SPECIAL GUEST EDITOR SECTION

Competitive Inhibition ELISA for Quantification of Ara h 1 and Ara h 2, the Major Allergens of Peanuts

DAVID A. SCHMITT, HSIAOPO CHENG, and SOHEILA J. MALEKI[1]
U.S. Department of Agriculture, Agricultural Research Service, Southern Regional Research Center, 1100 Robert E. Lee Blvd, New Orleans, LA 70124
A. WESLEY BURKS
Duke University Department of Pediatrics, Allergy and Immunology Division, Durham, NC 27710

Allergies to peanuts are becoming an increasingly important health problem as a result of the persistence and severity of the reaction in allergic individuals. Because no treatment currently is available, avoidance is the only option for peanut-allergic individuals. Avoidance of an abundant and often disguised food such as peanuts, however, is very difficult; therefore, competitive inhibition ELISAs were developed to detect and quantitate each of the major peanut allergens, Ara h 1 and Ara h 2. Under optimal conditions for each assay, the sensitivity of the Ara h 1 and Ara h 2 detection assays were 12 and 0.5 ng/mL, respectively. These assays were primarily devised to effectively compare the levels of Ara h 1 and Ara h 2 in a wide variety of peanuts or peanut products but can also be used to identify cross-reactive antigens. The method is simple and rapid, requiring only one allergen-specific antibody and, therefore, could be adapted specifically to detect the presence of these individual allergens in different foods.

Hypersensitivity to peanuts is an increasingly serious medical problem for 2 main reasons. First, peanut allergies are more likely to persist into adulthood, with approximately 1.3% of adults in the United States demonstrating a reaction to peanuts and/or tree nuts (1). Second, peanuts or tree nuts account for 80% of all food-induced anaphylactic events observed in emergency departments in the United States each year (2). To date, the only absolute way to prevent the allergic reactions or anaphylaxis associated with peanuts is avoidance. However, this is extremely difficult at times because peanuts or peanut products are pervasive ingredients in many foods, and labeling may not be complete (3). Therefore, therapeutic interventions and/or hypoallergenic peanuts are needed to prevent anaphylactic reactions caused by accidental ingestion of peanut-containing products by allergic individuals.

Several studies have demonstrated that the protein fraction of the cotyledon is the allergenic portion of the peanut (4, 5). Two of the allergens, Ara h 1 and Ara h 2, are classified as major peanut allergens because each is bound by serum IgE from 95% peanut-allergic individuals (6, 7). Therefore, the majority of peanut allergic individuals recognize both Ara h 1 and Ara h 2. Ara h 1 is a 62-kilodalton (kDa) protein that has significant homology with the vicilin seed storage protein family (8). Ara h 2 is a 17–20 kDa protein belonging to the conglutin family of proteins (9, 10). Other peanut allergens that have been characterized, such as Ara h 3/4, Ara h 5, and Ara h 6, have been described as minor allergens because they are recognized by serum IgE from 50% or less of the peanut- allergic individuals.

In our search for a peanut with reduced levels of allergens, we analyzed and developed multiple immunoassays. This paper describes the development of a competitive inhibition enzyme-linked immunosorbent assay (ELISA) method for the detection of the 2 major peanut allergens. In these assays, anti-Ara h 1 or anti-Ara h 2 antibodies are incubated with a known amount of the allergen (standards) or test samples (unknown). Any Ara h 1 or Ara h 2 present in the standard or samples will bind to the antibodies. When this mixture is added to plate-bound Ara h 1 or Ara h 2, the antibody that is not bound by free Ara h 1 or Ara h 2 in the sample (the standard or unknown) will bind to the allergen on the plate. Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG is then added, and addition of 3,3 , 5,5 -tetramethylbenzidene (TMB) causes a color change which is measured on a microplate reader. A high signal means that there was little allergen in the sample to inhibit binding of the antibody to the plate-bound allergen. Conversely, a low signal indicates that a high level of an allergen was present in the test sample that blocked the antibody from binding to the plate-bound allergen. The level of an allergen in the sample can be directly quantitated from a standard curve constructed by using crude peanut extract (CPE) with predetermined levels of allergens.

Guest edited as a special report on "Methodological Aspects of Food Allergens" by Kristina Williams.

[1] Author to whom correspondence should be addressed; e-mail: sjmaleki@srrc.ars.usda.gov.

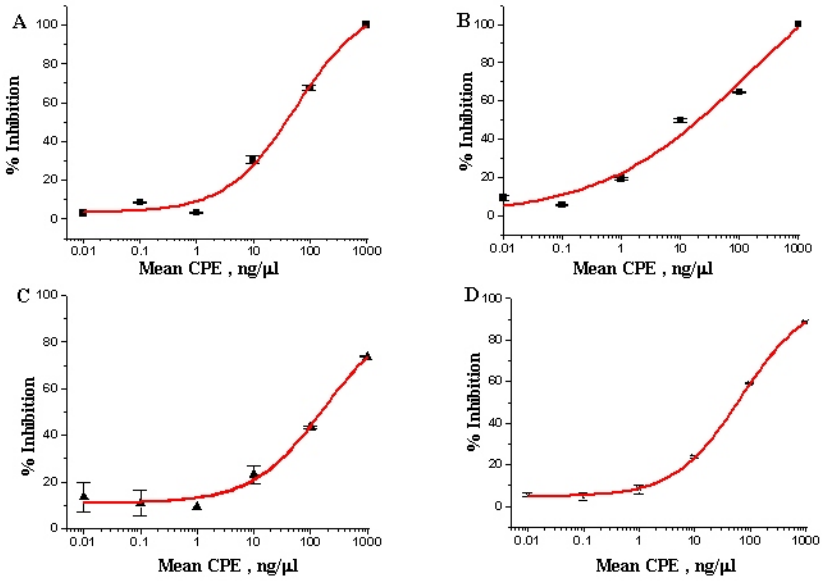

Figure 1. Optimization of Ara h 1 competitive inhibition ELISA. ELISAs were optimized for various anti-Ara h 1 antibody and HRP-conjugated anti-rabbit antibody concentrations. The x-axis represents CPE concentration and the y-axis represents % inhibition induced by each CPE concentration. (A) Ara h 1 = 25 ng, anti-Ara h 1 = 1:5000, and HRP-conjugated anti-rabbit IgG = 1:10 000; (B) Ara h 1 = 25 ng, anti-Ara h 1 antibody = 1:10 000, and HRP-conjugated anti-rabbit IgG = 1:10 000; (C) Ara h 1 = 25 ng, anti-Ara h 1 antibody = 1:5000, and HRP-conjugated anti-rabbit IgG = 1:15 000; (D) Ara h 1 = 25 ng, anti-Ara h 1 antibody = 1:10 000, and HRP-conjugated anti-rabbit IgG = 1:15 000.

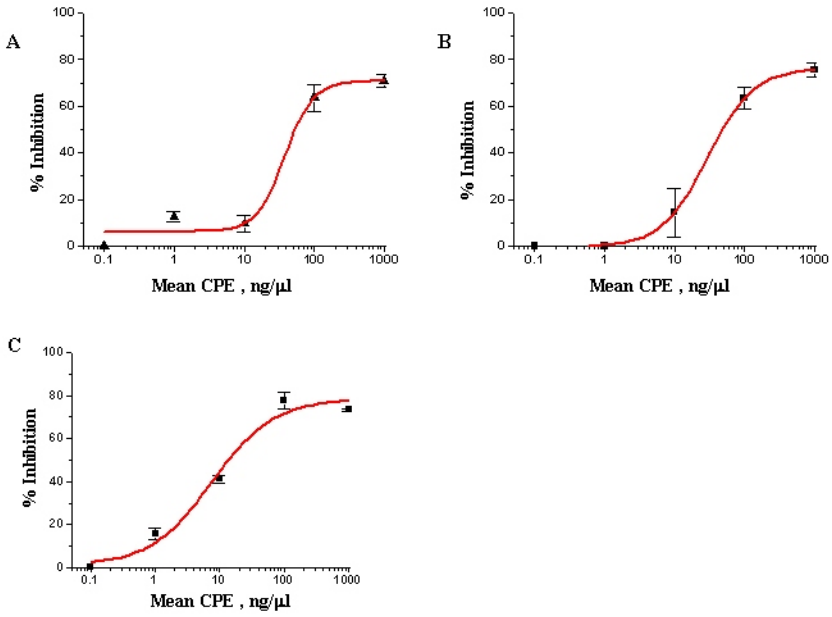

Figure 2. Optimization of Ara h 2 competitive inhibition ELISA. ELISAs were optimized by using various concentrations of anti-Ara h 2 antibody and a detection antibody. The x-axis represents CPE concentration and the y-axis represents % inhibition induced by each CPE concentration. (A) Ara h 2 = 25 ng, anti-Ara h 2 antibody = 1:5000, and HRP-conjugated anti-rabbit IgG = 1:10 000; (B) Ara h 2 = 25 ng, anti-Ara h 2 antibody = 1:10 000, and HRP-conjugated anti-rabbit IgG = 1:10 000; (C) Ara h 2 = 25 ng, anti-Ara h 2 antibody = 1:20 000, and HRP-conjugated anti-rabbit IgG = 1:10 000.

METHODS

Materials Required for the Assay

CPE, which was used to construct a standard curve in this assay, was prepared by solubilizing 50 mg partially defatted powder from the raw Florunner variety of peanut into 2 mL phosphate-buffered saline (PBS) using sonication. This variety of peanut has been used as the standard in several laboratories, including ours. The mixture was then centrifuged at 10 000 rpm to separate the soluble and insoluble fractions. The total protein of the soluble fraction was measured by the bicinchoninic acid (BCA) assay (Pierce, Rockford, IL) and was determined to be 2.5 mg/mL. The amounts of Ara h 1 and Ara h 2 in the CPE were predetermined by a similar assay (described below in the protocols for competitive ELISA) with purified Ara h 1 and Ara h 2 as standards and CPE as the unknown. Purified Ara h 1 and Ara h 2 were isolated from CPE as previously described (11, 12).

The polyclonal rabbit anti-Ara h 1 and the rabbit anit-Ara h 2 antibodies used for these protocols were custom-manufactured by Sigma Immunosys (The Woodlands, TX). Rabbits were immunizied with purified Ara h 1 and Ara h 2 to induce antibody production, and then bled to obtain serum antibodies against Ara h 1 and Ara h 2. HRP-conjugated goat anti-rabbit IgG was purchased from Sigma Chemical Co. (St. Louis, MO). The SureBlue^{TM} 3,3 , 5,5 -tetramethylbenzidine (TMB) microwell peroxidase 1 component substrate used in these assays was purchased from KPL (Gaithersburg, MD) and used according to manufacturer's instructions.

The ELISA plates used in this assay were Immulon 4HBX (VWR Scientific, West Chester, PA). The results of the ELISA were read at 450 nm using a Sunrise microplate reader (Tecan, Boston, MA). The plate washer used was the EL404 Microplate Washer (Bio-Tek Instruments, Winooski, VT). For coating the ELISA plates, purified Ara h 1 or Ara h 2 was diluted in 0.1M sodium bicarbonate, pH 9.6. The wash buffer used in the automated plate washer was PBS: 140mM NaCl, 2.7mM KCl, 3mM Na_2HPO_4, and 1.5mM KH_2PO_4 containing 0.05% Tween-20 (PBST). The competitor samples and the antibodies were diluted in PBS.

Protocol for Ara h 1 or Ara h 2 Competitive Inhibition ELISA: Coating and Blocking Steps

Purified Ara h 1 and Ara h 2 are diluted to 0.5 or 1 ng/ L in 0.1M $NaHCO_3$, pH 9.6, respectively. To every well of the 96-well ELISA plates, except the 3 used for noncoated control, 50 L of either Ara h 1 or Ara h 2 dilutions is added. The plates are then incubated for 30 min to 1 h at 37 C. After the incubation is complete, the plates undergo 3 washes with PBST on an automated plate washer. To block nonspecific binding sites, 200 L 3% bovine serum albumin (BSA) is added to every well on the ELISA plates and incubated overnight at 4 C, 2 h at room temperature, or 1 h at 37 C.

Protocol for Aha h 1 or Aha h 2 Competitive ELISA: Construction of Standard Curve

In these assays, CPE with known amounts of Ara h 1 and Ara h 2 is used to construct a standard curve. The amount of Ara h 1 or Ara h 2 in the CPE is determined by using purified Ara h 1 or Ara h 2 as the standard and determining the concentration of CPE required to induce equivalent levels of inhibition. In the anti-Ara h 1 competitive ELISA, CPE is diluted to 2000 ng/ L in PBS and 5-fold dilutions are made from this dilution (400, 80, 16, and 3.2 ng/ L). For the anti-Ara h 2 ELISA, CPE is diluted to 400 ng/ L and 5-fold dilutions are made from this concentration (80, 16, 3.2, and 0.64 ng/ L). A standard curve is constructed by plotting the concentration of the standard (CPE with predetermined levels of Ara h 1 and Ara h 2, as described above) versus the optical density (OD) at 450 nm or the percent inhibition of each concentration of the competitor as compared to the positive control. The positive control is defined by the amount of antibody that binds to plate-bound antigens in the absence of a competitor and results in a maximal signal. The % inhibition was calculated by using $1 - (A_2 - A_1/A_0)$ 100 = % inhibition for any given concentration of inhibitor. A_0 is the absorbance in the absence inhibitor (maximum absorbance); A_2 is the absorbance at any given concentration; A_1 is the average absorbance of control wells that do not contain any primary antibody (minimal signal). Because we used CPE as the standard, this graph represents the amount of allergen in CPE that can compete with the plate-bound allergen for binding of the antibody. Previous ELISA assays (as described above) demonstrated that Ara h 1 constitutes approximately 12% of the total protein present in our defatted CPE, whereas Ara h 2 constitutes approximately 1%. Therefore, in the Ara h 1 competitive ELISA, we multiplied the result obtained from the standard curve by 0.12 (12%) to obtain the estimated amount of Ara h 1 and by 0.01 (1%) to obtain the estimated amount of Ara h 2 in the standard curve for each of the unknown samples. The resulting data were fitted with a sigmoidal (power logistic) function and graphed by using Microcal Origin (Microcal Software, Inc., Northampton, MA) according to the equation described in detail in Czernik et al. (13).

Protocol for Ara h 1 and Ara h 2 Competitive ELISA: Incubation with Antibodies and Detection Step

Peanut samples with unknown levels of Ara h 1 and Ara h 2, which were previously normalized to 1 mg/mL total protein, are diluted to a concentration of 400 ng/ L. Equal volumes of either rabbit anti-Ara h 1 (1:5000 dilution) or anti-Ara h 2 (1:2500 dilution) are combined with either the CPE standards or the test samples and incubated for 30 min at 37 C to allow any Ara h 1 or Ara h 2 present in the sample to bind to the antibody. After the 30 min incubation, 50 L of each mixture is added in triplicate to the ELISA wells coated with either Ara h 1 or Ara h 2 (bound antigens) and incubated at 37 C for 1 h. After this incubation period is completed, the ELISA plates are washed 3 times on the automated plate washer. For both the anti-Ara h 1 and anti-Ara h 2 competitive

inhibition ELISA, a 1:10 000 dilution of the HRP-conjugated goat anti-rabbit IgG (Sigma) is made in PBS. Then, 50 L of this solution is added to every well on the ELISA plate and incubated at 37 C for 30 min to 1 h to allow the secondary antibody (HRP-conjugated goat anti-rabbit IgG) to bind to anti-Ara h 1 or anti-Ara h 2 antibodies attached to the plate-bound Ara h 1 or Ara h 2.

Upon completion of the incubation period, the ELISA plates are washed 3 times with PBST in the automated plate washer. Once the wash is complete, 100 L of the HRP substrate, TMB, is added to every well on the ELISA plate, according to manufacturer's instructions. The ELISA plates are allowed to incubate for 10–15 min or until a maximum color change in the positive control wells, with minimum color change in the blank wells, is seen. Finally, 100 L stop solution is added to every well on the ELISA plate and the absorbance is measured at 450 nm on a microplate reader. Using the standard curve, the amount of either Ara h 1 or Ara h 2 in each sample is determined.

SDS-PAGE and Western Blotting

In sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; Bio-Rad Laboratories, Hercules, CA), various amounts of crude extracts or purified allergens were loaded into polyacrylamide gels and the individual proteins separated according size. In some cases, the gels were stained with Gel-Code Blue staining solution (Pierce) in order to visualize the separated proteins. In other cases, the proteins were transferred to polyvinyl difluoride (PVDF) membrane and either anti-Ara h 1 or anti-Ara h 2 Western blots were performed. Briefly, the membrane was blocked by using 5% blotto (5% dry milk in PBST) for 1 h. Then, the membrane was placed in a sealable bag and either a 1:5000 dilution of anti-Ara h 1 antibody or a 1:3000 dilution of anti-Ara h 2 in 5% blotto was added (these were the same antibodies used in the ELISAs). The membrane was incubated for 1 h at room temperature and then removed from the bag and washed 3 times with PBST. A 1:100 000 dilution of HRP-conjugated goat anti-rabbit IgG was made, and the membrane was incubated in this mixture for 30 min. After washing 3 times in PBST and 2 more times in PBS, the membrane was exposed to ECLPlus Western blotting reagents (Amersham Bioscience Corp., Piscataway, NJ) for 5 min. The chemiluminescence was then measured on a FUJIFILM Luminescent Image Analyzer LAS-1000plus (Fuji Photo Film Co., Ltd., Duluth, GA).

Table 1. Determination of Ara h 1 and Ara h 2 levels in unknown samples using competitive inhibition ELISA

Sample No.	Total protein, ng	Ara h 1, ng	Ara h 2, ng
S1	10000	2395	10.3
S2	10000	911	11
S3	10000	358	13.6
S4	10000	223	9.3
S5	10000	23	15.7

Results and Discussion

Anti-Ara h 1 Competitive Inhibition ELISA

The search for an assay to quantify and compare the amount of allergens in a variety of peanut samples led us to develop a competitive inhibition ELISA for Ara h 1 and Ara h 2. In this assay, the amount of allergen present in a sample is determined by the inhibition of antibody binding to a plate-bound allergen by pre-incubating the sample (unknown) or standard (known) with an antibody against the allergen. A sample with no competitor present is also used as the positive control to determine the maximum amount of antibody binding to a plate-bound antigen. In this assay, a low signal indicates a higher amount of the allergen in the sample and vice versa. Figure 1 shows the optimization curves for 4 of the conditions tested for the anti-Ara h 1 competitive ELISA.

In this figure, the ELISA plates were coated with 25 ng Ara h 1. Then various concentrations of CPE were incubated with either a 1:5000 or a 1:10 000 dilution of anti-Ara h 1 antibody. After this incubation, either a 1:10 000 or a 1:15 000 dilution of HRP-conjugate anti-rabbit IgG was added. The data were plotted in a scattergram with % inhibition on the y-axis and the CPE concentration on the x-axis. The data were fit to a power logistic sigmoidal curve (13) using Microcal Origin software. Each concentration of the standard was performed in triplicate in each assay, and each assay was performed 3 times. The error bars in the graph represent the standard deviation (or average squared variance) for the 9 independent measurements of each point shown. The conditions chosen for future experiments were to coat each well of the microplate with 25 ng Ara h 1 protein, a primary antibody dilution of 1:10 000, and dilution of the HRP-conjugated anti-rabbit IgG to 1:10 000 (Figure 1B). These conditions were chosen because the curve was linear through most of the concentrations of CPE used in the standard curve, which meant that the assay was most sensitive

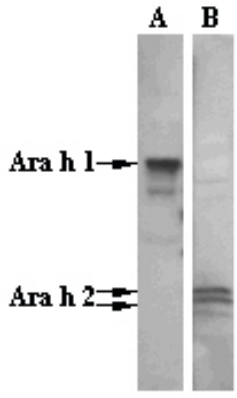

Figure 3. Specificity of anti-Ara h 1 and anti-Ara h 2 antibodies demonstrated by Western blot. Western blot analysis was performed on CPE using either (A) anti-Ara h 1 or (B) anti-Ara h 2 antibodies.

when these conditions were used. The concentration of CPE that inhibited antibody binding by 50% (IC_{50}) was 152 ng/ L, which was the lowest observed in any of the conditions tested. In addition, the sensitivity of the anti-Ara h 1 ELISA was determined to be 12 ng/mL.

Anti-Ara h 2 Competitive Inhibition ELISA

Optimization of the anti-Ara h 2 competitive inhibition ELISA is shown in Figure 2. In this figure, 3 optimization conditions are shown. Similar to Figure 1, the data here are plotted as % inhibition versus CPE concentration. Each concentration of the standard was performed in triplicate in each assay, and each assay was performed 3 times. The error bars in the graph represent the standard deviation (or average squared variance) for the 9 independent measurements of each point shown. The data are presented as a scattergram, and the data were fit to a power logistic sigmoidal curve (13) using Microcal Origin software. In one assay, the ELISA plates were coated with 25 ng Ara h 2 and the competitor was incubated with a 1:5000 dilution of anti-Ara h 2 antibody. Then, a 1:10 000 dilution of HRP-conjugated anti-rabbit IgG was used (Figure 2A). In the second assay, all of the conditions were the same except that a 1:10 000 dilution of anti-Ara h 2 was used (Figure 2B). The third assay used the exact same conditions except that a 1:20 000 dilution of anti-Ara h 2 antibody was used (Figure 2C). The optimal conditions selected for this assay were to coat each well of a microplate with 25 ng Ara h 2 protein, use a dilution of 1:5000 for the primary antibody (anti-Ara h 2 antibody), and a dilution of 1:10 000 for the HRP-conjugated anti-rabbit IgG antibody. These conditions were selected as the optimal conditions because the curve was linear at a lower CPE concentration than the other 2 conditions. In addition, the IC_{50} of this curve was lower than any of the other conditions (7.5 ng/ L). The sensitivity of this assay using these conditions was 0.5 ng/mL.

Determination of Ara h 1 and Ara h 2 Levels in Several Different Varieties of Peanuts

Table 1 presents data obtained from our study of Ara h 1 and Ara h 2 levels in several different varieties of peanuts. As observed in Table 1, the amounts of Ara h 1 and Ara h 2 present in different peanut varieties can fluctuate.

Western Blot Analysis

Western blot analysis was used to assess the specificity of the antibodies against Ara h 1 and Ara h 2. In Figure 3, the proteins in CPE were separated by SDS-PAGE (5 g/lane). The proteins were then transferred to PVDF membrane and probed with either anti-Ara h 1 antibody or anti-Ara h 2 antibody. As shown in Figure 3A, the blot probed with anti-Ara h 1 antibody demonstrates that the antibody only binds to Ara h 1 and not to Ara h 2. The opposite is observed when the blot is probed with anti-Ara h 2 antibody. This antibody only binds to Ara h 2 and not Ara h 1 (Figure 3B). The Western blots shown in Figure 3 demonstrate that the antibodies used in the competitive ELISA are specific.

The specificity anti-Ara h 2 antibody was further examined by testing its cross-reactivity to other proteins. In this case, proteins from crude extract of peanut, rice, soy, and casein as well as the purified peanut allergens Ara h 1 and Ara h 2 were separated by SDS-PAGE (15 g/lane for protein extracts and 2 g/lane for purified allergens). The gel was then stained with Gel-Code Blue staining solution to visualize the proteins (Figure 4A). A duplicate gel was transferred to PVDF membrane and an anti-Ara h 2 Western blot was performed (Figure 4B). The Western blot demonstrates the specificity of the antibody, showing that anti-Ara h 2 antibody bound only to the Ara h 2 in CPE (lane 1) and purified Ara h 2 (lane 3). A similar assay was used for determining the specificity of the anti-Ara h 1 antibody (data not shown).

Several immunoassays may be used to detect and quantitate peanut allergens from various sources. This paper describes the development of a competitive inhibition ELISA for the detection of Ara h 1 and Ara h 2. In the optimization assay, various concentrations of plate-bound allergen, CPE, anti-allergen antibody, and HRP-conjugated detection antibody were used in various combinations to determine the most sensitive assay conditions for detection of Ara h 1 and Ara h 2. For Ara h 1 detection, the optimum conditions led to the development of an ELISA with a sensitivity of 12 ng/mL, whereas the Ara h 2 ELISA developed here had a sensitivity of 0.5 ng/mL. The specificity of the polyclonal antibodies used in these assays was also determined with Western blot analysis, and no cross-reactivity was found.

The advantages of this type of ELISA are that it is a fairly rapid assay with relatively few steps, requires only one primary antibody, and can be adapted to many uses. This assay is the best

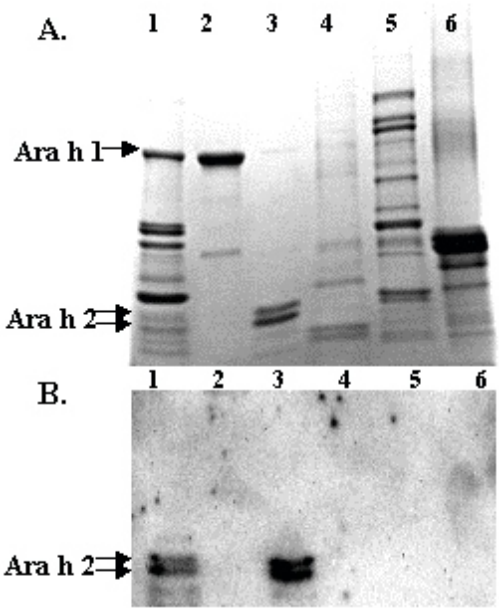

Figure 4. Specificity of the anti-Ara h 2 antibody. Proteins from crude extract (15 g/lane) of peanuts, rice, soy, and casein as well as the purified allergens Ara h 1 and Ara h 2 (2 g/lane) were separated by SDS-PAGE. (A) The SDS-PAGE was stained with Gel-Code Blue solution. (B) Western blot using anti-Ara h 2 antibody was performed. Lane 1 = CPE; Lane 2 = purified Ara h 1; Lane 3 = purified Ara h 2; Lane 4 = crude rice extract; Lane 5 = crude soy extract; Lane 6 = -casein.

method for comparative studies because differential plate binding of various unknown samples is not a factor that requires consideration. In fact, it is currently being used in our laboratory to compare levels of Ara h 1 and Ara h 2 in a large number of peanut samples and to examine the differences between differentially processed peanuts. An example of data obtained from this analysis is shown in Table 1. Competitive inhibition ELISA is also one of the best methods to analyze antigenic cross-reactivity. In contrast, one possible limitation is the sensitivity of this assay. Taylor and Nordlee (14) postulated that immunoassays for food contaminants must detect a minimum of 10 ppm. In our competitive inhibition ELISA, we detected specific peanut allergens to 12 and 0.5 ppm for Ara h 1 and Ara h 2, respectively. Commercially available assays for total peanut proteins have detection limits of 0.1 and 0.4 ppm (15, 16). Pomes et al. (17) described a sandwich ELISA developed for the detection of Ara h 1 in food products. The limit of detection in their assay was 30 ng/mL, with no cross-reactivity with proteins from other legumes. Although existing sandwich ELISA may be slightly more sensitive than our competitive inhibition ELISA in detecting total peanut proteins, it could be argued that the competitive ELISAs described here are detecting individual proteins and may therefore be more sensitive. Regardless, based on known sensitivity of commercialized ELISA kits (15, 16), we believe that the sensitivities of the 2 ELISAs are comparable.

One disadvantage of a competitive inhibition ELISA is that the matrix effect may impact the assay more than sandwich ELISA, which can lead to a decrease in sensitivity. This is especially true if the purified allergens, without a matrix, are used as the standards to detect allergens in an unknown sample within a matrix. In this case, the estimated amount of an individual allergen present in each sample (according to the standard curve) will be estimated to be higher than the amount of total protein originally added to each well, which is impossible. This is simply due to the fact that, without the presence of a mixture of components in a matrix, the antibody recognition of the antigen is unobstructed, and therefore the signal is amplified and estimates are elevated. When the level of a peanut/allergen in a certain matrix such as chocolate, peanut protein, or specific allergen can be combined with that matrix (also referred to as spiking) to provide a standard curve, the matrix effect should be minimized. However, the matrix effect is known to affect other types of ELISAs as well. In our case, where we are comparing the levels of allergens in different varieties of peanuts, it is currently not possible to obtain a peanut matrix without the allergens to use as a standard. For this reason, we chose to use CPE with predetermined amounts of Ara h 1 and Ara h 2 to construct our standard curve, rather than the purified allergens, to alleviate the problems with the matrix effect.

We have shown that competitive inhibition ELISA can be effectively used for the detection of Ara h 1 and Ara h 2. These assays are important because they allow rapid and sensitive detection of the 2 major allergens found in peanut, and may actually be more sensitive than assays that determine the presence of total peanut proteins. Because relatively few assays are available to measure the levels of individual allergens in food and avoidance is the only effective method to prevent the deleterious reactions to peanuts, it is important to develop rapid and reliable assays to detect allergen contamination in food. Although the assays described here were originally developed to detect and compare allergen levels in differentially processed peanuts and different peanut varieties, in the future they may be adapted to detect allergen levels or cross-reactive proteins in food products. This type of antigen-specific assay also may be useful in detecting cross-reactive proteins in genetically modified products. The availability of a variety of tests for the detection of trace amounts of food allergens may lead to the development of safer consumer products in the future.

References

(1) Sicherer, S.H., Munoz-Furlong, A., Burks, A.W., & Sampson, H.A. (1999) *J. Allergy Clin. Immunol.* **103**, 559–562

(2) Bock, S.A., Munoz-Furlong, A., & Sampson, H.A. (2001) *J. Allergy Clin. Immunol.* **107**, 191–193

(3) Wood, R.A. (2002) *J. Allergy Clin. Immunol*. **109**, 920–922

(4) Bush, R. K., Taylor, S.L., & Nordlee, J.A. (1989) *Allergy Proc*. **10**, 261–264

(5) Gillespie, D.N., Nakajima, S., & Gleich, G.J. (1976) *J. Allergy Clin. Immunol*. **57**, 302–309

(6) Stanley, J.S., King, N., Burks, A.W., Huang, S.K., Sampson, H., Cockrell, G., Helm, R.M., West, C.M., & Bannon, G.A. (1997) *Arch. Biochem. Biophys*. **342**, 244–253

(7) Burks, A.W., Shin, D., Cockrell, G., Stanley, J.S., Helm, R.M., & Bannon, G.A. (1997) *Eur. J. Biochem*. **245**, 334–339

(8) Burks, A.W., Williams, L.W., Helm, R.M., Connaughton, C., Cockrell, G., & O'Brien, T.J. (1991) *J. Allergy Clin. Immunol*. **88**,172–179

(9) de Jong, E.C., van Zijverden, M., Spanhaak, S., Koppelman, S.J., Pellegrom, H., & Penninks, A.H. (1998) *Clin. Exp. Allergy* **28**,743–751

(10) Burks, A.W., Williams, L.W., Connaughton, C., Cockrell, G., O'Brien, T.J., & Helm, R.M. (1992) *J. Allergy Clin. Immunol*. **90**, 962–969

(11) Koppelman, S.J., Bruijnzeel-Koomen, A.F.M., Hessing, M., & de Jongh, H.H.J. (1999) *J. Biol. Chem*. **274**, 4770–4777

(12) Sen, M., Kopper, R., Pons, L., Abraham, E.C., Burks, A.W., & Bannon, G.A. (2002) *J. Immunol.* **169**, 882–887

(13) Czernik, P.J., Peterson, C.A., & Hurlburt, B.K. (1996) *J. Biol. Chem.* **271**, 9141–9149

(14) Taylor, S.L., & Nordlee, J.A. (1995) *Food Technol*. **5**, 231–234

(15) Koppelman, S.J., Bleeker-Marcelis, H., van Duijn, G., & Hessing, M. (1996) *World Ingredients* **12,** 35–36

(16) Yeung, J.M., & Collins, P.G. (1996) *J. AOAC Int.* **79**, 1411–1416

(17) Pomes, A., Helm, R.M., Bannon, G.A., Burks, A.W., Tsay, A., & Chapman, M.D. (2003) *J. Allergy Clin. Immunol.* **111**, 640–645