Allergic Cross-reactivity Made Visible:  
NMR Structural Biology of Birch Pollen Allergens and 
Associated Food Allergens

Philipp Neudecker  
Lehrstuhl für Biopolymere  
Universität Bayreuth  
95440 Bayreuth, Germany

Cross-Reactivity Made Visible: Solution Structure of the Major Cherry Allergen Pru av 1, *J. Biol. 

P. Neudecker, K. Lehmann, and P. Rösch: Sequence-specific $^1$H, $^{13}$C and $^{15}$N resonance assignments 

P. Neudecker, K. Lehmann, J. Nerkamp, T. Haase, A. Wangorsch, K. Fötisch, S. Hoffmann, P. Rösch, 
S. Vieths, and S. Scheurer: Mutational epitope analysis of Pru av 1 and Api g 1, the major allergens of 
cherry and celery: correlating IgE reactivity with three-dimensional structure, *Biochem. J.*, in press 
(2003)

Schwarzinger, F. Ferreira, and P. Rösch: Solution Structure, Dynamics, and Hydrodynamics of the 
Calcium-bound Cross-reactive Birch Pollen Allergen Bet v 4 Reveal a Canonical Monomeric Two EF-
Allergy Types

Allergies are **hypersensitive immune reactions** to otherwise innocuous antigens, the so-called **allergens**. These hypersensitive immune reactions are commonly divided into **four types**:

<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
<th>Time course</th>
<th>Effectors</th>
<th>Antigens</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>IgE-mediated immediate-type hypersensitivity (allergy)</td>
<td>2 - 30 min</td>
<td>IgE bound to mast cells</td>
<td>pollen, food, animal dander, dust mite feces, insect venom</td>
<td>allergic rhinitis, asthma, food allergies, systemic anaphylaxis</td>
</tr>
<tr>
<td>II</td>
<td>Antibody-mediated cytotoxic hypersensitivity</td>
<td>5 - 8 h</td>
<td>IgM, IgG, complement</td>
<td>cell surface molecules</td>
<td>blood transfusion rejection (AB0), erythroblastosis fetalis (Rh)</td>
</tr>
<tr>
<td>III</td>
<td>Immune complex-mediated hypersensitivity</td>
<td>2 - 8 h</td>
<td>IgG, complement</td>
<td>DNA, non-self proteins</td>
<td>Arthus reaction, serum sickness, rheumatoid arthritis</td>
</tr>
<tr>
<td>IV</td>
<td>Cell-mediated delayed-type hypersensitivity</td>
<td>1 - 3 days</td>
<td>CD4+ T cells, CD8+ T cells, macrophages</td>
<td>M. tuberculosis, chromate, nickel</td>
<td>contact dermatitis, tuberculin reaction</td>
</tr>
</tbody>
</table>
Allergy Prevalence

Allergies nowadays affect an estimated 20 - 25 % of the population in industrialized countries. The prevalence of allergies is rising dramatically (75 % increase of the prevalence of asthma in the USA from 1980 to 1994).

Patient statistics clearly reveal a genetic predisposition for atopy. Children raised in rural areas are less affected than those raised in urban environments, and atopy is a rare phenomenon in developing countries. The prevalence of allergies in East Germany was lower than in West Germany (in spite of severe air pollution) and started to catch up after the reunification. Atopy therefore appears to be a pronounced civilization disorder.

=> What is the physiological basis of allergies?

=> What is the cause for the dramatic increase in the prevalence of allergies in industrialized countries in recent years?
**Immune Responses**

**Antigen-presenting cell**

- Somatic cell
- Cytosolic viral peptides

**T lymphocyte**

- MHC class I
- TCR
- CD8
- IFN-γ, TNF-α, TNF-β

**Effect (if co-stimulated via B7/CD28)**

- Activation of $T_c$ lymphocyte
  - $\Rightarrow$ Expression of Fas ligand
  - $\Rightarrow$ Induction of **apoptosis** via Fas
  - $\Rightarrow$ Containment of **viral** infection

- Activation of $T_{H1}$ lymphocyte
  - $\Rightarrow$ Expression of CD40 ligand
  - $\Rightarrow$ Activation of macrophage via CD40
  - $\Rightarrow$ **degradation** of endocytosed vesicular bacteria
  - $\Rightarrow$ Containment of **bacterial** infection

- Activation of $T_{H2}$ lymphocyte
  - $\Rightarrow$ Expression of CD40 ligand
  - $\Rightarrow$ Activation of B lymphocyte via CD40
  - $\Rightarrow$ Secretion of antibodies, isotype switching (also to IgE)
  - $\Rightarrow$ Neutralization of **toxins**
Atopy

The differentiation of naive CD4+ T_{H0} lymphocytes into either T_{H1} or T_{H2} lymphocytes is mainly determined by the cytokine pattern, particularly by IL-12 or IL-4, respectively.

A T_{H1}/T_{H2} imbalance favoring T_{H2} lymphocytes is called atopy. Atopic individuals therefore have a tendency to mount inadequate IgE-mediated immune responses and hence develop (often multiple) type I allergies.

(Figure 2 of M. Wills-Karp, J. Santeliz, and C. L. Karp: The germless theory of allergic disease: revisiting the hygiene hypothesis, *Nature Immunol.* 1, 69-75 (2001) is not included for copyright reasons)
The Hygiene Hypothesis

Due to lack of microbial exposition in utero the immune system of a newborn has primarily developed the $T_{H2}$-controlled response. Unlike healthy people, atopic individuals do not reverse this $T_{H1}$/$T_{H2}$ imbalance in their infancy.

The most promising hypothesis to explain the dramatic increase in the prevalence of allergies therefore postulates that improved hygiene conditions prevent sufficient stimulation of the normal $T_{H1}$-controlled immune response of children raised in industrialized countries. Although the typical food allergies (milk, eggs, bread) of atopic infants usually disappear upon growing up, they are more likely to develop the typical adulthood allergies (pollen, food, animal dander, dust mite feces, insect venom etc.) later on, usually as teenagers.

The actual mechanism of this immune modulation remains controversial. It has recently been proposed that insufficient microbial infection affects immune homeostasis by downregulation of the inhibitory immunoregulator IL-10 (counter-regulation hypothesis).
**Sensitization**

The physiological purpose of the IgE-mediated immune response appears to be the defence against parasitic pathogens.

Upon the first contact of a patient with a particular allergen, activated allergen-specific $T_{h2}$ lymphocytes activate allergen-specific B lymphocytes and secrete IL-4 to trigger isotype switching, resulting in the production of a set of polyclonal allergen-specific IgE antibodies. This procedure is called sensitization.

After secretion by the B lymphocytes, IgE antibodies are bound on the surface of mast cells, basophils, and eosinophils via the high-affinity receptor FcεRI. IgE serum titers are therefore five orders of magnitude lower than those of IgG.
The IgE-mediated Allergic Reaction

Upon further contacts of a sensitized patient with the allergen, cross-linking of surface-bound IgE antibodies by simultaneous allergen binding triggers mast cell degranulation, which releases preformed inflammatory mediators like histamine. Clinically relevant allergens therefore have to present at least two (patient-specific) IgE binding regions on their molecular surface, the so-called IgE epitopes.
Allergic Cross-reactions

Sensitization to an allergen A followed by contact with a different allergen B whose homology to A is so close that at least two of the IgE epitopes of A are conserved on the molecular surface of B

=> B will be recognized by A-specific IgE antibodies

=> Allergic cross-reaction between the allergens A and B

Due to the patient-specificity of the exact location of the IgE epitopes on the molecular surface the pattern of allergic cross-reactions is also patient-specific.

Food allergies of adult patients are usually based on cross-reactions after sensitization to pollen or skin allergens. Exceptions are food allergies to nuts or seafood, which are usually generic food allergies.

Examples: Latex with avocado, banana, kiwi

  Grass pollen with melon, banana, zucchini, cucumber

  Birch pollen with apple, pear, cherry, peach, apricot, plum, celery, carrot, soy bean, hazelnut
Diagnosis and Treatment of Allergies

Diagnosis:

- **Skin prick tests**
  Problems: Quality and composition of extracts, allergic cross-reactions
  Solution: Recombinant allergens for component-resolved diagnosis

Treatment:

- **Strict allergen avoidance** (e.g. diet)
  Problems: Immune response is sensitive enough to detect even traces of food impurities, variety of possible cross-reactions

- **Specific immunotherapy** (repeated allergen injection to improve immune tolerance via stimulation of $T_H^1$-controlled responses and production of competing IgG)
  Problems: Potentially severe anaphylactic side-effects, new sensitizations
  Solution: Modify allergen to destroy IgE epitopes while retaining T cell epitopes

- Relieve symptoms by **downregulation of inflammatory responses** (antihistamines, corticosteroids, epinephrine, other β-adrenergic drugs)

- Still in trial stage: **Cytokines or cytokine inhibitors to reverse $T_H^1/T_H^2$ imbalance**, anti-IgE antibodies
Biochemical and Biophysical Aspects

- Identification and cDNA cloning of allergens (including isoforms and variants)
- Recombinant production of allergens for standardizable component-resolved diagnosis and as vaccines for specific immunotherapy
- Characterization of posttranslational modifications and conformational transitions in the framework of the physiological function of an allergen which might contribute to the stabilization of the tertiary fold and/or to the IgE epitopes, especially glycosylations (so-called cross-reactive carbohydrate determinants (CCDs))
- Localization of clinically relevant IgE epitopes, which are usually conformational rather than sequential in nature and whose delineation therefore requires structural biology, to produce hypoallergenic allergen derivatives as vaccines for patient-tailored specific immunotherapy with reduced anaphylactic side-effects
- Identification of cross-reactive IgE epitopes and cross-reactivity patterns to allow a more reliable diagnosis and an allergen avoidance as specific as possible
- Allergic risk assessment of foodstuff, production of hypoallergenic food and ultimately an answer to the still unanswered question...
What makes an allergen an allergen?

Only a small portion of plant and animal protein families constitute clinically relevant allergens, although in principle each non-self protein can trigger an immune response. Promising explanations include

- High concentration
- Medium size for preferential processing by the immune system
- High solubility, especially for pollen allergens for increased penetration into the nasal mucosa
- Sufficient resistance to heat denaturation and proteinase digestion, especially for food allergens for resistance to food processing (cooking) and increased release from the gastrointestinal tract, where immune reactions are very limited
- Species-specific physiological function (e.g. plant pathogenesis-related proteins), which renders the existence of homologous proteins in the human organism and hence immune tolerance very unlikely
## Birch pollen allergens

Seven birch (*Betula verrucosa*) pollen allergens have been identified so far (http://www.allergen.org):

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Molar mass</th>
<th>IgE prevalence</th>
<th>Classification</th>
<th>Physiological function</th>
<th>Cross-reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bet v 1</td>
<td>17.4 kDa</td>
<td>95 %</td>
<td>Pathogenesis-related class 10 (PR-10)</td>
<td>Lipid-binding?</td>
<td><em>Fagales</em> pollen, fruits, vegetables, nuts</td>
</tr>
<tr>
<td>Bet v 2</td>
<td>14.3 kDa</td>
<td>10 - 20 %</td>
<td>Profilin</td>
<td>Actin-binding</td>
<td>Panallergen</td>
</tr>
<tr>
<td>Bet v 3</td>
<td>23.7 kDa</td>
<td>10 %</td>
<td>3 EF-hands</td>
<td><em>Ca²⁺</em>-binding</td>
<td>Various pollen</td>
</tr>
<tr>
<td>Bet v 4</td>
<td>9.4 kDa</td>
<td>5 - 20 %</td>
<td>Polcalcin, 2 EF-hands</td>
<td><em>Ca²⁺</em>-binding</td>
<td>Various pollen</td>
</tr>
<tr>
<td>Bet v 6</td>
<td>34 kDa</td>
<td>12 %</td>
<td>Isoflavone-reductase-like</td>
<td>Phenylcoumaran benzylic ether reductase</td>
<td>Pollen, fruits</td>
</tr>
<tr>
<td>Bet v 7</td>
<td>18 kDa</td>
<td>20 %</td>
<td>Cyclophilin</td>
<td>PPI</td>
<td>(likely panallergen)</td>
</tr>
<tr>
<td>Bet v 8</td>
<td>65.3 kDa</td>
<td>?</td>
<td>Pectin esterase</td>
<td>Pectin esterase?</td>
<td>(likely)</td>
</tr>
</tbody>
</table>
The Bet v 1 Allergen Family

More than 90% of birch pollinotics are sensitized to Bet v 1, often accompanied by allergic cross-reactions to the major cherry (Prunus avium) allergen Pru av 1 (59% sequence identity with Bet v 1.0101), the major soy bean (Glycine max) allergen Gly m 4 (47%), and/or the celery (Apium graveolens) allergen Api g 1.0101 (41%).

Properties: Monomers of 157-159 amino acids (17 kDa, 4 < pI < 6)
Constitutive expression of Bet v 1 and Pru av 1, but stress-inducible expression of Gly m 4
Unknown physiological function(s)

Sequence alignment:

<table>
<thead>
<tr>
<th>Position</th>
<th>1</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bet v 1.0101:</td>
<td>GV</td>
<td>FNYET</td>
<td>ETTSVIPAARLFKAFI</td>
<td>LDGDNLF</td>
<td>PKVAPQAIS</td>
<td>SVEG</td>
<td>NNGGPGTIKIKI</td>
</tr>
<tr>
<td>Pru av 1:</td>
<td>GV</td>
<td>FTYE</td>
<td>SFSIEI</td>
<td>PPRLFKA</td>
<td>FVLADNLVPK</td>
<td>IA</td>
<td>PQAIKHSB</td>
</tr>
<tr>
<td>Gly m 4:</td>
<td>GV</td>
<td>FTFD</td>
<td>EINSPVAPA</td>
<td>TLYKALVTD</td>
<td>DAVN</td>
<td>VPKALDSFK</td>
<td>SVE</td>
</tr>
<tr>
<td>Api g 1.0101:</td>
<td>GV</td>
<td>QTHV</td>
<td>LETSSV</td>
<td>AESKFQ</td>
<td>GFVIDV</td>
<td>TFLPKAPA</td>
<td>GAYKSVEI</td>
</tr>
</tbody>
</table>

Sequence:

- **Bet v 1.0101:**
  - Position 1-60: GVFN...KIKISFP
  - Position 70-120: GFPFK...YKV
  - Position 130-180: HTKG...

- **Pru av 1:**
  - Position 1-60: GVFTY...KIKITF
  - Position 70-120: GSFQ...G
  - Position 130-180: HTKG...

- **Gly m 4:**
  - Position 1-60: GVFTF...KIKITF
  - Position 70-120: GFSQ...G
  - Position 130-180: HTKG...

- **Api g 1.0101:**
  - Position 1-60: GVQ...KIKIT
  - Position 70-120: GFPFK...KIKI
  - Position 130-180: HTKG...
Structure Determination of Pru av 1 and Gly m 4

Bet v 1:
Crystal and solution structure of Bet v 1.2801 (Gajhede et al., 1996)

Pru av 1 (Bruker DRX600):
NMR experiments: 2D-TOCSY, 2D-NOESY, $^{15}$N-HSQC, HNHA, 3D-$^{15}$N-TOCSY-HSQC, HNCO, HNCA, HNCACB, CBCA(CO)NH, $^{13}$C-CTHSQC, H(C)CH-COSY, CCH-COSY, CP-HC(C)H-TOCSY, 3D-$^{15}$N-NOESY-HSQC, 3D-$^{13}$C-NOESY-HSQC, 3D-$^{15}$N/$^{15}$N-HMQC-NOESY-HSQC, 3D-$^{13}$C/$^{15}$N-HMQC-NOESY-HSQC
Experimental restraints: 2299 NOEs, 71 dihedral angles (scalar couplings), 34 hydrogen bonds ($D_2O$ exchange)
Three-stage simulated annealing using X-PLOR 3.851
=> Set of 22 accepted structures (out of 60)
  Average RMSD 0.41 Å for the backbone, 0.72 Å for all heavy atoms (well-ordered regions only); 82.4 % in most favored regions of Ramachandran plot

Gly m 4 (Bruker DRX600, DMX750):
NMR experiments: 2D-TOCSY, 2D-NOESY, $^{15}$N-HSQC, $^{15}$N-IPAP, HNHA, 3D-$^{15}$N-TOCSY-HSQC, HNCO, HNCA, HNCACB, HBHA(CBCACO)NH, CBCA(CO)NH, H(CCO)NH, C(CO)NH, $^{13}$C-CTHSQC, H(C)CH-COSY, CCH-COSY, CP-HC(C)H-TOCSY, 3D-$^{15}$N-NOESY-HSQC, 3D-$^{13}$C-NOESY-HSQC, 3D-$^{15}$N/$^{15}$N-HMQC-NOESY-HSQC, 3D-$^{13}$C/$^{15}$N-HMQC-NOESY-HSQC
Experimental restraints (so far): 64 dihedral angles (scalar couplings), 118 dipolar coupling constants, 141 $^{13}$Ca/$^{13}$Cβ chemical shifts
Homology model based on Pru av 1 and Bet v 1 already fulfills the 118 dipolar coupling constants to a quality factor of $Q = 49.5 \%$. 
Comparison of Bet v 1.2801, Pru av 1, and Gly m 4
**IgE Cross-reactivity of the Bet v 1 Allergen Family**

a) Immunoblot inhibition by Bet v 1.0101 (Lanes 3)

b) Immunoblot inhibition by Api g 1.0101 (Lane 2)

Solid Phase:
Bet v 1.0101 Api g 1.0101 Mal d 1 Pru av 1 Pyr c 1 Cor a 1.0401

Lanes 1: Negative control
Lanes 1, 4: Negative control
Lanes 2: Positive control
Lane 3: Positive control
IgE Reactivity of Pru av 1 and Api g 1.0101 Mutants
NMR and CD Spectra of Pru av 1 and Api g 1.0101 Mutants

Pru av 1

- wt
- Δ155-159
- E45W
- S112A
- S112P

Api g 1.0101

- wt
- Pru av 1 wt
- Pru av 1 S112A
- Pru av 1 E45W
- Pru av 1 Δ(155-159)
- Pru av 1 S112P
$^{15}$N-HSQC Spectra of Pru av 1 wt and Pru av 1 E45W
Comparison of Pru av 1 wt and Pru av 1 E45W

COOH

NH₂

W45 E45

COOH
Comparison of Pru av 1 and the START Domain of MLN64
NMR Spectroscopy of Pru av 1 with Brassinosteroids

Low solubility of brassinosteroids

=> Solvent:
  90% H$_2$O / 10% DMSO-d$_6$ (v/v)

28-Homocastasterone
The Large Internal Hydrophobic Cavity of Pru av 1

NH$_2$

COOH
Positions of Deoxycholate in Bet v 1.1001
The Bet v 4 Allergen Family (Polcalcins)

About 5 to 20% of birch pollinotics are sensitized to Bet v 4, often accompanied by allergic cross-reactions to other pollen allergens like the timothy grass (*Phleum pratense*) polcalcin Phl p 7 (69% sequence identity with Bet v 4).

Properties: Monomers or dimers of 77-84 amino acids (9 kDa, 4 < pI < 5)  
- Pollen-specific expression and therefore no cross-reactive food allergens  
- 2 EF-hands (bind Ca\(^{2+}\), but not Mg\(^{2+}\))  
- Unknown physiological function

Sequence alignment:

<table>
<thead>
<tr>
<th>Position:</th>
<th>1</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bet v 4:</td>
<td>ADDHPQDKAERERIFKRFDANGDGKISAAELGEALKTLGSI TPDEVKHMMAEIDTDGDGF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phl p 7:</td>
<td>ADDMERIFKRFDTNGDGKISLSELTDALRTLGSTSADEVQRMMAEIDTDGDGF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca2+:</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>CA2+</td>
<td>ISFQETFDFGRANRGLLKDVAKIF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDFNEFISFCNANPGLMKDVAKVF</td>
<td></td>
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<td></td>
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<tr>
<td>*</td>
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</tr>
</tbody>
</table>
The Structural Transition upon Ca$^{2+}$ Binding

**a) CD spectra**

- apo Bet v 4
- holo Bet v 4

\[ \Theta_{\text{diss}} / 10^9 \text{ cm}^2/\text{dmol} \]

\[ \lambda / \text{nm} \]

\[ 190, 200, 210, 220, 230, 240, 250, 260 \]

**b) $^{15}$N-HSQC spectra**

- +/- apo
- +/- holo

\[ F_1 / \text{ppm} \]

\[ F_2 / \text{ppm} \]

\[ T_m = 47 \, ^\circ \text{C} \]
Structure Determination of Bet v 4

Holo Bet v 4 (Bruker DRX600, DMX750):
NMR experiments: 2D-TOCSY, 2D-NOESY, $^{15}$N-HSQC, $^{15}$N-IPAP, HNHA, 3D-$^{15}$N-TOCSY-HSQC, H(N)CO, long-range H(N)CO, HNCO, HNCA, C(CO)NH, $^{13}$C-CTHSQC, H(C)CH-COSY, CCH-COSY, 3D-$^{15}$N-NOESY-HSQC, 3D-$^{13}$C-NOESY-HSQC, 3D-$^{15}$N/$^{15}$N-HMQC-NOESY-HSQC, 3D-$^{13}$C/$^{15}$N-HMQC-NOESY-HSQC
Experimental restraints: 1442 NOEs, 50 dihedral angles (scalar couplings), 22 hydrogen bonds (long-range H(N)CO, D$_2$O exchange), 61 dipolar coupling constants ($S^2_{\text{RSDM}} > 0.7$ only), 74 $^{13}$C$_\alpha$/$^{13}$C$_\beta$ chemical shifts

Modeling of Ca$^{2+}$ ligation based on holo calmodulin (supported by experimental data)
Three-stage simulated annealing using X-PLOR 3.851
=> Set of 25 accepted structures (out of 90)
  Average RMSD 0.22 Å for the backbone, 0.57 Å for all heavy atoms (well-ordered regions only);
  94.2 % in most favored regions of Ramachandran plot

Apo Bet v 4 (Bruker DRX600):
NMR experiments: $^{15}$N-HSQC
Working on it...
Solution Structure of Holo Bet v 4

COOH

NH₂
Secondary Structure Elements of Holo Bet v 4
Comparison of Holo Phl p 7 and Holo Bet v 4
Rotational Diffusion Tensor of Holo Bet v 4
# Hydrodynamic Properties of Apo and Holo Bet v 4

<table>
<thead>
<tr>
<th></th>
<th>NMR(^a)</th>
<th>Ultracentrifugation(^b)</th>
<th>Calculation(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo Bet v 4</td>
<td>18.9 Å ± 0.4 Å</td>
<td>18.2 Å ± 0.1 Å</td>
<td></td>
</tr>
<tr>
<td>Holo Bet v 4</td>
<td>17.8 Å ± 0.4 Å</td>
<td>17.9 Å ± 0.2 Å (≈ 85 %)</td>
<td>18.3 Å</td>
</tr>
<tr>
<td></td>
<td>22.2 Å ± 0.2 Å (≈ 15 %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rotational diffusion autocorrelation</td>
<td>6.1 ns</td>
<td>6.5 ns</td>
<td></td>
</tr>
<tr>
<td>times</td>
<td>5.9 ns</td>
<td>6.2 ns</td>
<td>5.4 ns</td>
</tr>
<tr>
<td></td>
<td>5.4 ns</td>
<td>5.4 ns</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Hydrodynamic radii from NMR translational diffusion measurements relative to internal dioxane and overall rotational diffusion autocorrelation times from NMR longitudinal and transverse relaxation measurements assuming a prolate axially symmetric rotational diffusion tensor.

\(^b\) Hydrodynamic radii from sedimentation velocity experiments assuming a hydration of 30 % (w/w). Values are average values over measurements at 3 different protein concentrations from 0.15 mM to 0.68 mM (apo Bet v 4) or 4 different protein concentrations from 0.12 mM to 0.60 mM (holo Bet v 4) in the form average value ± standard deviation. The resulting molecular mass estimates are 10.18 kDa ± 0.08 kDa, 10.52 kDa ± 0.22 kDa, and 20.3 kDa ± 1.0 kDa for apo Bet v 4, monomeric holo Bet v 4, and dimeric holo Bet v 4, respectively.

\(^c\) Values calculated on the basis of a prolate ellipsoid of revolution with semi-axes of 19.5 Å and 13.6 Å, which has the same tensor of inertia as holo Bet v 4 in the prolate axially symmetric approximation, adding a hydration layer of 2.8 Å.
Bet v 4 exhibits a pronounced reversible conformational transition upon Ca^{2+} binding, thereby exposing a hydrophobic binding groove for a potential ligand. This is the typical mechanism of EF-hand proteins with a regulatory function like calmodulin or troponin C, in contrast to those with a Ca^{2+} buffering function like parvalbumin or calbindin D_{9k}.
Conclusions

1. The secondary structure elements and the tertiary structure of Bet v 1, Pru av 1, and Gly m 4 are virtually identical. This renders the existence of cross-reactive IgE epitopes most likely and therefore explains the clinically observed allergic cross-reactions.

2. The P-loop around Glu45 constitutes one of the dominant cross-reactive IgE epitopes, which are highly patient-specific. The reduced IgE reactivity of Pru av 1 S112P is caused by disruption of the native fold, demonstrating a particularly effective way of producing hypoallergenic vaccines with retained T cell epitopes.

3. The hydrophobic cavity of Pru av 1 interacts with brassinosteroids in vitro. Its physiological function in vivo can therefore be expected to involve binding of plant sterols or other lipids.

4. In spite of the different oligomerization state the tertiary structure of holo Phl p 7 and holo Bet v 4 is virtually identical. Again, this renders the existence of cross-reactive IgE epitopes most likely and therefore explains the clinically observed allergic cross-reactions.

5. Bet v 4 exposes a hydrophobic groove upon Ca^{2+} binding, whose purpose can be expected to be the binding of a hypothetical ligand as part of a Ca^{2+}-controlled regulatory function.
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Andrea Hager, Dr. Kristian Schweimer (NMR spectrometers)
Beate Diaw, Dr. Rainer Haeßner, PD Dr. Gerd Gemmecker, Prof. Dr. Horst Kessler (750 MHz NMR spectrometer, Technische Universität München, Germany)
Anke Eisenmann, Markus Zeeb, Dr. Stephan Schwarzinger, PD Dr. Jochen Balbach (NMR relaxation and diffusion)
Rainer Hofmann, Prof. Dr. Heinrich Sticht (bioinformatics)
Thomas Lauber, Dr. Amanda Nourse (analytical ultracentrifugation)
Prof. Dr. Paul Rösch (principal investigator)

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