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Can we explain the allergenicity of peanuts on the basis of the three-dimensional structure of its allergens and use the information to devise means of eliminating peanut allergy?

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ABSTRACT

Helical bundles are found in all the known structures of peanut allergens. The peptide fragments, which survive gastrointestinal digestion of the allergens and are absorbed intact, are hypothesized to reassociate and form stable helical bundles in the circulation, which could elicit a specific IgE response resulting in peanut allergy. The hypothesis is supported by the finding of a diminished allergenicity of an isoform of the peanut allergen, Ara h 3, which has a major deletion. A very probable consequence of this deletion is the reduced tendency to form a stable helix bundle. The discovery of structurally disrupted isoforms of the other peanut allergens and the breeding of plants that contain only those isoforms could lead to the elimination of peanut allergy.

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Introduction

Peanut allergy is a growing epidemic, currently affecting 0.6% of adults and children and is the most common cause of fatal and near fatal food-induced anaphylaxis in the United States. Previously considered a lifelong allergy, current evidence suggests that up to 20% of peanut-allergic individuals will tolerate peanuts at some point in the future. Sensitization to peanut allergens is usually through the gastrointestinal route, but cutaneous exposure with peanut-containing lotions and creams has also been implicated.

Peanut allergic individuals usually recognize more than one allergenic protein, and allergens recognized by more than 50% of sensitized individuals are considered major allergens. The major peanut allergens include Ara h 1, a member of the vicilin family of seed storage proteins, Ara h 2, a member of the conglutin family of seed storage proteins, and peanut agglutinin allergens along with the minor allergens including profilin (Ara h 5), glycinin seed storage proteins (Ara h 3,4), and other conglutin proteins (Ara h 6,7) [1]. Ara h 3, a minor allergen, has a molecular weight of 60 kDa and is recognized by 44% of peanut allergic patients. Ara h 3 shares homology with other glycinin family proteins including G1 and G2 glycinins and beta-conglycinin in soybean allergens, potentially contributing to the cross-reactivity between peanuts and other legumes. Varied processing of peanuts throughout the world has been associated with a variable incidence of peanut

allergy. Roasting of peanuts seems to confer increased allergen stability and increased allergenicity versus boiling or frying [2,3]. For example, peanuts are most commonly boiled or fried in China, but peanut allergy is much less prevalent in China despite comparable amounts of peanut consumption. Beyer et al. [2] evaluated IgE binding intensity to Ara h 1, Ara h 2, and Ara h 3 after roasting, frying, or boiling peanuts and noted decreased binding intensity of all three peanut allergens after frying or boiling although total protein quantities were unaltered.

In order to elicit a specific immune response, antigens must present stable structures that persist in the circulation during the course of affinity maturation. For food allergy, the allergen, or fragments thereof, must survive gastrointestinal digestion and be absorbed. Protein allergens are unlikely to survive enzymatic digestion whole, so that the stable structures that are required to elicit specific IgE must be formed by the digestion products, some of which could be sizeable peptides. Examples of stable protein structures are helices and helix bundles, beta-sheets and beta-sheet structures, and combinations thereof. Stability is provided by disulfide bonds, favorable charge–charge interactions, hydrogen bonds, and hydrophobic interactions.

Attempts have been made to subject peanut allergens to the action of gastric and intestinal enzymes *in vitro* and the reactivity of the resulting products with specific IgE has been investigated (see, for example [4,5]). Indeed, digestion products of some peanut allergens have been found to resist further degradation and to react with specific IgE from patients with peanut allergy [4]. Whether or not those digestion products are absorbed into the circulation whole is subject to speculation. Alternatively, the

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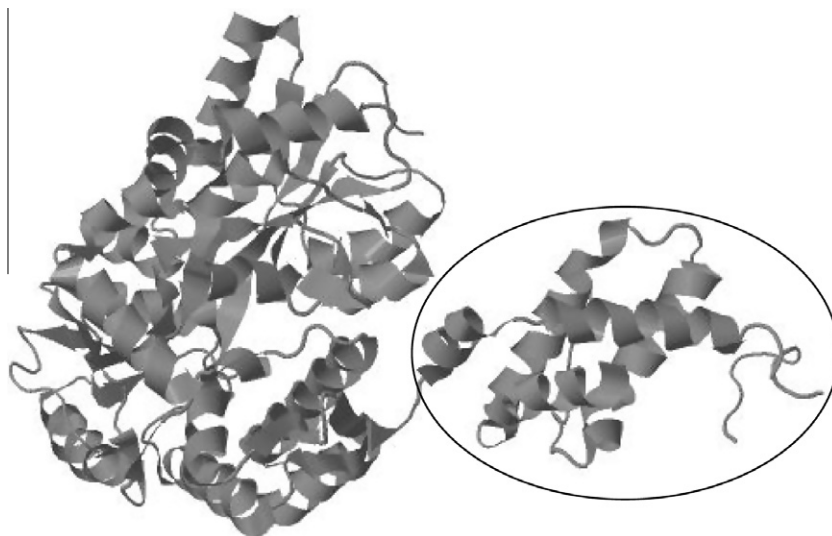


Fig. 1. Ara h 2 (encircled on the right) fused with maltose-binding protein (on the left) (PDB Entry 3OB4).



Fig. 2. Ara h 6 (PDB Entry 1W2Q, first molecule in the entry).

structures that are presented to the immune system are probably the result of the spontaneous association into stable structures of peptide fragments which are more likely to be absorbed through the gastro-intestinal lining.

We propose that the understanding of the allergenicity of certain foods could be approached by examining the three-dimensional structure of the allergens and predicting the possible stable structures, which the fragments resulting from gastrointestinal digestion, might spontaneously form. We have used this approach in a study of peanut allergy that utilizes the three-dimensional structure of peanut allergens currently available in the Protein Data Bank (PDB) (<http://www.ncbi.nlm.nih.gov/>). Our earlier study to explain the difference in allergenicity of tropomyosins from various animals [6] invokes differences in the size of the fragments which are expected to result from gastric digestion.

The amino acid sequences of isoforms of several of the major peanut allergens have been determined. One isoform of the peanut allergen, Ara h 3, has been found to have diminished allergenicity [7]. A plausible explanation for the diminished allergenicity of that isoform is presented here. The explanation is based on a prediction of the effect of the altered amino-acid sequence on the allergenic structure that would be presented to the immune system.

The structure of peanut allergens

The available three-dimensional structures of peanut allergens were obtained from the NCBI database (<http://www.ncbi.nlm.nih.gov/>). X-ray crystallographic structures are available for the peanut allergens, Ara h 1 [8] (PDB Entry 3S7I), Ara h 2 fused with maltose-binding protein [9] (PDB Entry 3OB4), and Ara h 3 [10] (PDB Entry



Fig. 3. An Ara h 3 monomer. The helices, which form a helix bundle in the trimeric structure, are labeled (PDB Entry 3C3V).

3C3V). In addition, an NMR structure is available for the peanut allergen, Ara h 6 [5] (Lehmann et al., 2006; PDB Entry 1W2Q).

The structure of Ara h 2 (from PDB Entry 3OB4) is shown in Fig. 1. The solution structure of Ara h 6 (PDB Entry 1W2Q) is shown in Fig. 2. The principal structure in both is a helix bundle.

The crystal structure of a monomer of Ara h 3 is shown in Fig. 3. Ara h 3 normally exists as a hexamer, consisting of two trimers

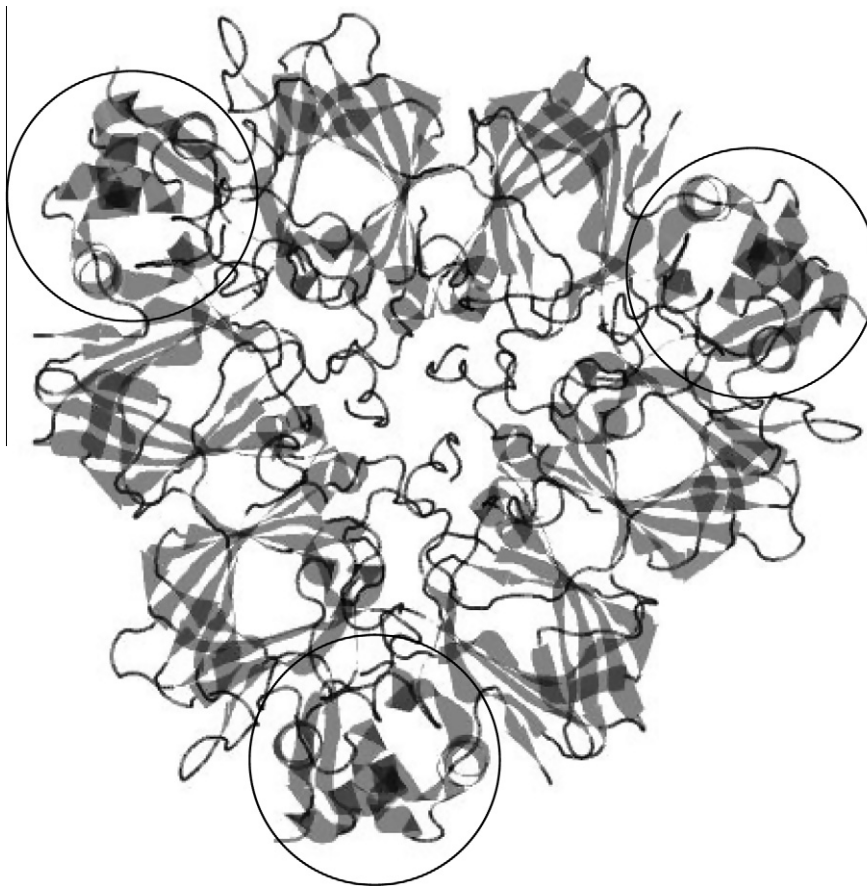


Fig. 4. Ara h 3 trimer with the helix bundles circled (PDB Entry 3C3V).

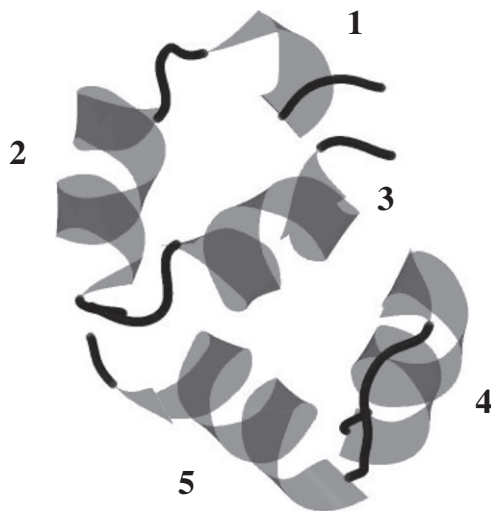


Fig. 5. The helix bundle in Ara h 3. The helices are labeled as in Fig. 3 (PDB Entry 3C3V).

[10]. The proposed structure of an Ara h 3 trimer is shown in Fig. 4. The helices in the Ara h 3 monomer do not form a helix bundle among themselves. But, when monomers associate to form a trimer (Fig. 4), the helices at the two ends of the molecule form a helix bundle (Fig. 5). Similar structures are found for Ara h 1 [8] and these are shown in Figs. 6 and 7. The helix bundle in Ara h 1 is shown in Fig. 8. The residue contacts within the helix bundle in Ara h 3 are presented in Table 1.



Fig. 6. A monomer of Ara h 1 (PDB Entry 3S7I).



Fig. 7. Ara h 1 trimer (PDB Entry 3S7I).

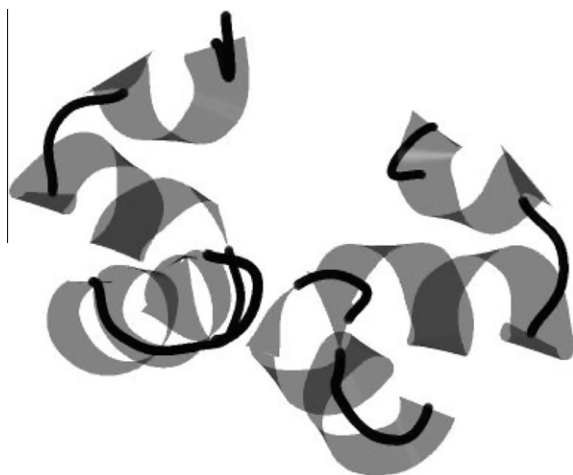


Fig. 8. The helix bundle in Ara h 1 (PDB Entry 3S7I).

The helix bundle is formed by a continuous stretch of residues in Ara h 2 and Ara h 6 (Figs. 1 and 2); in Ara h 1 and Ara h 3, the bundle is formed by helices from distant parts of the molecule. The helix bundles in Ara h 2 and Ara h 6 are stabilized by several disulfide bonds (see, PDB Entries 1W2Q [5] and 3OB4 [9]); there are no disulfide bonds in the bundles of Ara h 1 and Ara h 3 (see, PDB Entries 3S7I [8] and 3C3V [10]).

The amino sequences of the allergenic and less-allergenic isoforms of Ara h 3 are compared in Tables 2a and 2b. Amino-acid,

as well as length, differences are found between the two isoforms, including a 24-residue deletion in the less-allergenic isoform. Indicated in Tables 2a and 2b are the secondary structure elements found in the crystal structure of Ara h 3. Note that the deletions do not occur in the beta-sheet elements (Table 2a), but instead in the loop regions and in a segment that contributes to the helix bundle (Table 2b). In fact, the 24-residue deletion completely removes one of the helices, as well as the N-terminal part of the next helix (Table 2b).

The proposed explanation for the diminished allergenicity of Ara h 3

The analysis of the three-dimensional structures of Ara h 1, Ara h 2, Ara h 3, and Ara h 6 shows a common structural motif – a helix bundle. In Ara h 2 and Ara h 6, the bundle is formed by a continuous stretch of amino acids, while in Ara h 1 and Ara h 3, the bundle is formed by distant stretches of residues.

Helix bundles have been found in many proteins and are stable structures (see, for example, [11]). Their interiors are often formed by hydrophobic residues found at the fourth and seventh positions of the constituent helices [12,13]. Loops of different lengths and sequences have been found in the interhelical regions and those differences do not hinder the formation of the bundle, attesting to the stability of the bundle.

The helix bundles in Ara h 3 and in Ara h 1 are formed normally in the biologically active form of these molecules. This natural tendency of the helices to associate suggests that helix bundles can also form from the digestion fragments in the absence of the rest of the structure.

Table 1
Residue contacts in the helix bundle of Ara h 3.

[2]									
Leu 271				2					
Phe 275		3							
[3]									
Leu 286		5			1	1			
Arg 287			4	2					
[4]									
Tyr 499		2					1		
[5]									
Arg 503								1	
Gln 505					2				
Ala 506									1
Arg 507								18	
Gln 508						1			
Leu 509				3	1	1			
	Phe 263	Phe 266	Pro 268	Leu 271	Phe 275	Ile 282	Leu 286	Glu 492	Ala 496
	[1]			[2]		[3]		[4]	



In this matrix of contacts, the element $c(i,j)$ represents the number of interacting atom pairs, one from residue i and the other from residue j . Two atoms are said to be in contact if the distance between them is at most 4 Ångströms. The double-headed arrow indicates the residues which are absent in the less-allergenic isoform. The helices where the residues are located are indicated by bracketed numbers.

Table 2a
Amino acid sequences of the allergenic and less-allergenic isoforms of Ara h 3 in relation to the beta sheets in the three-dimensional structure of the molecule.

30	40	50	60	70	80	90	
ISFRQQPEENACQFQRLNAQRPD	<u>NRI</u> SEGGYIETWNPNNQEFECAGV	<u>ALS</u> R <u>LV</u> LRR <u>N</u> <u>AL</u> RRPFYSNAP <u>Q</u>	ACH91862				
VTFRQGGREENECQFQRLNAQRPD	NRISEGGYIETWNPNNQEFQCAGVALSRTVLRN <u>AL</u> RRPFYSNAPL	AAT39430					
100	110	120	130	140	150		
<u>EIFIQ</u> QGRGY <u>FGLI</u> FPGCPST <u>YEE</u> PAQQGRRYQSQRPPRLQ-EEDQ-SQQ <u>QDS</u> HQKV <u>HRF</u> NEG <u>DL</u> IAV	ACH91862						
EIVVQQGSGYFGLIFPGCPSTYEEPAQEGRRYQSQKPSRRFQVGQDDPSQQ <u>QDS</u> HQKVH <u>RF</u> DEG <u>DL</u> IAV	AAT39430						
160	170	180	190	200	210	220	
PTGV <u>AF</u> WLYNDHDTD <u>VVA</u> VS <u>LT</u> DTNNNDNQLDQFPR <u>FN</u> LAGNHEQEFLRYQQQSRQSRRRSLPYSPYSP	ACH91862						
PTGVAFWMYNDSDTDVTVTLSDTSSIHNQLDQFPRRFYLAGNQEQEFLRYQQQGSRPYRQISPRVRG	AAT39430						
230	240	250	260	270	280	290	
QSQPRQEEREFSPRGQHSRRERAGQEEHEGGNIFSGFTPEFLAQAFQVDDRQIVQNLRGENESEEQG <u>AI</u>	ACH91862						
DEQENEGSNIFSGFAQ-----EFLQHAFQV-DRQTVENLRGENEREQ <u>GAI</u>	AAT39430						
300	310	320	330	340	350	360	
<u>VT</u> VRGGLRILSPD---RKRGADEEEYDEDEYDEEDRRRGRGRSGNGIEETICTATV <u>KKN</u> IGRNR	ACH91862						
VTVKGGLRILSPDEEDESRRSPSRREEFDEDRSRPQQRGKYDENRRGYKNGIEETICSASV <u>KKN</u> LGRSS	AAT39430						
370	380	390	400	410	420	430	
SPD <u>IYN</u> PQ <u>GSL</u> K <u>TAN</u> ELNLLILRWLGL <u>SAEYGN</u> LYRN <u>LFV</u> PHYNTNAH <u>SI</u> IYAL <u>RG</u> AHVQVVD <u>SNGN</u>	ACH91862						
NPDIYNPQAGSLRSVNELDLPILGWLGLSAQHGTIYRNAMFVPHYTLNAHTIVVALNGRAHVQVVD <u>SNGN</u>	AAT39430						
440	450	460	470	480	490	500	
<u>RVYDEEL</u> QEGH <u>VLVVP</u> QNF <u>AVAG</u> KSQSD <u>NFEYVAF</u> KTDSRPS <u>IAN</u> LAGENSVIDNLPEEVVANSYGLPRE	ACH91862						
RVYDEELQEGHVLVVPQNF <u>AVAAKAQ</u> SENYEYLAFTKDSRPS <u>IAN</u> LAGENSIIDNLPEEVVANSYRLPRE	AAT39430						
510	520	530					
QARQLKNNNPFFK <u>FV</u> PP-SQQSPRAVA	ACH91862						
QARQLKNNNPFFK <u>FV</u> PPFDHOSMREVA	AAT39430						

Numbering follows that in PDB Entry 3C3V. The segments which are in beta sheets are shown bold and underlined. Beta sheet designations are from PDB Entry 3C3V. The allergenic isoform is identified by the GenBank Accession Code ACH91862; the less allergenic isoform by AAT39430.

Table 2b

Amino acid sequences of the allergenic and less-allergenic isoforms of Ara h 3 sequences in relation to the alpha helices in the three-dimensional structure of the molecule.

30	40	50	60	70	80	90	
ISFRQQPEENACQFQRLNAQRPDNRIESEGYYIETWNPNNQEFECAGVALSRLVLRNALRRPFYSNAPQ	ACH91862						
VTFRQQGEENECQFQRLNAQRPDNRIESEGYYIETWNPNNQEFQAGVALSRTVLRNALRRPFYSNAPL	AAT39430						
100	110	120	130	140	150		
EIFIQQGRGYFGLIFPGCPSTYEPAQQGRRYQSQRPPRLQ-EEDQ-SQQQQDSHQKVHRFNEGDLIAV	ACH91862						
EIYVQQGSGYFGLIFPGCPSTYEPAQEGRRYQSQKPSRRFQVGQDDPSQQQQDSHQKVHRFDEGDLIAV	AAT39430						
160	170	180	190	200	210	220	
PTGVAFWLYNDHDTDVAVSLTDTNNNDNQLDQFPRRFNLAGNHEQEFLRYQQQSRQSRRLSPYSPYSP	ACH91862						
PTGVAFWMYNDHDTDVVTVTLSDTSSIHNLQDQFPRRFYLAGNQEQEFLRYQQQSGSRPHYRQISPRVRG	AAT39430						
230	240	250	260	270	280	290	
QSQPRQEEREFSPRGQHSRRERAGQEEHEGGN <u>IFSGFTPEFLAQAFQVDRQIVQNLR</u> GENESEEEQGAI	ACH91862						
DEQENEGSNIFSGFAQ-----EFLQHAFQV-DRQTVENLRGENEREQQGAI	AAT39430						
<1> <- 2 -> <- 3 ->							
300	310	320	330	340	350	360	
VTVRGGLRILSPD---RKRGADEEEYDEDEYDEEDRRRGRSGRSGNGIETICTATVKKNIGRNR	ACH91862						
VTVKGGLRILSPDEDESSRSPSRREEFDEDRSRPQQRKYDENRRGYKNGIETICSASVKKNLGRSS	AAT39430						
370	380	390	400	410	420	430	
SPDIYNPQAGSLKTANELNLLILRWLGLSAEYGNLYRNALFVPHYNTNAHSIIYALRGRAHVQVVDNSGN	ACH91862						
NPDIYNPQAGSLRSVNELDLPLGLWLGLSAQHGTIYRNAMFVPHYTLNAHTIVVALNGRAHVQVVDNSGN	AAT39430						
440	450	460	470	480	490	500	
RVYDEELQEGHVLVVPQNFVAVAGKSQSDNFEYVAFKTDSPRSIANLAGENSVIDNL <u>PEEVVANSYGLPRE</u>	ACH91862						
RVYDEELQEGHVLVVPQNFVAVAAKAQSENYEYLAFTKTDSPRSIANLAGENSIIDNLPEEVVANSYRLPRE	AAT39430						
<- 4 -> <-							
510	520	530					
<u>QARQLKNN</u> NPFFKFFVPP-SQQSPRAVA	ACH91862						
QARQLKNNNPFFKFFVPPFDHQSMEVA	AAT39430						
5	->						

Numbering follows that in PDB Entry 3C3V. The segments which form the helix bundle are shown bold and underlined. Helix designations are from PDB Entry 3C3V. The allergenic isoform is identified by the GenBank Accession Code ACH91862; the less allergenic isoform by AAT39430.

The major deletion found in the less-allergenic Ara h 3 isoform results in the loss of helix 1 and the N-terminal part of helix 2 of the bundle (Table 1). The deletion of the amino acids Phe263, Phe266, and Pro268 will result in a major loss of the contact (16 of the 49 residue pairs, or 32.7%) in the bundle (Table 1). Those three residues are thus major contributors to the contact and their loss is predicted to cause a major disruption in the structure of the bundle and its stability. Of particular importance is the loss of the two phenylalanines, Phe263 and Phe266. These residues contribute to the hydrophobic interior of the bundle and are expected to facilitate the formation of a helix bundle from digestion fragments of Ara h 3.

We propose that the diminished antigenicity of the Ara h 3 isoform is due to the distinct possibility that gastrointestinal digestion of the isoform will result in fragments that cannot form a stable helix bundle for presentation to the immune system.

Our hypothesis can be confirmed by synthesizing fragments of the allergenic Ara h 3 isoform that include the segments which form the helix bundle. We predict that those fragments will form stable helix bundles that would be recognized by specific IgE from individuals who have peanut allergy. We further predict that corresponding fragments from the less-allergenic Ara h 3 isoform will not form stable helix bundles and will not be recognized by specific IgE.

Conclusion

Peanut allergy is increasing in prevalence throughout the world yet certain countries, such as China, continue to have a low incidence of peanut allergy despite high levels of peanut consumption. Varied methods of processing peanuts, whether roasting, frying, or boiling, seem to confer different allergenic properties on peanuts, possibly due to altered tertiary structures or binding sites of allergens. Determining the protein variations in the major and minor allergens may lead to the development of “hypoallergenic” or therapeutic plant hybrids in the future.

There are three independently determined sequences for the less-allergenic isoform of Ara h 3 (GenBank Entries AAT39430 [7], AB117154 [14], and ADQ53859 [15]). This strongly suggests that the existence of this isoform is not uncommon. This also suggests that the differences observed between the less-allergenic and the more common isoform of Ara h 3 do not interfere with the biological function of the molecule. There may be isoforms of the other peanut proteins that are also less allergenic. Finding such isoforms may lead to the generation of plant hybrids that will not produce allergenic peanuts. We believe that the most attractive isoforms in this regard are those which result in the destabilization of the allergenic structure. The same strategy may apply to other food allergies as well.

Conflict of interest statement

There are no conflicts of interest. The views expressed in this article are those of the authors and do not reflect the official policy of the Department of Army/Navy/Air Force, Department of Defense, or US Government.

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