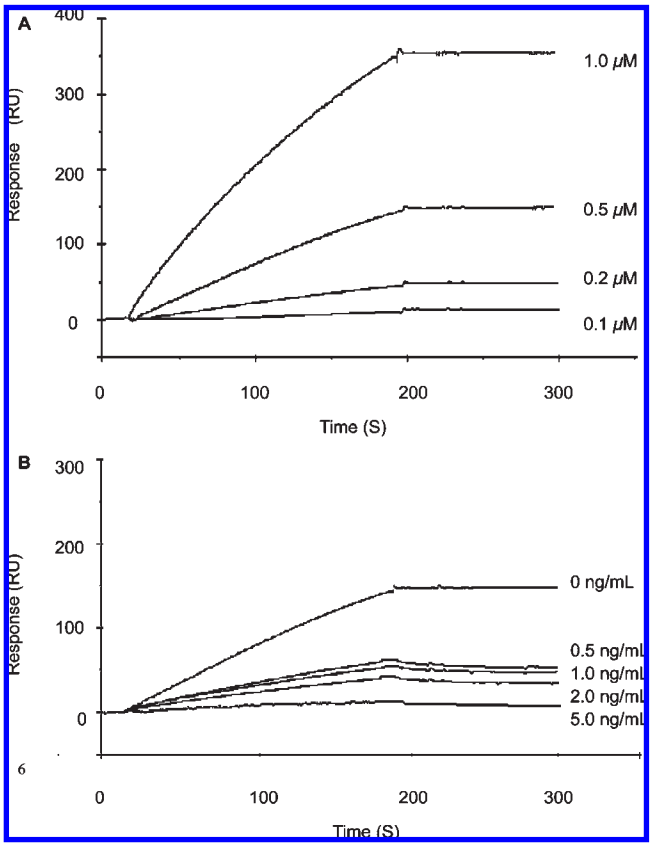

Figure 5. Binding analysis of scFv by surface plasmon resonance. (A) The dose-dependent binding of scFv−H4 with concentrations ranging from 0.1 to 1.0 μM . (B) Competition between AFB_1−BSA conjugate and free AFB_1 in various concentrations (0−2.0 ng/mL) as indicated.

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKCLEWVSS
TGAGGGTRYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCVKHKTT
FDYWGQGTLVTVSSGGGGSGGGGSGGGGSTDIQMTQSPSSLSASVGDRV
TITCRASQSISSYLNWYQQKPGKAPKLLIYRASHLQSGVPSRFSGSGSGTDF
TLTISSLQPEDFATYYCQQATASPVTFGCGTKVE

Figure 6. Amino acid sequence of H4. The shaded areas depicted CDRs.

be isolated against these two antigens, respectively. The entire coding sequence of H4 was presented in **Figure 6** (GenBank accession No. 1176324). We used both Kabat and Chothia definitions to identify the complementary determining regions (CDRs) of our recombinant antibodies (*24*−*26*). Sequence blasting indicated that the light chain of H4 belongs to subgroup I of human κ chain while the heavy chain belongs to subgroup III of the human heavy chain.

With purified scFv of H4, we examined the direct binding of AFB_1 to Maxisorp plates. AFB_1 standard solutions with concentrations ranging from 0.01 to 1000 μM were incubated in Maxisorp plates at 4 °C overnight, and ELISA was performed as described in the Materials and Methods. AFB_1−BSA of equivalent concentrations was examined at the same time. The clear correlation between AFB_1 concentration and ELISA signal indicated that AFB_1 could directly bind to Maxisorp plates (**Figure 7**). The detailed mechanism of the binding AFB_1 to the Maxisorp plate was not clear yet. Hofstetter suggested that an aromatic amino functional group appeared to be important for the binding. Maxisorp plates were routinely pretreated by the

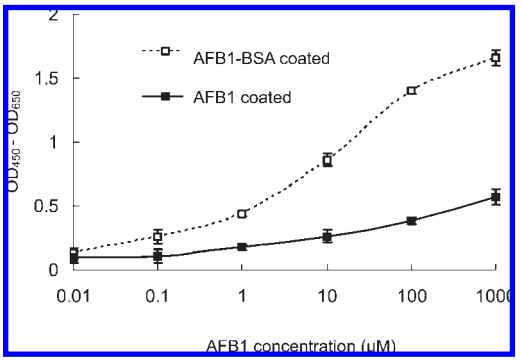

Figure 7. Influence of AFB_1 concentration in direct coating on the ELISA signal. The solid line was the ELISA signal on AFB_1 coated wells while the dashed line was the ELISA signal on AFB_1−BSA coated wells. The concentration of AFB_1−BSA was converted into AFB_1 concentration with the conjugated ratio of 8.1. The BSA-blocked blank wells served as blank sample. Absorbance signals were presented with standard deviation of duplicates, and the blank value was 0.076 ± 0.03.

manufacturer by γ-irradiation using ^{60}Co to induce the formation of reactive functional groups (*16*). Besides the functional groups, Maxisorp plates had a hydrophobic surface as well as many hydrophilic binding sites, which resulted in a fine patch work of hydrophobic and hydrophilic binding sites (specification of the Maxisorp plates). When molecules were small, van der Waals mediated bonds were relatively weak; therefore, they might be insufficient for stable binding. Hydrophobic interaction between Maxisorp plates and AFB_1 might be the predominate force in the binding. The signal of AFB_1 coated wells was much lower than that of AFB_1−BSA coated wells (**Figure 7**). This might indicated that the binding force of AFB_1 to the Maxisorp plate was weaker than those interactions of functional groups between AFB_1−BSA and the plate. In addition, single-site binding of AFB_1 facilitated its dissociation from Maxisorp plates while the dissociation was more difficult in the case of AFB_1−BSA with multisite binding. The weak binding of AFB_1 to the Maxisorp plate with the weak ELISA signal might not be suitable for good characterization of the selected antibody and development of immunoassays; however, the binding is strong enough to immobilize AFB_1 as antigen in the antibody selection.

Generally, the directly binding of AFB_1 to the Maxisorp plate was applicable in the anti-AFB_1 scFv panning from the recombinant library, as we described above. Selection with these AFB_1-coated plates avoided the interference of carrier in the selection; thus, it was highly specific to the anti-AFB_1 antibodies. ELISA and SPR measurement confirmed that the clone of H4 selected against AFB_1 had a high affinity, which was better than the results in previously reports (*7*, *8*). We would extend the direct coating approach to other haptens in further studies. Meanwhile, the high affinity of scFv−H4 set a solid foundation for the immunological analysis of AFB_1 contamination in food and the affinity column prior to HPLC. The high yield expression of H4 in *E. coli* resulted in cost-saving preparation of antibody. In addition, the tags in the recombinant antibody facilitate purification, immobilization, and detection of scFv in application.

ABBREVIATIONS USED

scFv, single chain fragment variables; BSA, bovine serum albumin; PCR, polymerase chain reaction; DGGE, denaturing gradient gel electrophoresis; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HPLC, high performance liquid chromatograph; ELISA, enzyme-linked immunosorbent assay; IAC, immunoaffinity chromatograph; Protein A/HRP, protein

A-horseradish peroxidase conjugate; anti-M13/HRP, anti-M13 monoclonal antibody/horseradish peroxidase conjugate; PBS, phosphate buffered saline (pH7.2); MPBS, 2% Marvel milk powder in PBS; PBST, PBS containing 0.05% Tween-20; TMB, 3,3′,5,5′-tetramethylbenzidine; IC_{50}, half maximal inhibitory concentration; SDS−PAGE, sodium dodecyl sulfate polyacrylamid gel electrophoresis; SPR, surface plasmon resonance; CDR, complementary determining region.

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LITERATURE CITED

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