Allergen protein families – what makes them so peculiar?

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(Food) Allergens belong to a limited number of protein families – sharing structural determinants and displaying sequence similarity - the molecular basis of cross reactivity!

Allergens in ALLFAM Database
(http://www.meduniwien.ac.at/allergens/allfam/)

- **1.043 Allergens from 180 Allfam Protein** families
  (out of 12.273 Pfam protein families) – 2% of all known domains

Cross-reactivity

- IgE cross-reactivity among members of an allergen family does not always correlate with clinical significance
Food and Pollen Allergens: Examples

- nsLTPs (lipid transfer proteins)
- 2S Albumins
- Parvalbumins
- PR10 (Bet v 1 homologues)
- Profilin
Non specific lipid transfer proteins

- Monomeric proteins 7-9 kDa
- Robust structure due to 4 disulfide bridges
- Lipid transport across membranes / pathogen defense
- Accumulation of nsLTPs in outer layers of plant organs
- Primary sensitization via food allergen
- First description as food allergen from peach peel Pru p 3
Allergenic nsLTPs in ALLFAM Database

- Pollen: 9
- Plant Food: 27

- Cross-reactivity within Rosaceae fruits high
- North-South Difference in Sensitization
- Eliciting severe symptoms
Cross reactivity and clinical relevance

4 IgE binding epitopes of nsLTPs from Rosaceae (covering 40% of the molecular surface)

_Borges et al._ Biochem Biophys Res Comm. 2008
Cross reactivity and clinical relevance

Cross reactivity between Api g 2 (celery), Art v 3 (mugwort) and Pru p 3 (peach)

Gadermaier et al. Plos One 2011;
nsLTP: physicochemical properties and allergenicity

- Resistance to proteolysis ✓
- Stable 3D structure ✓
- Abundancy ✓/
- Solubility ✓
- Matrix - protein interaction ✓
- Primary sensitizer ✓
2S Albumins

- Heterodimeric proteins (subunits ~4 and 9 kDa resp.)
- Characteristic fold (4 disulfide bridges)
- Seed storage proteins
- Stable proteins
- First description of an allergenic 2S albumin from soybean
Structural homology within the protein family is high – sequence similarity across plant species is rather low - IgE cross-reactivity across distantly related botanical families rather low

*Moreno & Clemente Open Biochem J 2008,*
Cross reactivity and clinical relevance

Cross reactive epitopes among 2S albumins from tree nuts: Car i 1 (pecan), Jug r 1 (walnut) and Ana o 3 (cashew)

• Sharma et al. J Agricult Food Chem 2011
Cross-reactivity and clinical relevance

• Major allergens (peanut, tree nuts) – in some foods minor allergens
• Elicitor of severe systemic reactions

IgE cross-reactivity: peanut – lupine; mustard – rapeseed; sesame – poppy seeds;

2S Albumins: physicochemical properties and allergenicity

- Resistance to proteolysis ✓
- Stable 3D structure ✓
- Abundancy ✓
- Solubility ✓/
- Matrix - protein interaction* ✓
- Primary sensitizer ✓

*Ber e 1 – allergenic activity dependant on binding to lipid fraction
Parvalbumin

- Ca$^{2+}$ binding protein (12 kDa)
- Characteristic helix-loop-helix (EF-hand) motif
- Major allergens in fresh and salt water fish
- AllFam Database: 25 Allergens
- Present in muscle tissue
- Resistant against heat treatment and proteolysis

Griesmeier et al. Allergy 2009
Summary Parvalbumins

- **Parvalbumins** are
  - major fish allergens and contribute to clinical cross reactivity among various fish and frog species
  - Sequence similarity of parvalbumins and abundancy determine level of cross-reactivity
  - Point mutations at Ca-binding sites affect IgE binding capacity

Parvalbumins: physicochemical properties and allergenicity

- Resistance to proteolysis  ✓
- Stable 3D structure  ✓
- Abundancy  ✓
- Solubility  ✓
- Matrix - protein interaction ?
PR 10 (Bet v 1 homologues)

- 17 kDa proteins (154 – 160 aa) present in many plant species with high sequence similarity
- Plant steroid carrier
- First allergen identified from birch pollen (Bet v 1)
- Proteins with low stability (pH extremes, enzymatic treatment)
• Allergenic PR10 proteins in ALLFAM Database
• Pollen: 12
• Plant Food: 18

• Primary sensitization via the pollen allergen
• (approx. 70% of Fagaceae pollen allergics develop related plant food allergies)
• Prevalence: North – South difference (Fagaceae distribution)
• Eliciting mild symptoms – exemptions exist:
• Gly m 4, Api g 1, Dau c 1 – induces severe symptoms in allergic patients

• Ballmer-Weber & Hoffmann-Sommergruber Curr Opin Allergy Clin Immunol 2011
Relevant IgE Epitopes

- Bet v 1 and Mal d 1 share ~56% sequence identity
- 75% of Mal d 1 surface is likely to bind anti-Bet v 1 antibodies*

Hypoallergenic molecules with point mutations**

- *Jenkins et al. 2005, J Allergy Clin Immunol.;
- **Ma et al, 2005, Int Arch Allergy Immunol
### Bet v 1: physicochemical properties and allergenicity

<table>
<thead>
<tr>
<th>Property</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance to proteolysis</td>
<td>-</td>
</tr>
<tr>
<td>Stable 3D structure</td>
<td>-</td>
</tr>
<tr>
<td>Abundancy</td>
<td>✓</td>
</tr>
<tr>
<td>Solubility</td>
<td>✓</td>
</tr>
<tr>
<td>Matrix - protein interaction</td>
<td>✓</td>
</tr>
<tr>
<td>Primary sensitizer (Bet v 1)</td>
<td>✓</td>
</tr>
<tr>
<td>No individual sensitizing capacity:</td>
<td></td>
</tr>
<tr>
<td>Bet v 1 homologous food allergens)</td>
<td>✓</td>
</tr>
</tbody>
</table>
Profilin

- 12–15 kDa monomeric proteins (125 – 133 aa); pI 4.3 – 9.2;
- Actin binding, involved in various cell signalling pathways (e.g. cytokinesis)
- First allergen description: Bet v 2 (birch pollen)
- Plant derived profilins share overall three dimensional structure and sequence similarity of 75%
Since then numerous allergenic profilins identified from various plant species (Fruits, vegetables, spices).

PanAllergen!

Allergenic profilins in ALLFAM Database

Pollen: 24

Plant Food: 25
Profilin in pollen-plant food cross-reactivities

- Primary sensitization via pollen profilin
- Sensitization in various plant foods 10 – 20%
- Protein with intermediate stability
- However, North-South difference in sensitisation patterns
Epitope analysis of Profilin

- Hev b 8 structure as a model

Molecular surface of profilins contain conserved and variable residues. Epitope 7 and central part of epitope 1 are classified as conserved whereas epitope 8 is rather variable – Evidence for range of cross reactivity

Radauer et al, Clin Exp Allergy, 2006
Clinical relevance

minor food allergen in a range of vegetables, fruits and nuts (apple, carrot, celeriac).

Major food allergen in melon, orange/citrus, banana and tomato

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<tr>
<td>Solubility</td>
<td>√</td>
</tr>
<tr>
<td>Matrix - protein interaction</td>
<td>?</td>
</tr>
<tr>
<td>Primary sensitizer: Pollen Profilin</td>
<td>√</td>
</tr>
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</table>
Allergens
Purification and Authentication

• Knowledge on purified allergens has exploded within the past 10 years –

• Impact on
• Component resolved Diagnosis
• Options for Immunotherapy
• Identification of allergens in foods (labelling)

• However: no standardised procedure – how to assess integrity of purified molecules?

Sancho, et al.: 2010 Clin Exp Allergy
## Physico-chemical Characterization of Allergens

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Methods</th>
</tr>
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<tbody>
<tr>
<td>Sequence verification</td>
<td>MALDI-TOF, Q-TOF MS, N-terminal sequencing</td>
</tr>
<tr>
<td>Isoforms</td>
<td>2D-PAGE, LC-MS</td>
</tr>
<tr>
<td>Folding</td>
<td>CD, FT-IR, NMR</td>
</tr>
<tr>
<td>Aggregation</td>
<td>Size exclusion chromatography (SEC)</td>
</tr>
<tr>
<td>Purity (proteolysis)</td>
<td>SDS-PAGE, SEC, ES-MS</td>
</tr>
<tr>
<td>Biological activity</td>
<td>Lipid binding, enzymatic activity</td>
</tr>
<tr>
<td>IgE reactivity</td>
<td>ELISA, RAST, CAP, Chip; Immunoblotting (IB), inhibition assay; (human reference sera), His release assay;</td>
</tr>
</tbody>
</table>

_Hoffmann-Sommergruber, Mills & Vieths, 2008 “Mol Nutr Food Res_
Mass spectrometry rPru p 1

Sequenced peptides: (trypt. digest):

- GVFTYESEFTSEIPPPRLFKAFVLDADNLVPKIAPOAIKHSEILELEGDGPGTIKKITFG 60
  EGSQYGYVKHKIDSIDKENHSYSYTLEGDALGDNLKEKSYETKLVASPSSGISIKSTSH 120
  YHTKGDVIEIKEEHVKAEGKEKASNLFKLIETYLKGGPDAYN 159

rPru p 1

Theoretical: 17516.6 Da
Measured: 17516 Da

Gaier et al. Mol Nutr Food Res Epub 2008 April
Thermally induced unfolding (95°C) and refolding is pH dependant

- CD at pH 7.5
- .......CD at pH 3.0
CD Pru p 3

Stepwise thermally induced unfolding (95°C) shows irreversible denaturation—pH dependant (pH 7.5 versus pH 3).
Novel methods

- 1D-1H-NMR spectroscopy
- Tertiary structure of proteins determined at atomic resolution under physiological conditions (0.5 mg/ml)
- Spectrometry was carried out at 700 MHz
- Pru p 3 – nsLTP from peach
- Fingerprint for protein family
LTP Protein family

rCor a 8
0.15 mM, 115 AA, 11.8 kDa, 256 scans

nMal d 3
0.08 mM, 115 AA, 11.4 kDa, 6504 scans

nPru p 3
0.39 mM, 91 AA, 9.2 kDa, 256 scans

LTP Protein family
1H-NMR Spectra. Amide-aromatic region enlarged and rescaled.
Allergic Sensitization

- Allergen uptake by dendritic cells (DCs):
  Ber e 1 – versus SFA8:

Murine DCs in co-culture with T-cells:
Th1-cytokines induced by both proteins, Th2-type cytokines only by Ber e 1;*

Bet v 1 versus Api g 1:
Bet v 1 induced Th2 cytokine production; Api g 1 a Th1 cytokine pattern;**

Smole et al. Allergy 2010
Smole et al. Allergy 2010
Conclusions

• It is only a minority of protein families that possess an allergenic activity
• Several factors contribute to make a protein an allergen
• Novel efficient and high throughput methods are needed for a reliable allergenic risk assessment
Food Allergy and Anaphylaxis Campaign: June 2012 – June 2013

EAACI Meeting Geneva

Food Allergy Training Course Aug 2012, Vienna

Food Allergy and Anaphylaxis Meeting (FAAM) in Feb 2013, Nice

EAACI/WAO Meeting Milano, June 2013
Substantial Equivalence: valid for immunologic studies? (Glycosylation patterns, endotoxins).

Range of variability within one line known?

Relevance of an animal model for allergenicity testing

So far no validated animal model available for allergenic risk assessment
EFSA: Scientific opinion on allergenicity assessment of GM plants

- Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed 2010; EFSA Journal 2010; 8(7):1700:

- Up to date scientific evidence and state of the art methodology compiled and reviewed
- Formulation of recommendations based on scientific evidence

http://www.efsa.europa.eu
Novel aspects

- In silico analysis: cut off 35% sequence identity (80 aa window); include structural aspects of GM proteins, food processing and food matrix and impact on allergenicity
- Post market monitoring optional
- Survey of different routes of exposure recommended
- Cell based assays useful
- Up to date proteomics should be applied
- So far no validated animal model for allergenicity testing available