Production and Characterization of Monoclonal and Polyclonal Antibodies against the Mycotoxin Cyclopiazonic Acid

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Polyclonal antibodies (Pabs) against cyclopiazonic acid (CPA) were obtained from both mice and rabbits after they were immunized with CPA–bovine serum albumin (BSA) or ethylenediamine-modified BSA. An indirect enzyme-linked immunosorbent assay (ELISA) was developed for antibody titer determination and for CPA assay. Antibody titer reached 9000 9 weeks after immunization. Competitive ELISA revealed that the concentrations causing 50% inhibition binding (ID_{50}) of the rabbit Pabs to the solid-phase CPA–keyhole limpet hemocyanin by CPA and CPA-imine were 62 and 3388 ng/mL, respectively. Four hybridoma cell lines (5C8B2, 5C8D1, 5C8D3, 5C8F3) that produced IgG1 and κ-chain isotype monoclonal antibodies (Mabs) with a binding constant in the range 6.1×10^8 to 2.0×10^{10} L/mol for CPA were generated. The ID_{50} of the Mab elicited by these clones ranged from 0.014 to 0.17 ng/mL for CPA and from 3.34 to 44.2 ng/mL of CPA-imine, respectively. The linear portion of the standard curve for the Mab-based ELISA (Mab from clone 5C8B2) for CPA was in the range 0.001–0.5 pg/assay. Neither Mabs nor Pabs cross-reacted with tenuazonic acid, D-lysergic acid, lysergol, and tryptophan.

INTRODUCTION

Cyclopiazonic acid (CPA), an indole tetramic acid, is a toxic secondary metabolite produced by a number of *Aspergilli* and *Penicillia* (Cole and Cox, 1981; Holzapfel, 1968). This mycotoxin is toxic to several animal species, causing degeneration and necrosis of the liver, lesions of the myocardium, and neurotoxic effects (CAST, 1989; Cole and Cox, 1981; Nishie et al., 1985; Peden, 1990; Porter et al., 1988; Purchase, 1971). Biochemically, CPA alters calcium homeostasis, induces charge alterations in plasma membranes and mitochondria, and also behaves as an antioxidant (Riley and Goeger, 1991; Riley et al., 1992). Recent discoveries on the specific inhibitory effect of CPA to calcium-dependent ATPases of sarcoplasmic reticulum and other systems (Seidler et al., 1989) have led to a wide use of CPA as a powerful tool in elucidating the molecular mechanisms for cellular Ca^{2+} influx (Demaurex et al., 1992; Ishii et al., 1992; Low et al., 1992; Riley et al., 1992). Cyclopiazonic acid has been conidered to be potentially hazardous to humans and animals (CAST, 1989) because it is produced by fungi, including the *Aspergillus* species *flavus*, *oryzae*, *tamarii*, and *versicolor* and *Penicillium* species *camembertii*, *crustosum*, *cyclopium*, *griseofulvum*, *patulum*, and *viridicatum*, that are frequently isolated from foods and feeds (Cole and Cox, 1981; Gallagher et al., 1978; Holzapfel, 1968). CPA has also been found in corn, cheese, peanuts, and other foods and feed (Chang-Yen and Bidasee, 1990; Hahnau and Weiler, 1991; Lansden, 1984; Le Bars, 1979; Still et al., 1978).

Interest in developing more rapid, sensitive, and specific methods for the analysis of CPA in foods and feeds has always been high because detection of this mycotoxin in foods and feeds is an analytical challenge since it has neither fluorescence nor strong UV absorption. Derivatization of CPA is generally necessary for most chemical analyses (Chang-Yen and Bidases, 1990); thus, the detection limits of most methods for this mycotoxin are generally high (Lansden, 1984; Norred et al., 1987). Recently, immunoassays have been developed for mycotoxin analysis (Chu, 1991; Morgan and Lee, 1990; Pestka, 1988; Martibauer et al., 1991). Hahnau and Weiler (1991) have produced polyclonal antibodies (Pabs) against CPA and developed a sensitive enzyme-linked immunoassay. Such a development has led to a demand for specific, well-characterized, and uniform antibodies against this mycotoxin. Monoclonal antibodies (Mabs) are suitable for this purpose. In the present study, attempts were made to produce Pabs and high-affinity Mabs. Four hybridoma cell lines eliciting high-affinity monoclonal antibodies against CPA were obtained. Details for the production and characterization of both Pabs and Mabs, as well as a comparison of their properties, are reported in this paper.

MATERIALS AND METHODS

A. Materials. Cyclopiazonic acid (CPA), polylysine (PLL, molecular weight 50 000) bovine serum albumin (BSA, RIA grade), *o*-phenylenediamine (OPD), Tween 20, CPA–BSA conjugate (C-3291, lot 89F-4045), D-lysergic acid, and lysergol were obtained from Sigma Chemical Co. (St. Louis, MO). Cyclopiazonic acid imine was kindly provided by Dr. R. T. Riley of USDA. Tenuazonic acid (TA) was prepared as the copper salt using the procedures described previously (Griffin and Chu, 1983). Ethylenediamine-modified BSA (EDA-BSA) was prepared according to the method of Chu et al. (1982). Goat anti-rabbit IgG–peroxidase conjugate was purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN). Keyhole limpet hemocyanin (KLH), and goat anti-mouse IgG + IgM–peroxidase conjugate (ELSA grade) were obtained from Pierce Chemical Co. (Rockford, IL). Polyethylene glycol 1000 was a J. T. Baker Chemical Co. (Phillipsburg, NJ) product. The murine myeloma cell line P3/NS-1/1-AG4-1 was obtained from American Type Culture Collection (Rockville, MD). Virus-free BALB/c mice were obtained from Harlan Sprague-Dawley (Madison, WI). *Pasteurella*-free female New Zealand rabbits were purchased from LSR Industries (Union Grove, WI). Freund's complete adjuvant containing *Mycobacterium tuberculosis* H37 Ra and Freund's incomplete adjuvant were obtained from Difco Laboratories (Detroit, MI). Dulbecco modified Eagle's medium, fetal calf serum, and penicillin–streptomycin were obtained from GIBCO Laboratories (Grand Island, NY). All other chemicals and organic solvents used were of reagent grade or better.

B. Preparation of Immunogens. Conjugation of CPA to PLL or KLH was achieved via the Mannisch reaction using formaldehyde as the cross-linking reagent under the conditions

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previously described (Chu et al., 1992). For example, in a typical experiment, 1.74 mg of PLL in 0.5 mL of 0.1 M sodium acetate buffer (pH 4.1) was reacted with 0.32 mg of CPA (in 32 μL of dimethyl sulfoxide) in the presence of 25 μL of 37% formaldehyde at room temperature for 72 h and stored at 4 °C overnight. The reaction mixture was dialyzed against 2 L of distilled water at 4 °C for 72 h with two changes of distilled water. In a similar manner, 0.19 mg of CPA was reacted with 2 mg of KLH.

Conjugation of CPA to EDA-BSA was carried out under acidic conditions according to the procedures described by Arens and Zenk (1980) for the conjugation of ergotamine to proteins. Briefly, a CPA solution (2.4 mg of CPA in 1.5 mL of dimethyl sulfoxide and 0.5 mL of acetic acid) was added dropwise to a solution containing 10 mg of EDA-BSA in 1 mL of distilled water, 0.5 mL of 3 M sodium acetate, and 0.5 mL of 37% formaldehyde. After reaction at room temperature for 24 h, the mixture was dialyzed against distilled water under the conditions described above.

C. Production of Polyclonal Antibodies in Rabbits. The immunization schedule and methods of immunization were essentially the same as those described for T-2 toxin (Chu et al., 1979) by a multiple-injection technique. Three rabbits were each injected intradermally with 500 μg of CPA–HCHO–EDA-BSA in 1.0 mL of 0.01 M phosphate buffer (PB, pH 7.5) containing 0.85% NaCl (PBS), emulsified with 2.0 mL of Freund's complete adjuvant. For booster injections, 300 μg of immunogen in 1.0 mL of PBS and 2.0 mL of Freund's incomplete adjuvant was used for each rabbit. The collected antisera were precipitated with $(NH_4)_2SO_4$ to a final saturation of 35% by mixing 2 mL of antisera with 1 mL of saturated $(NH_4)_2SO_4$ solution. The precipitates were redissolved in water and reprecipitated twice. Finally, the precipitates were reconstituted to half of the original volume with distilled water, dialyzed against distilled water for 0.5–1 h followed by 0.01 M PB overnight (all at 4 °C), and lyophilized.

D. Production of Monoclonal Antibody. Immunization procedures and tissue culture protocols were similar to those described by Fan et al. (1988).

1. Immunization. The immunogen was prepared by dissolving an appropriated amount of CPA–HCHO–BSA (Sigma) in 0.01 M PBS and emulsified with an equal volume of Freund's complete adjuvant. Three 6-week-old BALB/c mice were each injected intraperitoneally with 0.5 mL of the emulsified solution containing 50 μg of immunogen. Booster injections were made every 2 weeks after the initial immunization with 20–50 μg of immunogen in 0.01 M PBS containing no adjuvant. Blood samples were removed from the tails at a biweekly interval after each booster injection, and serum was obtained after incubation at 4 °C for 30 min and centrifugation at 11 000 rpm for 12 min. Antibody titers were determined by an indirect ELISA as described below.

2. Cell Line and Culture Media. The myeloma cell line (P3/NS-1/1-AG4-1) was grown in HT medium, which consisted of Dulbecco modified Eagle's medium with 20% fetal calf serum, 1 mM sodium pyruvate, 0.375% sodium bicarbonate, 60 μM hypoxanthine, 20 μM thymidine, and penicillin–streptomycin at a final concentration of 100 units/mL of each. Cloning medium consisted of HT medium plus normal mouse erythrocytes at a concentration of 0.5%. Hybridomas were selected by growth in HAT medium, which consisted of HT medium plus 0.5 μM aminopterin (Fan et al., 1988; Oi and Herzenberg, 1980).

3. Fusion and Cloning. The mouse with the highest polyclonal antibody titer (i.e., 200; 7 weeks after immunization with two booster injections) was selected for fusion. Four days before fusion, the mouse was primed with a total of 50 μg of immunogen (20 μg twice via ip injection and 10 μg once via iv injection). The mouse was sacrificed by cervical dislocation; the spleen was aseptically removed, mashed with a glass pestle, passed through a Cellector tissue sieve, and resuspended in a medium similar to HT medium but without fetal calf serum, hypoxanthine, and thymidine to produce a single-cell suspension. The suspension was then mixed with 10^7 myeloma cells at a ratio of 5:1. After centrifugation, fusion was carried out by incubation with 1 mL of 50% polyethylene glycol 1000 at room temperature for 1 min, followed by addition of 8 mL of HT medium for another 10 min. The cells were pelleted, suspended in HAT medium plus normal mouse erythrocytes at a concentration of 0.5%, and plated in 96-well tissue culture plate (Corning plate 25860; Corning, NY). The colonies were fed every 5 days with freshly prepared HAT medium. When the colonies reached at least half-confluency in the well (approximately 9–12 days after fusion), hybridomas were screened for specific antibodies against CPA using the indirect ELISA described below. Positive cells were cloned by the limiting dilution method (Oi and Herzenberg, 1980) into 96-well tissue culture plates. The supernatant fluids of the late stationary phase of the positive clone cell cultures were collected for subsequent purification and characterization.

E. Purification of Monoclonal Antibodies. Purification of the antibodies obtained from culture supernatant fluids was achieved by precipitation with $(NH_4)_2SO_4$ to a final saturation of 50% twice reconstituted to $^1/_{10}$ of original volume with deionized water and dialyzed against 0.5 L of distilled water for 0.5 h and then against 2 L of 0.01 M PB overnight.

F. Monitoring of Polyclonal Antibody Titers by Indirect ELISA. The antibody titers were determined by an indirect ELISA. For rabbit serum, CPA–HCHO–PLL was used as the solid-phase test antigen. For mouse serum and cultural supernatant fluids, CPA–HCHO–KLH was coated on each well of the ELISA plate. In general, 0.1 mL of CPA–HCHO–PLL (1 μg/mL) or CPA–HCHO–KLH (2 μg/mL) in 0.01 M PBS (pH 7.5) was added to each well of a 96-well ELISA microtiter plate (Nunc plate 2-69620; Nunc, Roskilde, Denmark). The plate was kept at 4 °C overnight. After the solution was removed, the wells were washed four times (0.35 mL/well) with PBS–Tween buffer (0.01 M phosphate saline buffer, pH 7.5, with 0.5% Tween 20). This was followed by incubation with 0.17 mL of 0.1% gelatin (blocking agent) in 0.01 M PBS at 37 °C for 30 min. The plate was washed four times with 0.35 mL of PBS–Tween to remove the excess blocking agent. To each well was added 0.1 mL of various dilutions of anti-CPA serum or supernatant fluid; the contents were gently mixed and incubated at 37 °C for 1 h. The plate was washed with 0.35 mL of PBS–Tween four times, and 0.1 mL of goat anti-rabbit IgG-HRP at 1:20 000 dilution or goat anti-mouse IgG + IgM–HRP at 1:5000 dilution in 0.01 M PBS was added to each well. After incubation at 37 °C for 1 h, the plate was washed, and 0.1 mL of freshly prepared OPD substrate solution (10 mg of OPD plus 13 μL of 30% hydrogen peroxide in 25 mL of 0.05 M of citrate–phosphate buffer, pH 5.0) was added. Ten minutes after incubation at room temperature in the dark, the reaction was terminated by adding 0.1 mL of 1 N HCl. The absorbance at 490 nm was determined in an automatic ELISA reader (Thermomax microplate reader, Molecular Devices, Co., Menlo Park, CA).

G. Competitive Indirect ELISA. For characterization of antibody specificity regarding CPA derivatives, a competitive indirect ELISA was used. The protocol was similar to those for monitoring antibody titers as described above, except that a constant amount of antibody (50 μL of antiserum or cultural supernatant at appropriate dilution) together with 50 μL of CPA or CPA-related derivatives at different concentrations was added to the CPA–HCHO–KLH-coated microplate well. The optimum dilution of CPA–HCHO–KLH (2.0 μg/mL, 0.1 mL/well) coated on the microtiter plate was determind by titration against various serum or cultural supernatant dilutions.

H. Characterization of Antibody. *1. Determination of Isotype.* A commercially made kit (mouse typer, Bio-Rad Laboratories, Richmond, CA), based on a sandwich-type ELISA, was used to determine the isotypes of the monoclonal antibodies produced by various hybridoma cell lines. In this assay, each well of the ELISA plate was coated with 0.1 mL of CPA–HCHO–KLH (2 μg/mL in 0.01 M PBS) for the binding of specific immunoglobulin. Identification of specific immunoglobulin was then carried out by using the protocol described by the manufacturer.

2. Analysis of Antibody Specificity. The competitive ELISA described above was used for the determination of the specificity of both polyclonal and monoclonal antibodies. Cyclopiazonic acid, CPA-imine, D-lysergic acid, lysergol, tenuazonic acid, and tryptophan were used in the test.

3. Determination of Affinity Constants. The affinity constants of monoclonal antibodies obtained by various hybridoma cell lines to CPA were determined by an equilibrium dialysis method (Chu, 1971). The free CPA concentration in this analysis was determined by ELISA. A computer program (InPlot4,

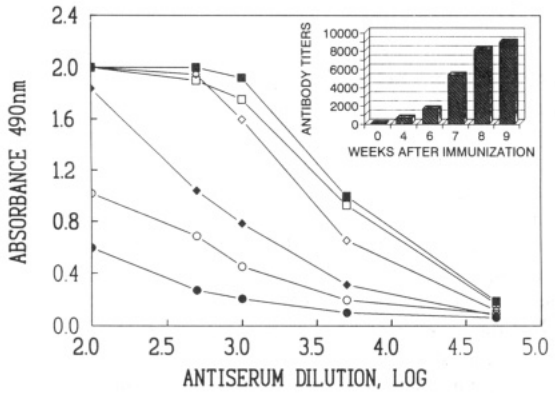

Figure 1. Determination of antibody titers by indirect ELISA. CPA–HCHO–PLL (1.0 μg/mL, 0.1 mL/well) was coated on the ELISA plate. The symbols ●, ○, ◆, ◇, □, and ■ indicate serum collected at 0, 4, 6, 7, 8, and 9 weeks after initial injections, respectively. The insert shows antibody titers of rabbit 18. Booster injections were made 4 and 8 weeks after initial immunization.

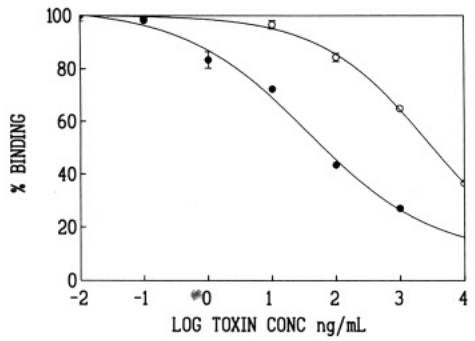

Figure 2. Competitive indirect ELISA of CPA using rabbit Pab against CPA–HCHO–BSA. CPA–HCHO–KLH (2.0 μg/mL, 0.1 mL/well) was coated on the ELISA plate. The antiserum (rabbit 19, 43rd week bleeding) dilution was 1:1000. The symbols ● and ○ represent CPA and CPA-imine, respectively. Data with no error bars indicate that the errors were within the size of the symbols. D-Lysergic acid, lysergol, TA, and tryptophan did not cause any inhibition of binding at concentrations of 10, 10, 100, and 100 μg/mL, respectively.

GraphPad Software, Inc. San Diego, CA) was used for the calculation of the binding constants based on the Scatchard plot method (Scatchard, 1949).

RESULTS

A. Production and Characterization of Polyclonal Antibodies. Results of the titration of antibody titers for a representative rabbit (no. 18) immunized with CPA–EDA-BSA are shown in Figure 1. Since nonspecific binding of preimmune serum to the coated microtiter plate was observed, a preselected absorbance of 0.6 was arbitrarily used in the estimation of antibody titer. Thus, the antibody titer was defined as the reciprocal of the antiserum dilution that gives an absorbance of 0.6 at 490 nm under the indirect ELISA conditions described. The antibody titers for the preimmune serum varied around 100. Among three rabbits tested, only two gave good response. The immune response for a typical rabbit (no. 18) after immunization with CPA–EDA-BSA is shown in the insert of Figure 1. The rabbit started to elicit antibodies as early as 4 weeks after immunization. Antibody titers reached 9000 9 weeks after initial immunization and two booster injections.

Results of the specificity of polyclonal antibodies, as determined by a competitive indirect ELISA, are shown in Figure 2. The concentrations causing 50% inhibition

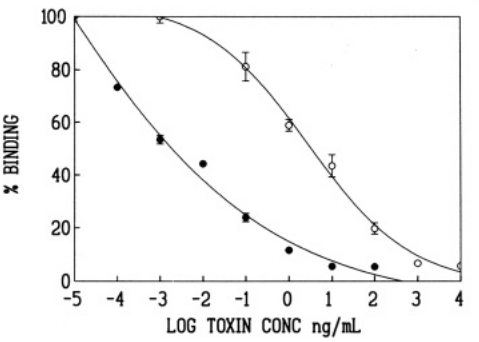

Figure 3. Competitive indirect ELISA of CPA using Mab against CPA–HCHO–BSA antiserum. CPA–HCHO–KLH (2.0 μg/mL, 0.1 mL/well) was coated on the ELISA plate. Supernatant fluid obtained from cell line 5C8B2 after ammonium sulfate precipitation and dialysis was diluted 100 times with PBS. Fifty microliters of the diluted Mab was used in each well. The symbols ● and ○ represent CPA and CPA-imine, respectively. Data with no error bars indicate that the errors were within the size of the symbols. D-Lysergic acid, lysergol, TA and tryptophan did not cause any inhibition of binding at concentrations of 0.1, 0.1, 0.1, and 10 μg/mL, respectively.

of binding (IC_{50}) of polyclonal antibodies to the solid-phase antigen CPA–HCHO–KLH by CPA and CPA-imine were found to be 62 and 3388 ng/mL (3.1 and 169 ng/assay), respectively. Thus, the relative reactivities of CPA and CPA-imine with the polyclonal antibodies are 100 and 1.8%. D-Lysergic acid, lysergol, TA, and tryptophan did not cause any inhibition of binding of the antibodies to the solid-phase CPA at concentrations of 10, 10, 100, and 100 μg/mL, respectively. The affinity contant of the polyclonal antibodies for CPA was found to be 2.2×10^7 L/mol.

B. Production of Monoclonal Antibody. Fourteen days after fusion, an average of 0.7 colony/well was found. Initial screening of 161 wells showed 36 master cell lines producing antibodies that were reactive with CPA–HCHO–KLH coated on the ELISA plate. The binding of antibodies elicited by two master cell lines (5A10 and 5C8) to the solid-phase CPA–KLH was diminished when free CPA was present in the screening wells. Master clone 5C8 was then selected for further cloning and expansion because antibody produced by this clone had the highest binding capacity with CPA (absorbance of 0.6 at 490 nm at a dilution of 1:1000; CPA–HCHO–KLH coated on the ELISA plate at a concentration 2 μg/mL). Further cloning and selecting of clones by limiting dilution method led to the isolation of four stable hybridoma cell lines (5C8B2, 5C8D1, 5C8D3, and 5C8F3), producing high titers of monoclonal antibodies against CPA.

C. Characterization of Monoclonal Antibodies. *1. Isotyping.* A commercially available kit was used to determine the isotypes of the monoclonal antibodies. Monoclonal antibodies from all four cell lines were found to be immunoglobulin G1, κ-light chain. The concentration of IgG1 produced by the cell lines was in the range 126–169 μg/mL of culture supernatant solution.

2. Specificity. The specificity of monoclonal antibodies produced by the four cell lines was determined by a competitive indirect ELISA. The typical inhibition curves, in which CPA or CPA-imine was competing with solid-phase CPA–HCHO–KLH for the binding of the monoclonal antibody obtained from cell line 5C8B2, are shown in Figure 3. The IC_{50} of monoclonal antibodies obtained from 5C8B2 were 0.014 and 3.34 ng/mL (0.7 and 167 pg/assay) for CPA and CPA-imine, respectively. The relative cross-reactivities of CPA and CPA-imine with this monoclonal antibody are 100 and 0.4%. The IC_{50} data for

Table I. Affinity Constants and IC_{50} Values of Monoclonal Antibodies Produced against CPA

cell line	IC_{50}, ng/mL		affinity constant for CPA, L/mol
	CPA	CPA-imine	
5C8B2	0.014	3.34	2.0×10^{10}
5C8D1	0.17	42.1	1.7×10^{9}
5C8D3	0.036	4.5	8.6×10^{8}
5C8F3	0.08	7.2	6.1×10^{8}
Pab	62	3388	2.2×10^{7}

monoclonal antibodies obtained from four cell lines regarding CPA and CPA-imine are compared in Table I. D-Lysergic acid, lysergol, tryptophan, and TA did not cause inhibition of binding of the four monoclonal antibodies to the solid-phase CPA at concentrations of 0.1, 0.1, 0.1, and 10 μg/mL, respectively.

3. Association Constants. The association constants of antibodies produced by the four cell lines with CPA are shown in Table I. They ranged from 6.1×10^8 to 2.0×10^{10} L/mol.

DISCUSSION

Antibodies, both polyclonal and monoclonal, with high specificity against CPA were obtained in the present studies. The antibodies were highly specific for CPA with some cross-reactivity with CPA-imine. There was practically no cross-reaction with other related naturally occurring alkaloids. Specific antibodies, both Mab and Pab, against ergot alkaloids from *Claviceps* have been obtained by several investigators (Arens and Zenk, 1980; Shelby and Kelley, 1990, 1991, 1992). Our results on the failure of cross-reaction of anti-CPA antibodies with D-lysergic acid and lysergol suggest that the anti-CPA antibodies are unlikely to be reactive with ergot alkaloids produced by *Claviceps* species. The present results are also consistent with the data reported by Hahnau and Weiler (1991), who demonstrated that the Pab against CPA had little reaction with ergocristine, tryptophan, TA, and several other indole mycotoxins.

Data obtained from both competitive indirect ELISA and equilibrium dialysis methods revealed that the affinity of the polyclonal antibodies (2.2×10^7) for the CPA was much lower than the monoclonal antibodies (6.1×10^8 to 2.0×10^{10}). All four hybridoma cell lines yielded monoclonal antibodies with similar affinities for CPA. However, it is of interest to note that whereas the affinity constant of Mab obtained from cell line 5C8D1 to CPA is higher than that of the Mab obtained from cell lines 5C8D3 and 5C8F3, the ID_{50} concentrations of CPA for the latter two Mabs were lower than that for Mab 5C8D1. It is possible that Mab obtained from cell line 5C8D1 has a higher affinity to CPA in solution than with the CPA in the solid phase. Polyclonal antibodies against CPA were also obtained by Hahnau and Weiler (1991) in an earlier study using the same immunogen. Although the affinity constant of the polyclonal antibodies obtained by Hahnau and Weiler (1991) has not been reported, it is reasonable to speculate that it might also be lower than that of the monoclonal antibodies obtained in present studies, because their ID_{50} value was about 0.6 pmol (201 pg) of CPA/assay, compared to a range of 0.014–0.17 ng of CPA/mL (0.7–8.5 pg/assay) for the monoclonal antibodies in the present study. It is reasonable to assume that the apparent binding constant of the polyclonal antibodies obtained by Hahnau and Weiler (1991) is about 100–1000 times lower than the monoclonal antibodies obtained in the present study. Hahnau and Weiler's value for Pab would probably compare in affinity with our Pab.

Since the monoclonal antibodies in this study had a much higher affinity for CPA, as well as other advantages over polyclonal antibodies, they would be a better choice for use in various immunoassays. The capability of using a monoclonal antibody for the analysis of CPA could be estimated from the standard curve in the competitive indirect ELISA (Figure 3). The linear range of the ELISA was found to be between 0.001 and 0.5 ng/mL or 0.05–25 pg/assay, which is one of the most sensitive ELISA systems that has been reported for a mycotoxin assay (Chu, 1991). Using polyclonal antibodies against CPA and a direct ELISA using the alkaline phosphatase system, Hahnau and Weiler (1991) found that the linear range of the standard curve for CPA analysis was in the range 33 pg–2 ng/assay. Since it is not known how different matrices will affect the monoclonal antibody-based ELISA, vigorous recovery tests with CPA added to various foods and feeds will have to be carried out to determine the ultimate sensitivity of this assay.

Because the affinity of the monoclonal antibodies to CPA was almost 250 times higher than that to CPA-imine and because only small amounts of CPA-imine are naturally occurring in raw agricultural products, ELISA of CPA should not be affected by CPA-imine. However, in view of the fact that antibody affinities to CPA-imine were also high, the monoclonal antibodies could be used for the analysis of CPA-imine if CPA and CPA-imine were separated by a chemical method, such as TLC or HPLC. The monoclonal antibody-based immunoassay could be used as a postcolumn monitoring system for both toxins. Alternatively, a monoclonal antibody-based affinity column could be used to separate CPA and CPA-imine before ELISA.

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