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Detection of Abrin in Food Using Enzyme-Linked Immunosorbent Assay and Electrochemiluminescence Technologies

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ABSTRACT

Abrin is a toxic ribosome-inactivating protein present in beans of *Abrus precatorius,* also known as rosary peas. The possibility that abrin could be used to adulterate food has made the development of assays for the detection of abrin a priority. Rabbit-derived polyclonal antibodies and mouse monoclonal antibodies were prepared against a mixture of abrin isozymes. The specificity and cross-reactivity of the antibodies were evaluated against a challenge library of 40 grains, nuts, legumes, and foods. An enzyme-linked immunosorbent assay (ELISA) and an electrochemiluminescence (ECL)–based assay were assembled and optimized. Polyclonal (capture) and polyclonal (detection) ELISAs, polyclonal and monoclonal ELISAs, and polyclonal and monoclonal ECL assays had limits of detection (LODs) of 0.1 to 0.5 ng/ml for abrin in buffer. The LOD for abrin dissolved into juices, dairy products, soda, chocolate drink, and condiments and analyzed with the ECL assay ranged from 0.1 to 0.5 ng/ml in the analytical sample. In contrast, the LODs for the ELISAs ranged from 0.5 to 10 ng/ml in the analytical sample.

Abrin is a class II toxic ribosome-inactivating protein present in the beans of *Abrus precatorius,* a plant commonly known as rosary pea, jequirite bean, and crab's eyes *(13, 17, 19). A. precatorius* is ubiquitous in subtropical climates and has been used in jewelry and medicine for centuries. The discovery of abrin, historic uses of rosary peas, and the biochemical properties of abrin have been extensively reviewed *(4, 20).* The hardness of shelled rosary peas prevents intoxication when ingested unless the integrity of the shell is damaged, as when threaded to make a necklace.

Abrin is a 64-kDa glycoprotein whose structure has been elucidated at 2.18 Å with an R factor of 18.9% *(24).* Abrin is composed of two subunits, an A chain and a B chain, linked by a disulfide bond. The A chain is a 251–amino acid *N*-glycosidase that catalyzes the depurination of adenin-4324 of 28S rRNA *(6, 14, 21).* The B chain is a 268–amino acid lectin that targets the toxin to cells and facilitates uptake. Despite structural and functional homology between abrin and ricin, these two ribosome-inactivating proteins are immunologically distinct; the antibodies raised against one protein do not cross-react with those raised against the other *(7, 12).* Animals immunized against abrin are not protected against intoxication by ricin and vice versa *(3, 5, 9, 20).*

Abrin purified from rosary peas as a single mixture of isozymes and administered intraperitoneally to mice had an LD_{50} (50% lethal dose) of 20 μg/kg of body weight *(17).* Fractionation of abrin according to the method of Hedge et al. *(11)* by diethylaminoethyl-Sephacel chromatography resulted in abrin I, abrin II, and abrin III, which had intraperitoneal LD_{50} values of 22, 2.4, and 10 μg/kg of body weight, respectively. Abrin is slightly more toxic than ricin *(10)* and has been studied for use in cancer therapy *(16).*

Rosary peas also contain isozymes of a 120-kDa agglutinin. The agglutinin consists of two A chains and two B chains and is immunologically indistinguishable from abrin. Despite these similarities, the rosary pea agglutinin is considerably less toxic than abrin, and extensive research has been conducted examining the basis for this difference *(1, 2, 18, 23).* Toxicity is not correlated with the glycosidase activity of the A chain. Chemically reduced (activated) castor bean agglutinin has a 50% inhibitory concentration (IC_{50}) of 0.05 nM for the inhibition of protein synthesis by rabbit reticulocyte lysate, and reduced abrin and reduced ricin have IC_{50} values of 0.5 and 0.1 nM, respectively. The native protein, with the disulfide bond between the subunits, is virtually inactive; castor bean agglutinin, abrin, and ricin have IC_{50} values of >100, 88, and 84 nM, respectively *(2).*

Despite the toxicity of abrin, the ubiquitous presence of rosary peas, and a lengthy history dating back to the 19th century, very little has been published on in vitro methods for the detection of abrin, other than functional assays measuring inhibition of protein synthesis or deadenylase activity *(15).* Recently, detection methods based on liquid chromatography–electrospray ionization with tandem mass spectrometry (LC-ESI MS-MS) identification of tryptic peptides *(22),* toxin complexation with aptamers *(25),* and human monoclonal antibodies *(26)* were published. The LC-ESI MS-MS method allowed detection of 1 pmol (equivalent to 64 ng of protein) per injection of the domi-

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nant peptides derived from abrin and ricin. The aptamer assay, which employed a ruthenium tag that was intercalated into the aptamer, displayed a limit of detection (LOD) of 1 nM, equivalent to 64 ng/ml. The binding of toxin to human monoclonal antibodies, as assayed with surface plasmon resonance (Biacore, Piscataway, N.J.), displayed an LOD of 35 ng/ml with the human monoclonal antibody Fab E12.

An enzyme-linked immunosorbent assay (ELISA) and an electrochemiluminescence (ECL) assay were developed using rabbit polyclonal and mouse monoclonal antibodies against abrin. The ELISA and ECL assay had LODs of 0.1 to 0.5 ng/ml for abrin isozymes in buffer. Food samples analyzed after a 5-fold dilution had LODs of 0.1 to 0.5 ng/ml in the ECL assay, whereas the ELISA LODs were approximately 10-fold higher. The LODs for both the ELISA and ECL assay were lower than those published for other assays and lower than the level associated with health risks.

MATERIALS AND METHODS

Abrin C *(13)* and phosphate-buffered saline (PBS; catalog no. P3813) were obtained from Sigma Chemical Company (St. Louis, Mo.). Abrin and the agglutinin from *A. precatorius* were also prepared under contract to the U.S. Food and Drug Administration (FDA) according to the method of Hedge et al. *(11).*

Rabbit polyclonal and mouse monoclonal antibodies against abrin were prepared under contract to the FDA at Tetracore, Inc. (Rockville, Md.). Biotinylated antibodies were prepared using biotin-X 2,4-dinitrophenyl-X-L-lysine, succinimidyl ester (SE catalog no. B-2604, Molecular Probes, Invitrogen, Eugene, Oreg.) according to the manufacturer's recommendations. Goat anti-mouse immunoglobulin (Ig) G–horseradish peroxidase (HRP) and streptavidin-HRP were purchased from Accurate Chemical and Scientific Corp. (Westbury, N.Y.). HRP substrate, 2,2′-azino-di(3-ethylbenzthiazoline-6-sulfonate), was purchased from KPL, Inc. (Gaithersburg, Md.) in a two-component format (ABTS 2-Component). Food products were purchased locally. Food samples spiked with abrin were allowed to stand for a minimum of 15 min for absorption of the toxin into the sample and then diluted fivefold with 200 mM sodium phosphate, pH 6.8 (NaPi) prior to analysis.

ELISAs. Sandwich ELISAs consisting of rabbit polyclonal capture and monoclonal detector antibodies (poly-mono) and rabbit polyclonal capture and biotinylated rabbit polyclonal detector antibodies (poly-poly) were developed. Optimum performance of the poly-mono ELISA entailed the use of 20 μg/ml polyclonal capture antibody to coat the wells, 2.5 μg/ml monoclonal antibody to detect the presence of captured abrin, and 80 ng/ml goat anti-mouse IgG-HRP conjugate. The poly-poly ELISA gave optimum results with the 2.5 μg/ml capture antibody, 10 μg/ml biotinylated rabbit polyclonal detector antibody, and 200 ng/ml streptavidin-HRP. In both assays, the plates were coated with capture antibody for 16 h at 4°C and then blocked with PBS, 0.1% Tween 2, and 5% nonfat skim milk at 37°C for 1 h. Sample, detector, and conjugate incubations were conducted at 37°C for 1 h. The plates were read at 405 nm after incubation with 100 μl of HRP substrate for 30 min at room temperature.

ECL assays. All ECL assays entailed coating Sector PR100 Streptavidin Multi-Array plates with 2.5 g/ml biotinylated rabbit polyclonal capture antibody for 16 h at 22°C. Sequential sample and 5-μg/ml ruthenium-labeled monoclonal detector antibody incubation steps were carried out for 1 h at 22°C, with shaking for the initial 27 min. A simultaneous incubation procedure for sample with detector antibody entailed diluting the sample 2.5-fold with NaPi and then mixing 1:1 with the detector antibody for a net 5-fold dilution, comparable to the sequential procedure. ECL measurements were made using the MSD-T read buffer and the 96-well format, Sector PR100 ECL plate reader (Meso Scale Discovery, Gaithersburg, Md.), with a read time of approximately 2 min per plate.

Determination of LODs. LODs were determined using the lowest concentration of analyte that generated a response greater than the background plus four times the standard deviation.

RESULTS AND DISCUSSION

Antibody specificities and ELISA cross-reactivities. Antibody specificities and cross-reactivities of the poly-mono and poly-poly ELISAs with 40 plant products at concentrations of 2% (20 mg/ml), 0.2% (2 mg/ml), and 0.02% (0.2 mg/ml) (wt/vol) were examined (Table 1). The results are presented as the equivalent amount of abrin necessary to generate a comparable response above background. The poly-mono ELISA produced a positive response with 0.02% celiac (nongluten) flour, equivalent to an abrin concentration of 3 ng/ml, only slightly above the cutoff (Table 1). The lack of positive response with samples containing more celiac flour (0.2 and 2%) indicates that the slight positive response with 0.02% celiac flour was probably not due to cross-reactivity.

In contrast, the poly-poly ELISA produced slight positive responses with several plant products, although only five products (cocoa, corn, boiled green beans, hazelnuts, and walnuts) had positive responses equivalent to >4 ng/ml. The positive responses observed with hazelnuts and boiled green beans probably did not indicate cross reactivity. The responses observed with hazelnuts were not correlated with concentration (7, <2, and 3 ng/ml for 0.02, 0.2, and 2%, respectively), and the single positive response observed with 2% boiled green beans was not confirmed with raw green beans. In contrast, the responses with cocoa, corn, and walnuts were positively correlated with the 0.02, 0.2, and 2% sample concentrations, with 6, 8, and 9 ng/ml, 2, 3, and 8 ng/ml, and <2, 2, and 6 ng/ml, respectively. The responses with cocoa, corn, and walnuts were characteristic of saturation at approximately 9 ng/ml abrin, probably reflecting the saturation of the poly-poly ELISA observed at 10 ng/ml (see Fig. 1B).

ELISAs. Figure 1 illustrates the results obtained using the poly-mono and poly-poly ELISA to detect abrin C, abrin fraction I, abrin fraction II, abrin fraction III, and agglutinin in buffer. The data plotted are the responses generated after subtracting the background responses, which averaged 0.060 ± 0.008 ng/ml ($n = 12$) and 0.127 ± 0.017 ng/ml ($n = 10$) for the poly-mono and poly-poly ELISAs, respectively. The LODs for the abrin isozymes ranged from 0.1 to 0.5 ng/ml, with fraction II ≤ abrin C < fraction III < fraction I. The agglutinin had LODs of 20 and 2 ng/ml with the poly-mono and poly-poly ELISAs, respectively.

Responses characteristic of saturation were observed using the poly-mono ELISA at abrin concentrations of 100

TABLE 1. *ELISA cross-reactivities with three concentrations (2, 0.20, and 0.02%, wt/vol) of plant products*

Plant product	ELISA reactivities (ng/ml)[a]					
	Poly-mono			Poly-poly		
	2%	0.20%	0.02%	2%	0.20%	0.02%
Adzuki beans	<2[b]	<2	<2	<2	2	2
Almond nuts	<2	<2	<2	<2	<2	<2
Amaranth (flour)	<2	<2	<2	<2	<2	<2
Arrowroot	<2	<2	<2	2	<2	<2
Barley	<2	<2	<2	<2	<2	<2
Black-eyed peas	<2	<2	<2	4	2	2
Brazil nuts	<2	<2	<2	<2	<2	<2
Buckwheat (flour)	<2	<2	<2	4	2	<2
Cashew nuts	<2	<2	<2	<2	<2	<2
Chestnuts	<2	<2	<2	<2	<2	<2
Cocoa	<2	<2	<2	9	8	6
Corn (flour)	<2	<2	<2	8	3	2
Celiac (flour)	<2	<2	3	2	2	3
Gluten-free cereal	<2	<2	<2	2	<2	<2
Green beans, raw	<2	<2	<2	<2	<2	<2
Green beans, boiled	<2	<2	<2	7	<2	<2
Green peas	<2	<2	<2	<2	<2	<2
Hazelnuts	<2	<2	<2	3	<2	7
Kamut	<2	<2	<2	<2	<2	<2
Lentils	<2	<2	<2	<2	<2	<2
Lima beans	<2	<2	<2	<2	<2	<2
Macadamia nuts	<2	<2	<2	<2	<2	<2
Millet (flour)	<2	<2	<2	<2	<2	<2
Mung beans	<2	<2	<2	<2	<2	<2
Oat	<2	<2	<2	<2	<2	<2
Peanuts	<2	<2	<2	<2	<2	<2
Pecan	<2	<2	<2	<2	<2	<2
Pinto beans	<2	<2	<2	<2	<2	<2
Pistachio nuts	<2	<2	<2	<2	<2	<2
Poppy seeds	<2	<2	<2	<2	<2	<2
Pumpkin seeds	<2	<2	<2	<2	2	<2
Rice (flour)	<2	<2	<2	<2	<2	<2
Rye (flour)	<2	<2	<2	2	<2	<2
Sesame seeds	<2	<2	<2	2	<2	2
Soy (flour)	<2	<2	<2	<2	<2	<2
Spelt (flour)	<2	<2	<2	<2	<2	<2
Sunflower seeds	<2	<2	<2	4	2	<2
Teff	<2	<2	<2	<2	<2	<2
Walnuts	<2	<2	<2	6	2	<2
Wheat, whole (flour)	<2	<2	<2	<2	<2	<2

[a] Response after subtracting background abrin response based on standard curves. Poly-mono ELISA negative control antibody (NCA) ranged from 0.06 to 0.1 optical density units (<3 ng/ml). Poly-poly ELISA NCA typically was equivalent to <4 ng/ml; one sample was equivalent to 6 ng/ml.

[b] <2, response was less than the lowest abrin standard of 2 ng/ml.

to 500 ng/ml, whereas the poly-poly ELISA underwent saturation at approximately 10 ng/ml depending on the abrin isozyme. The saturation of the poly-poly ELISA at lower abrin concentrations than those needed to saturate the poly-mono ELISA probably reflected the greater avidity of the polyclonal detector antibody for abrin. The dynamic range and utility of the ELISAs are dependent on LODs, the concentration of abrin that saturates the assay, and the background responses and their associated standard deviations. The wider dynamic range of the poly-mono ELISA makes it potentially useful for quantitative determinations, and the narrow dynamic range and greater avidity of the poly-poly ELISA might provide a better qualitative assay.

The detection of abrin fraction II in food using the poly-mono and poly-poly ELISAs is presented in Figure 2. The average background absorbances for PBS, vegetable juice, orange juice, soy-based infant formula, salsa, whole milk, Caesar salad dressing, hazelnut-flavored chocolate milk, and cola-flavored soda, when analyzed using the poly-mono and poly-poly ELISAs (which entailed fivefold dilution with NaPi), were comparable at 0.055 ± 0.005 ng/ml ($n = 14$) and 0.052 ± 0.003 ng/ml ($n = 14$), respectively. A slightly greater background that averaged 0.08 ± 0.01 ng/ml was consistently observed with chipotle mustard. Of the foods examined, the highest LOD for abrin was that for the cola-flavored soda, at 10 ng/ml for both assays, whereas the LOD for abrin in the other foods ranged from 0.5 to 2 ng/ml. Although the foods reduced the sensitivity of the ELISAs, the increases in LODs did not effect the utility of the assays to detect the toxin at levels less than those that would pose a health threat.

ECL assays. Figure 3 depicts the detection of the various abrin isozymes and agglutinin in NaPi using ECL assays based on a poly-mono configuration with the samples incubated simultaneously with the detector antibody. With a measured background of 143 ± 27 counts ($n = 12$), the LODs for the four abrin isozymes ranged from 0.1 to 0.5 ng/ml, with abrin fraction II = abrin C < abrin fraction III = abrin fraction I. The LOD for agglutinin was approximately 10 ng/ml. Though the ECL assay had LODs comparable to those of the poly-mono ELISA, the dynamic range was considerably greater, as indicated by only a slight change in the slope of the titration curve at 500 ng/ml abrin (see Figs. 1A and 3). The greater dynamic range of the ECL assay probably reflected the advantage associated with photon counting versus the limitations associated with measuring absorbance. Though the optics of the plate reader used for the ELISAs were excellent, a plate reader with a more sensitive detector might display a larger dynamic range. In the ECL assay, if the detector antibody were added after the sample, with a wash step between, the dynamic range may have been greater. The simultaneous incubation of large quantities of analyte with detector antibody results in a hook effect on the titration curve, which is more dramatic than the saturation profile obtained when a sequential protocol is employed.

Although both the ECL assay and the ELISAs employed poly-mono configurations, it was impossible to compare the effectiveness of the two platforms on a per capture antibody or capture antibody-antigen complex basis. The two platforms required the use of different types of plates with different absorption properties, and it was not possible to either coat or determine the amount of capture antibody immobilized on the surface of the wells.

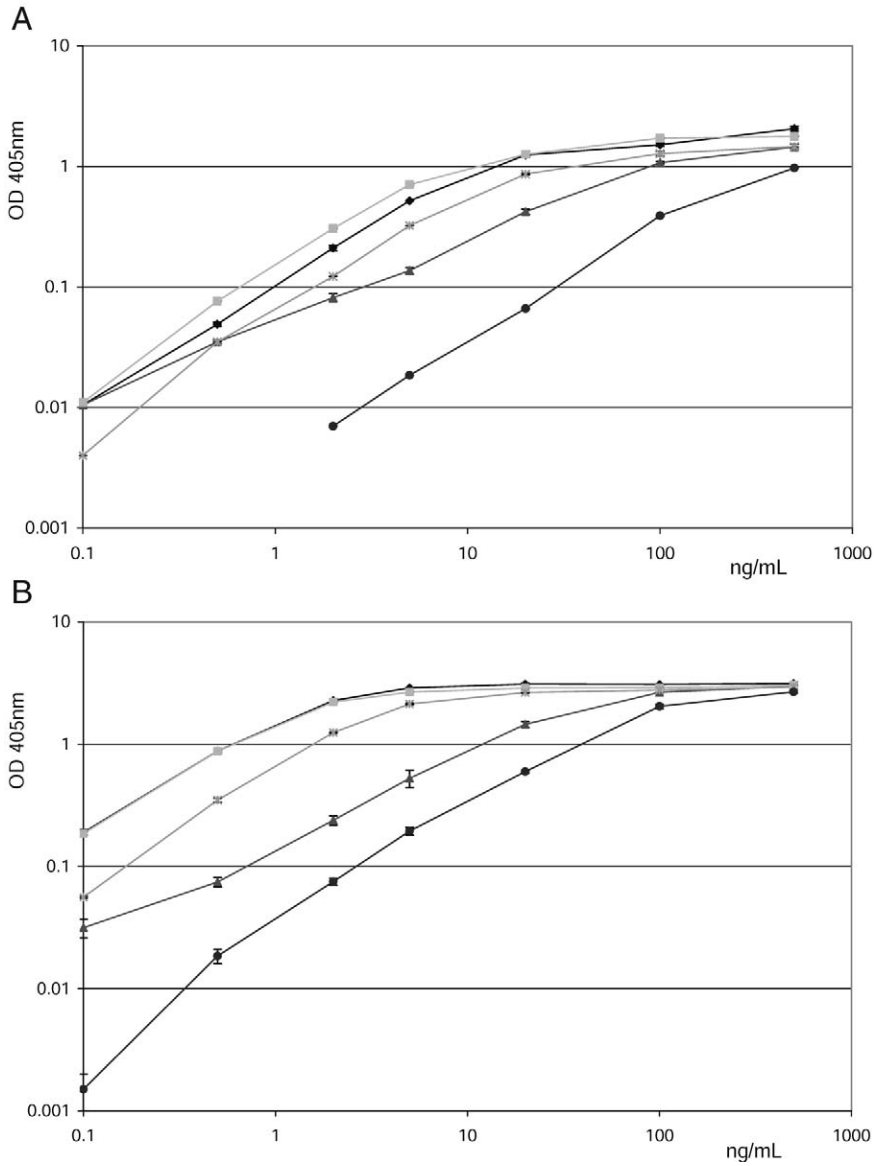

FIGURE 1. *Detection of abrin isozymes and agglutinin using (A) polyclonal capture–monoclonal detection and (B) polyclonal capture–polyclonal detection ELISAs.* ◆*, Abrin C;* ▲*, abrin fraction I;* ■*, abrin fraction II; *, abrin fraction III;* ●*, agglutinin from Abrus precatorius in 200 mM sodium phosphate, pH 6.8. The responses plotted are the average of duplicate analyses after subtracting background: 0.060 ± 0.008 ng/ml (*n *= 12) for the poly-mono and 0.127 ± 0.017 ng/ml (*n *= 10) for the poly-poly ELISAs. The error bars represent the range for duplicate analyses.*

ECL assay detection of abrin in food. The utility of the ECL assay to detect abrin spiked into food is presented in Figure 4. The detection of abrin in vegetable juice, orange juice, soy-based infant formula, salsa, chipotle mustard, Caesar salad dressing, hazelnut-flavored chocolate milk, and cola-flavored soda using a sequential procedure similar to that used in the poly-mono ELISA is presented in Figure 4A. Figure 4B depicts the results obtained when the same food samples were analyzed using a protocol in which the sample and detector antibody were incubated together. In both configurations, the food was diluted fivefold. Whether the samples were analyzed using the sequential or simultaneous protocol had only a slight effect on the average ECL background of the food, 209 ± 42 counts (n = 16) versus 239 ± 71 counts (n = 16), respectively. In contrast, a significant difference was noticed between the ECL assay results for salsa, chipotle mustard, vegetable juice, and cola-flavored soda versus those for dairy products and orange juice at 267 ± 39 counts (n = 15) versus 184 ± 43 counts (n = 16), respectively. The basis of the difference in results for salsa, vegetable juice, chipotle mustard, and cola-flavored soda (group 1) versus dairy products and orange juice (group 2) is not clear. The 2.5-fold dilution of the samples with NaPi prior to analysis eliminated any differences in pH. The presence of lycopenes from processed tomatoes and pepper does not explain the elevated ECL counts observed with cola-flavored soda.

Significant differences also were observed between the two groups when the background responses were compared based on whether the sample and detector antibodies were incubated simultaneously or sequentially, separated by a wash step. The sequential protocol displayed background responses of 245 ± 24 counts (n = 8) and 174 ± 15 counts (n = 8) for the two groups of food items, and the simultaneous protocol yielded backgrounds of 292 ± 39 counts (n = 7) and 193 ± 60 counts (n = 8), respectively. As a result of this difference, the LOD for the sequential ECL protocol was 0.1 ng/ml for all of the food items examined, and the LOD using a simultaneous protocol was 0.5 ng/ml for all foods except vegetable juice, for which the LOD was 0.1 ng/ml.

Owing in part to the large ECL signal at abrin concen-

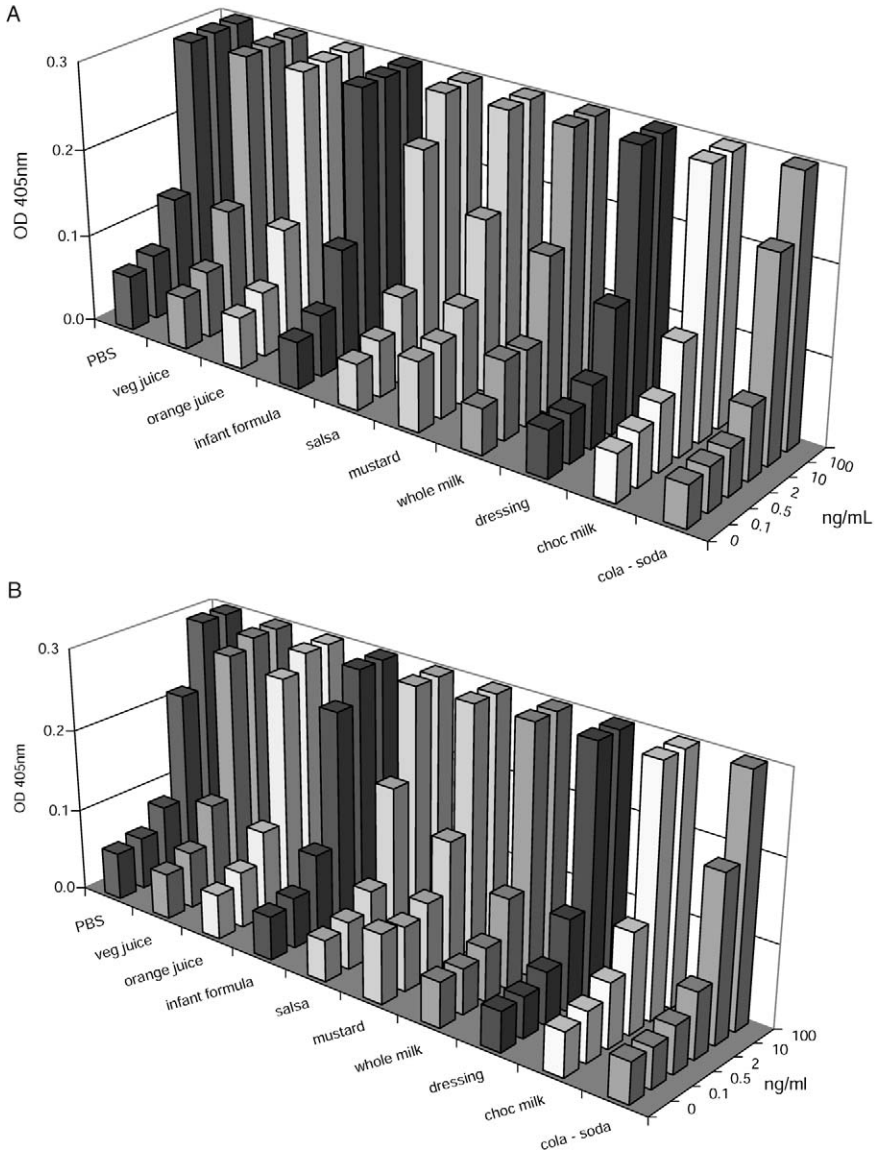

FIGURE 2. *ELISA detection of abrin fraction II spiked into PBS, vegetable juice, orange juice, soy-based infant formula, salsa, chipotle mustard, whole milk, Caesar salad dressing, hazelnut-flavored chocolate milk, and cola-flavored soda at concentrations of 0, 0.1, 0.5, 2, 10, and 100 ng/ml. The spiked samples were analyzed using (A) polyclonal capture–monoclonal detection and (B) polyclonal capture–polyclonal detection ELISAs. The data plotted represent the absorbances measured at 405 nm not corrected for background. The ordinate was truncated at 0.3 absorbance units to provide better scaling and comparison of the samples at lower concentrations of abrin.*

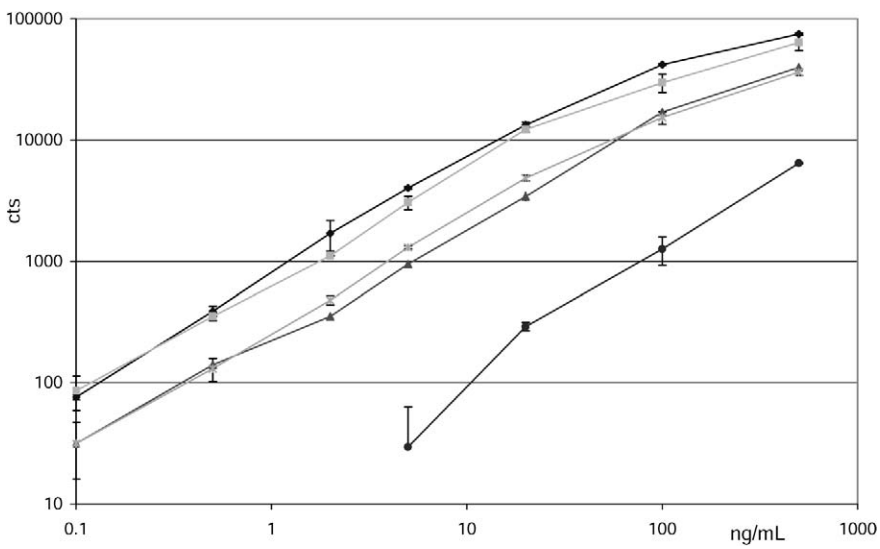

FIGURE 3. *ECL assay detection of abrin isozymes and agglutinin. ♦, Abrin C; ▲, abrin fraction I; ■, abrin fraction II; *, abrin fraction III; ●, agglutinin from* Abrus precatorius *in 200 mM sodium phosphate, pH 6.8. The data were generated using a protocol in which the samples were incubated simultaneously with the detector antibody. The responses plotted are the average of duplicate analyses after subtracting the background: 143 ± 27 counts (*n *= 12). The error bars represent the range for duplicate analyses.*

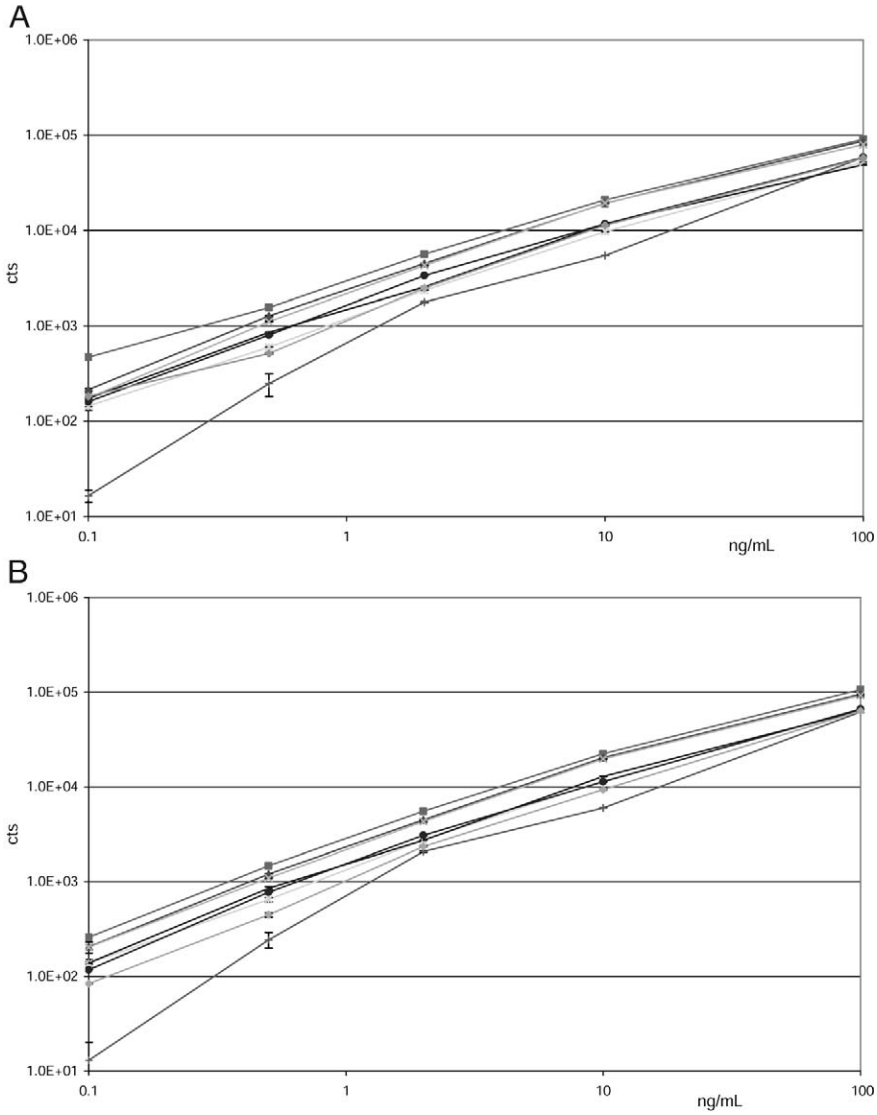

FIGURE 4. *ECL assay detection of abrin fraction II spiked into vegetable juice (×), orange juice (▲), soy-based infant formula (■), salsa (■), chipotle mustard (◆), Caesar salad dressing (|), hazelnut-flavored chocolate milk (*), and cola-flavored soda (◆) at concentrations of 0, 0.1, 0.5, 2, 10, and 100 ng/ml. The samples were analyzed using either (A) a sequential protocol in which the sample and detector antibodies were incubated separately for 1 h or (B) a simultaneous protocol in which the sample and detector antibody were incubated together. All analyses entailed coating the wells of avidin MA100 PR Plates (Meso Scale Discovery) with 2.5 μg/ml biotinylated rabbit polyclonal capture antibodies and detecting the presence of captured abrin with 5 μg/ml monoclonal antibody. The data plotted represent the ECL counts generated in the presence of MSD-T buffer. The responses plotted are the average of duplicate analyses after subtracting background. The error bars represent the range for duplicate analyses.*

TABLE 2. *Comparison of simultaneous versus sequential ECL detection of abrin in food products*

Food product	% response[a]	*n*
Vegetable juice	104 ± 8	8
Orange juice	102 ± 8	8
Infant formula	105 ± 10	4
Salsa	112 ± 13	8
Chipotle mustard	94 ± 12	8
Caesar salad dressing	109 ± 14	8
Chocolate milk	117 ± 6	8
Cola-flavored soda	100 ± 9	8

[a] ECL responses (minus background) generated by the simultaneous incubation of sample with detector antibody expressed as the percentage of the ECL counts signal generated when the detector antibody was added after the sample had been incubated. Abrin concentration ranged from 0.5 to 100 ng/ml. The responses generated with 0.1 ng/ml (typically between 20 and 250 counts above background) were not included in the average because of the high variance resulting from the low signal-to-noise ratio.

trations exceeding the LOD, the differences between the two groups of foods and whether the detector antibody was incubated with the sample simultaneously or sequentially were inconsequential (Table 2 and Fig. 4). In previous studies with ricin *(8),* increasing concentrations of analyte resulted in a loss of ECL signal when the sample and detector antibodies were incubated simultaneously, referred to as a hook effect, whereas the sequential incubation of sample and detector leveled off at a constant ECL signal (saturation). Onset of a hook effect or saturation are dependent on the affinity of the antibodies with the analyte and the concentration of the analyte. The large signal-to-noise of the ECL assay prevented the hook effect from resulting in an ECL signal comparable to background with ricin. Because the data presented in this study indicate a dynamic range of concentrations greater than that for the ricin assay, it is unlikely that this assay will generate false-negative results.

Rabbit polyclonal and mouse monoclonal antibodies were raised against abrin and used to develop diagnostic ELISAs and ECL assays. The specificity of the antibodies was demonstrated with 40 plant products. Both types of assays were capable of detecting abrin in food. LODs for food with the ELISAs ranged from 0.5 to 10 ng/ml abrin

in the analytical sample and that for the ECL assays ranged from 0.1 to 0.5 ng/ml. The ELISAs and ECL assays were able to detect abrin in food at concentrations that were orders of magnitude lower than those that would pose a health concern.

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