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Using patient serum to epitope map soybean glycinins reveals common epitopes shared with many legumes and tree nuts

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ABSTRACT

Soybean consumption is increasing in many Western diets; however, recent reviews suggest that the prevalence of soy allergy can be as high as 0.5% for the general population and up to 13% for children. The immunoglobulin-E (IgE) binding of sera from six soy-sensitive adult human subjects to soybean proteins separated by 2D gel electrophoresis was studied. Synthetic peptide sets spanning the mature glycinin subunit A2 and A3 primary sequences were used to map the IgE-binding regions. Putative epitopes identified in this study were also localized on glycinin hexamer models using bioinformatics software. We identified linear IgE-binding epitopes of the major storage protein Gly m 6 by screening individual soy-sensitive patient sera. These epitopes were then further analysed by 3D *in silico* model localization and compared to other plant storage protein epitopes. Web-based software applications were also used to study the ability to accurately predict epitopes with mixed results. A total of nine putative IgE-binding epitopes were identified in the glycinin A3 (A3.1–A3.3) and A2 (A2.1–A2.6) subunits. Most patients' sera IgE bound to only one or two epitopes, except for one patient's serum which bound to four different A2 epitopes. Two epitopes (A3.2 and A2.4) overlapped with a previously identified epitope hot spot of 11S globulins from other plant species.

Most epitopes were predicted to be exposed on the surface of the 3D model of the glycinin hexamer. Amino acid sequence alignments of soybean acidic glycinins and other plant globulins revealed one dominant epitope hot spot among the four reported hot spots. This study may be helpful for future development of soy allergy immunotherapy and diagnosis.

1. Introduction

Food allergy is an important public health concern with eight major foods accounting for 90% of all food allergies: milk, eggs, fish, Crustacean shellfish, tree nuts, peanuts, wheat, and soybeans (US Food and Drug Administration, 2004; Verma et al., 2013). In addition to high rates of soy allergy among children and adult populations (Katz et al., 2014), the consumption of soy is rising in Western diets mainly as a result of newly described health and wellness attributes and the desire of some to consume plant-based proteins (Chen et al., 2014). Therefore, the potential for accidental exposure is an increasing concern.

Soybean is valued nutritionally for its high seed protein content (approximately 36–38% w/w). The storage proteins, glycinins (11S globulins) and β-conglycinins (7S globulins), are major contributors (80%) to the seed storage protein content of this plant. These seed proteins also have a major impact on the nutritional value and functional quality of soybean food products (Poysa et al., 2006).

At least 19 different soybean proteins have been reported to bind to human sera IgE from soy-allergic patients, including Gly m 1 to Gly m 6, Gly m Bd 28K, Basic 7S globulin precursor (SBg7S) and its low MW subunit, Gly m Bd30K (P34), a 15-kDa LEA group III protein, and sucrose-binding protein homologue S-64 (L'Hocine and Boye, 2007; Holzhauser et al., 2009; Gagnon et al., 2010).

The allergenic properties of soy glycinin (Gly m 6) have been extensively described (Holzhauser et al., 2009,b; Helm et al., 2000a,b; Zhao et al., 2015). A European study found that 86% (6/7) of patients with anaphylaxis to soy had IgE that bound to Gly m 5 or Gly m 6, compared to 55% (6/11) of patients with moderate, and 33% (4/12) with mild soy-related symptoms (Holzhauser et al., 2009).

Abbreviations: AA, amino acid; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; HS, hot spot; IgE, immunoglobulin E; RAST, radioallergosorbent test; SDAP, structural database of allergenic proteins; SDS, sodium dodecyl sulfate; TBS-BSA 2%, 2% bovine serum albumin in tris buffered saline; TMB, Tetramethylbenzidine.

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Table 1
Clinical characteristics of soy allergic/sensitive patients.

Patient	Sex/Age	ImmunoCAP scores (kU/L)				
		Soy	Peanut	Almond	Hazelnut	Walnut/Pecan
1	M/33	>100	15.9–45.5[d]	9.13–13.2[d]	8.41–12.7[d]	–
2[*]	F/25	4+[b]	Self-reported	–	–	–
3[a]	M/18	15	>100[d]	0.59[d]	0.3[d]	–
4	F/38	9.08	–	22.1	–	–
5	F/28	7.85	6.29	–	48.4	35.1/23
6	F/23	3+[b] 68x[c]	Self-reported	–	–	–

[a] DPC immulite score.
[b] Skin Prick Test.
[c] RAST score.
[d] Determined in other bleeds, (–) not determined.
[*] Anaphylactic.

In Japan, where soybean has traditionally been a significant component of the daily food intake, studies revealed that the majority of the population (allergic or not) had elevated IgE levels reactive to Gly m 5 and Gly m 6 (Komei et al., 2011).

Glycinins belong to the large functionally diverse cupin storage protein superfamily named after a conserved β-barrel domain (Dall'Antonia et al., 2014). The cupin protein family has previously been shown to represent one of the largest groups of plant food allergens (Jenkins et al., 2005). According to the AllFam database of allergen families, there are 43 cupin allergens in plants (http://www.meduniwien.ac.at/allergens/allfam/factsheet.php?allfam_id=AF045) of which, 18 are 11S globulins. Examples include peanut Ara h 3 (Rabjohn et al., 1999); English Walnut Jug r 4 (Wallowitz et al., 2006); pecan Car i 4 (Sharma et al., 2011); hazelnut Cor a 9 (Beyer et al., 2002); cashew Ana o 2 (Wang et al., 2003); and almond Pru du 6 (Willison et al., 2011).

Linear epitopes have been previously mapped for soy glycinin A2 (Helm et al., 2000b; Xiang et al., 2002) and A1a subunits (Beardslee et al., 2000) using human sera IgE and for A1a and A5A4 subunits using swine sera IgG (Taliercio and Kim, 2013). In this study, we described and compared the linear epitopes found in the glycinin A2 and A3 subunits using individual serum IgE from a panel of adults with soybean sensitivity. Patient serum was selected based on western blot screening for those individuals reacting to either of the A2 or A3 subunits. We then compared these epitopes to those described in other legumes and tree nut species containing 11S globulin allergens (cupins) to characterize both unique and shared domains. We present data suggesting that many cupin allergens share protein folds, which contain epitope hot spots (HS) or common domains primarily on their exposed hydrophilic surfaces. However, we also show that protein sequence similarity alone is not necessarily the best predictor of storage protein allergenicity, nor of epitope HS within this limited number of patient sera thus far analysed.

2. Materials and methods

2.1. Patient serum

Soy-sensitive sera were selected from a panel of 36 adult patients, of which 32 have previously been described by Gagnon et al., (2010) and four additional sera were obtained from PlasmaLab International (http://www.plasmalab.com). Informed consent was obtained from all patients prior to serum collection. ImmunoCAP scores for soy and other legumes and nuts of these subjects are detailed in Table 1. Patient cross-reference to Gagnon et al., (2010) is as follows: 1(CS2), 2(DP), 3 (DG), 4(RB), 6(LM). Patient 6 has previously been clinically diagnosed with soybean allergy (Herian et al., 1990), whereas other patients were sensitized with unconfirmed clinical manifestations. The median age of patients was 30 years (interquartile range 18–55 years).

2.2. Immunoblot analysis

Western blotting and immune screening of human sera was conducted as previously described (Gagnon et al., 2010). Membranes were hybridized with patient serum dilutions ranging from 1/50 to 1/500 (see Fig. 1 in Saeed et al., submitted).

2.3. Epitope mapping

Two peptide sets representing the mature amino acid sequences of glycinin A2 (P04405, 90 peptides) and A3 (BAB15802, 104 peptides) were synthesized and biotinylated by Mimotopes (http://www.mimotopes.com) *via* parallel array platform. Quality Control Assurance was provided for both peptide synthesis and biotinylation by reverse phase HPLC (RP-HPLC), and by mass spectrometry (MS) respectively. The biotinylated 12-mer peptides, frame-shifted by three residues were used as per manufacturer's instructions (Application/Method PT3013). The epitope mapping experiments are described in Saeed et al., submitted.

2.4. Sequence alignments

Genebank accessions used in the sequence alignments for Figs. 1 and 2 were BAB15802 (A3), P04405 (A2), P04776 (A1a), AAM46958.1 (Ara h 3), AAW29810.1 (Jug r 4), ABW86978.1 (Car i 4), AAL73404.1 (Cor a 9), AAN76862.1 (Ana o 2), CAA55009.1 (Pru du 6.01), and CAA55010.1 (Pru du 6.02). Alignments were performed using Clustal W (http://www.ebi.ac.uk/Tools/msa/clustalw2).

Epitopes reported by others in Fig. 2 were as follows: A2: Helm et al., 2000b and Xiang et al., 2002; A1a: Beardslee et al., 2000; Arah3: Rabjohn et.al., 1999; Flinterman et al., 2008; and Rougé et al., 2009; Jug r 4 and Cor a 9: Robotham et al., 2009; Car i 4: Sharma et al., 2011; Ana o 2: Wang et al., 2003; Pru du 6.01/6.02: Willison et al., 2011.

2.5. B-cell epitope prediction servers

Three popular B-cell epitope prediction servers (ABCpred (Saha and Raghava, 2006), BepiPred 1.0 (Larsen et al., 2006) and SVMTriP (Yao et al., 2012)) were tested with the A2 and A3 sequences and the results were compared to the epitopes obtained in this study. For further details, see Table 3 (Saeed et al., submitted).

2.6. Molecular modeling

Molecular graphics and analyses were performed with the UCSF Chimera package. Chimera was developed by the Resource for