Allergic cross−reactivity made visible: The solution structure of the major cherry allergen Pru av 1

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Birch pollinosis is often accompanied by hypersensitivity to fruit as a consequence of the cross−reaction of pollen−allergen specific immunoglobulin E (IgE) antibodies with homologous food proteins. To provide a basis for examining the cross−reactivity on a structural level we used heteronuclear multidimensional nuclear magnetic resonance (NMR) spectroscopy to determine the first high−resolution three−dimensional structure of a pollen−related food allergen, a well−defined structure of the major cherry allergen Pru av 1 in solution. The secondary structure elements and the tertiary fold of Pru av 1 are virtually identical to the major birch pollen allergen Bet v 1. The fact that the P−loop around Glu45, which is known as one of the IgE antibody binding epitopes of Bet v 1, is structurally conserved in Pru av 1 suggests this region to constitute a cross−reactive epitope. This might also explain why Api g 1.0101, the major celery allergen where Glu45 is replaced by lysine, fails to efficiently reduce IgE binding to Pru av 1 in immunoblot inhibition experiments. The large hydrophobic cavity expected to be important for the still unknown physiological function of Bet v 1 is also conserved in Pru av 1. Structural homology to a domain of the human protein MLN64 that is associated with cholesterol transport suggests phytosteroids as ligands for Pru av 1. NMR spectroscopy provides first experimental evidence that Pru av 1 indeed interacts with phytosteroids, and molecular modeling shows that the hydrophobic cavity is large enough to accomodate two such molecules.

Introduction

Up to 70 % of birch pollen allergic patients who suffer from clinical syndromes like rhinitis, asthma, and dermatitis also show hypersensitivity to fresh fruit or vegetables. The allergic reactions after ingestion of foodstuff are predominantly oropharyngeal, for example itching and swelling of lips, tongue and throat, but in rare cases even severe anaphylactic reactions are possible. The symptoms of these type I allergies are caused by an immune response which is triggered when two receptor−bound immunoglobulin E (IgE) antibodies on the surface of a mast cell or basophil are cross−linked by simultaneous binding of an otherwise harmless antigen, the so−called allergen. Pollen−associated food allergies are a consequence of the cross−reaction of pollen−allergen specific IgE antibodies with highly homologous proteins contained in foodstuff. The 17.4 kDa major birch (*Betula verrucosa*) pollen allergen Bet v 1 is responsible for IgE binding in more than 95 % of birch pollen allergic patients. A series of allergens with high sequence identity to Bet v 1 have been reported, pollen allergens from other trees belonging to the *Fagales* order as well as food allergens like Api g 1.0101 from celery (*Apium graveolens*), Mal d 1 from apple (*Malus domestica*), Pru av 1 from cherry (*Prunus avium*), Pyr c 1 from pear (*Pyrus communis)*, and Cor a 1.0401 from hazelnut (*Corylus avellana*) (Fig. 1).

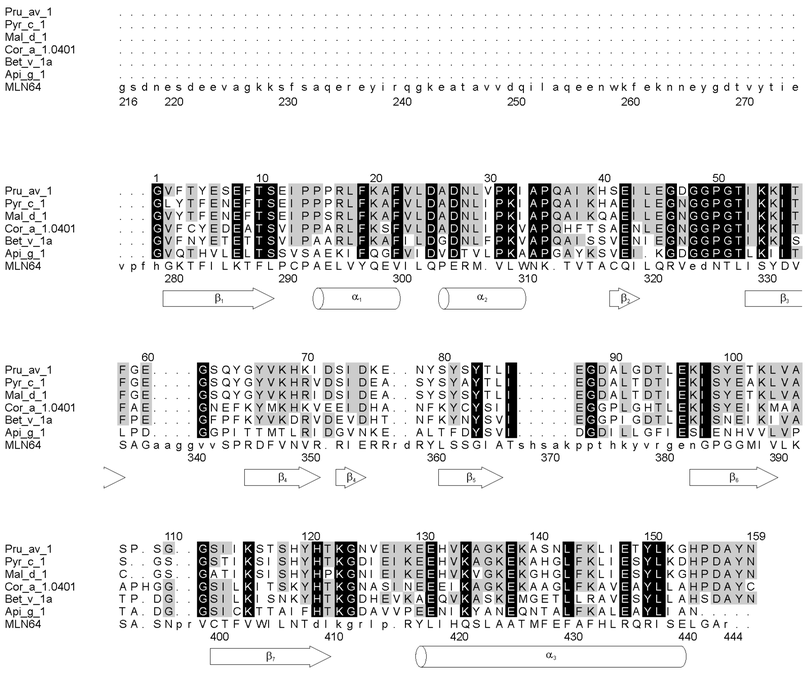

Fig. 1: Structure−based sequence alignment with Pru av 1 of Pyr c 1 (83.5% sequence identity to Pru av 1), Mal d 1 (82.9%), Cor a 1.0401 (64.4%), Bet v 1 isoform a (59.1%), Api g 1.0101 (41.2%), and the START domain of MLN64 (8.5%). The sequence positions above and below the sequences correspond to Pru av 1 and MLN64, respectively. Gaps in the alignment are indicated by dots. Residues conserved in at least 4 of the 6 allergens are highlighted by grey boxes, residues conserved in all 6 allergens by black boxes. The secondary structure elements of Pru av 1 are shown below the alignment. The alignment of the allergens with Pru av 1 is based on homology models created by SWISS−MODEL, the alignment of the START domain of MLN64 with Pru av 1 on a Dali server comparison; the 129 MLN64 residues used for the alignment are printed in uppercase, residues not used for the alignment in lowercase.

Methods and Results

In contrast to Bet v 1[1, 2, 3] as yet no high−resolution three−dimensional structure of any of the corresponding food allergens is available. Since this is a prerequisite for a detailed understanding of the observed immune cross−reactivity on a structural level, we determined the three−dimensional structure of the 17.5 kDa major cherry allergen Pru av 1 in solution based on a series of mostly heteronuclear multidimensional NMR experiments performed on both uniformly ^{15}N− and ^{13}C/^{15}N−labeled samples[4, 5]. Pru av 1 shows a well−defined structure in solution (Fig. 2) with average atomic root mean square deviations (RMSDs) from the average structure of 0.60 Å for the backbone and 0.93 Å for all heavy atoms. A schematic representation is shown in Fig. 3. A backbone overlay with the crystal structure of Bet v 1[1] (Fig. 4) confirms that both the secondary structure elements and the tertiary fold of these two allergens are virtually identical with a backbone atomic RMSD of 1.85 Å. Together with the considerable sequence identity between Pru av 1 and Bet v 1, the conserved backbone conformation leads to a very similar molecular surface as far as shape and charge distribution are concerned, rendering the existence of cross−reactive IgE binding epitopes most likely. Preincubation with Bet v 1a indeed inhibits binding of pooled serum IgE to the related food allergens almost completely in immunoblot experiments (Fig. 5), supporting the experience that these food allergies are usually a consequence of cross−reaction after sensitization to birch pollen.

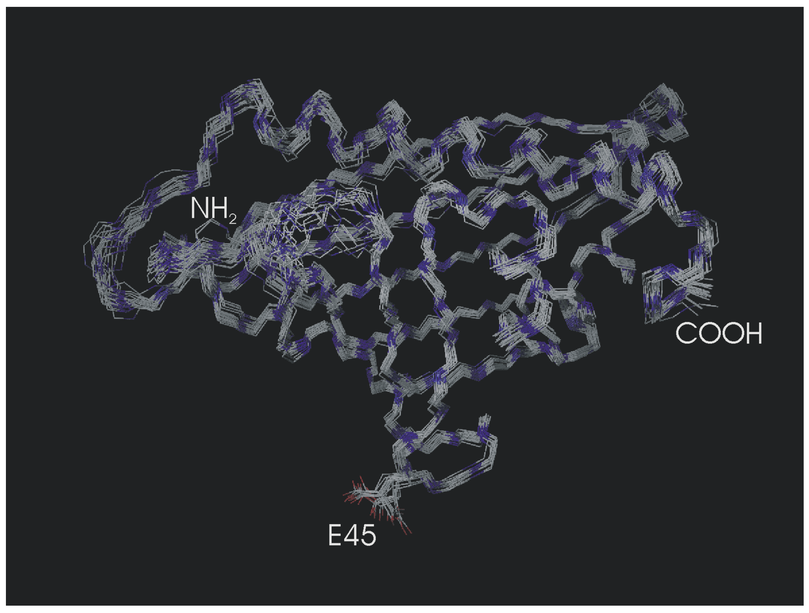

Fig. 2: Backbone overlay of the 22 accepted structures of Pru av 1. C, N, and O atoms are color−coded gray, blue, and red, respectively. The NH_2−terminus on the left−hand side is hidden by the loop from Ile86 to Glu96, the COOH−terminus can be seen on the right−hand side. The structures are in excellent agreement, especially as far as the β−strands are concerned. The side−chain of Glu45 shown at the bottom is clearly solvent−exposed in all structures.

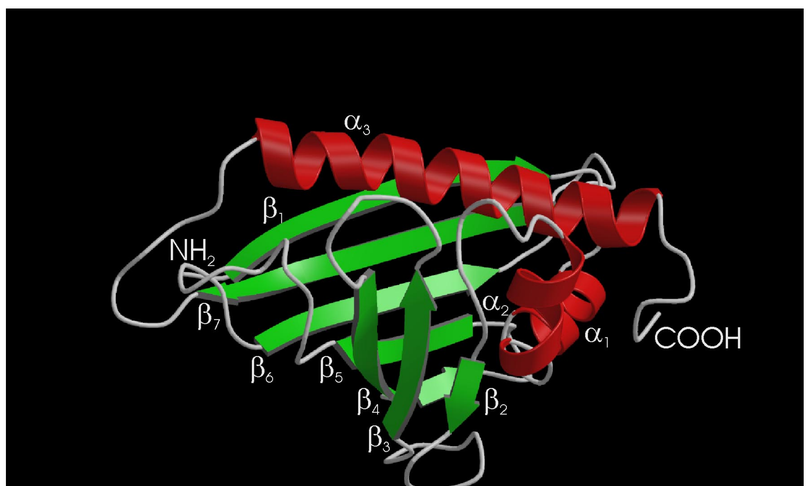

Fig. 3: Schematic representation of the secondary structure elements of Pru av 1. Same view as in Fig. 2. A folded seven−stranded antiparallel β−sheet and two short α−helices arranged in a V−shaped manner wrap around a long COOH−terminal α−helix to form a basket−like structure with the long helix resembling a handle, thus creating a large hydrophobic cavity in the center, which is very unusual for proteins.

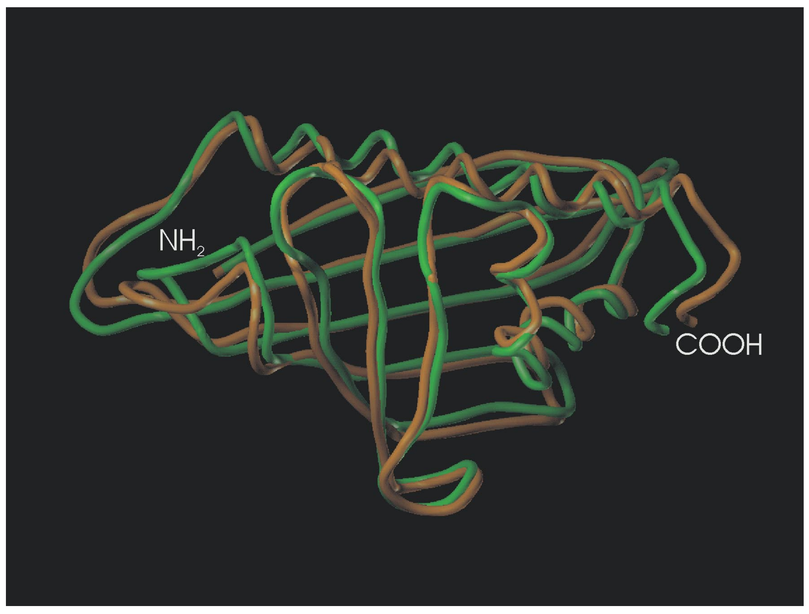

Fig. 4: Backbone overlay of the solution structure of Pru av 1 (green) and the crystal structure of Bet v 1[1] (orange). Same view as in Fig. 2 and 3. The tertiary fold is almost identical.

For Bet v 1, the glycine−rich P−loop around Glu45 was recently identified as the binding epitope of a monoclonal murine IgG antibody with high capacity to inhibit binding of serum IgE from allergic patients to Bet v 1[6]. Introduction of four point mutations including E45S indeed reduced the IgE binding capacity by severalfold[3]. In the crystal structure of the complex of Bet v 1 with the IgG Fab fragment the negatively charged side−chain of Glu45 is located in a binding pocket of the antibody with a positive electrostatic potential, where it forms two hydrogen bonds. In addition to Glu45, which is found to be solvent−exposed in all 22 accepted structures of Pru av 1 (Fig. 2), 14 of the remaining 15 residues forming the interface between Bet v 1 and the IgG Fab fragment are conserved or substituted conservatively in Pru av 1 (Fig. 1), which strongly suggests this region to be a cross−reactive IgE binding epitope. This is supported by the significantly decreased binding of serum IgE to the Pru av 1 mutants G46P and ΔT52 observed for some patients[7]. It can also provide an explanation why IgE binding to Pru av 1 is only weakly inhibited by preincubation with Api g 1.0101 (Fig. 6), where the P−loop is shorter by a residue and the negatively charged Glu45 is substituted by a positively charged lysine (Fig. 1).

The physiological function of all these allergens is still unknown. They show high sequence similarity to pathogenesis−related proteins, but most of them seem to be expressed constitutively. The striking structural homology with the recently determined crystal structure of the START domain of human MLN64[8] (Fig. 7) in spite of low sequence identity (Fig. 1) suggests phytosteroids as putative ligands for Bet v 1 and Pru av 1, which is supported by first NMR spectroscopic data (Fig. 8).

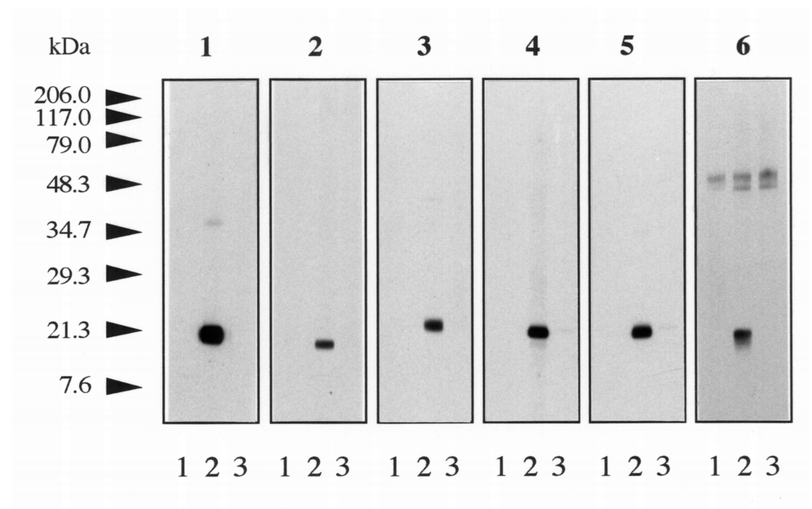

Fig. 5: Immunoblot inhibition of IgE binding to Bet v 1a (1), Api g 1.0101 (2), Mal d 1 (3), Pru av 1 (4), Pyr c 1 (5), and Cor a 1.0401 (6) on the solid phase. A serum pool from birch pollinotic patients with associated food allergy was preincubated with Bet v 1a (3). Serum from a non−allergic subject (1) and samples without inhibitor (2) were used as controls.

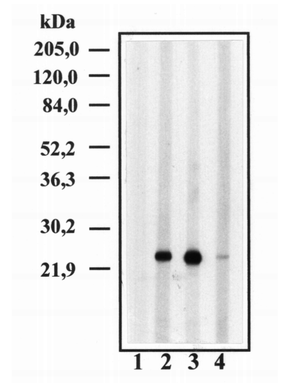

Fig. 6: Immunoblot inhibition of IgE binding to Pru av 1 on the solid phase with Pru av 1 (1) and Api g 1.0101 (2) as inhibitors. A sample without inhibitor (3) and serum from a non−allergic subject (4) were used as controls. Preincubation of the serum pool with Api g 1.0101 only leads to a small reduction of IgE binding to Pru av 1.

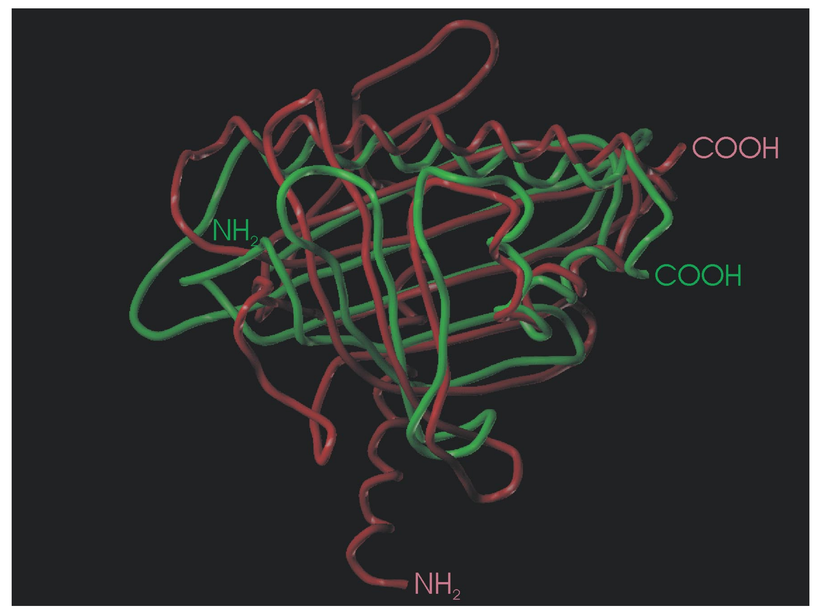

Fig. 7: Backbone overlay of the solution structure of Pru av 1 (green) and the crystal structure of the START domain of MLN64[8] (red), a protein that is associated with cholesterol transport. Same view as in Fig. 2, 3 and 4. The backbone atomic RMSD over the 129 residues used for the alignment is only 2.89 Å, even though the sequence identity over these 129 residues is only 8.5 % (Fig. 1).

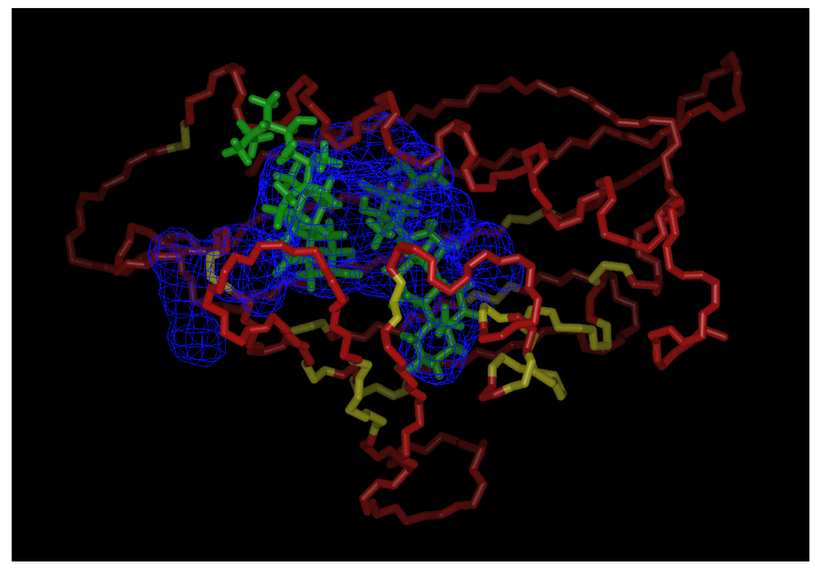

Fig. 8: Visualization of the hydrophobic cavity. Similar view as in Fig. 2, 3, 4 and 7. The backbone of the solution structure of Pru av 1 is shown in red, the cavity is indicated by blue lines. Residues whose amide proton resonances disappeared from the NMR spectra upon presence of homocastasterone due to intermediate exchange processes are colored yellow. The location of two castasterone molecules modeled into the cavity is shown in green.

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