JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2004,** *52,* 4903−4907 **4903**

The Major Peanut Allergen Ara h 1 and Its Cleaved-off N-Terminal Peptide; Possible Implications for Peanut Allergen Detection

HARRY J. WICHERS,*,† THOMAS DE BEIJER,† HUUB F. J. SAVELKOUL,§ AND AART VAN AMERONGEN†

Agrotechnology and Food Innovations, Wageningen University and Research Center, Bornsesteeg 59, 6708 PD Wageningen, The Netherlands, and Cell Biology and Immunology Group, Wageningen University and Research Center, Marijkeweg 40, 6709 PG Wageningen, The Netherlands

Ara h 1 was purified from raw peanuts (*Arachis hypogaea* L.) in the presence or absence of protease inhibitors. N-Terminal amino acid sequences were determined after western blotting. Both purification procedures proved to be very consistent and resulted in identical chromatographic and electrophoretic behavior of Ara h 1 and in the isolation of identical proteins of ∼64 kDa with RS/H_PPGERTRG as the N-terminal amino acid sequence. Consequently, purified Ara h 1 appears to be truncated at the N-terminal side. The observations strongly suggest that Ara h 1 occurs physiologically as a protein of which the first 84 and 78 amino acids, respectively, are cleaved off in planta upon maturation of the protein. On the basis of epitope mapping, the cleaved-off N-terminal peptide contains three allergenic epitopes, of which two are major. These truncated epitopes will go undetected in assays when purified Ara h 1 from peanuts is used as reference material. Patients' sera, however, contain IgE-type antibodies against the epitopes that are contained in the cleaved-off peptide, implying that the peptide, or part of it, is still present in peanuts that are consumed. Possible consequences of this exposure to these three epitopes are discussed. On the basis of literature data the cleaved-off peptide is hypothesized to have antifungal activity.

KEYWORDS: ***Arachis hypogaea*; Ara h 1; allergen; assay; peanut; truncation**

INTRODUCTION

Peanut (*Arachis hypogaea* L.) allergy is among the most important food allergies in Western societies, not only because of incidence [0.6% of children in the United States (*1*)] but also because of the severity of the symptoms: peanut allergy may result in anaphylactic shock and life-threatening conditions. In sensitive individuals 50% of an allergic population may respond to as little as 3 mg or $^1/_{50}$ of a peanut. Threshold levels can be as low as 100 μg (*2*). Peanut allergies result in 50−100 casualties in the United States annually (*3*) and likely a similar number in the whole of the European Union. An important risk is posed by so-called hidden peanut allergens: peanut fragments can be present in products where they are not expected, for instance, via cross-contamination on production lines or via the use of peanut-derived ingredients in nonpeanut food products (e.g., peanut-derived frying oil).

The peanut contains a set of allergenic proteins, designated Ara h 1−7. Most predominant allergens are Ara h 1 and Ara h 2, to which (depending on the population under study) 70−90% of patients' sera respond (*4*, *5*).

Ara h 1 (vicilin-like protein) is well characterized from a biochemical and genetic perspective. Ara h 1 is a globular 7S storage glycoprotein (*6*) and occurs as a highly stable trimer in peanut. Such multimers are composed of monomers of ∼64 kDa, which are held together via hydrophobic interactions. Hydrophobic amino acids that contribute to trimer formation are at the distal ends where monomer−monomer interactions occur and where also the majority of IgE-binding epitopes are located. These epitopes are therefore more or less hidden in the native protein trimer complex that may protect the monomers from degradation, thus resulting in enhanced allergenicity (*5*). Ara h 1 possesses 23 allergenic epitopes, of which 4 are immunodominant (defined as epitopes that are recognized by >80% of the patients' sera), as evidenced by peptide mapping. The epitopes do not share any obvious sequence motif (*7*).

Ara h 1 is very abundant in peanut, where it may account for 12−16% of total protein, corresponding to 3.1−4.6% of peanut mass. Ara h 1 levels do not vary significantly among varieties or among peanuts that are grown in different regions (*8*).

Two genes have been described for Ara h 1, encoding 626 and 614 amino acids, respectively, and both showing an N-terminal 25 amino acid signal sequence (*9*).

* Author to whom correspondence should be addressed (e-mail harry.wichers@wur.nl).
† Agrotechnology and Food Innovations.
§ Cell Biology and Immunology Group.

10.1021/jf049697o CCC: $27.50
Published on Web 07/02/2004

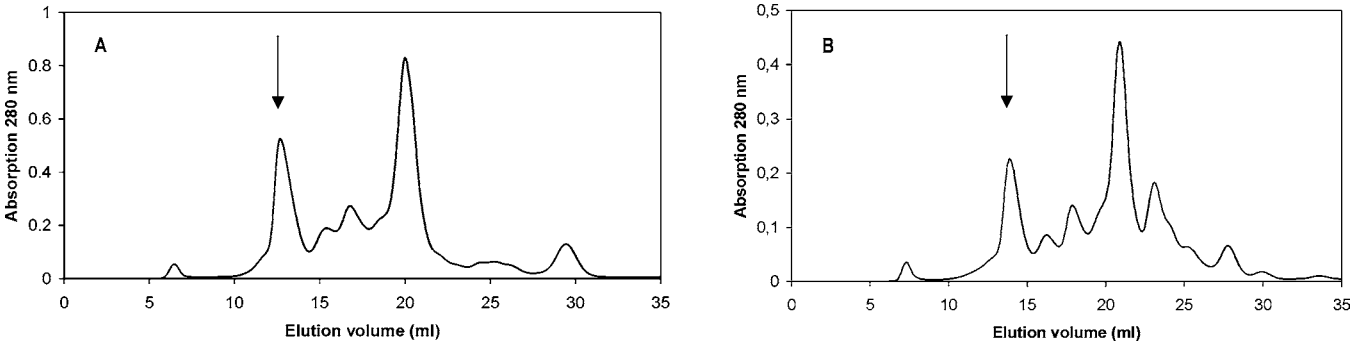

Figure 1. Superose 6 chromatography of a peanut extract prepared and fractionated in the absence (left) or presence of a protease inhibitor cocktail (right). Arrows: elution of Ara h 1.

Various reports have appeared on the purification and identification of Ara h 1. Usually, the identity of purified Ara h 1 is checked by determining part of its amino acid sequence. Often, internal sequences obtained from tryptic digests have been used (*10*). De Jong et al. (*4*) report the N-terminal sequence of the isolated product as RSPPGERTRG, suggesting that from the amino acid sequence of Ara h 1, as deduced from its nucleotide sequence (*9*), not only is a 25 amino acid signal sequence cleaved off but so is a 53−59 amino acid peptide (length dependent on isoform) that bears at least three allergenic epitopes, of which two are major (*7*). A similar observation has been reported by Buschmann et al. for roasted peanuts of var. Virginia (*11*).

The development of adequate methods for the analysis, diagnosis, and treatment requires a thorough understanding of the structure of allergens as they are consumed. For instance, it is essential that at least the major allergenic epitopes be present in protein isolates that are used for the calibration of immunochemical assays, to ensure that no epitopes go undetected in such assays. If not, a product's allergenic potential may be substantially underestimated. This may pose unexpected reactions upon ingestion of food products that were analyzed and declared safe on the basis of such an assay.

This paper describes the isolation and characterization of Ara h 1 from raw peanuts of variety Common Natal under various extraction and purification conditions, to shed light on the biochemical and immunochemical characteristics of physiologically occurring forms of this protein.

MATERIALS AND METHODS

Protein Extraction. Raw peanuts (variety Common Natal, from Safrinut, Gauteng, South Africa) were obtained from a local Oriental specialty shop and stored frozen (−18 °C) until use. After defrosting, peanuts were ground with a pestle and mortar, at 0 °C, until a fine structure was obtained. Subsequently, the peanuts were further homogenized in a blender (Analysemuhle A 10, Janke und Kunkel) into a sticky slurry.

Protein was extracted by suspending 10 g of peanut slurry in 100 mL of 50 mM Tris-HCl and 0.2 M NaCl, pH 8.2, and stirred for 2 h at room temperature. When extraction was performed under protease-inhibitory conditions, the extraction buffer contained 1 mM EDTA, 1 mM PMSF, and 0.5% (v/v) protease inhibitor cocktail (Sigma catalog no. P9599). Under these conditions, no protease activity was detectable in extracts of raw peanuts (not shown).

Subsequently, the extract was centrifuged in an SS34 rotor (Sorvall RC 5B plus) at 15000 rpm for 20 min. After centrifugation, the top layer of the tubes contained peanut oil, which was removed first. The supernatant was filtered over a 0.45 μm membrane (Cornering, Germany). The pellet was discarded.

To the supernatant was added 0.01% dithiotreitol (v/v), and it was stored in a cold room at 4 °C overnight. Subsequently, the extract was divided in aliquots of 2 mL and stored in the freezer (−18 °C) until further use.

Chromatography. *Gel Permeation Chromatography.* A 1.5 mL sample of the extract was thawed, filtered through a 0.20 μm membrane (Cornering, Germany), applied to a Superose 6 HR 10/30 column, and eluted on an Äkta purifier (both from Pharmacia Biotech, Uppsala, Sweden) at 0.5 mL/min with 50 mM Tris-HCl and 0.2 M NaCl, pH 8.2. Fractions of 1 mL were collected. Buffers were filtered through a 0.45 μm membrane (Millipore) prior to use. Under protease-inhibitory conditions, the elution buffer contained 1 mM EDTA and 1 mM PMSF. Protein was detected by reading the absorbance at 280 nm. Ara h 1 was detected by analyzing the fractions with SDS-PAGE (see below).

Affinity Chromatography. Two milliliters of the Ara h 1 containing Superose fractions were applied to a Sepharose 4B column (13 × 50 mm, bed volume = 7 mL) to which concanavalin A was linked (Pharmacia Biotech). Under protease-inhibitory conditions, EDTA was first removed from the extract by dialyzing it against 5 × 100 mL of 50 mM Tris-HCl, 0.2 M NaCl, and 1 mM PMSF, pH 8.2. The column was eluted at 0.5 mL/min with 2 column volumes of 50 mM Tris-HCl and 0.2 M NaCl, pH 8.2, and with a linear gradient in 4 column volumes of 50 mM Tris-HCl and 0.2 M NaCl, pH 8.2, and 1 M α-methyl mannopyranoside in water. Under protease-inhibitory conditions, all elution fluids contained 1 mM PMSF. Protein was detected by reading the absorbance at 280 nm. Ara h 1 was detected by analyzing the fractions with SDS-PAGE (see below). Ara h 1 containing fractions were pooled and stored at −18 °C until further use.

SDS-PAGE, Blotting, and Sequencing. SDS-PAGE analyses under denaturing conditions on 10% acrylamide gels and Western blotting were performed as described elsewhere (*12*). After electrophoresis, gels or blots were stained with CBB R250.

Amino acid sequencing was performed on protein bands that were cut out of the Western blots after the blots had been stained with CBB R250, by Midwest Analytical Inc., St. Louis, MO, following the Edman degradation protocol.

Analysis of the Ara h 1 Cleaved-off Peptide. The Ara h 1 cleaved-off peptide was analyzed for its predicted biochemical characteristics by several tools available at http://www.expasy.org/ (ProtParam, ProtScale, PeptideCutter). Entries P43237 and P43238 in the Swiss-Prot database were used for sequence information. Structural elements were predicted according to the methods of Chou and Fasman (*13*).

RESULTS

Isolation of Ara h 1 from Raw Peanuts in the Absence and Presence of Protease Inhibitors. Ara h 1 was purified from raw peanuts (var. Common Natal), obtained from a local retail seller, in two steps according to the protocol as outlined under Materials and Methods.

Figure 1A shows the elution profile of a peanut extract that was prepared in the absence of protease inhibitors and fractionated on Superose 6 HR. **Figure 1B** shows a chromatography profile of a peanut extract that was prepared in the presence of 1 mM EDTA, 1 mM PMSF, and protease inhibitor cocktail from Sigma and fractionated in the presence of 1 mM EDTA and 1

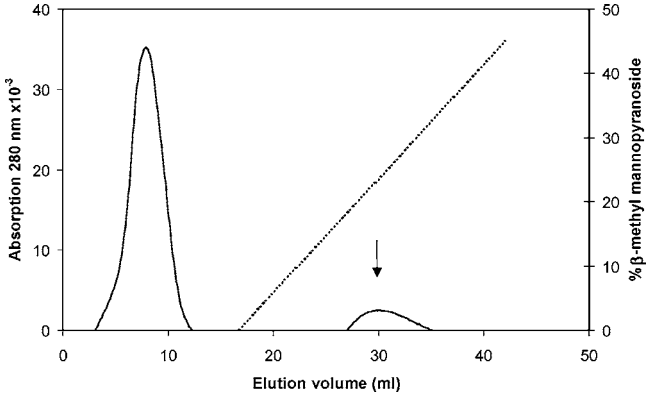

Figure 2. Elution of Ara h 1 from Con-A–Sepharose 4B, using a gradient of α-methyl D-mannopyranoside. Arrow: elution of Ara h 1.

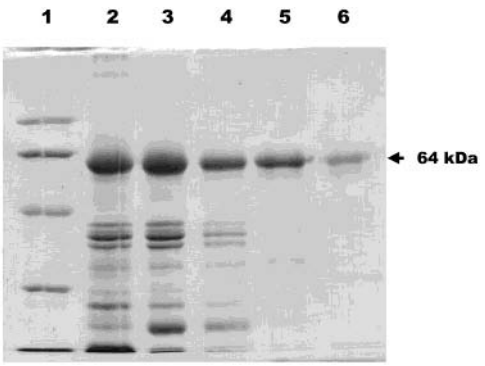

Figure 3. Denaturing SDS-PAGE of various fractions from the purification of Ara h 1: lane 1, markers [respectively, 97.4, 66.2, 45, 31, and 21.5 (on front) kDa from top]; lane 2, crude extract (absence of protease inhibitors); Ara h 1 containing Superose-6 fractions in, respectively, the absence (lane 3) and presence (lane 4) of protease inhibitors; Ara h 1 containing Con-A fractions in, respectively, the absence (lane 5) and presence (lane 6) of protease inhibitors.

mM PMSF. Under both chromatographic conditions, that is, the presence or absence of protease inhibitors, Ara h 1 eluted after 12−14 mL, as determined by SDS-PAGE (see below).

Figure 2 shows the elution profile of the Ara h 1 containing Superose 6 peak in Con-A affinity chromatography under protease-inhibiting conditions. When Ara h 1 was isolated under these conditions, the elution buffers contained 1 mM PMSF. EDTA could not be used in this case as it would interfere with Con-A affinity. Isolation of Ara h 1 in the absence or presence of 1 mM PMSF yielded identical elution profiles. Under both conditions, Ara h 1 eluted at ∼0.23 M α-methyl D-mannopyranoside.

The isolation procedure was repeated several times and yielded very consistent results. In conclusion, preparation of the peanut extract in the presence of protease inhibitors and subsequent fractionation and isolation from, respectively, Superose 6 and Con-A−Sepharose 4B essentially yielded identical chromatographic behaviors of Ara h 1 when the operations were performed with or without incorporation of specific protease-inhibiting precautions throughout the isolation procedure.

SDS-PAGE and Blotting of Ara h 1 Fractions. Figure 3 shows the behavior of various fractions from the purification procedure for Ara h 1 in either the presence or absence of protease-inhibiting circumstances, in SDS-PAGE under denaturing conditions.

The molecular masses of ∼64 kDa of the eventually isolated proteins (**Figure 3**, lanes 5 and 6) correspond to what is reported in the literature for monomeric Ara h 1 as isolated from peanuts (*11*, *14*). Importantly, the molecular masses are identical regardless of whether the proteins were isolated under protease-inhibitory conditions or not.

Identification of Isolated Proteins as Ara h 1 by N-Terminal Amino Acid Sequencing. N-Terminal amino acid sequence analysis, after Western blotting, of the proteins shown in lanes 5 and 6 (**Figure 3**) demonstrated their sequence as R-S/H_-P−P-G-E-R-T-R-G. This result identifies the isolated proteins as Ara h 1 and as a mixture of both isoforms as cloned by Burks et al. (*9*), albeit that the first 84 and 78 amino acids of the cloned sequences, respectively, appear to be missing.

Analysis of the Ara h 1 Cleaved-off Peptide. The Ara h 1 cleaved-off peptide was analyzed for its biochemical characteristics. The predicted molecular mass of the 26−79 peptide from Clone P17 (entry P43237 in the Swiss-Prot database) is 6285 Da, and its isoelectric point (p*I*) is 8.29. For Clone P41B (entry P43238) the molecular mass of the 26−84 peptide is predicted to be 6794 Da with a p*I* of 8.29. The Ara h 1 cleaved-off peptide contains six cysteine residues. Although the linear sequence of the cleaved-off peptide is predicted to be unstable by in silico proteolytic methods, it is expected that the peptide will form internal S−S bridges, leading to a rigid and much more stable conformation. Predicted structural elements are shown in **Figure 4**. In the peptide, here numbered from 1 to 59, two β-sheets are predicted that enclose an α-helix around amino acid 30.

DISCUSSION

Isolation of Ara h 1 from raw peanuts in the absence or presence of protease-inhibiting conditions leads to isolation of identical forms of Ara h 1, with identical chromatographic and electrophoretic behavior and identical N-terminal amino acid sequences. However, the protein appears to be N-terminally truncated when the N-terminal amino acid sequence is compared to the full-length sequence as deduced from the Ara h 1 open reading frame (*9*). The isolation procedure, consisting of gel permeation chromatography and Con-A affinity chromatography, is not likely to separate possibly coexisting truncated and nontruncated Ara h 1 forms. The molecular mass differences are too small to be resolved in gel permeation chromatography, and both forms are likely to be glycosylated. Therefore, our observations strongly suggest that the first 84 and 78 amino acids, respectively, have been cleaved off during maturation of the protein in planta. Of these truncated amino acids, 25 represent a signal sequence, as predicted with the SignalP program (http://us.expasy.org/tools).

The current observations, in particular the determination of the N-terminal amino acid sequences of the isolated proteins, demonstrate that three allergenic epitopes do not occur on the part of the protein that generally is purified as and considered to be Ara h 1 (*11*, *14*). Yet, as Ara h 1's allergenic epitopes have been identified on the basis of peptide mapping and interaction with patients' sera, it is obvious that also the truncated peptide occurs in some form in peanuts that are consumed (*7*). Other authors only describe internal sequences when verifying the identity of isolated Ara h 1 (*10*). This may lead to misinterpretations of analytical data if such Ara h 1 is, for instance, used as reference or calibration in analytical setups, as the three epitopes on the truncated peptide (of which two are major) will not be involved in the analytical reactions. The N-terminal amino acid sequence of Ara h 1 purified from peanuts has been described similarly before (*4*, *11*). The likely physiological origin of the truncation *in planta*, as described in this paper, was pointed out by De Jong et al. (*4*). These authors

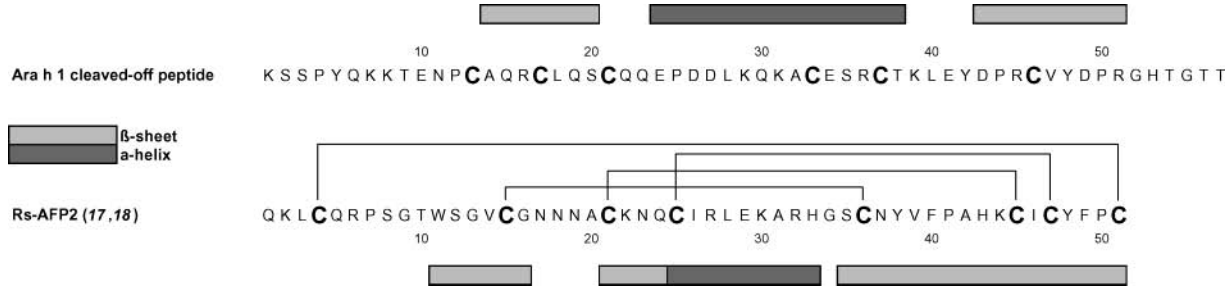

Figure 4. Prediction of structural elements for the Ara h 1 cleaved-off peptide and the characteristics of the radish (*R. sativus*) antifungal protein Rs-AFP2 (*17*, *18*).

hypothesized that the product from the gene as cloned by Burks et al. (*9*) needs to be processed upon maturation to yield mature Ara h 1 as it is found in peanuts. However, De Jong et al. did not purify Ara h 1 under protease-inhibitory conditions, whereas in the present study we have compared both protease-inhibitory and -noninhibitory conditions. Our observations, therefore, substantiate the likelihood of in planta processing, rather than truncation being an isolation artifact. The truncation has been described now as occurring in three varieties of peanuts [Runner Jumbo 38/42 (*4*), roasted Virginia (*11*), and Common Natal, this paper] and therefore very likely represents a physiological phenomenon in the maturation of Ara h 1 that occurs in many, if not all, peanut varieties.

The truncated peptide of Ara h 1 has not been isolated until now. The occurrence of IgE-type antibodies against the three allergenic epitopes that it contains (*7*) is most easily explained by assuming that this peptide occurs in peanuts in a form that is able to provoke an antibody response upon ingestion. Alignment of the truncated peptide with the other allergenic proteins from peanut indicates some sequence similarity between the first major Ara h 1 epitope and a sequence in Ara h 2. This Ara h 2 sequence is, however, not described as allergenic (*15*), making it unlikely that in this case sequence similarity explains the occurrence of IgE antibodies against this epitope in humans.

A further analysis of the cleaved-off peptide reveals that the molecular mass is 6285 and 6794 Da, respectively (depending on isoform), and that the predicted isoelectric point is 8.29 for both isoforms. It is questionable whether the various authors that separated peanut proteins by SDS-PAGE have been able to identify oligopeptides of this size. There are two reasons for this: in general, electrophoretic conditions were such that only proteins having molecular masses of 10 kDa and higher were separated. Second, upon immunoblotting of electrophoresed peanut proteins, all authors used conditions according to the method of Towbin et al. (*16*). In this procedure the pH of the transfer buffer is 8.3 and, considering the predicted isoelectric point of the cleaved-off peptide, that is, 8.29, inefficient transfer of this peptide from the polyacrylamide gel to the membrane may have played a role as well. In conclusion, it is likely that none of the authors have been able to detect the truncated peptide using immunoblots with specific IgE antibodies against epitopes 1 and 3, that is, the two major epitopes on the cleaved-off peptide (*7*). Furthermore, in ELISA and similar assays the use of purified peanut allergens (i.e., with Ara h 1 not containing the cleaved-off peptide), or a peanut protein extract (i.e., containing the cleaved-off peptide) will potentially lead to differences in IgE reactivity as well.

Recently, a peptide designated hypogin, shown to have antifungal activity, was isolated from peanut (*17*). The first 40 amino acids of this 7.2 kDa peptide have been sequenced, and it appears that hypogin has a substantial homology with the Ara h 1 cleaved-off peptide. The first major and second allergenic epitopes attributed to Ara h 1 (*7*) also occur in the sequenced part and, on the basis of the level of homology, could easily give rise to cross-reactive antibodies. However, some striking differences exist. As far as sequence, hypogin does not contain cysteine residues, whereas the Ara h 1 cleaved-off peptide contains six. This is very remarkable, because the mature Ara h 1, that is, the purified protein, contains only one cysteine. It is, therefore, tempting to speculate that the cleaved-off peptide has a rigid structure based on three internal cystine bridges. The proposed rigid structure would also imply that the cleaved-off peptide could be quite stable under digestive conditions, explaining the induction of IgE antibodies against epitopes 1−3 (*7*). The Ara h 1 cleaved-off peptide resembles a class of antifungal oligopeptides from plant seeds in having a relative abundance of cysteine residues (*18*, *19*). In **Figure 4**, the Ara h 1 cleaved-off peptide sequence is shown together with a typical example of an antifungal peptide from radish (*Raphanus sativus*), and the predicted structural elements are indicated. On the basis of the structural similarity to hypogin and to the radish peptide, antifungal activity for this Ara h 1 peptide may be hypothesized. Current research is directed at the isolation of the truncated peptide and verification of its postulated antifungal activity.

In conclusion, after purification from peanuts, Ara h 1 is obtained as a truncated protein, in which the first 85 and 78 amino acids, respectively, as expected on the basis of nucleotide sequence, are missing. This truncation very likely takes place in the plant and therefore represents a physiological mechanism in the maturation of Ara h 1. Yet the peptide that is cleaved off is able to provoke an IgE-type antibody response, as three allergenic epitopes were identified on it by making use of patients' sera. Apparently, the peptide is still present in peanuts, possibly as an antifungal compound. These findings have implications for the analysis of Ara h 1 allergenicity, in particular when Ara h 1 purified from peanuts is used as a reference.

ABBREVIATIONS USED

CBB R250, Coomassie Brilliant Blue R 250; Con-A, concanavalin A; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethanesulfonyl fluoride; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; Tris, tris(hydroxymethyl)aminomethane.

LITERATURE CITED

(1) Pomes, A.; Helm, R. M.; Bannon, G. A.; Burks, A. W.; Tsay, A.; Chapman, M. D. Monitoring peanut allergen in food products by measuring Ara h 1. *J. Allergy Clin. Immunol.* **2003**, *111*, 640−645.

(2) Wensink, M.; Penninks, A. H.; Hefle, S. L.; Koppelman, S. J.; Bruijnzeel-Koomen, C. A.; Knulst, A. C. The distribution of individual threshold doses eliciting allergic reactions in a population with peanut allergy. *J. Allergy Clin. Immunol.* **2002**, *110*, 915−920.

(3) Sampson, H. A. Peanut allergy. *N. Engl. J. Med.* **2002**, *346*, 1294−1299.

(4) De Jong, E. C.; Van Zijverden, M.; Spanhaak, S.; Koppelman, S. J.; Pellegrom, H.; Penninks, A. H. Identification and partial characterization of multiple major allergens in peanut proteins. *Clin. Exp. Allergy* **1998**, *28*, 743−751.

(5) Maleki, S. J.; Kopper, R. A.; Shin, D. S.; Park, C. W.; Compadre, C. M.; Sampson, H.; Burks, H. W.; Bannon, G. A. Structure of the major peanut allergen Ara h 1 may protect IgE-binding epitopes from degradation. *J. Immunol.* **2000**, *164*, 5844−5849.

(6) Van Ree, R.; Cabanes-Macheteau, M.; Akkerdaas, J.; Milazzo, J. P.; Loutelier-Bourhis, C.; Rayon, C.; Villalba, M.; Koppelman, S.; Aalberse, R.; Rodriguez, R.; Faye, L.; Lerouge, P. β(1,2)-Xylose and α(1,3)-fucose residues have a strong contribution in IgE binding to plant glycoallergens. *J. Biol. Chem.* **2000**, *275*, 11451−11458.

(7) Burks, A. W.; Shin, D.; Cockrell, G.; Stanley, J. S.; Helm, R. M.; Bannon, G. A. Mapping and mutational analysis of the IgE-binding epitopes on Ara h 1, a legume vicilin protein and a major allergen in peanut hypersensitivity. *Eur. J. Biochem.* **1997**, *245*, 334−339.

(8) Koppelman, S. J.; Vlooswijk, R. A. A.; Knippels, L. M. J.; Hessing, M.; Knol, E. F.; Van Reijsen, F. C.; Bruijnzeel-Koomen, C. A. F. M. Quantification of major peanut allergens Ara h 1 and Ara h 2 in the peanut varieties Runner, Spanish, Virginia and Valencia, bred in different parts of the world. *Allergy* **2001**, *56*, 132−137.

(9) Burks, A. W.; Cockrell, G.; Stanley, J. S.; Helm, R. M.; Bannon, G. A. Recombinant peanut allergen Ara h 1 expression and IgE binding in patients with peanut hypersensitivity. *J. Clin. Invest.* **1995**, *96*, 1715−1721.

(10) Burks, W.; Sampson, H. A.; Bannon, G. A. Peanut allergens. *Allergy* **1998**, *53*, 725−730.

(11) Buschmann, L.; Petersen, A.; Schlaak, M.; Becker, W. M. Reinvestigation of the major peanut allergen Ara h 1 on molecular level. *Monogr. Allergy* **1996**, *32*, 92−98.

(12) Soler-Rivas, C.; Möller, A. C.; Arpin, N.; Olivier, J. M.; Wichers, H. J. Induction of a tyrosinase mRNA in *Agaricus bisporus* upon treatment with a tolaasin preparation from *Pseudomonas tolaasii*. *Physiol. Mol. Plant Pathol.* **2001**, *58*, 95−99.

(13) Chou, P. Y.; Fasman, G. D. Empirical predictions of protein conformation. *Adv. Enzymol.* **1978**, *47*, 45−148.

(14) Burks, A. W.; Williams, L. W.; Helm, R. M.; Connaughton, C.; Cockrell, G.; O'Brien, T. J. Identification of a major peanut allergen, Ara h 1, in patients with atopic dermatitis and positive peanut challenges. *J. Allergy Clin. Immunol.* **1991**, *88*, 172−179.

(15) Stanley, J. S.; King, N.; Burks, A. W.; Huang, S. K.; Sampson, H.; Cockrell, G.; Helm, R. M.; West, C. M.; Bannon, G. A. Identification and mutational analysis of the immunodominant IgE epitopes of the major peanut allergen Ara h 2. *Arch. Biochem. Biophys.* **1997**, *342*, 244−253.

(16) Towbin, H.; Staehelin, T.; Gordon, J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedures and some applications. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 4350−4354.

(17) Ye, X. Y.; Ng, T. B. Hypogin, a novel antifungal peptide from peanuts with sequence similarity to peanut allergen. *J. Pept. Res.* **2001**, *57*, 330−336.

(18) Osborn, R. W.; Broekaert, W. F. Antifungal proteins. In *Seed Proteins*; Shewry, P. R., Casey, R., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1999; pp 727−751.

(19) Fant, F.; Vranken, W.; Broekaert, W.; Borremans, F. Determination of the three-dimensional solution structure of *Raphanus sativus* antifungal protein 1 by H-1 NMR. *J. Mol. Biol.* **1998**, *279*, 257−270.

Received for review February 23, 2004. Revised manuscript received May 27, 2004. Accepted June 2, 2004. We acknowledge the support of the European Commission via the PROTALL project (FAIR-CT98-4356) and the INFORMALL project (QLRT-2001-02284) and of the Allergy Consortium Wageningen "Allergy Matters".

JF049697O