

Homology modelling of the major peanut allergen Ara h 2 and surface mapping of IgE-binding epitopes

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Received 17 January 2005; received in revised form 7 March 2005; accepted 18 March 2005

Available online 28 April 2005

Abstract

Three-dimensional models built for the peanut Ara h 2 allergen and other structurally-related 2S albumin allergens of dietary nuts exhibited an overall three-dimensional fold stabilized by disulphide bridges well conserved among all the members of the 2S albumin superfamily. Conformational analysis of the linear IgE-binding epitopes mapped on the molecular surface of Ara h 2 showed no structural homology with the corresponding regions of the walnut Jug r 1, the pecan nut Car i 1 or the Brazil nut Ber e 1 allergens. The absence of epitopic community does not support the allergenic cross-reactivity observed between peanut and walnut or Brazil nut, which presumably depends on other ubiquitous seed storage protein allergens, namely the vicilins. However, the major IgE-binding epitope identified on the molecular surface of the walnut Jug r 1 allergen shared a pronounced structural homology with the corresponding region of the pecan nut Car i 1 allergen. With the exception of peanut, 2S albumins could thus account for the IgE-binding cross-reactivity observed between some other dietary nuts, e.g. walnut and pecan nut.

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Keywords: Peanut allergen; 2S albumin; Three-dimensional model; Epitope mapping; IgE-binding cross-reactivity

1. Introduction

Peanut allergy has been recognized as the most worrying food hypersensitivity responsible for extremely severe allergic reactions in children, adolescents and adults [1]. In fact, peanut and peanut-containing food products cause more fatal anaphylactic shocks than any other foodstuffs [2]. Sensitization to peanut generally occurs during the infancy and very often persists throughout the life [3]. In this respect, peanut allergy actually differs from other current food allergies, e.g.

to milk or egg proteins, which prevail in children but usually vanish in adults [4]. Until now, the strict avoidance of peanut and peanut by-products into the diet constitutes the best way to prevent the allergic manifestations in sensitized individuals. However, peanut proteins often occur as hidden allergens in many (inadequately labelled) food products and the accidental consumption of offending food allergens is very difficult to avoid. In addition, closely-related allergens occurring in other dietary seeds, e.g. tree nut [5], are susceptible to trigger allergic reactions in individuals previously sensitized to peanut proteins. Obviously, this allergic cross-reactivity drastically complicates the food avoidance rules of peanut-sensitive patients [6] and inevitably impacts upon their quality of life [7]. Peanut allergy depends on three allergens Ara h 1 [8], Ara h 2 [9] and Ara h 3 [10] that occur in seeds as storage proteins. Ara h 1 and Ara h 2 are of paramount importance since serum IgE from >90% of peanut-sensitive individuals recognize these two major allergens [11,12]. Linear IgE-binding B-cell epitopes have been identified along

Abbreviations: Ana o 3, *Anacardium occidentale* (cashew) allergen 3; Ara h 2, *Arachis hypogea* (peanut) allergen 2; Ber e 1, *Bertholletia excelsa* (Brazil nut) allergen 1; Cari 1, *Carya illinoensis* (pecan) allergen 1; CDD, cross-reactive carbohydrate determinant; HCA, hydrophobic cluster analysis; IgE, immunoglobulin E; Jug r 1, *Juglans regia* (walnut) allergen 1; LTP, lipid transfer protein; Ses i 3, *Sesamum indicum* (sesame) allergen 3

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the amino acid sequence of Ara h 1 [11] and Ara h 2 [12]. Ara h 2 consists of an extremely ubiquitous 2S albumin seed storage protein also present in many other dietary seeds, including soybean [13], walnut [14], cashew nut [15], Brazil nut [16], sunflower [17] or sesame seeds [18] (see Fig. 3). Although these nut allergens are moderately similar ($\leq 35\%$ of amino acid sequence similarity) to Ara h 2, they apparently share a conserved three-dimensional fold [19]. Some of them could therefore exhibit B-cell epitopes very similar to those recognized on the surface of Ara h 2 and thus account for the IgE-binding cross-reactivity observed between peanut and other dietary nuts. To check this hypothesis, three-dimensional models of Ara h 2 and other nut allergens were built by homology modelling and checked for the presence of epitopes with conformation similar to the Ara h 2 B-cell epitopes. The major IgE-binding epitopes of the walnut Jug r 1 and pecan Car i 1 allergens shared a very similar conformation but exhibited no structural homology with the corresponding region of the peanut allergen. Although 2S albumins offer a molecular basis for the IgE-binding cross-reactivity observed between some dietary nuts, e.g. walnut and pecan nut, they apparently do not account for the cross-reactions reported between peanut and other dietary nuts.

2. Materials and methods

Multiple amino acid sequence alignments were carried out with CLUSTAL-X [20] and displayed with ES-Prpt [21]. The hydrophobic cluster analysis (HCA) [22] was performed to delineate the conserved secondary structural features (stretches of α -helix) along the amino acid sequence of Ara h 2 and other 2S albumins by comparison with the castor bean (*Ricinus communis*) 2S albumin allergen Ric c 3 [23] used as a model. HCA plots were generated using the program drawhca of L. Canard (<http://www.lmcp.jussieu.fr/~soyer/www-hca/hca-form.html>).

Molecular modelling of Ara h 2 and other 2S albumins was carried out on a Silicon Graphics O2 R10000 workstation, using the programs InsightII, Homology and Discover 3 (Accelrys, San Diego CA, USA). The atomic coordinates of the

castor bean allergen Ric c 3 [23] (RCSB Protein Data Bank code 1PSY) were used to build the three-dimensional model of the allergens. The percentages of both identity ($\sim 35\%$) and homology ($\sim 75\%$) Ric c 3 shares with Ara h 2 (Fig. 1) and other dietary 2S seed albumins allowed us to build rather accurate three-dimensional models using the RMN coordinates of the castor bean 2S albumin as a template. The extended N-terminal region, which is extremely disordered in the 20 RMN models, was necessarily omitted in the model building. Accordingly, the model built for Ara h 2 starts at residue Glu5. Two out of the 10 linear IgE-binding epitopes identified along the amino acid sequence of Ara h 2 fall into the N-terminal region that has been neglected in the model building. However, they do not correspond to the three immunodominant epitopes characterized in Ara h 2. Ara h 2 readily differs from Ric c 3 by an extra-loop corresponding to a Pro-rich repeat occurring in the N-terminal portion of the polypeptide. Another less extended extra-loop occurs at the C-terminal end of the polypeptide chain. These two extra-loops were tentatively modelled using the best geometrical fitting with the core domain 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 [24] and the search algorithm implemented in the Homology program [25] to maintain proper side-chain orientation. The geometry of loop regions was corrected using the refine option of TurboFrodo [26]. An energy minimization of the final models was carried out by 150 cycles of steepest descent using Discover 3. The program TurboFrodo was run to draw the Ramachandran plot and to perform the superposition of the model with the template protein. PROCHECK [27] was used to assess the geometric quality of the three-dimensional models. As an example, 62% of the residues of Cari i 1 (54% for 1PSY used as template) 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 Glu57 which occurs in the non allowed region (result not shown). Cartoons were drawn with PyMOL W.L. DeLano (<http://www.pymol.org/>).

Electrostatic potentials were calculated and displayed with GRASP using the parse3 parameters [28]. The solvent probe radius used for molecular surfaces was 1.4 Å and a standard

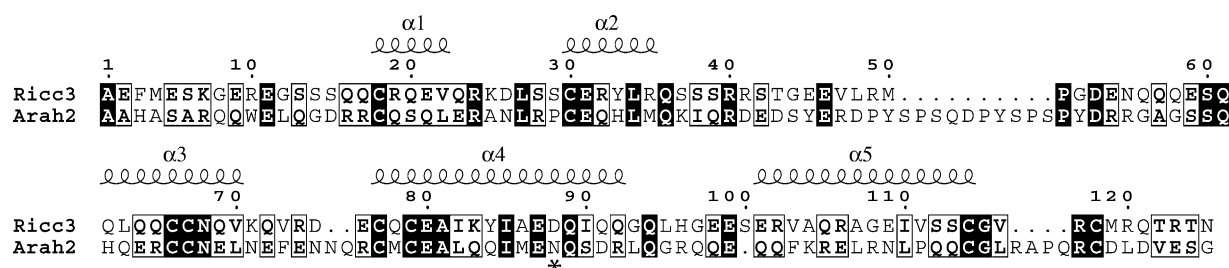


Fig. 1. Structural alignment of Ara h 2 to the castor bean Ric c 3 2S albumin. Stretches of α -helix ($\alpha 1$ – $\alpha 5$) occurring along the polypeptide chain of Ric c 3 have been indicated. The putative N-glycosylation site of Ara h 2 is indicated by an asterisk (*). The alignment was performed with CLUSTAL-X [20], manually modified according to the HCA plots [22], and represented with ESPrpt [21].

2.0 Å-Stern layer was used to exclude ions from the molecular surface [29]. 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. No even distribution of the net negative charge of the carboxylic group of negatively charged residues was performed between their two oxygen atoms prior to the calculations.

B-cell epitopes were predicted from the hydropathic profiles as being the most hydrophilic, flexible and surface exposed regions. Different scales of hydrophilicity [30], flexibility [31], exposition to the solvent [32] and antigenicity [33], were used to build the hydropathic profiles with the MacVector (Kodak) software.

The surface occupied by the previously identified or predicted sequential B-cell epitopes [14] along the amino acid sequence of Ara h 2 was calculated and displayed on the molecular surface of the proteins with PyMOL. The overall conformation of the sequential B-cell epitopes on the molecular surface of the 2S albumins was displayed with PyMOL.

3. Results and discussion

Homology-based molecular modelling of Ara h 2 and other 2S seed albumin allergens showed that seed storage 2S albumins share a common three-dimensional structural scaffold made of five α -helices arranged in a right-handed superhelix and connected by more or less extended loops (Fig. 2A and B). This three-dimensional conformation, stabilized by four conserved disulphide bridges, is reminiscent of that found in type 1 LTP which constitute another group of important food allergens [34]. However, 2S albumins differ from LTP by the topology of the disulphide bridges [23]. Ara h 2 readily differ from other 2S albumins by two additional loops located at the N- and C-terminal ends of the polypeptide chain, respectively. The more extended N-terminal extra loop roughly corresponds to a tandem repeat of a Pro-rich hexapeptide DPYSPS which has no equivalent in Ric c 3 or coincides with the less conserved region of all other 2S albumins sequenced so far. Ara h 2 contains a putative *N*-glycosylation site Asn88-Gln-Ser. Although well exposed, its location at the end of the main α 4 helix suggests it is not actually glycosylated. Other modelled nut allergens and, especially, Jug r 1 and Car i 1, exhibited a very similar three-dimensional fold but the N-terminal extra loop corresponding to the tandem Pro-rich repeat of Ara h 2 is less extended (results not shown).

Molecular mapping of the 10 linear B-cell epitopes previously characterized along the polypeptide chain of Ara h 2 [12] showed a fairly well exposition on the molecular surface of the three-dimensional model (Fig. 2D and F). This is especially true for the two major epitopes #6 and 7 which correspond to the protruding N-terminal Pro-rich extra loop of Ara h 2. This extra loop has no counter-part in Ric c 3

(Fig. 2C and D) and possess an overall three-dimensional conformation distinct from that found in the corresponding shorter extra loops of other nut allergens, e.g. Jug r 1 or Car i 1. Even though they are better conserved in other nut allergens (see Fig. 3), other minor epitopes #8, 9 and 10 located at the C-terminus of the Ara h 2 polypeptide also differ by their three-dimensional conformation from the corresponding regions of Ric c 3 (Fig. 2E and F) and other allergens (results not shown). Most of these epitopes seems specific for Ara h 2 and hence unsuitable for promoting IgE-binding cross-reactivity with other nut allergens.

Similarly, no conformational similarity could be detected between the experimentally characterized or predicted B-cell epitopes of other nut allergens and the major or minor B-cell epitopes of Ara h 2. In this respect, although B-epitope 8 103RLQGRQQEQQFK114 of Ara h 2 overlaps the core domain (bold letters) of the immunodominant linear B-cell epitope QGLRGEEMEEMV characterized in Jug r 1 [35], both regions exhibit rather distinct amino acid sequences and overall conformations (Fig. 4). However, this linear B-cell epitope of Jun r 1 is well conserved (identical or homologous residues are underlined) in the pecan nut Car i 1 allergen (EGIRGEEMEEMV), the cashew nut Ana o 3 allergen (EQIKGEEVRELY), and the Brazil nut Ber e 1 allergen (EM(Q)PRGEQMRRMM) (Fig. 3). Accordingly, its overall conformation is very similar in Car i 1 but rather different in Ana o 3 or in the castor bean Ric c 3 allergen (94GQLHGEESEVA104) (Fig. 4). This absence of epitopic community among 2S albumins from distinct plant families does not support the allergenic cross-reactivity observed between peanut (Fabaceae) and Brazil nut (Juglandaceae), showing that pre-incubation of sera from peanut-sensitive patients with a Brazil nut extract resulted in a decrease in the IgE-binding to peanut extract in ELISA measurements [36]. Similarly, no decrease occurred after pre-incubation with a cashew nut (Anacardiaceae) extract.

Seed storage 2S albumins thus appear as ubiquitous allergens distributed in plants belonging to distinct monocot and dicot families. Although their overall three-dimensional structure stabilized by disulphide bridges is well conserved among all the members of the 2S albumin superfamily, their amino acid sequences is highly variable, especially in the N-terminal part of the polypeptide chain. Consequently, they do not share common or consensus surface-exposed B-cell epitopes except for those belonging to the same family, e.g. the walnut Jug r 1 and the pecan nut Car i 1, susceptible to account for the currently observed allergenic cross-reactivity between peanut and other tree nuts. Other seed storage protein allergens largely distributed in dietary seeds, namely vicilins, should be responsible for this allergenic cross-reactivity. However, some IgE-binding cross-reactivity among 2S albumins from peanut and other tree nuts could depend on IgE directed against cross-reactive carbohydrate determinants (anti-CDD IgE). A single putative *N*-glycosylation site 94Asn-Gln-Ser exists along the amino acid sequence of Ara h 2 but it is located into helix α 3 that occurs at the C-terminal

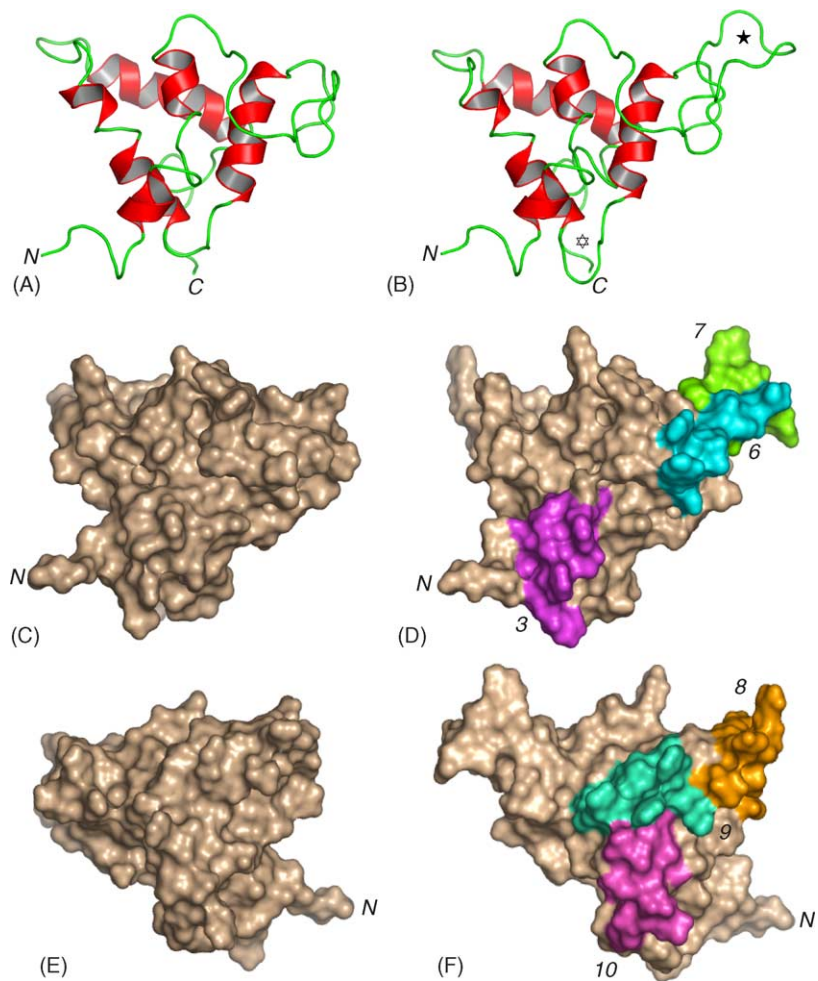


Fig. 2. Ribbon diagram of the castor bean 2S albumin Ric c 3 allergen (A) and Ara h 2 (B). The two N- and C-terminal extra loops of Ara h 2 are indicated by stars (★ and ☆). N and C indicate the N- and C-termini of the polypeptide chains, respectively. Molecular surfaces of Ric c 3 (C) and Ara h 2 (D) showing the areas occupied by the major B-epitopes 3 (pink), 6 (blue) and 7 (green); N-termini are oriented left. Molecular surfaces of Ric c 3 (E) and Ara h 2 (F) showing the areas occupied by the major B-epitopes 8 (orange), 6 (green blue) and 7 (light pink); N-termini are oriented right.

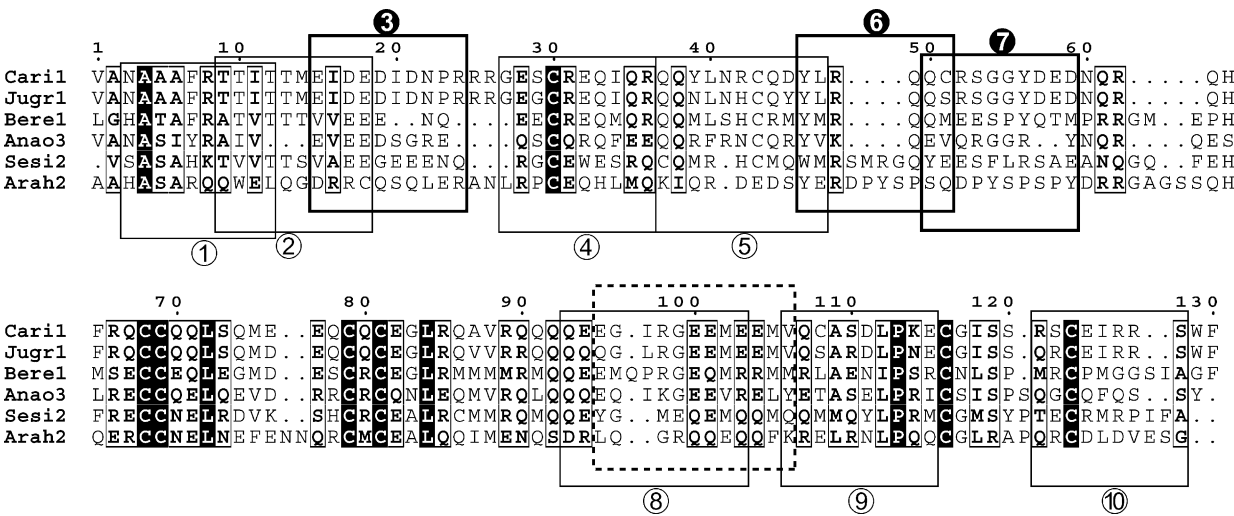


Fig. 3. Structural alignment of Ara h 2 with Ses i from Sesame seeds and other tree nut allergens Ana o 3, Ber e 1, Car i 1 and Jug r 1. The major B-cell epitopes (numbered 3, 6 and 7) and the minor B-cell epitopes (numbered 1, 2, 4, 5, 8–10) of Ara h 2 and the corresponding regions of other 2S allergens are open boxed. The major B-cell epitope of Jug r 1 and the corresponding regions of other allergens is dashed boxed.

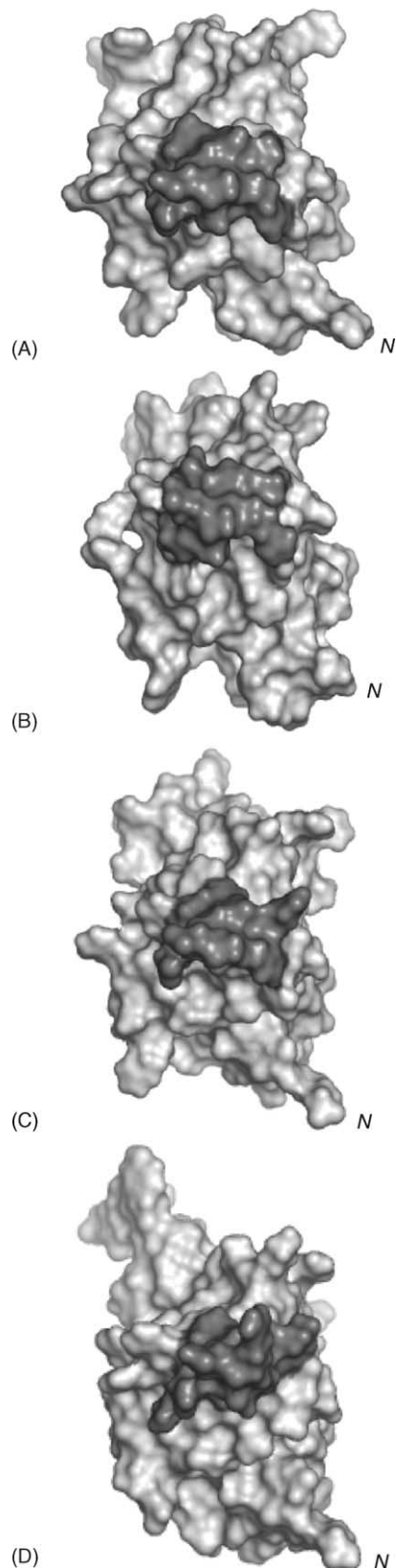


Fig. 4. Molecular surfaces of Jug r 1 (A), Car i 1 (B), Ric c 3 (C) and Ara h 2 (D) showing the three-dimensional conformation of the major B-cell epitope (gray) characterized on the surface of Jug r 1 and the corresponding regions on the surface of other 2S albumin allergens. All 2S albumins are similarly oriented and N indicates the N-terminal end of the polypeptide chains.

end of the molecule. In addition, it is poorly exposed on the molecular surface in such a way that Ara h 2 may be supposed to be unglycosylated. According to [12] the allergenic character of Ara h 2, which do not contain significant amounts of carbohydrate, appears to be solely due to epitopes. Type O-glycans have also been shown to behave as CCD of the Mugwort pollen allergen Art v 1 [37]. However, sera of patients allergic to grass pollen with significant levels of anti-peanut IgE but without positive skin prick tests and no symptom of peanut allergy were extremely poorly reactive when tested for the presence of anti-CDD IgE [38]. Similarly, one cannot exclude that true conformational B-cell epitopes distinct from continuous epitopes participate in some cross-reactivity between Ara h 2 and other tree nut allergens even though their continuous epitopes do not share sequence or structural similarities. In this respect, the use of mimotopes from phage display libraries, allowed to identify compact patches of sequence motifs on the molecular surface of the Bet v 1 [39] and Phl p 5 [40] allergens that most likely represent the true conformational B-cell epitopes. The coalescence of linear epitopes 6–7 and 9–10 on the molecular surface of Ara h 2 (see Fig. 2D and F) could create more surface-extended conformational epitopes responsible for some cross-reactivity with other tree nut allergens.

Acknowledgement

The financial support of CNRS is gratefully acknowledged.

References

- [1] Sampson HA, Mendelson LM, Rosen JP. Fatal near-fatal anaphylactic reactions to foods in children and adolescents. *New Engl J Med* 1992;327:380–4.
- [2] Bock SA, Muñoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol* 2001;107:191–3.
- [3] Bock SA, Atkins FM. The natural history of peanut allergy. *J Allergy Clin Immunol* 1989;83:900–4.
- [4] Bock SA. Natural history of severe reactions to foods in young children. *J Pediatr* 1985;107:676–80.
- [5] Teuber SS, Comstock SS, Sathe SK, Roux KH. Tree nut allergy. *Curr Allergy Asthma Rep* 2003;3:54–61.
- [6] Muñoz-Furlong A. Patient's perspective and public policy regarding anaphylaxis. *Norvartis Found Symp* 2004;257:165–74.
- [7] Avery NJ, King RM, Knight S, Hourihane JO. Assessment of quality of life in children with peanut allergy. *Pediatr Allergy Immunol* 2003;14:378–82.
- [8] Burks AW, Williams LW, Helm RM, Connaughton C, Cockrell G, O'Brien TJ. Identification of a major peanut allergen, Ara h I, in patients with atopic dermatitis and positive peanut challenges. *J Allergy Clin Immunol* 1991;88:172–9.
- [9] Burks AW, Williams LW, Connaughton C, Cockrell G, O'Brien TJ, Helm RM. Identification and characterization of a second major peanut allergen, Ara h II, with use of the sera of patients with atopic dermatitis and positive peanut challenge. *J Allergy Clin Immunol* 1992;90:962–9.

- [10] Rabjohn P, Helm RM, Stanley JS, West C, Sampson HA, Burks AW, et al. Molecular cloning and epitope analysis of the peanut allergen Ara h 3. *J Clin Invest* 1999;103:535–42.
- [11] Shin DS, Compadre CM, Maleki SJ, Kopper RA, Sampson H, Huang SK, et al. Biochemical structural analysis of the IgE-binding sites on Ara h 1, an abundant and highly allergenic peanut protein. *J Biol Chem* 1998;273:13753–9.
- [12] Stanley JS, King N, Burks AW, Huang SK, Sampson H, Cockrell G, et al. Identification mutational analysis of the immunodominant IgE binding epitopes of the major peanut allergen Ara h 2. *Arch Biochem Biophys* 1997;342:244–53.
- [13] Shibasaki M, Suzuki S, Tajima S, Nemoto H, Kuroume T. Allergenicity of major component proteins of soybean. *Int Arch Allergy Appl Immunol* 1980;61:441–8.
- [14] Teuber SS, Dandekar AM, Peterson WR, Sellers CL. Cloning and sequencing of a gene encoding a 2S albumin seed storage protein precursor from English walnut (*Juglans regia*), a major food allergen. *J Allergy Clin Immunol* 1998;101:807–14.
- [15] Teuber SS, Sathe SK, Peterson WR, Roux KH. Characterization of the soluble allergenic proteins of cashew nut (*Anacardium occidentale* L.). *J Agric Food Chem* 2002;50:6543–9.
- [16] Ampe C, Van Damme J, de Castro LA, Sampaio MJ, Van Montagu M, Vandekerckhove J. The amino acid sequence of the 2S sulphur-rich proteins from seeds of Brazil nut (*Bertholletia excelsa* H.B.K.). *Eur J Biochem* 1986;159:597–604.
- [17] Kelly JD, Hefle SL. 2S methionine-rich protein (SSA) from sunflower seed is an IgE-binding protein. *Allergy* 2000;55:556–60.
- [18] Tai SS, Wu LS, Chen EC, Tzen JT. Molecular cloning of 11S globulin and 2S albumin, the two major seed storage proteins in sesame. *J Agric Food Chem* 1999;47:4932–8.
- [19] Pantoja-Uceda D, Bruix M, Santoro J, Rico M, Monsalve R, Villalba M. Solution structure of allergenic 2S albumins. *Biochem Soc Trans* 2002;30:919–24.
- [20] Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tool. *Nucl Acid Res* 1997;15:4876–82.
- [21] Gouet P, Courcelle E, Stuart DI, Metoz F. ESPript: analysis of multiple sequence alignments in PostScript. *Bioinformatics* 1999;15:305–8.
- [22] Gaboriaud C, Bissery, Benchetrit T, Mornon JP. Hydrophobic cluster analysis: an efficient new way to compare and analyse amino acid sequences. *FEBS Lett* 1987;224:149–55.
- [23] Pantoja-Uceda D, Bruix M, Giménez-Gallego G, Rico M, Santoro J. Solution structure of Ric c 3, a 2S albumin storage protein from *Ricinus communis*. *Biochemistry* 2003;42:13839–47.
- [24] Ponder JW, Richards FM. Tertiary templates for proteins. Use of packing criteria in the enumeration of allowed sequences for different structural classes. *J Mol Biol* 1987;193:775–91.
- [25] Mas MT, Smith KC, Yarmush DL, Aisaka K, Fine RM. Modeling the anti-CEA antibody combining site by homology and conformational search. *Proteins Struc Func Genet* 1992;14:483–98.
- [26] Roussel A, Cambillau C. In: Committee SG, editor. TURBO-FRODO: Silicon graphics geometry partners directory. Mountain View, California: Silicon Graphics; 1989.
- [27] Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK: a program to check the stereochemistry of protein structures. *J Appl Cryst* 1993;26:283–91.
- [28] Nicholls A, Sharp KA, Honig B. Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons. *Proteins Struc Func Genet* 1991;11:281–96.
- [29] Gilson MK, Honing BH. Calculation of electrostatic potential in an enzyme active site. *Nature* 1987;330:84–6.
- [30] Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. *J Mol Biol* 1982;157:105–32.
- [31] Karplus PA, Schulz GE. Prediction of chain flexibility in proteins. *Naturwis* 1985;72:212–3.
- [32] Lee B, Richards FM. The interpretation of protein structures: estimation of static accessibility. *J Mol Biol* 1971;55:379–400.
- [33] Hopp TP, Woods KR. Prediction of protein antigenic determinants from amino acid sequences. *Proc Natl Acad Sci USA* 1981;78:3824–8.
- [34] Pastorello EA, Pompei C, Pravettoni V, Brenna O, Farioli L, Trambaioli C, et al. Lipid transfer proteins and 2S albumins as allergens. *Allergy* 2001;56:45–7.
- [35] Robotham JM, Teuber SS, Sathe SK, Roux KH. Linear IgE epitope mapping of the English walnut (*Juglans regia*) major food allergen Jug r 1. *J Allergy Clin Immunol* 2002;109:143–9.
- [36] de Leon MP, Glaspole IN, Drew AC, Rolland JM, O'Hehir RE, Suphioglu C. Immunological analysis of allergenic cross-reactivity between peanut and tree nuts. *Clin Exp Allergy* 2003;33:1273–80.
- [37] Leonard R, Petersen BO, Himly M, Kaar W, Wopfner N, Kolarich D, et al. Two novel types of O-glycans on the mugwort pollen allergen Art v 1 and their role in antibody binding. *J Biol Chem* 2005;280:7932–40.
- [38] van der Veen MJ, van Ree R, Aalberse RC, Akkerdaas J, Koppelman SJ, Jansen HM, et al. Poor biologic activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins. *J Allergy Clin Immunol* 1997;100:327–34.
- [39] Ganglberger E, Sponer B, Schöll I, Wiedermann U, Baumann S, Hafner C, et al. Monovalent fusion proteins of IgE mimotopes are safe for therapy of type I allergy. *FASEB J* 2001;15:2524–6.
- [40] Hantusch B, Krieger S, Untersmayr E, Schöll I, Knittelfelder R, Flicker S, et al. Mapping of conformational IgE epitopes on Phi p 5a by using mimotopes from a phage display library. *J Allergy Clin Immunol* 2004;114:1294–300.