NMR spectroscopic structural characterization of SAM22, an allergenic pathogenesis−related protein from soy bean

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Birch pollinosis is often accompanied by food hypersensitivity because of the cross−reaction of pollen−allergen specific IgE antibodies with homologous food proteins. The solution structure of the 159 amino acid major cherry allergen Pru av 1 revealed that the cross−reactive IgE epitopes are a consequence of a tertiary structure that is virtually identical to the major birch pollen allergen Bet v 1. Both allergens show high sequence identity to the PR−10 family of pathogenesis−related plant proteins, but the physiological function of all these proteins is unknown. Structural homology to a human protein associated with cholesterol transport suggests that Bet v 1 and Pru av 1, which both form a large hydrophobic cavity, are lipid−binding proteins, which is supported by our NMR experiments showing that Pru av 1 interacts with steroids. SAM22 from soy bean, a stress−induced PR-10 protein sharing approximately 50% sequence identity with the constitutively expressed Bet v 1 and Pru av 1, was recently observed to cause anaphylactic reactions of birch pollinotic patients as well. To provide a basis for a detailed analysis of the allergenic and functional relationship between the Bet v 1 family and the PR−10 family, the determination of the high−resolution structure of SAM22 by heteronuclear NMR experiments is currently under way in our laboratories. Evaluation of the chemical shift indices, scalar coupling constants and medium−range NOE patterns already indicates that its secondary structure and tertiary fold are similar to Bet v 1 and Pru av 1.

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).

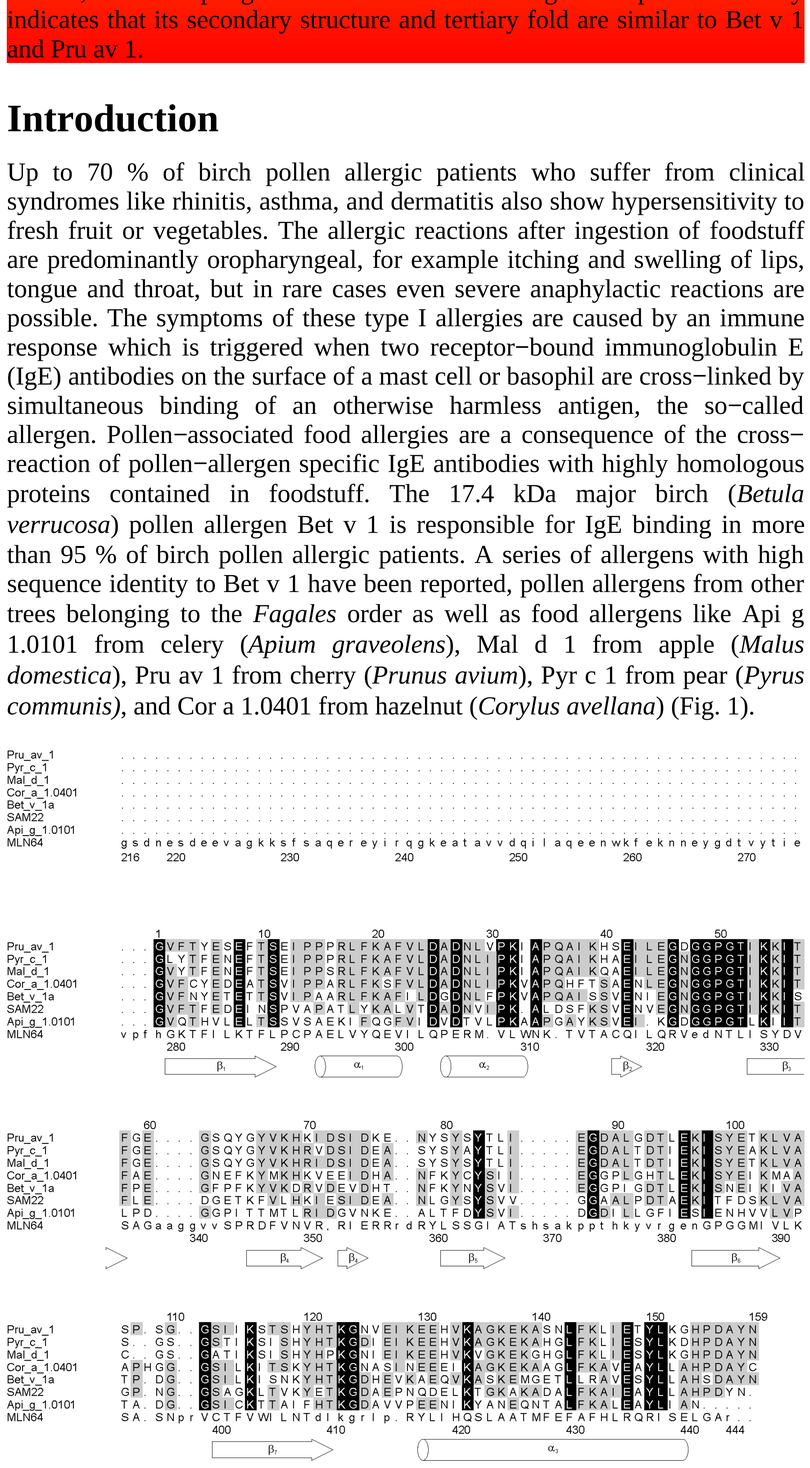

Fig. 1: Structure−based sequence alignment with Pru av 1 of Pyr c 1 (83.5% sequence identity to Pru av 1), Mal d 1 (82.9%), Cor a 1.0401 (64.4%), Bet v 1 isoform a (59.1%), SAM22 (53.5 %), Api g 1.0101 (41.2%), and the START domain of MLN64 (8.5%). The sequence positions above and below the sequences correspond to Pru av 1 and MLN64, respectively. Gaps in the alignment are indicated by dots. Residues conserved in at least 4 of the 7 allergens are highlighted by grey boxes, residues conserved in all 7 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] 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 2438 experimental restraints derived from a series of mostly heteronuclear multidimensional NMR experiments performed on both uniformly ^{15}N− and uniformly ^{13}C/^{15}N−labeled samples[3, 4]. 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 the secondary structure elements and the tertiary fold 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.

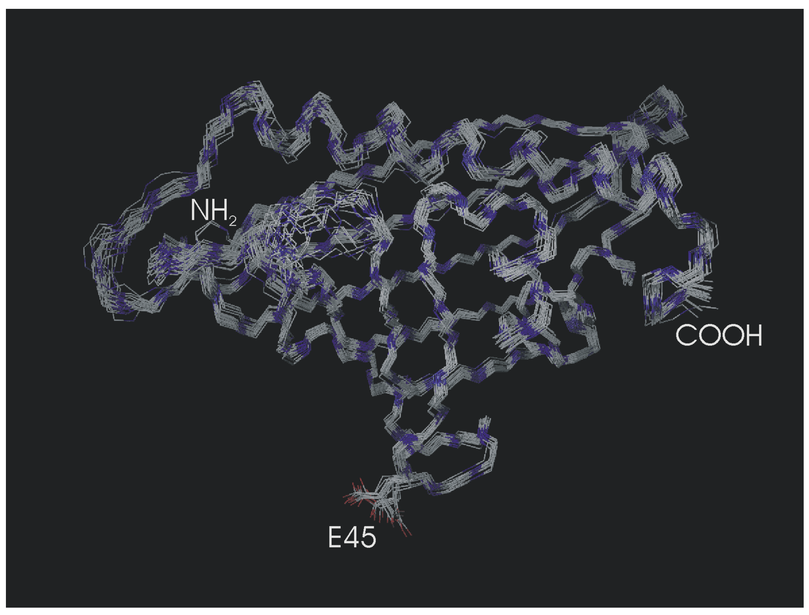

Fig. 2: Backbone overlay of the 22 accepted structures of Pru av 1. C, N, and O atoms are color−coded gray, blue, and red, respectively. The NH_2−terminus on the left−hand side is hidden by the loop from Ile86 to Glu96, the COOH−terminus can be seen on the right−hand side. The structures are in excellent agreement, especially as far as the β−strands are concerned. The side−chain of Glu45 shown at the bottom, which is known as a key residue of one of the IgE binding epitopes of Bet v 1[6], is clearly solvent−exposed in all structures.

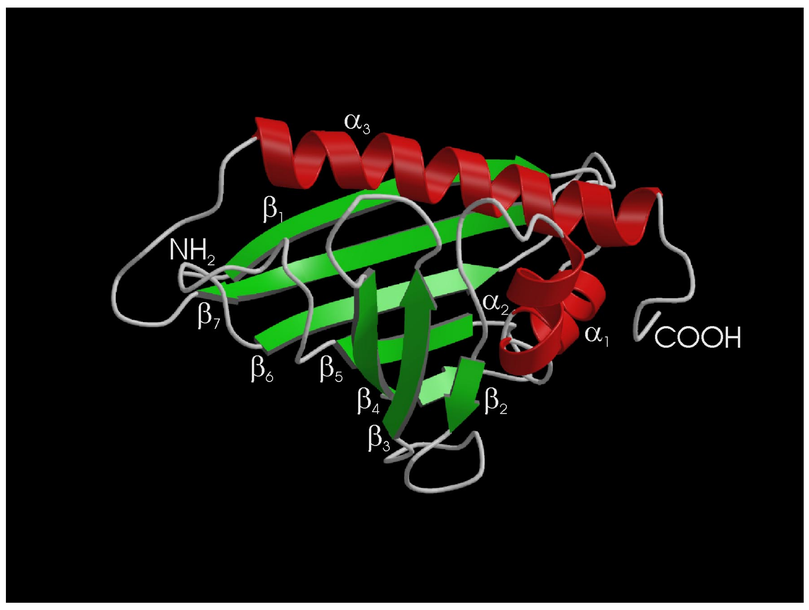

Fig. 3: Schematic representation of the secondary structure elements of Pru av 1. Same view as in Fig. 2. A folded seven−stranded antiparallel β−sheet and two short α−helices arranged in a V−shaped manner wrap around a long COOH−terminal α−helix to form a basket−like structure with the long helix resembling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

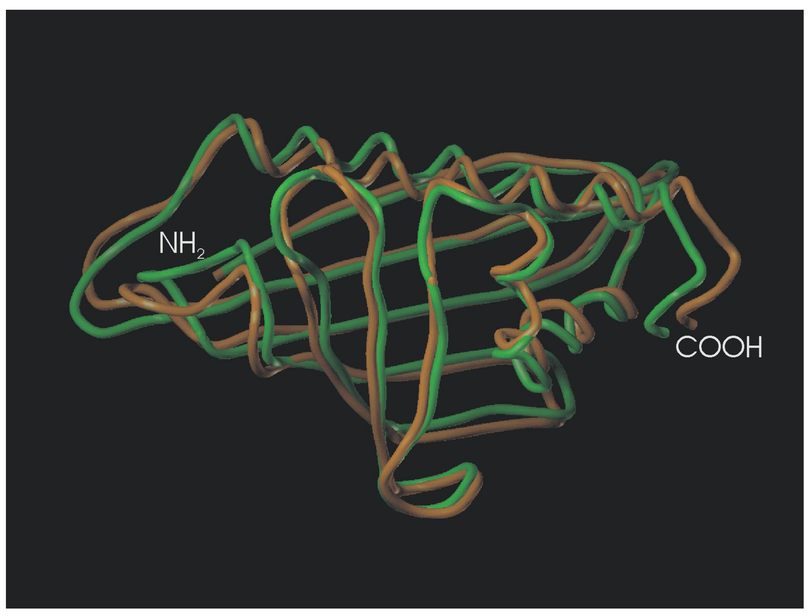

Fig. 4: Backbone overlay of the solution structure of Pru av 1 (green) and the crystal structure of Bet v 1[1] (orange). Same view as in Fig. 2 and 3. The tertiary fold is almost identical.

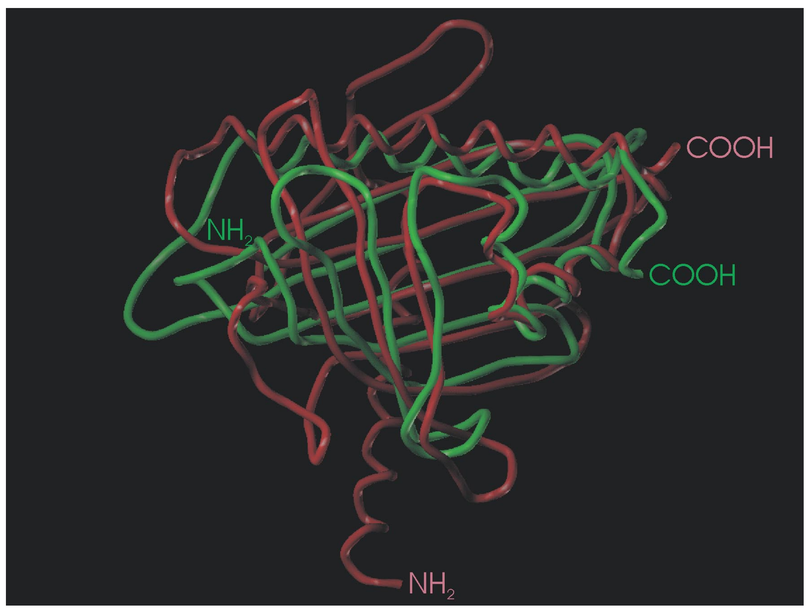

Fig. 5: Backbone overlay of the solution structure of Pru av 1 (green) and the crystal structure of the START domain of MLN64[5] (red), a protein that is associated with cholesterol transport. Same view as in Fig. 2, 3 and 4. The backbone atomic RMSD over the 129 residues used for the alignment is only 2.89 Å, even though the sequence identity over these 129 residues is only 8.5 % (Fig. 1).

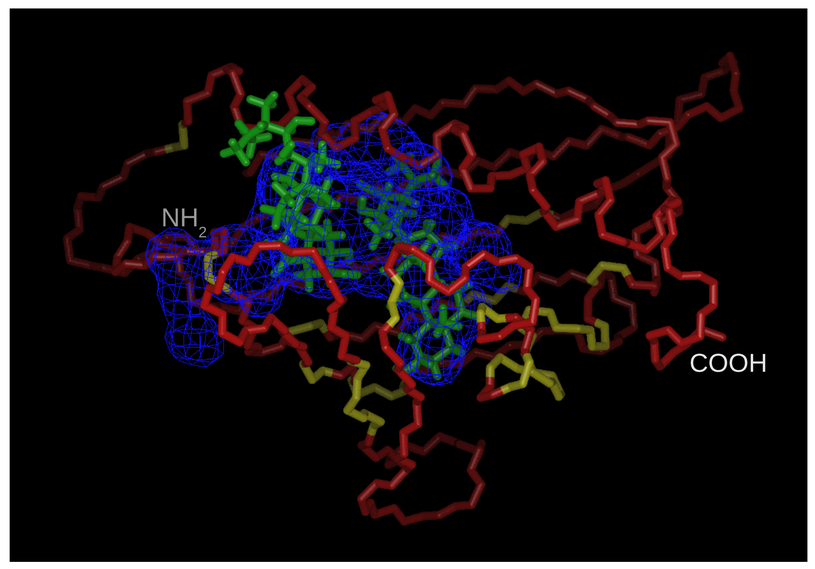

Fig. 6: Visualization of the hydrophobic cavity. Similar view as in Fig. 2, 3, 4 and 5. The backbone of the solution structure of Pru av 1 is shown in red, the cavity is indicated by blue lines. The location of two castasterone molecules modeled into the cavity (top) is shown in green, residues whose amide proton resonances disappeared from the [^{1}H,^{15}N] HSQC upon presence of homocastasterone due to intermediate exchange processes are colored yellow.

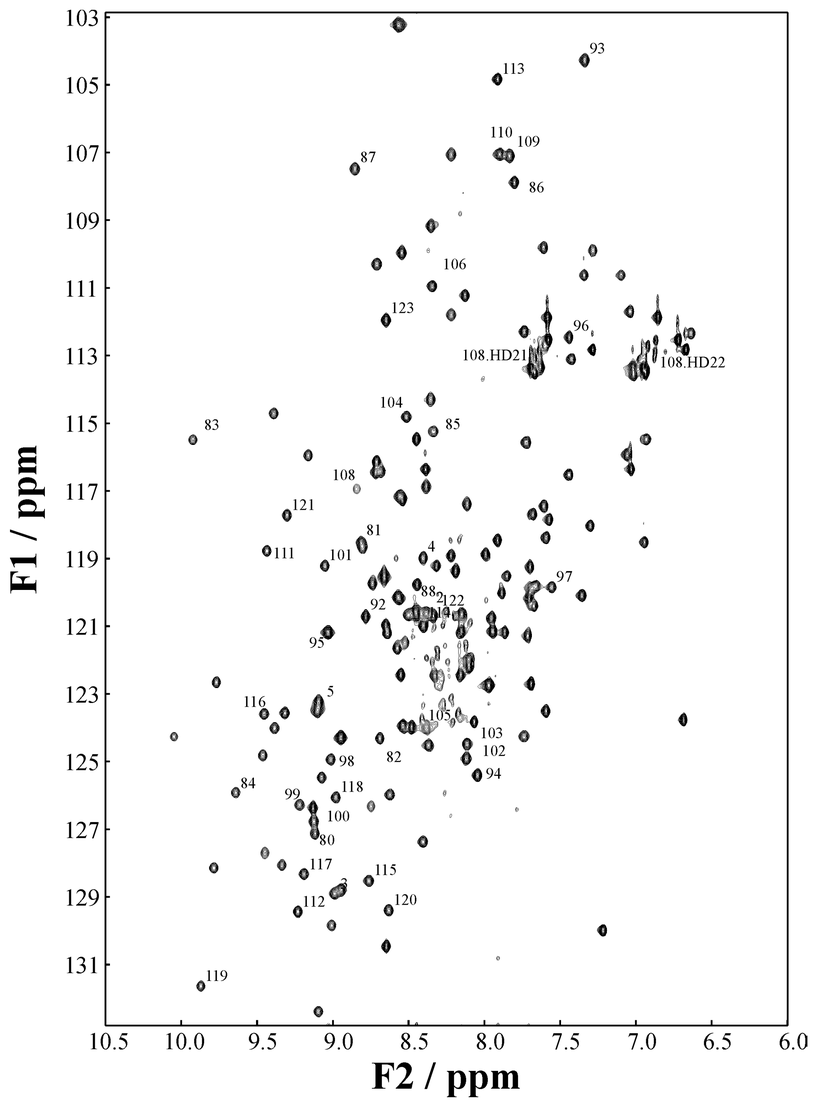

Fig. 7: [^{1}H,^{15}N] HSQC spectrum of 1.5 mM uniformly ^{15}N−labeled SAM22 in 50 mM potassium phosphate, pH = 7.0, 10 % (v/v) D_2O, recorded at T = 298 K on a Bruker DRX600 NMR spectrometer, showing the well−dispersed amide proton resonances labeled according to their residue numbers if already assigned.

The physiological function of all these allergens is still unknown. They show high sequence similarity to the PR−10 family of pathogenesis−related proteins, but most of them are expressed constitutively. The striking structural homology with the crystal structure of the START domain of human MLN64[5] (Fig. 5) 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. 6). Several other lipids have also been reported to bind to the hydrophobic cavity of Bet v 1 with moderate affinity in fluorescence assays[7]. To provide a basis for a detailed analysis of the allergenic and functional relationship between the Bet v 1 family of allergens and the PR−10 family of pathogenesis−related proteins, we performed a series of mostly heteronuclear multidimensional NMR experiments (Fig. 7) on both uniformly ^{15}N− and uniformly ^{13}C/^{15}N−labeled samples of SAM22 from soy bean[8], a stress−induced PR−10 protein sharing approximately 50% sequence identity with Bet v 1 and Pru av 1 (Fig. 1) that was recently observed to cause anaphylactic reactions of birch pollinotic patients as well and thus may be considered the "missing link" between these two protein families. Evaluation of the chemical shift indices, scalar coupling constants and medium−range NOE patterns already indicates that its secondary structure and tertiary fold are similar to Bet v 1 and Pru av 1.

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