



Vicilin allergens of peanut and tree nuts (walnut, hazelnut and cashew nut) share structurally related IgE-binding epitopes

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Abstract

Surface-exposed IgE-binding epitopes of close overall conformation were characterized on the molecular surface of three-dimensional models built for the vicilin allergens of peanut (Ara h 1), walnut (Jug r 2), hazelnut (Cor a 11) and cashew nut (Ana o 1). They correspond to linear stretches of conserved amino acid sequences mainly located along the C-terminus of the polypeptide chains. A glyco-epitope corresponding to an exposed *N*-glycosylation site could also interfere with the IgE-binding epitopes. All these epitopic regions should participate in the IgE-binding cross-reactivity commonly reported between tree nuts or between peanut and some tree nuts in sensitized individuals. Owing to this epitopic community which constitutes a risk of cross-sensitization, the avoidance or a restricted consumption of other tree nuts should be recommended to peanut-sensitized individuals.

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1. Introduction

Peanut and tree nut allergy has now become a major public health concern, especially in children and adolescents and, particularly, in industrialized countries (Burks, 2003). Anaphylactic shocks to peanut and tree nut allergens are extremely severe and sometimes fatal in sensitized individuals (Sampson et al., 1992). Seed storage proteins of peanut and tree nuts have been recognized as the major allergens responsible for both the sensitization and the anaphylactic responses of susceptible individuals (Burks et al., 1995). In peanut (*Arachis hypogaea*), they

consist of the well-characterized vicilin (Ara h 1) (Viquez et al., 2003), 2S albumin (Ara h 2) (Viquez et al., 2001), and 11S globulin (Ara h 3) (Dodo et al., 2004). Very similar protein allergens occur in other tree nuts, e.g. the vicilin allergens Jug r 2 in walnut (*Juglans regia*) (Teuber et al., 1999), Cor a 11 in hazelnut (*Corylus avellana*) (Lauer et al., 2004), and Ana o 1 in cashew nut (*Anacardium orientale*) (Wang et al., 2002).

The exact relationship between peanut and tree nut allergy is still a matter of controversy but remains of paramount importance to recommend the (strict) avoidance of tree nuts to peanut-sensitized individuals and vice versa (Sicherer et al., 1998). Even though tree nut allergy is sometimes associated to peanut allergy, it is still unclear whether these associated sensitivities reflect a specific response to cross-reacting IgE-binding allergens or correspond to an enhanced propensity of sensitized individuals to develop an IgE response towards unrelated food allergens (Sicherer et al., 1998). To clarify these issues, the major seed storage allergens of peanut and tree nuts have to

Abbreviations: Ana o 1, *Anacardium orientale* (cashew nut) vicilin allergen; Ara h 1, *Arachis hypogaea* (peanut) vicilin allergen; CCD, cross-reacting carbohydrate determinant; Cor a 11, *Corylus avellana* (hazelnut) vicilin allergen; HCA, hydrophobic cluster analysis; IgE, immunoglobulin E antibody; Jug r 2, *Juglans regia* (walnut) vicilin allergen.

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be carefully checked for the occurrence of surface epitopes of similar conformation susceptible to cross-react with the IgE synthesized in response to the sensitizing allergen. Obviously, the major vicilin allergens Ara h 1 of peanut, Jug r 2 of walnut, Cor a 11 of hazelnut or Ana o 1 of cashew nut share very similar amino acid sequences, especially at their very C-termini where are concentrated most of the minor IgE-binding epitopes (Burks et al., 1997; Shin et al., 1998) (see Fig. 1). Although these pronounced amino acid sequence similarities suggest some epitopic community among the vicilin allergens they

remain insufficient to support their IgE-binding cross-reactivity. In fact, IgE-binding B-cell epitopes are essentially conformational in nature and only exposed epitopes of closely related three-dimensional conformations are susceptible to specifically interact with an IgE antibody. Accordingly, three-dimensional models of the vicilin allergens from peanut (Ara h 1), walnut (Jug r 2), hazelnut (Cor a 11) and cashew nut (Ana o 1) were built by homology modelling and checked for the occurrence of surface-exposed epitopes of similar conformation susceptible to account for some IgE-binding cross-reactivity.

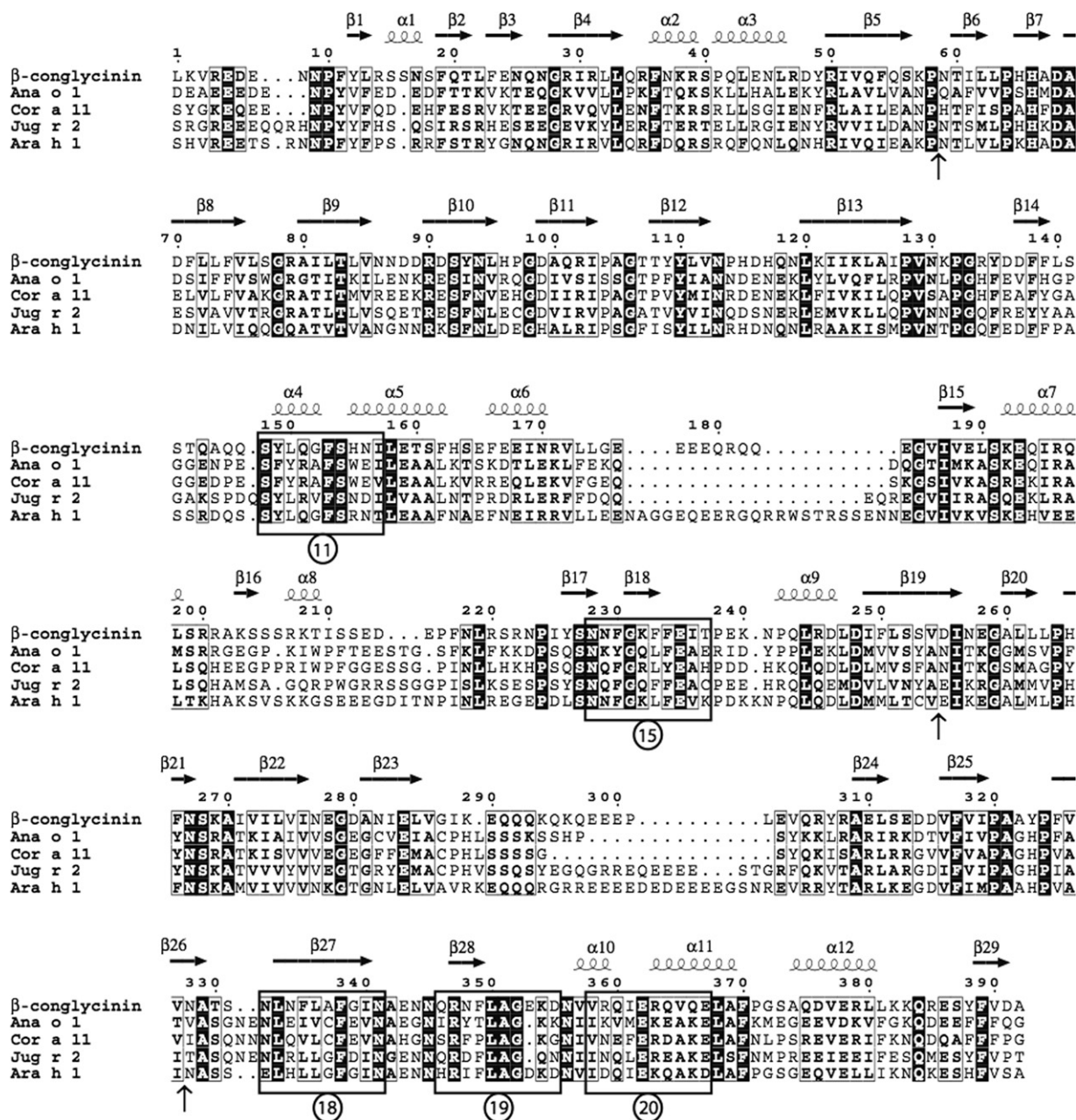


Fig. 1. Structural alignment of Ara h 1, Jug r 2, Cor a 11 and Ana o 1 to β-conglycinin (amino acid sequence of 11PJ) of soybean. Strands of β-sheet (β1-β29) and stretches of α-helix (α1-α12) occurring along the polypeptide chain of β-conglycinin have been indicated. The putative N-glycosylation sites are indicated by vertical arrows. Linear B-cell epitopes #11, 15, 18, 19 and 20 identified in Ara h 1 and the homologous regions of β-conglycinin and other tree nut allergens are open boxed. The alignment was performed with CLUSTAL-X (Thompson et al., 1997), manually modified according to the HCA profiles (Gaboriaud et al., 1987), and represented with ESPript (Gouet et al., 1999) using a structural homology matrix (Risler et al., 1988).

2. Materials and methods

2.1. Analytical methods

The whole protein extracts of peanut, soybean, walnut, hazelnut and cashew nut seeds were obtained from mixing 1 g of finely ground flour with 10 mL of 20 mM Tris, 150 mM NaCl buffer (pH 7.6) (TBS) and stirring for 4 h at room temperature. After centrifugation at $15,000 \times g$ for 30 min, the supernatant was extensively dialyzed against several changes of TBS during 48 h at 4 °C. The supernatant obtained after centrifugation at $15,000 \times g$ for 30 min was filtered through 0.2 μ m membrane and stored frozen at –80 °C until used.

The protein content of the seed samples was estimated using the bicinchoninic acid kit reagent (Pierce) (Smith et al., 1987) with bovine serum albumin as a standard.

Seed proteins were separated by SDS-PAGE in standard 12.5% polyacrylamide gels and stained with Coomassie blue (Fig. 1). Coomassie blue stained bands were digested with trypsin in the gel and mass mapped by MALDI-TOF analysis as previously described (Shevchenko et al., 1996). The software Protein Prospector was used for the identification of the protein using the NCBI non-redundant database.

For MALDI-TOF-MS analysis, protein samples of 0.75 μ L of a 0.5 mg mL^{–1} protein solution in 50 mM Tris buffer (pH 7.5) were co-crystallized on the MALDI plate with 0.75 μ L of 0.6 mM of 3,5-dimethoxy-4-hydroxy cinnamic acid (sinapinic acid) in 50% (w/v) of aceto nitrile containing 0.1% (w/v) trifluoroacetic acid used as a matrix solution. Desorption and ionization of crystallized samples were carried out on a Voyager-DETM STR (Perspective Biosystems, USA) mass spectrometer in positive linear mode using an accelerating voltage of 25 kV, a grid voltage of 90% and an extrac-

tion delay time of 300 ns. Acquisition mass was performed between 3000 and 50,000 Da using a mixture of three proteins of known mass molecular weight (insulin: 5,734 Da, thioredoxin: 11,674 Da and apomyoglobin: 16,952 Da) for the internal calibration.

Following 1D SDS-PAGE, proteins were transferred onto a Protran nitrocellulose 0.2 μ m membrane (Schleicher & Schuell) at 20 V during 45 mn using a 48 mM Tris/39 mM glycine/20% (v/v) methanol mixture. After an overnight incubation in 10 mM PBS (pH 7.4) containing 0.2% (v/v) Tween and 5% (v/v) skimmed milk, the membrane was soaked in the patient IgE-containing sera diluted 1:10 in the same buffer and incubated for 2 h in a moist chamber. After three washings of 10 min each with the same buffer, the membrane was soaked in rabbit HRP-labelled anti-human IgE diluted 1/5000 in the buffer and incubated for 1 h under gentle stirring. Following three washings of 10 min each with buffer, the immunolabelled spots were detected using the ECL Plus detection (Amersham) after 3 min exposure in cassette. All the handlings were carried out at room temperature.

Sera from patients allergic to peanut, that had been previously challenged by skin prick tests and oral provocation tests with the offending food, were used as a IgE source in immunoblotting experiments to reveal the capacity of IgE from peanut-allergic patient to cross-react with similar allergens from other tree nut extracts.

2.2. Molecular modelling

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 Risler structural matrix for homologous residues (Risler et al., 1988).

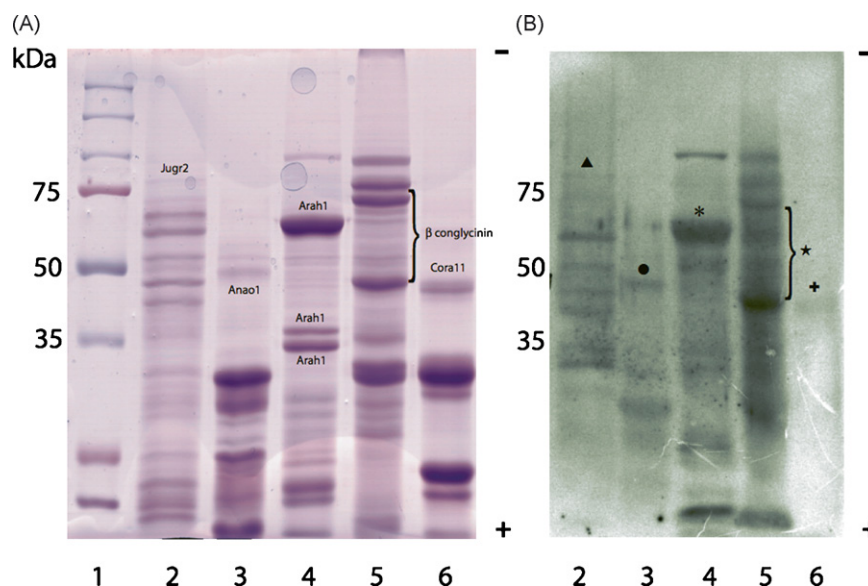


Fig. 2. (A) SDS-PAGE of protein extracts from walnut (line 2), cashew nut (line 3), peanut (line 4), soybean (line 5) and walnut (line 6). The vicilin allergen fractions for each protein extract are indicated. Molecular weight markers (kDa) migrated on line 1. (B) Western blot corresponding to the SDS-PAGE migration shown in (A). The vicilin allergens cross-reacting with a IgE-containing serum from a peanut-allergic patient are indicated by ▲ (Jug r 2), ● (Ana o 1), * (Ara h 1), ★ (β-conglycinin) and + (Cor a 11).

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 sequence of Jug r 2, Cor a 11 and Ana o 1 by comparison with the soybean (*Glycine max*) β -conglycin (Maruyama et al., 2001) used as a model. HCA plots were generated using the HCA server (<http://bioserv.rpbs.jussieu.fr>).

Ara h 1 has been modelled as previously described (Barre et al., 2005). Molecular modelling of other vicilin allergens was carried out on a Silicon Graphics O2 R10000 workstation, using the programs InsightII, Homology and Discover3 (Accelrys, San Diego CA, USA). The atomic coordinates of the soybean β -conglycinin (Maruyama et al., 2001) (RCSB Protein Data Bank code 1IPJ) were used to build the three-dimensional model of the allergens. The high percentages of both identity and similarity β -conglycinin shares with the vicilin monomers of Jug r 2 (40%/85.5%), Cor a 11 (36%/81%) and Ana o 1 (31.5%/79%), respectively (Fig. 1), allowed us to build rather accurate three-dimensional models using the X-ray coordinates of the soybean protein as a template. The extended loop regions corresponding to the two X-ray unsolved, and sequence stretches of β -conglycinin were tentatively modelled. The best geometrical fitting with the core domain compatible with the homotrimeric organization (absence of steric clashes) was used as a criterion for choosing among the different loop conformations available in the PDB. 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, 1989). An energy minimization of the final models was carried out by 150 cycles of steepest descent using the cvff forcefield 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, about 80% of the residues of Ara h 1 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 Asp222 and His410 which occur in the non-allowed region (result not shown). Cartoons were drawn with Molscript (Kraulis, 1991) and displayed with Bobscript (Esnouf, 1997) and Raster 3D (Merritt and Bacon, 1997).

Electrostatic potentials were calculated and displayed with GRASP using the parse3 parameters (Nicholls et al., 1991). 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 (Gilson and Honing, 1987). 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.

Sequential IgE-binding epitopes were predicted from the hydropathic profiles as being the most hydrophilic, flexible and surface-exposed regions. Different scales of hydrophilic-

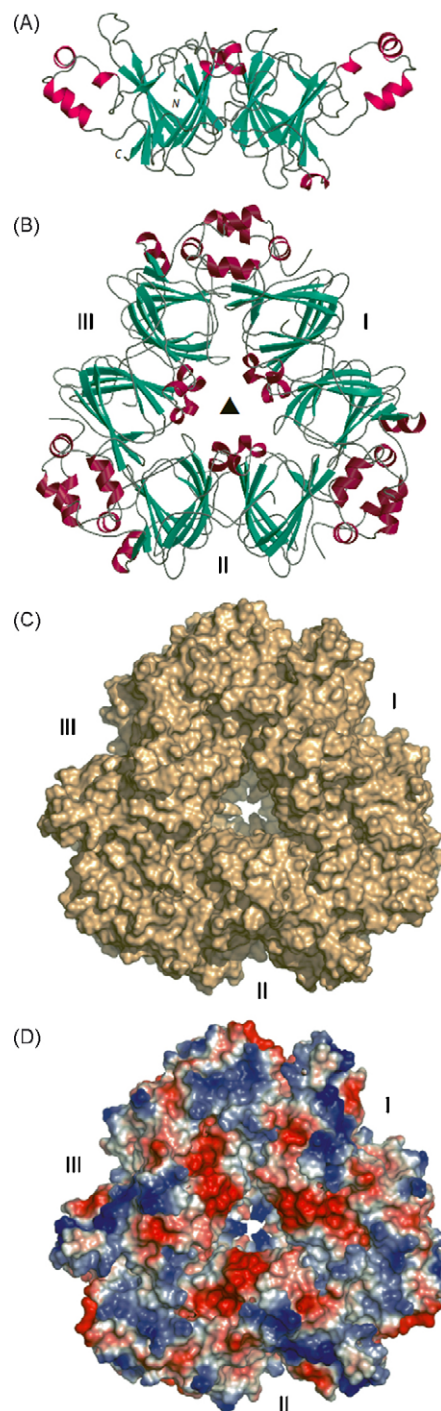


Fig. 3. (A) Ribbon diagram of the three-dimensional model of the Ana o 1 monomer. Strands of β -sheet and stretches of α -helix are colored green and red, respectively. Coil structures and loops are colored light grey. N and C indicate the N- and C-terminus of the polypeptide, respectively. (B) Ribbon diagram of the Ana o 1 homotrimer. The black triangle (▲) indicates the three-fold symmetry axis of the prism formed by the three vicilin monomers I, II and III. The cartoon was drawn with Molscript (Kraulis, 1991) and displayed with Bobscript (Esnouf, 1997) and Raster v3D (Merritt and Bacon, 1997). (C) Molecular surface of the Ana o 1 homotrimer (front face). The molecular surface were calculated and displayed with PyMOL. (D) Mapping of the electrostatic potentials (calculated with GRASP) on the molecular surface of the Ana o 1 homotrimer (front face). Electropositive and electronegative regions are colored blue and red, respectively. Neutral regions are colored white. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 1

Amino acid sequences of IgE-binding epitopes exposed at the surfaces of the vicilin allergens closely related to Ara h 1

	epitope 11	epitope 15	epitope 18	epitope 19	epitope 20
Ara h 1	* SYLQGF [*] SRNT	* * * NNFGKLF [*] EVK	* ELHLLGFGIN	* * * * HRIFLAGDKD	* * * * IDQIEKQAKD
Ana o 1	* * SFYRAF [*] SWEI	* * * * NKYQ [*] LF [*] EA [*] E	* * NLEIVC [*] FEVN	* * IRYTLA [*] G-KK	* * * * IKVMEKEAKE
Cor a 11	* * SFYRAF [*] SWEV	* * NQFGRIYE [*] AH	* * NLQVLC [*] FEVN	* * SRFPLA [*] G-KG	* * * * VNEFERDAKE
Jug r 2	* * SYLRVFSNDI	* * NQFGQFF [*] EAC	* * NLRLLGFDIN	* * QRDFLAG-QN	* * * * INQLEREAKE
β-conglycinin	* SYLQGF [*] SHNI	* * NNFGK [*] FEIT	* NLN [*] FLAFGIN	* * * * QRN [*] FLAGEKD	* * * * VRQIERQVQE

Identical residues are in black boxed white letters and charged residues are indicated by asterisks.

ity (Kyte and Doolittle, 1982), flexibility (Karplus and Schulz, 1985), exposition to the solvent (Lee and Richards, 1971) and antigenicity (Hopp and Woods, 1981), were used to build the hydropathic profiles with the MacVector (Kodak) software. However, the linear IgE-binding epitopes that contain either electropositively (Arg, Lys) or electronegatively (Asp, Glu) residues or both, and that are sufficiently exposed on the molecular surface of the modelled allergens were retained as epitopes. Other predicted epitopes that do not fulfil these requirements were not considered as accurate IgE-binding epitopes and were discarded.

The surface occupied by the previously identified or predicted sequential IgE-binding epitopes (Shin et al., 1998) along the amino acid sequence of Ara h 1, Jug r 2, Ana o 1 and Cor a 11 was calculated using the amino acid conformers of lowest free energy that do not introduce any steric conflict. The overall conformation of these epitopes on the molecular surface of the vicilin proteins was displayed with PyMOL (W.L. DeLano, <http://pymol.sourceforge.net>).

The xylosylated *N*-glycan Man(3)XylGlcNAc(2) chain previously identified at the Asn residue of Ara h 1 (Kolarich and Altmann, 2000) was modelled using the GlyProt server of Glyco-SCIENCES (<http://www.glycosciences.de/modeling/glyprot/php/main.php>) and represented in CPK. Man(3)XylGlcNAc(2) was also identified as the main glycan structure in Cor a 11 allergen (Lauer et al., 2004). Assuming that *N*-glycosylation sites occurring at the surface of other vicilins are similarly glycosylated, the corresponding Man(3)XylGlxNAc(2) chain was modelled on Ana o 3 and Jug r 2.

3. Results and discussion

Sera of peanut-allergic patients that strongly interact with the Ara h 1 vicilin often reacted with the corresponding vicilin allergens (Ana o 1, Cor a 11, Jug r 2) from other tree nuts as shown in Western blot experiments (Fig. 2). In addition, β conglycinin (vicilin) from soybean seeds also interacted with the IgE-containing sera. However, this cross-reactivity greatly varies among the tested sera with a few sera that interacted with

Ara h 1 but did not show any cross-reaction with other tree nut vicilin allergens.

The three-dimensional models built for the Jug r 2, Cor a 11 and Ana o 1 monomers exhibit an overall cupin fold very similar to that of β-conglycinin (Fig. 3A). They all possess the cupin motif made of two tandemly arrayed modules. Each module consists of a β-barrel core domain of two walls of antiparallel β-sheet associated to a loop domain containing two extended α-helices. Both modules are connected by an α-helix-containing linker region. Like Ara h 1, these vicilin monomers differ from β-conglycinin by a more (Ara h 1) or less extended (other tree nut vicilins) extra-loop protruding out between β-strands β7–β8 of the C-ter cupin module. In agreement with the apparent molecular mass of ~kDa of the native vicilin allergens, the three cupin motifs are arranged around a three-fold symmetry axis via the C-terminal helix exchange of the monomers in a homotrimeric structure in which the cupin motifs occupy the three faces of a flattened prism (Fig. 3B). The extra-loops of the monomers all extend on the same face of the homotrimer that is referred as the back face of the trimer.

Most of the identified (Shin et al., 1998) or predicted IgE-binding epitopes sharing similar amino acid sequences essentially occur on the back face of the vicilin homotrimers. Except for epitope #11 (the labelling refers to the Ara h 1 epitopes) located on the front face of the homotrimers (Fig. 4A–C), they correspond to epitopes #15, 18, 19 and 20 (Fig. 4D–F). The identified/predicted epitopes contain identical or structurally homologous amino acids (Table 1). All these epitopic regions are rather well exposed on the surface of the homotrimers and mostly correspond to electropositively charged regions rich in basic Arg or Lys residues (Fig. 3C). However, their exposition to the solvent is better when checked on the monomers (not shown) instead of the trimers. In this respect, the C-terminal helix exchange responsible for the trimeric arrangement of the monomers restricts the accessibility of those epitopic regions located in the vicinity. In addition, the extra-loop occurring in these allergens exerts some masking effect on previously exposed epitopes such as epitope #18 of Cora h 11 or Ana o 1. As shown in Fig. 5, some of these epitopes identified in Ara

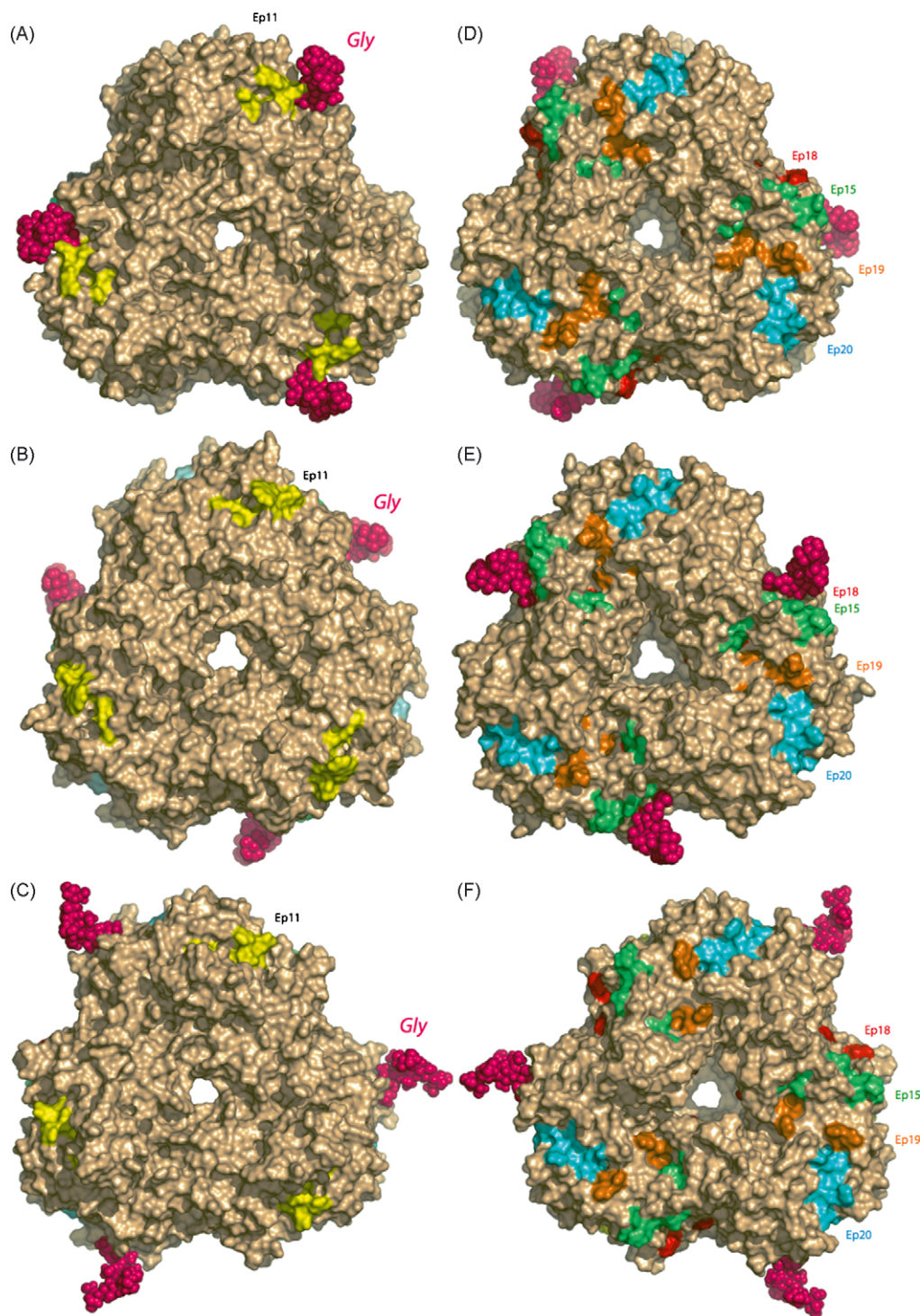


Fig. 4. (A–C) Location of the characterized (Ara h 1) or predicted (Cor a 11, Jug r 2) homologous B-cell epitopes #11 (yellow) on the molecular surface (front face) of the Ara h 1 (A), Cor a 11 (B) and Jug r 2 (C) homotrimeric. (D–F) Location of the characterized (Ara h 1) or predicted (Cor a 11, Jug r 2) homologous B-cell epitopes #11 (yellow), 15 (green), 18 (red), 19 (orange) and 20 (cyan) on the molecular surface (back face) of the Ara h 1 (D), Cor a 11 (E) and Jug r 2 (F) homotrimeric. The modelled *N*-glycan chains corresponding to the putative *N*-glycosylation sites are represented in magenta CPK. The molecular surface were calculated and displayed with PyMOL. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

h 1, e.g. epitopes #11, 15, 18, 19 and 20, exhibit a very similar three-dimensional conformation in other tree nut allergens and, especially, in Jug r 1. Rather surprisingly, epitopes #15 of Cor a 11 and Ana o 1 appeared as more structurally similar to that of epitopes #11 of both Ara h 1 and Jug r 2 instead of mimick-

ing epitopes #15 of these allergens. Conserved epitopes of Jug r 2 appear as being the most closely structurally related from Ara h 1 epitopes. Except for epitopes #15 and 20, the other corresponding regions of both Cor a 11 and Ana o 1 exhibited a rather different conformation from the homologous Ara h 1 epi-

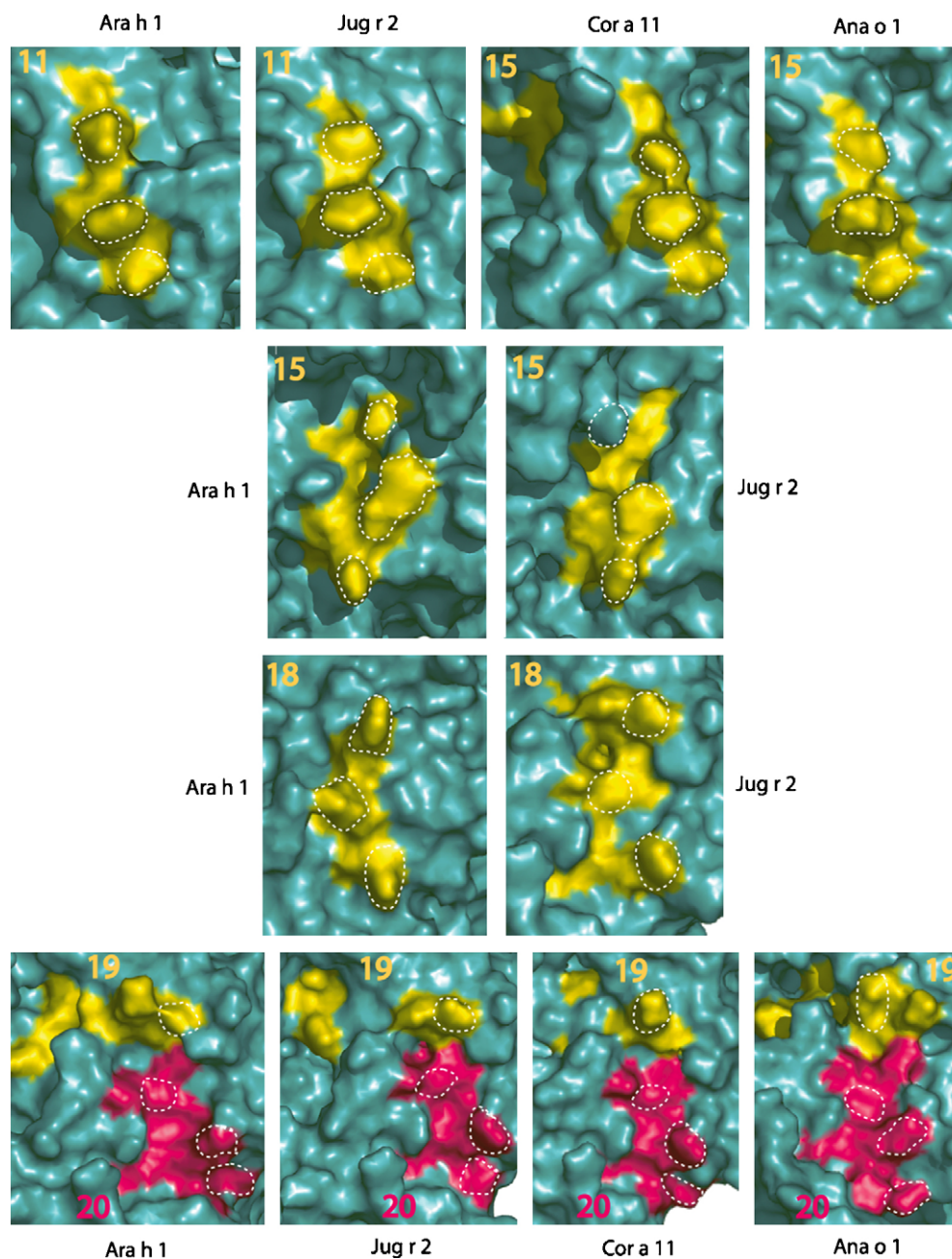


Fig. 5. Apparent three-dimensional conformation of the major B-cell epitopes #11, 15, 18, 19 and 20 characterized on the molecular surface surfaces of Ara h 1 homotrimer and of the homologous regions on the molecular surface of Jug r 2, Cor a 1 and Ana o 1 homotrimers. Epitopic regions 11–15–18–19 and 20 are colored yellow and pink, respectively. Homologous residues protruding on the surface of epitopes are white dashed circled. The molecular surfaces (colored cyan) were calculated and displayed with PyMOL. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

topes but readily resemble the corresponding allergenic regions of β -conglycinin.

The occurrence of cross-reactive B-cell epitopes in peanut and tree nut extracts has been already demonstrated using Western blot and ELISA measurements with IgE-containing sera from individuals with or without peanut and tree nut allergy (de Leon et al., 2003). These epitopes of rather similar conformation should thus be able to specifically interact with the IgE antibodies and could account for IgE-binding cross-reactivity between peanut and other tree nuts (Pumphrey et al., 1999; Sutherland et al., 1999; Teuber et al., 2003). However, in agreement with

our predictions, little cross-reactivity was previously observed between peanut and cashew nut allergens (Gillespie et al., 1991). In this respect, the cross-reactivity reported among tree nuts, e.g. between cashew nut and pistachio nut (Fernandez et al., 1995), seems much more frequent than that occurring between peanut and other tree nuts (Goetz et al., 2005).

Obviously, the predicted IgE-binding cross-reactivity on the basis of the conformational similarity between the surface-exposed epitopes of peanut and other tree nuts allergens is apparently unrelated to their botanical position. Both the closely related Jug r 1 and Cor a 11 which share some epitopic similarity

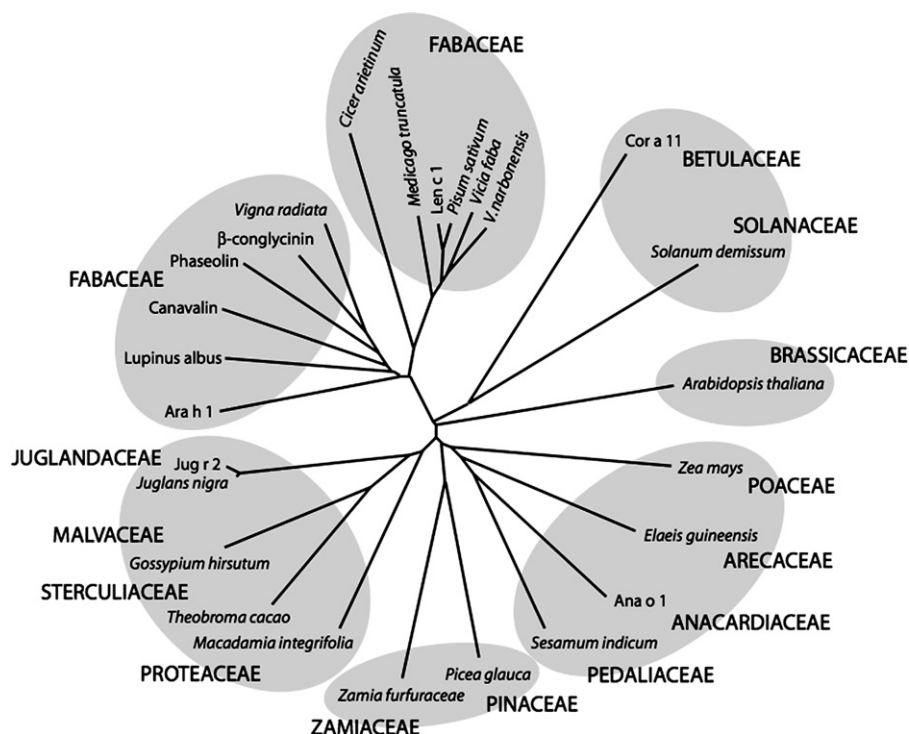


Fig. 6. Parsimony tree built from the available amino acid sequences of plant vicilins. Genuine vicilin allergens are indicated by their abbreviations and other plant vicilins are indicated by the Latin names of the corresponding species. The plant families to which the vicilins belong are indicated. Clusters are grey circled.

with Ara h 1 occur in the species *J. regia* and *C. avellana* belonging to the family Juglandaceae, very distinct from the Fabaceae which contains Ara h 1 of *A. hypogaea*. Conversely, Ana o 1 of *A. orientale* which belongs to the so phylogenetically remote family of Anacardiaceae exhibits less epitopic similarity with Ara h 1 (Fig. 6).

The seed storage vicilin allergens consist of glycoproteins that are posttranslationally *N*-glycosylated during their processing along the endoplasmic reticulum and the Golgi apparatus before they accumulate into the protein bodies of the ripening seeds. Ara h 1 and Cor a 11 have been reported to contain a xylosylated *N*-glycan chain Man(3)XylGlcNAc(2) (Kolarich and Altmann, 2000; Lauer et al., 2004) and other vicilin allergens exhibit putative *N*-glycosylation sites that are predicted to be actually glycosylated. The modelled *N*-glycans of Ara h 1 and other vicilins readily protrude from the molecular surface of the homotrimers thus becoming more exposed than the corresponding peptidic IgE-binding epitopes (Fig. 4). They most probably act as conformational epitopes (glyco-epitopes) susceptible to account for some cross-reactivity among the vicilin allergens even though the degree of glycosylation is essentially variable from one allergen to another due to its posttranslational character (Altmann, 2007). Moreover, these glyco-epitopes exert some masking effect on the IgE-binding epitopes that should drastically reduce their accessibility to the corresponding IgE antibodies. This is especially the case for the glyco-epitopes of Ara h 1 and Cor a 11 towards IgE-binding epitopes 1 and IgE-binding epitopes 15 and 18, respectively (Fig. 5). The recently reported activation *in vitro* of monocyte-derived dendritic cells by glycosylated Ara h 1 sheds a new light on the capacity of

glyco-epitopes to orient the Th1/Th2 balance towards responding Th2 cells (Schreffler et al., 2006).

In addition to Ara h 1 and other orthologous vicilin proteins, both peanut and tree nuts contains highly allergenic glycoallergens like 2S albumins (Ara h 2 of peanut, Jug r 1 of walnut) or legumins (Ara h 3 of peanut, Cor a 9 of hazelnut) that also account for some cross-reactivity between peanut and other tree nuts (Beardslee et al., 2000; de Leon et al., 2007). In this respect, Ara h 2 was recognized as being the most important peanut allergen from IgE Western blotting, basophil-histamine release and intracutaneous testing experiments (Koppelman et al., 2004).

Besides the serological aspect of the cross-reactivity between peanut and tree nuts, the clinical relevance of cross-reacting IgE in allergic individuals still remains a puzzling question (van Ree, 2004). The discrepancy observed between cross-reactivity and cross-allergenicity, that discriminates between sensitized individuals without clinical reactivity and allergic patients, is far from being understood but, most probably, results from multiple factors (level of the synthesized IgE, polymorphism of the FcεRI receptors, balance of T regulatory cells, Th1/Th2 cells, etc.) (Sicherer, 2002; Bousquet et al., 2006). The possible occurrence of cross-reacting carbohydrate determinants (CCD) or glyco-epitopes on structurally related vicilin allergens appears as an additional source of clinically irrelevant results (Mari et al., 1999).

In spite of the scarcity of clinical studies on cross-reactivity between peanut and tree nuts, peanut-specific IgE cross-reacting with tree nut allergens were demonstrated to cause effector-cell activation that might trigger tree nut allergic manifestations in peanut-allergic subjects (de Leon et al., 2005). Accordingly, both

the predicted occurrence of structurally related epitopes in nuts and their immunological assessment in ELISA tests suggest to recommend the avoidance of some other tree nuts to peanut-allergic individuals. Along this line, great care must be taken to avoid cross-contamination with improperly labelled foods or food products containing hidden allergens once sensitivity is diagnosed.

Acknowledgement

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