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Linear IgE-epitope mapping and comparative structural homology modeling of hazelnut and English walnut 11S globulins

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ABSTRACT

Allergic reactions to walnuts and hazelnuts can be serious. The 11S globulins (legumins) have been identified as important allergens in these and other nuts and seeds. Here we identify the linear IgE-binding epitopes of walnut and hazelnut 11S globulins, and generate 3D 11S globulin models to map the locations of the epitopes for comparison to other allergenic homologues. Linear IgE-epitope mapping was performed by solid-phase overlapping 15-amino acid peptides probed with IgE from pooled allergic human sera. Several walnut (Jug r 4) and hazelnut (Cor a 9) 11S globulin peptides with reactivity to patient IgE were identified. Comparative alignment with cashew (Ana o 2), peanut (Ara h 3), and soybean G1 (Gly m 6.0101) and G2 (Gly m 6.0201) allergenic homologues revealed several shared allergenic 'hot spots'. Homology modeling was performed based on the atomic structure of the soybean glycinin. Surface map comparisons between the tree nut and peanut homologues revealed structural motifs that could be important for IgE elicitation and binding and show that, contrary to predictions, the reactive epitopes are widely distributed throughout the monomeric subunits, both internally and externally, including regions occluded by quaternary subunit association. These findings reveal structural features that may be important to allergenicity and cross-reactivity of this protein class.

1. Introduction

Allergies to tree nuts and peanuts can be severe, are generally not outgrown, and appear to be increasing in prevalence (Bock et al., 2001; Sicherer et al., 2003). Sensitivity to walnuts, cashews and hazelnut are the first, second, and sixth most frequent tree nut allergies in the US, respectively (Sicherer et al., 2003). Most identified nut allergens are seed storage proteins, many of which are members of the evolutionarily related cupin family 11S and 7S globulins, also termed legumins and vicilins, respectively (Breiteneder and Radauer, 2004). The 11S globulins are synthesized as single polypeptides which are most often post-translationally cleaved into acidic (MW ~ 50–60 kDa) and basic (MW ~ 20 kDa) subunits that remain joined by an intermolecular disulfide bond (Shewry et al., 2003). These proteins are typically deposited in high quantities in the maturing nut as non-glycosylated trimers or dimers of trimers (hexamers) with molecular masses of up to 450 kDa (Shewry et al., 2003).

Recombinant 11S proteins of walnut (Jug r 4) (Wallowitz et al., 2006), hazelnut (Cor a 9) (Beyer et al., 2002), cashew (Ana o 2) (Wang et al., 2003), and peanut (Ara h 3) (Rabjohn et al., 1999) have been identified as important allergens. The amino acid (aa) sequence of this family of proteins is fairly well conserved, and IgE cross-reactivity between tree nuts and, to a lesser extent, between tree nuts and peanuts has been reported (Poltronieri et al., 2002; Asero et al., 2004; Wallowitz et al., 2006; de Leon et al., 2003; Goetz et al., 2005; de Leon et al., 2005). Our recent studies show that the 11S globulins of these nuts are likely to be involved in this cross-reactivity (Wallowitz et al., 2006, 2007). Based on a comparison of peanut Ara h 3 (Rabjohn et al., 1999) and cashew Ana o 3 (Wang et al., 2003) linear epitopes, aa homologies and molecular modeling, Barre et al. (2007) predicted that certain peptide segments in the walnut and hazelnut would be IgE targets. Here, we identify the linear IgE-binding legumin epitopes of hazelnut Cor a 9 and English walnut Jug r 4, compare them with those previously identi-

Abbreviations: MW, molecular weight; aa, amino acid.

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fied on cashew Ana o 2 and peanut Ara h 3, and, through homology modeling, generate 3D epitope maps of all 4 allergens for structural comparisons.

2. Methods

2.1. Human sera

Blood samples were drawn after informed consent from patients with self-reported reactions to walnut and hazelnut and the sera stored at −70 °C until use. The study was approved by the relevant institutional review boards. The presence of walnut- and hazelnut-reactive IgE was confirmed by CAP FEIA assay (Phadia, Inc., Uppsala, Sweden) and specific Jug r 4 or Cor a 9 reactivity was confirmed by immunoblotting against nut protein extract. Clinical characteristics of the subjects are shown in Table 1.

2.2. Solid-phase peptide (SPOTs) synthesis and binding to IgE

SPOTs membranes displaying either the 508 aa sequence of Jug r 4 or the 515 aa sequence of Cor a 9, in the form of 62 or 63 15-aa peptides, respectively, each offset by 8 residues, were synthesized on derivatized cellulose sheets and probed as previously described (Wang et al., 2003).

2.3. Sequence analysis and molecular modeling

Primary sequences were aligned using BLAST 2.0. For modeling, aa sequences were aligned with the sequence of the soybean glycinin A3B4 homohexamer (Adachi et al., 2003) (PDB 1od5, http://www.rcsb.org/pdb/home/home.do) using CLUSTALX (Thompson et al., 1997) and adjusted manually to allow modeling of target sequence regions not resolved in the crystal structure. Models were constructed by submitting the threaded and aligned PDB files to the automatic homology server SWISS-MODEL (Schwede et al., 2003). The resultant PDB models were subjected to successive rounds of Steepest Descent energy minimization (until delta E was <0.050 kJ/M) using GROMOS96 (van Gunsteren and Berendsen, 1987) to relieve local conflicts and bond length anomalies. A single monomer from each target was selected and superimposed over the remaining two subunits to impose symmetry in the final trimer model using THESEUS (Theobald and Wuttke, 2006; Theobald and Wuttke, 2008). Final models for imaging were created using PyMol (http://www.pymol.org) by merging a solid surface model of the templated regions with a ribbon diagram of the un-templated regions. Swiss-PdbViewer v4.0.1 (http://www.expasy.org/spdbv/) was used to generate electrostatic charge models. Solvent-exposed residues (1.4 Å probe) on the monomer, trimer and hexamer were determined based on modeling of the soybean proglycinin A3B4 homohexamer (PDB 1od5) and analysis using the GETAREA program (http://curie.utmb.edu/getarea.html).

3. Results

3.1. Jug r 4 linear epitopes

The aa sequence of recombinant (r)Jug r 4 was screened for IgE-binding linear epitopes by probing 62 overlapping solid-phase synthetic peptides with sera from 17 walnut-allergic (Table 2), rJug r 4-IgE reactive patients (Wallowitz et al., 2006). Each was assigned to one of four pools (3–4 sera each). As presented in Table 2, the 17 serum samples, distributed among four pools, collectively reacted strongly with 3, moderately with 7, and weakly with 12 peptides. It should be noted that differences in the intensity of the reaction may reflect the affinity of the reactants, the relative amount of IgE antibody present in the sera or a combination of the two parameters. Many of the reactive peptides partially overlap adjacent reactive peptides and thus may together represent single epitopes.

Table 1
Characteristics of walnut and hazelnut-allergic subjects.

No.[a]	Sex	Age	Allergy to walnut (W)/hazelnut (H)	IgE reactivity on immunoblot to W or H	Other atopy	Other known food reactivity
1	M	25	W, H	W = weak	asthma	cashew, pecan
3	F	26	W	W = weak	AD, AR asthma	peanut, cashew pistachio
4	M	27	W, H	W = mod H = weak	asthma	cashew, brazil, coconut
5	F	54	W, H	H = weak	AR, asthma	cashew, pecans
7	F	30	W, H	W = mod H = strong	AD,AR	peanut, cashew
8	F	43	W, H	W = strong	AD, AR, asthma	peanut, unspecified tree nuts
9	F	35	W	W = strong	AD, AR, asthma	cashew, pecans, almond
10	F	31	W, H	H = strong	AR, asthma	cashew, sunflower
14	F	39	W, H	W = strong	asthma	unspecified tree nuts
20	F	48	W, H	W = weak H = weak	AD, AR asthma	peanut, cashew, chestnut
32	M	38	W, H	W = mod	AD, asthma	cashew, pecan, pistachio
44	F	50	W, H	W = strong H = strong	AD, AR asthma	almond, pecan, pine nut
48	F	62	W	W = weak	none	peanut, cashew, almond
49	F	22	W	W = weak	asthma	pecan, macadamia
81	M	13	W	W = mod	asthma	almond, NE other tree nuts
82	F	24	W	W = weak	none	peanut, walnut, almond, pine nut, NE other tree nuts
83	M	6	W	W = strong	Asthma	walnut, NE other tree nut
91	F	34	W, H	H = strong	AD	cashew
92	M	4	H	H = strong	AD, AR	NE other tree nuts
93	F	11	W, H	H = strong	AD, AR	peanut, NE other tree nuts
94	M	20	H	H = mod	AD, AR	peanut
95	M	2	W, H	H = mod	AD, AR	NE other tree nuts
96	M	10	W, H	H = weak	AD, AR	none reported

[a] Patient #s 1–49 correspond to those in previous publications (Wang et al., 2002, 2003; Robotham et al., 2005) and 81–96 represent new patients. Weak = patient IgE bound with weak intensity, mod: moderate, and strong: strong. NE: never eaten.