



Homology modelling and conformational analysis of IgE-binding epitopes of Ara h 3 and other legumin allergens with a cupin fold from tree nuts

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Abstract

Linear IgE-binding epitopes identified in legumin allergens of peanut (Ara h 3) and other allergenic tree nuts (Jug r 4 of walnut, Cor a 9 of hazelnut, Ana o 2 cashew nut) were mapped on three-dimensional models of the proteins built up by homology modelling. A conformational analysis revealed that consensual surface-exposed IgE-binding epitopes exhibited some structural homology susceptible to account for the IgE-binding cross-reactivity observed among peanut and tree nut allergens. This structurally related cross-reactivity seems irrespective of the botanical origin of the allergens and thus demands that persons allergic to peanut avoid other three nuts to prevent possible allergic reactions. IgE-binding epitopes similar to those found in 11S globulin allergens do not apparently occur in other vicilin allergens with the cupin fold from peanut (Ara h 1) or tree nuts (Jug r 2 of walnut, Cor a 1 of hazel nut, Ana o 3 of cashew nut).

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1. Introduction

Allergy to peanut (*Arachis hypogaea*), which has dramatically increased in industrialized countries during the past 10 years (Sicherer et al., 2003; Sampson, 2004; Sicherer and Sampson, 2006; Palmer and Burks, 2006), is considered as a potentially severe food allergy susceptible to trigger anaphylactic shocks in sensitized subjects. The major peanut allergens responsible for the IgE-binding reactivity of sensitized patients have been identified as seed storage proteins including a 7S globulin or vicilin (Ara h 1) (Burks et al., 1995), a 2S water-soluble albumin (Ara h 2) (Viquez et al., 2001) and a 11S globulin or legumin (Ara h 3) (Rabjohn et al., 1999). Linear IgE-binding epi-

topes have been characterized using the Spot technology along the amino acid sequences of Ara h 1 (Burks et al., 1997; Shin et al., 1998), Ara h 2 (Stanley et al., 1997) and Ara h 3 (Rabjohn et al., 1999). Our knowledge on these IgE-binding epitopes is of paramount importance to develop new tools for both the identification of trace amounts of peanut in food products, the clinical diagnosis and treatment of peanut allergy, especially in children. In this respect, the availability of mutated allergens with modified IgE-binding epitopes that retain their T-epitope reactivity but fail to interact with the corresponding IgE should allow the development of vaccines for the immunotherapy of peanut allergy (Pons et al., 2005). Other legume seeds (soybean, sesame) or tree nuts (cashew nut, Pecan nut, walnut) also contain seed storage protein orthologs of the globulins (Ara h 1, Ara h 3) and 2S albumins (Ara h 2) of peanut, susceptible to provoke allergic reactions in sensitized people (Teuber, 2003). With respect to these protein homologies, some IgE-binding cross-reactivity has been observed among tree nuts and between peanut and other tree nuts (Fig. 1) (Teuber and Peterson, 1999; Beardslee et al., 2000; de Leon et al., 2003; Asero et al., 2004; Nguyen et al., 2004; de Leon et al., 2005; Goetz et al., 2005). Beside their clinical relevance (van Ree, 2004), these IgE-binding cross-reactions bring to recommend the avoidance of tree nuts and tree nut-containing food to individuals allergic to

Abbreviations: Ana o 2, Ana o 3, allergens 2 (legumin) and 3 (vicilin) from *Anacardium orientale* (cashew nut); Ara h 1, Ara h 3, allergens 1 (vicilin) and 3 (legumin) from *Arachis hypogaea* (peanut); Ber e 2, allergen 2 (legumin) from *Bertholletia excelsa* (Brazil nut); Cor a 1, Cor a 9, allergens 1 (vicilin) and 9 (legumin) from *Corylus avellana* (hazel nut); Fag e 1, allergen 1 (13S globulin) from *Fagopyrum esculentum* (buckwheat); HCA, hydrophobic cluster analysis; Jug r 2, Jug r 4, allergens 2 (vicilin) and 4 (legumin) from *Juglans regia* (wall nut).

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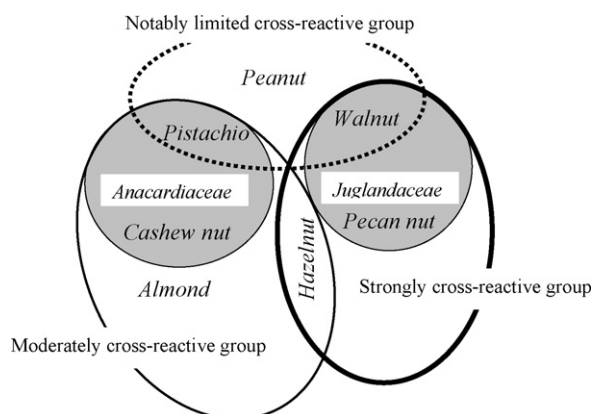


Fig. 1. Cartoon illustrating some of the IgE-binding cross-reactivities observed among tree nuts and between peanut and other tree nuts (Goetz et al., 2005). Species belonging to the same botanical family are grey circled.

peanut and *vice versa*. Among the major peanut allergens, Ara h 3, that had been classically considered as a less potent allergen, might be more allergenic than previously thought, particularly in children (Restani et al., 2005). This prompted us to build three-dimensional models of Ara h 3 and related legumin allergens from other tree nuts (Rout and Chrungoo, 1996; Helm et al., 2000; Beardslee et al., 2000; Beyer et al., 2002; Asero et al., 2002; Wang et al., 2003) and perform a detailed conformational analysis of their IgE-binding epitopes to assess some possible IgE-binding cross-reactivity among these seed allergens. Our results reveal the occurrence of sterically related IgE-binding epitopes on the molecular surface of 11S globulin allergens that possibly accounts in part for the cross-reactivity reported between peanut and other tree allergens. They also support the existence of extended conformational IgE-binding epitopes in these allergenic proteins.

2. Materials and methods

Multiple amino acid sequence alignments were carried out with CLUSTAL-X (Thompson et al., 1997) and displayed with ESPript (Gouet et al., 1999) using the structural Risler's matrix for homologous residues (Risler et al., 1998).

The hydrophobic cluster analysis (HCA) (Gaboriaud et al., 1987) was performed to delineate the conserved secondary structural features (strands of β -sheet and stretches of α -helix) along the amino acid sequences of Ara h 3, Ana o 2, Ber e 1, Cor a 9 and Jug r 1, by comparison with the soybean (*Glycine max*) glycinin A3B4 [32] used as a model. HCA plots were generated using the HCA server (<http://www.lmcp.jussieu.fr>).

Molecular modelling of the legumin allergens was carried out on a Silicon Graphics O2 R10000 workstation, using the programs InsightII, Homology and Discover3 (Accelrys, San Diego CA, USA). The X-ray solved cupin structure available at the PDB for the legumin A3B4 glycinin from soybean (PDB code 1OD5) (Adachi et al., 2003), share percentages of both identity (from 42% up to 51%) and homology (from 77% up to 86%) with other legumins (Fig. 1) and thus could be used as a template to build rather accurate three-dimensional models for the

legumin allergens. Desordered regions for which X-ray coordinates are not available in 1OD5 were omitted in the models built for other allergens. Steric conflicts were corrected during the model building procedure using the rotamer library (Ponder and Richards, 1987) and the search algorithm implemented in the Homology program (Mas et al., 1992) to maintain proper side-chain orientation. The geometry of loop regions was corrected using the refine option of TurboFrodo (Roussel and Cambillau, 1990). An energy minimization of the final models was carried out by 200–300 cycles of steepest descent using the cvff force-field of Discover. The program TurboFrodo was run to draw the Ramachandran plot and to perform the superposition of the model with the template protein. PROCHECK (Laskowski et al., 1993) was used to assess the geometric quality of the three-dimensional models. As an example, 77% of the residues of Ana o 2 were correctly assigned on the best allowed regions of the Ramachandran plot, the remaining residues being located in the generously allowed regions of the plot except for Arg323 which occurs in the non allowed region (result not shown).

Ara h 3 and other legumin allergens were tentatively assigned as homotrimers displaying an arrangement of the cupin motifs similar to that found in the soybean proglycinin A1aB1b homotrimer (PDB code 1FXZ) (Adachi et al., 2001). Most of the amino acid residues occurring at the interfaces of the cupin motifs of the proglycinin homotrimer are conserved in other legumins. Accordingly, no steric clash was observed upon the homotrimeric assembly of the cupin protomers of Ara h 3, Ana o 2, Ber e 1, Cor a 9 and Jug r 4.

Electrostatic potentials were calculated as previously described (Nicholls et al., 1991) and displayed with GRASP using the parse3 parameters (Gilson and Honing, 1987). The solvent probe radius used for molecular surfaces was 1.4 Å and a standard 2.0 Å-Stern layer was used to exclude ions from the molecular surface. The inner and outer dielectric constants applied to the protein and the solvent were, respectively, fixed at 4.0 and 80.0 and the calculations were performed keeping a salt concentration of 0.145 M.

Surface areas of the previously characterized linear IgE-binding epitopes on the molecular surface of the modelled legumin allergens were calculated using GRASP. The overall conformation of the linear IgE-binding epitopes on the molecular surface of the legumin allergens was displayed with PyMOL of W.L. DeLano (<http://www.pymol.org>).

The Conformational Epitope Prediction (CPE) server (<http://bioinfo.ernet.in/cep.htm>) (Kulkarni-Kale et al., 2005) was used to predict the conformational epitopes on the surface of the modelled allergens.

An unrooted tree was built from the available legumin amino acid sequences using the parsimony algorithm of the PHYLIP package (available from J. Felsenstein at <http://evolution.genetics.washington.edu/phylip.html>). The tree was rendered with TreeView (Page, 1996).

3. Results

The three-dimensional models built from the X-ray coordinates of the A3B4 glycinin (PDB code 1OD5) for Ara h 3 and

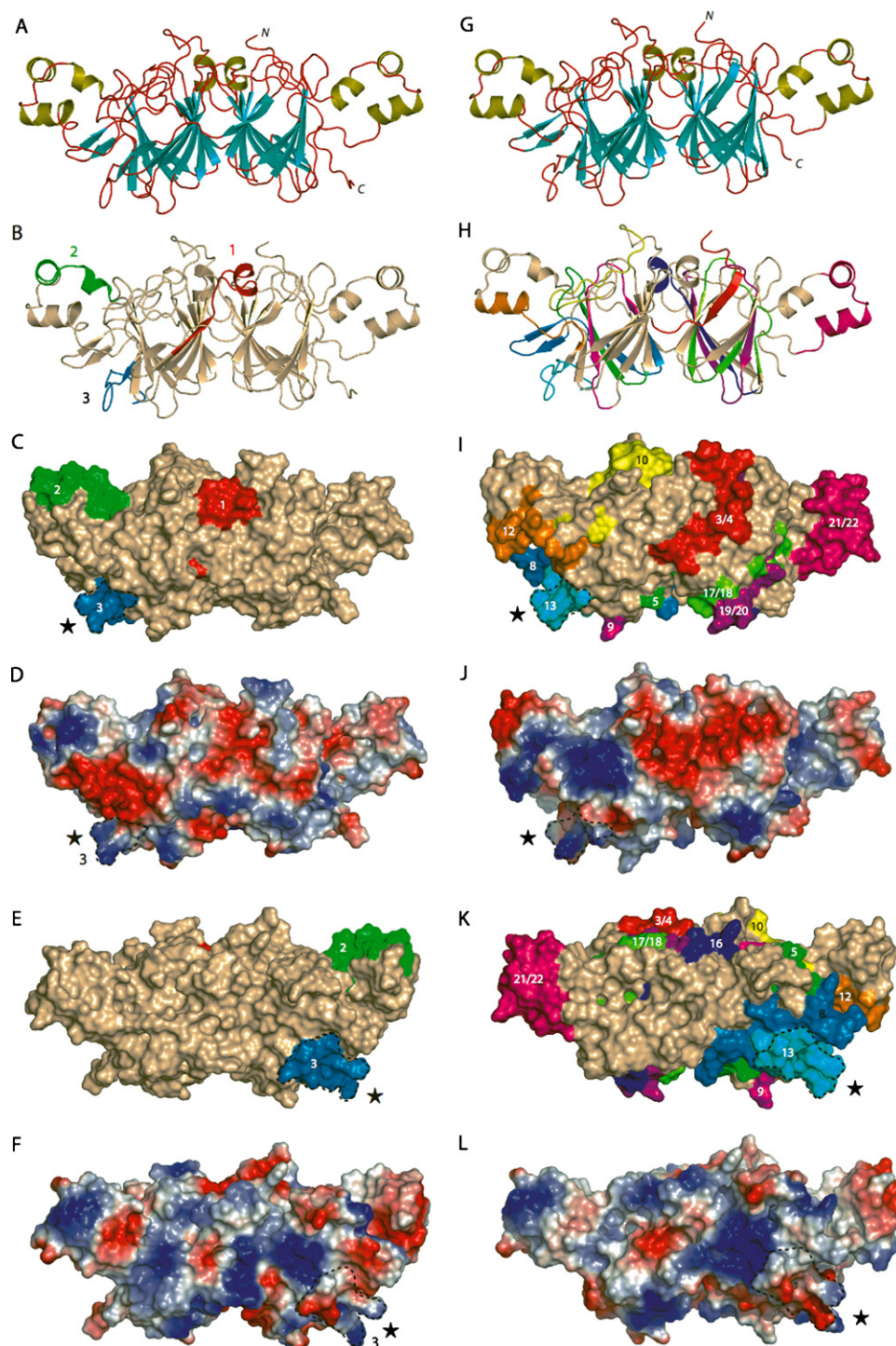


Fig. 2. Ribbon diagram of Ara h 3 (A) and Ana o 2 (G) protomers. α -Helices, β -strands and coil structures are colored olive green, gree-blue and red, respectively. N and C indicate the N- and C-terminal ends of the polypeptide chains. Localization of linear IgE-binding epitopes on the ribbon diagram of Ara h 3 (B) and Ana o 2 (H). Localization of linear IgE-binding epitopes on both faces of the molecular surface of Ara h 3 (C, E) and Ana o 2 (I, K). Linear IgE-binding epitopes are numbered according to their identification along the polypeptide chain of allergens. Mapping of electrostatic potentials on both faces of the molecular surface of Ara h 3 (D, F) and Ana o 2 (J, L). The positive and negative electrostatic potentials are displayed at -5 kT and $+5$ kT levels and colored blue and red, respectively. Neutral surfaces are white. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

other tree nut legumin allergens Ana o 2, Ber e 1, Cor a 9 and Jug r 4, all consist of a cupin motif built from two tandemly arrayed modules related by a pseudo-dyad axis (Fig. 2A and G). In each module, two walls of antiparallel β -strands forming the core structure of the module associate to an extended loop

containing three α -helices. Both modules are connected by a linker region made of two short stretches of α -helix to form the legumin protomer. According to the conserved character of 11S globulins, all of these proteins are assumed to possess a similar cupin fold for their protomers with, however, a few discrepan-

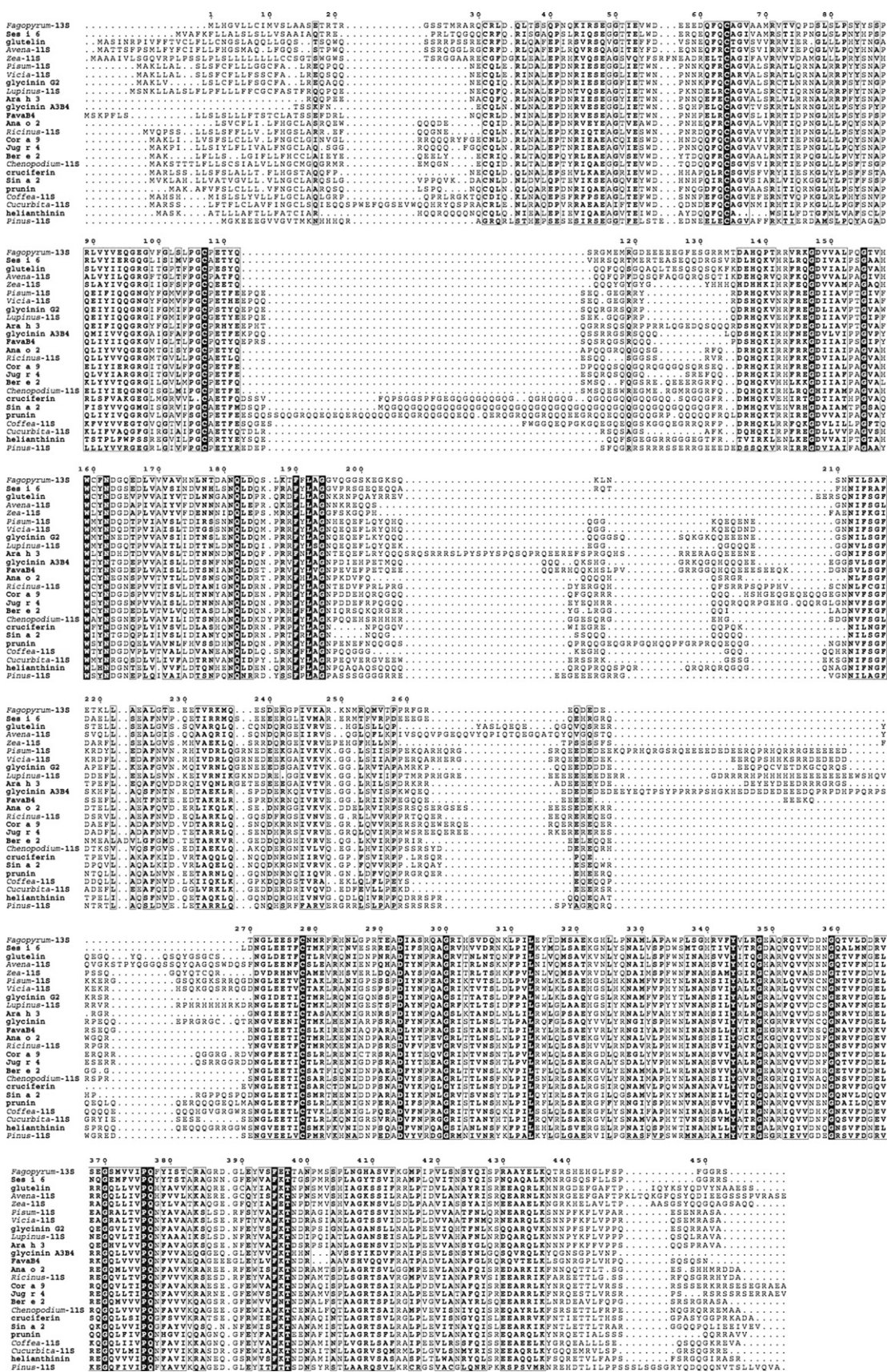


Fig. 3. Multiple amino acid sequence alignment of 11S globulins of different origins. Identical residues are in black-boxed white letters and structurally homologous residues are in open boxed black letters. Gaps are indicated by points (.). 11S globulins are identified by their allergen code or their botanical (plant genus name) origin.

Table 1
Amino acid sequence of the linear IgE-binding epitopes characterized on Ara h 3

epitope 1	30 I E T W N P N N Q E F E C A G 44	(-3)
epitope 2	237 G N I F S C F T P E F L E Q A 251	(-2)
epitope 3	276 V T V R C G L R I L S P D R 289	(3)

The best and moderately exposed residues at the surface of Ara h 3 are in black-boxed white letters and grey-boxed black letters, respectively. Residues recognized as critical to the IgE-binding activity of epitopes are indicated by an asterisk (*). Residues are numbered according to the amino acid sequence of Ara h 3. No data is available for epitope 4, which is lacking in the three-dimensional model. Net charges are indicated in brackets.

cies concerning the occurrence of some extra-loops, e.g. in Ara h 3 or Sin a 2, as shown from the amino acid sequence alignments (Fig. 3).

The putative arrangement of three Ara h 3 or Ana o 2 protomers around a three-fold symmetry axis did not create any steric hindrance and gave a homotrimeric structure in which the three cupin motifs occupy the three faces of a flattened prism (Fig. 4A and F). This prism-shaped homotrimeric arrangement of 11S globulin similar to that found in glycinin A3B4 is supposed to occur in other legumins of different origins. In this respect, both the hydrophilic and hydrophobic amino acid residues ensuring the connection of the three protomers to form the glycinin A3B4 homotrimer are usually well conserved in other legumins. Mapping of the electrostatic potentials on the molecular surface of the Ara h 3 and Ana o 2 homotrimers indicates that both faces of the trimers definitely differ by their overall charges. One face is predominantly electronegatively charged whereas the opposite face is essentially electropositive (Fig. 4C, E, H and J). A very similar repartition of charged residues was observed in the glycinin A3B4 homotrimer (Adachi et al., 2001) and other modelled Cor a 9, Jug r 4 or Ber e 2 homotrimers, which also exhibit two differently charged faces (result not shown). These charge discrepancies apparently result from the occurrence of two differently exposed acidic and basic subunits in the legumin allergens.

The homohexameric models proposed for the Ara h 3 and other tree nut 11S globulins Ana o 2, Ber e 1, Cor a 9 and Jug r 4 takes into account the electrostatic attraction as a driving force for the fast association of two homotrimers and the conserved residues (compared to those found in the glycinin A3B4 homohexamer (Adachi et al., 2003)) responsible for the decrease in the accessible surface area of both homotrimers upon their association around a three-fold axis. The homohexameric structures (result not shown) resulting from the stacking of two similarly oriented homotrimers thus exhibit a electropositive face and an opposite electronegative face like in the glycinin A3B4 hexamer (Adachi et al., 2003).

The three linear epitopes characterized on the surface of the Ana o 2 protomer (Wang et al., 2003) exhibited very different shapes with accessible surface areas ranging from 320 Å² (epitope 9) up to 660 Å² (epitopes 19–20) (Fig. 2H, I and K). Most of these epitopes contain either negatively (Asp, Glu) or positively (Arg, Lys) charged residues (Fig. 2J and L) but predominantly occur on the acidic face of the Ana o 2 trimer (Fig. 4G and

H). In this respect, the quaternary association of the protomers restricts the surface exposition of some linear epitopes on the homotrimer and, especially, of epitope 21/22 which is located at one end of the protomers and becomes partly buried upon their trimeric association (Figs. 2I, K and 4G, I). Quite surprisingly, the Ara h 3 protomer exhibits only three well identified linear IgE-binding epitopes (Rabjohn et al., 1999) (Fig. 2B, C and E). The amino acid residues that have recognized as essential for the IgE-binding activity (Table 1) mostly coincide with the best-exposed residues at the surface of the epitopes. These epitopes essentially locate on the acidic face of the homotrimer (Fig. 4B–E).

The linear IgE-binding epitope 13 of Ana o 2 (stretch 231–247) well coincides with epitope 3 (stretch 276–289) of Ara h 3 (Fig. 5A and G). Both epitopes form a strongly charged area on the molecular surface of the corresponding allergens and exhibit a rather conserved overall conformation characterized by some protruding/charged residues (Fig. 5B and H). An inspection of the corresponding region of other legumin allergens and, especially, of other tree nut allergens (Cor a 9, Jug r 4), revealed that despite a moderate degree of amino acid sequence conservation (Table 2) this region possesses a very similar overall conformation and often exhibit similarly protruding/charged residues (Fig. 5C and E). However, the overall conformation of this region in Ber e 2 readily differs from Ara h 3 and other legumin allergens (result not shown). From these common structural features it can be concluded that linear IgE-binding epitope 13 of Ana o 2 correspond to a consensual epitope occurring in other three nut legumin allergens like Ara h 3, Cor a 9 and Jug r 4 but not Ber e 1. This unique epitopic community apparently extends beyond the group of tree nut legumin allergens since the homologous region of the G2 glycinin allergen has been previously recognized as a IgE-binding epitope (Helm et al., 2000). However, this region exhibit rather different structural features and often lacks charged residues in other legumins from different species, e.g. glutelin from rice (*Oryza sativa*) (Fig. 5I and J).

Epitope 2 (linear stretch 237–251) of Ara h 3 has been previously predicted to have counterparts in other tree nut legumins, especially in Cor a 9 (Beyer et al., 2002). This IgE-binding epitope located on two α-helical segments is nicely exposed at one extremity of the Ara h 3 protomer (Fig. 2C and E) but weakly exposed at the surface of the Ara h 3 homotrimer due to the end-to-end association of the protomers into the trimer (Fig. 4B and D). Actually, counterparts of this epitopic region with homolo-

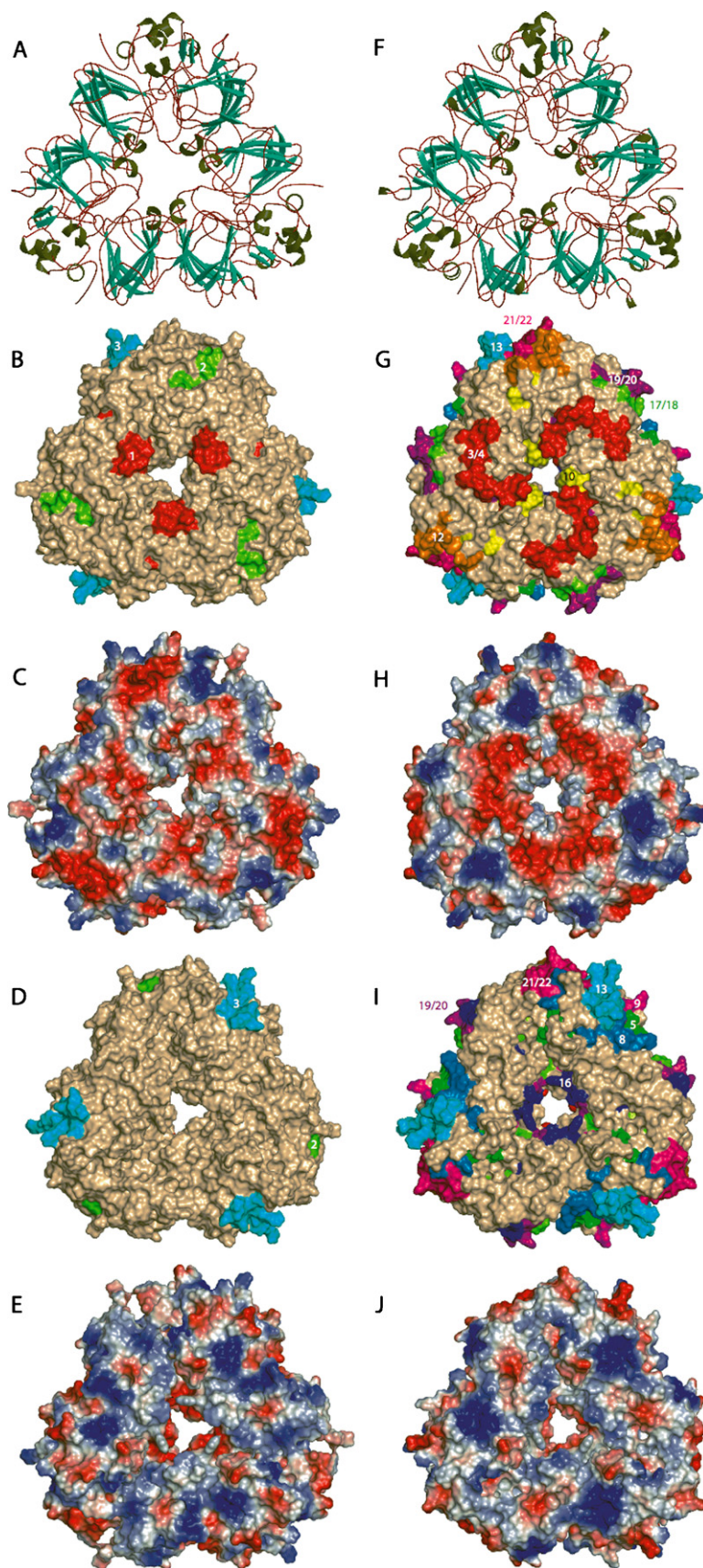


Table 2

Comparison of the amino acid sequence of linear IgE-binding epitope 13 characterized on Ana o 2 (stretch 231–247) with homologous regions of other 11S legumins

Ana o 2	I	V	K	V	K	D	D	E	-	L	R	V	I	R	P	S	R	S	(2)
Cor a 9	I	V	K	V	E	G	R	-	-	L	Q	V	V	R	P	E	R	S	(2)
Jug r 4	I	V	R	V	E	G	R	Q	-	L	Q	V	I	R	P	R	W	S	(3)
Ber e 2	I	V	R	V	E	Q	G	-	-	L	K	V	I	R	P	P	R	I	(3)
glycinin A3B4	I	V	T	V	E	G	G	-	-	L	S	V	I	S	P	K	W	Q	(0)
Ara h 3	I	V	T	V	R	G	G	-	-	L	R	I	L	S	P	D	R	K	(4)
FabaB4	I	V	R	V	E	G	G	-	-	L	R	I	I	N	P	E	G	Q	(0)
Pisum-11S	I	V	K	V	K	G	G	-	-	L	S	I	I	S	P	P	E	K	(2)
cruciferin	I	I	R	V	Q	G	P	-	-	F	S	V	I	R	P	P	-	L	(2)
prunin	I	I	Q	V	R	G	N	-	-	L	D	F	V	Q	P	P	R	G	(1)
Ricinus-11S	I	V	N	V	E	G	R	-	-	L	L	V	V	R	P	P	R	T	(2)
Coffea-11S	T	V	R	A	E	K	-	-	-	L	Q	L	F	L	P	E	Y	S	(-1)
Fagopyrum-13S	I	V	K	A	R	K	N	M	-	R	Q	M	V	T	P	P	R	F	(5)
glutelin	I	V	R	V	E	H	G	-	-	L	S	L	L	Q	P	Y	A	S	(0)
Zea-11S	I	V	R	V	E	P	E	H	G	F	H	L	L	N	P	T	P	S	(-1)

Identical residues are in black-boxed white letters and structurally homologous residues are grey boxed. Net charges are indicated in brackets.

Table 3

Comparison of the amino acid sequence of IgE-binding epitope 2 of Ara h 3 with the corresponding regions of other 11S legumins

Ara h 3	237	G	N	I	F	S	G	F	T	P	E	F	L	E	Q	A	F	252	(-2)
Ana o 2		R	N	L	F	S	G	F	D	T	E	L	L	A	E	A	F		(-2)
Ber e 2		D	N	V	F	K	G	F	N	M	E	A	L	A	D	V	L		(-2)
Cor a 9		N	N	V	F	S	G	F	D	A	E	F	L	A	D	A	F		(-3)
Jug r 4		N	N	V	F	S	G	F	D	A	D	F	L	A	D	A	F		(-3)
Glutelin		Q	N	I	F	S	G	F	S	T	E	L	L	S	E	A	L		(-2)
Glycinin G1	217	G	S	I	L	S	G	F	T	L	H	F	L	E	H	A	F	S V 235	(-1)

*

The IgE-binding epitope stretch characterized in glycinin G1 from soybean is indicated by a line and an asterisk (*). Identical residues are in black-boxed white letters and structurally homologous residues are grey boxed. Net charges are indicated in brackets.

gous sequences exist in other tree nut allergens including Ana o 2, Cor a 9 and Jug r 4 (Table 3) and this region almost completely overlaps a linear IgE-binding epitopic sequence stretch characterized in glycinin G1 (Beardslee et al., 2000). A very homologous sequence stretch also occurs in the 11S glutelin from rice. A detailed surface analysis performed on the modelled 11S allergens revealed a very similar conformation for this region, which in addition, possesses a marked electronegative character (Fig. 6A–E). Obviously, the linear IgE-binding epitope 2 of Ara h 3 corresponds to another sterically conserved epitope occurring in other three nut legumin allergens (Ana o 2, Cor a 9, Jug r 4) and even in glutelin from rice, a remotely related monocot plant. However, like for epitope 13 of Ana o 3 (epitope 3 of Ara h 3), the corresponding region in Ber e 2 exhibited a quite different conformation even though it displays a strongly electronegative character (Fig. 6F).

4. Discussion

Even though the homotrimeric vicilin (7S globulin) and homo-hexameric legumin (11S globulin) allergens of peanut and other tree nuts possess a similar cupin fold, they definitely differ

by their IgE-binding epitopes. Some of these epitopes are similarly exposed at the surface of the trimers but exhibit a rather distinct amino acid sequence and strikingly differ by their overall three-dimensional conformation. The different distribution of charged residues (Asp, Glu, Arg, Lys) on the molecular surface of the allergens, which significantly occur in IgE-binding epitopes of the cupin allergens, is apparently responsible for these discrepancies.

A detailed conformational analysis of the two consensus IgE-binding epitopes common to Ara h 3 and other tree nut allergens – with the exception of Ber e 2 – using the most energetically favourable conformers for the surface-exposed residues, revealed that bulked amino acid residues protrude at identical positions conferring the epitope a very similar spatial conformation. In addition, many of the protruding residues correspond to either electropositively (Arg, Lys) or electronegatively (Asp, Glu) residues even though the location of positive and negative charges slightly differ depending on the amino acid changes observed among the allergens (Figs. 5 and 6).

Compared to the 22 linear IgE-binding epitopes characterized on both faces of the Ana o 2 homotrimer, the occurrence of only three epitopes on the sole acidic face of the Ara h 3 trimer is

Fig. 4. Ribbon diagram of Ara h 3 (A) and Ana o 2 (F) homotrimers. The same color code has been used as in Fig. 2. Localization of linear IgE-binding epitopes on the electronegative face of Ara h 3 (B) and Ana o 2 (G) homotrimers. Localization of linear IgE-binding epitopes on the electropositive face of Ara h 3 (D) and Ana o 2 (I) homotrimers. Mapping of electrostatic potentials on both faces of Ara h 3 (C, E) and Ana o 2 (H, J) homotrimers showing the electronegative (C, H) and electropositive (E, J) of both homotrimers. The positive and negative electrostatic potentials are displayed at -5 kT and $+5$ kT levels and colored blue and red, respectively. Neutral surfaces are white. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

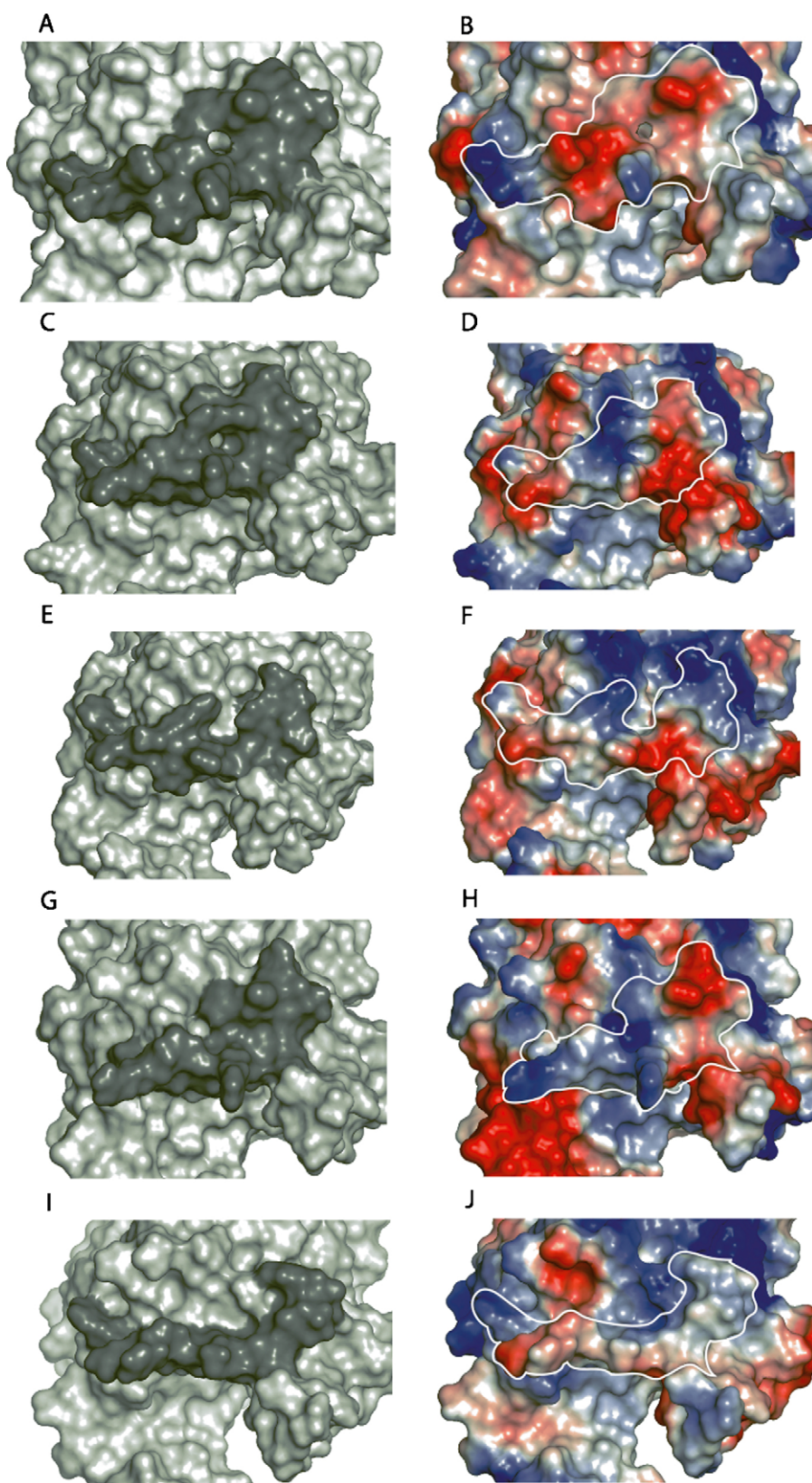
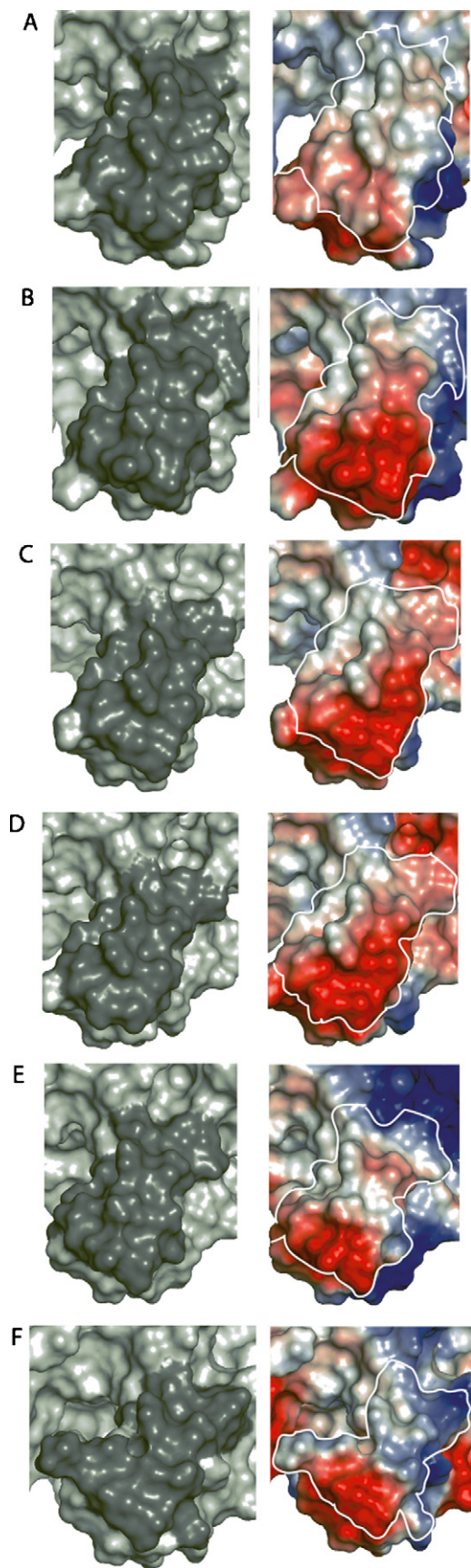


Fig. 5. Molecular surface of IgE-binding epitope 13 (deep grey) of Ana o 2 (A) and of the corresponding regions of Cor a 9 (C), Jug r 4 (E), Ara h 3 (G) and rice glutelin (I). The corresponding electrostatic potential maps of these epitopic regions of Ana o 2 (B), Cor a 9 (D), Jug r 4 (F), Ara h 3 (H) and rice glutelin (J) are white circled. The positive and negative electrostatic potentials are displayed at -5 kT and $+5$ kT levels and colored blue and red, respectively. Neutral surfaces are white. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)



rather puzzling in regard to a recent investigation that pointed out the dominant immunoreactivity of the basic subunit of Ara h 3 in children allergic to peanuts (Restani et al., 2005). In addition, Western blotting analyses using IgG directed against the Ara h 3 basic subunit showed the occurrence of some cross-reactive polypeptides common to Ara h 3, lupin conglutin and soybean Bg7S protein (Magni et al., 2005a). The cross-reactivity among the 11S globulin basic subunits was further confirmed using IgE from patients sensitized to lupin (Magni et al., 2005b). The three Ara h 3 epitopes most probably correspond to the major IgE-binding epitopes but some other epitopic regions should also occur on the surface of the Ara h 3 allergen.

The involvement of linear IgE-binding epitopes as exclusive supports for the IgE-binding cross-reactivity of 11S globulins has to be toned down by the possible occurrence on the surface of these allergens of conformational epitopes resulting from the coalescence of several linear epitopes susceptible to develop significant IgE-binding activity. Such a situation should occur on both faces of the Ana o 2 trimer where linear epitopes (epitopes 3, 4, 10 and epitope 12 + parts of epitopes 8, 21 and 22 on the acidic face) and (epitopes 8, 13 + parts of epitopes 5 and 9 on the basic face) cluster to form more extended IgE-binding areas (Fig. 4G and I). However, the area (often $>1000 \text{ \AA}^2$) covered by these confluent epitopes on the molecular surface exceeds by far the binding area ($600\text{--}700 \text{ \AA}^2$) of the corresponding IgE paratopes suggesting that only a few well exposed/protruding residues from each linear epitope should participate in the conformational epitope. Accordingly, exposed residues belonging to epitopes 13, 5 and 9 were predicted to participate in a conformational epitope using the CEP server facilities (<http://bioinfo.ernet.in/cep.htm>). Epitopes 21 and 22 were similarly predicted to participate in a unique conformational epitope located at one extremity of the Ana o 2 legumin protomer.

Other vicilin allergens with a cupin motif are presumably involved in the IgE-binding cross-reactivity between peanut (Ara h 1) and tree nuts (Ana o 3, Cor a 1, Jug r 4). Even though vicilin and legumin allergens from peanut and other tree nuts share a common trimeric organization, they definitely differ by the localization and extent of the linear IgE-binding epitopes characterized on their molecular surfaces. As an example, epitope mapping on both faces of the Ana o 2 (legumin) and Ana o 3 (vicilin) trimers reveals striking differences (result not shown). In addition, the localization of charged residues as shown from the mapping of electrostatic potentials on their molecular surfaces significantly differ and roughly coincide with the location of epitopic areas. This observation suggests that the IgE-binding cross-reactivity observed among peanut and other tree nuts more probably originate from the occurrence

Fig. 6. Molecular surface of IgE-binding epitope 2 (deep grey) of Ara h 3 (A), Ana o 2 (B), Cor a 9 (C), Jug r 4 (D), rice glutelin (E) and Ber e 2 (F). The corresponding electrostatic potential maps are white circled on the right panel. The positive and negative electrostatic potentials are displayed at -5 kT and $+5 \text{ kT}$ levels and colored blue and red, respectively. Neutral surfaces are white. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

of sterically similar epitopes on similar allergens rather than truly different target allergens sharing sterically similar epitopes. However, using a different approach based on the calculation of a property distance (PD) value deduced from the comparison of the physico-chemical properties and structure of peptides with sequences in other allergenic proteins (Schein et al., 2005) showed that the 498–507 major IgE-binding epitope (RRYTAR-LKEG) of Ara h 1 has two similar peptides in its one sequence (252-NRKSFNLDEG-261, 351-RRWSTRSSEN-360), two in Ara h 3 (124-HQKVHRFDEG-133, 412-RVYDEELQEG-421) and one in Ara h 4 (435-RVYDEELQEG-444) and Ara h 2 (28-RRCQSQLERA-37). According to these criteria, only 7 out of the 31 IgE-binding epitopes identified in both Ara h 1 and Ara h 2 using the SPOT method are truly unique, that seems to be overestimated. On our three-dimensional models built for Ara h 1 and Ara h 3, the major 498–507 epitope of Ara h 1 and the corresponding regions 124–133 and 412–421 of Ara h 3 exhibited quite different sizes and conformations that does not support the suspected epitopic correspondence. In addition, the amino acid sequence stretch 124-HQKVHRFDEG-133 appears as poorly accessible on the surface of Ara h 3 that should prevent its recognition by the corresponding IgE.

The occurrence of structurally related epitopes in legumin proteins from different edible seeds is in accordance with the recently observed *in vitro* cross-reactivity of serum IgE that bind a recombinant Jug r 4 fusion protein with protein extracts from hazelnut (Cor a 9), cashew (Ana o 2) and peanut (Ara h 3) (Wallowitz et al., 2006). Interestingly, the single serum sample (over three) that cross-reacted with a peanut extract was from a patient who tolerated peanuts while no cross-reaction occurred with a serum sample from another patient with anaphylaxis to peanut. As a possible clue for these unexpected results, it was suggested that whether Ara h 3 is not always clinically relevant in peanut allergy or is not sufficient to trigger an allergenic response in susceptible individuals when not associated to other allergens, e.g. Ara h 1 and Ara h 2. Co-sensitization to peanuts and tree nuts is a common clinical observation, most probably because of peanut-specific serum IgE cross-reacting with closely related tree nut allergens. However, it is unclear whether this cross-reactivity correlates to clinical cross-allergenicity. The frequent sensitization of peanut allergic patients to other tree nuts (estimated to 20–50% depending of the incriminated tree nuts (Moneret-Vautrin et al., 1998)) is scarcely associated to clinical symptoms due to the consumption of the cross-reacting tree nuts (estimated to 5% according to (Sicherer, 2001)). However, peanut-specific IgE that cross-react with tree nut allergens have been demonstrated to cause *in vitro* basophil activation that might contribute to tree nut allergic symptoms in peanut allergic subjects (de Leon et al., 2005). Conversely, peanut-lupin IgE and IgG1 cross-reactivity induced no clinical symptoms of cross-allergenicity in peanut-sensitized mouse strains (Liffrani et al., 2005). The discrepancy observed between cross-reactivity and cross-allergenicity that separates asymptomatic subjects from patients most likely depends of multiple factors including the level and polyclonality of the synthesized IgE, the polymorphism of the FcεRI receptors, the balance of T regulatory cells and Th1/Th2 cells, etc. (Bousquet et al., 2006).

Although allergies to peanut and tree nuts usually persist to adulthood, clinical investigations performed in peanut-allergic children showed that approximately 20% of the cases either outgrew their allergy or subsequently developed oral tolerance (Crespo et al., 2006). Some clinical case reports exist on patients previously sensitized but tolerant to peanut that have developed either tree nut or sesame allergy (Beausoleil and Spergel, 2006). Although these patients had negative results to peanut skin challenges, they exhibited systemic reactions and positive skin tests to the incriminated tree nut and sesame. In fact, the immunologic mechanism responsible for developing tolerance to one food group and not another still remains poorly understood. The fact that tree nut and sesame allergies can exist or develop in patients in spite of the development of tolerance to peanut suggests that distinct T cell epitopes occurring in closely related allergens of different origins, e.g. vicilins, are responsible for these discrepancies with respect to the balance between T regulatory cells, Th1 and Th2 cells. In addition, the allergen doses should be decisive in the development of either tolerance or allergic response (Akdis, 2006). Alternatively, tree nut sensitivity might simply reflect the greater propensity of previously peanut allergic patients to form IgE in response to allergenic proteins.

As shown from the phylogenetic tree built up from the available amino acid sequences of 11S globulins, proteins that have been well documented as genuine allergens occur in plant families as distantly related as Fabaceae (Ara h 3, lupin 11S globulin, soybean 11S globulin), Brassicaceae (Sin a 2) and Polygonaceae (Fag e 1) (Fig. 7). However, some allergenic 11S globulins, e.g. Ana o 2 (Anacardiaceae), Cor a 9 (Betulaceae) and Jug r 4 (Juglandaceae), belong to rather closely related families in the Fagales group. These phylogenetical relationships among allergenic 11S proteins have to be, however, interpreted with caution since many of them need to be more deeply investigated as possible allergens, especially in the Poaceae (maize), Asteraceae (sunflower) and Pedaliaceae (sesamum).

Deciphering the structural basis for the IgE-binding cross-reactivity among the major peanut and tree nut allergens brings new insights on the mutations that might be introduced in recombinant allergens to use the mutated molecules as possible vaccines. This immunotherapeutic approach should offer an innocuous alternative to the classical desensitization of allergic subjects with native allergens. In agreement with the new status of major allergen proposed for Ara h 3 (Restani et al., 2005), this peanut allergen thus appears as being as potentially interesting as other allergens Ara h 1 (vicilin) and Ara h 2 (2S albumin) for investigating its use in peanut immunotherapy. In addition to recombinant allergens with modified IgE-binding epitopes that retain their T-epitope reactivity but fail to interact with the corresponding IgE (Pons et al., 2005), synthetic peptides representing T cell epitopes should offer substantial advantages over mutated allergens because of their reduced ability to cross-link IgE at the surface of histamine-releasing activated cells (King et al., 2005). Synthetic T cell epitopic stretches identified on the amino acid sequences of the major peanut allergens, in particular Ara h 1, Ara h 2 and Ara h 3, should be used for down-regulate the allergic response and induce some tolerance to peanut allergens (de

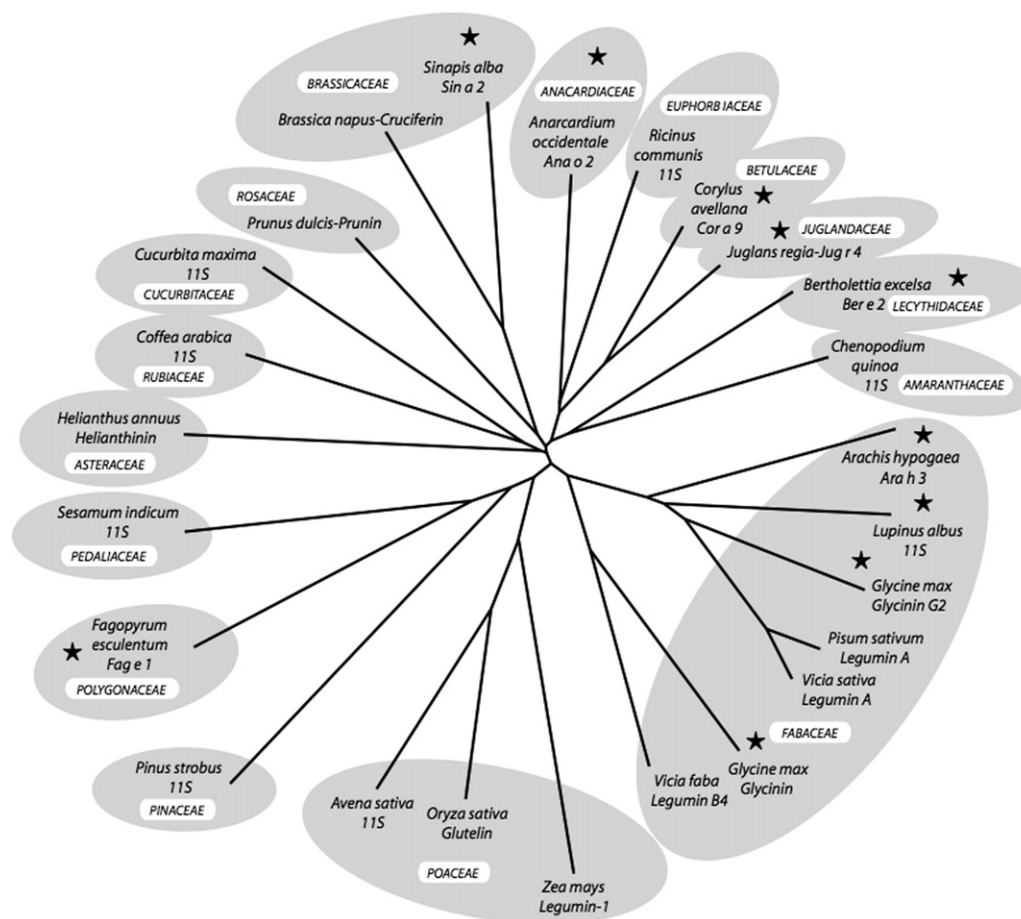


Fig. 7. Parsimony tree built from the 11S globulins of known amino acid sequences. The binomial names of plant species were used and species belonging to the same family are grey circled. 11S globulins, which have been well documented as food allergens are indicated by a star (*).

Leon et al., 2007). These synthetic vaccines might also result in some tolerance toward other closely related tree nut allergens that share closely related T cell epitopes with the cupin and 2S albumin allergens from peanut. In this respect, eight of the seventeen synthetic overlapping 20-mer peptides covering the entire sequence of Ara h 2 were identified as T cell epitopes with two highly reactive epitopes corresponding to the 19–47 and 73–119 sequence stretches of Ara h 2 (Glaspole et al., 2005). The second epitopic region is rather well conserved in other 2S albumin allergens from tree nuts ($\leq 60\%$ similarity) (Barre et al., 2005) and might also share T cell epitopes in common with Ara h 2. Recently, however, a novel Ara h 3 isoform (Ara h 3-im) lacking most of the fourth IgE-binding epitope previously characterized in Ara h 3 was identified in peanut (Kang and Gallo, 2007). This Ara h 3 isoform that displays a reduced allergenicity, might be used as a natural hypoallergen for vaccination purpose or to create hypoallergenic peanut lines through silencing of the major active allergens and selection by mutational breeding.

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