Food Hypersensitivity of Many Birch Pollen Allergic Patients Can Be Explained by Highly Similar Molecular Structures of the Corresponding Allergens

Philipp Neudecker[1], Jörg Nerkamp[1], Kristian Schweimer[1], Heinrich Sticht[1], Markus Boehm[1], Stefan Vieths[2] and Paul Rösch[1]

[1] Lehrstuhl für Struktur und Chemie der Biopolymere, Universität Bayreuth, Bayreuth, Germany

[2] Abteilung für Allergologie, Paul−Ehrlich−Institut, Langen, Germany

Phone: +49−921−553869; Fax: +49−921−553544; E−mail: philipp.neudecker@uni−bayreuth.de

About 70 % of birch pollen allergic patients exhibit oral syndroms after ingestion of fruit and vegetables. The high sequence identity between the major birch pollen allergen Bet v 1 (17.4 kDa) and several food allergens suggests common structural features. Multidimensional heteronuclear NMR was used to determine the first high−resolution three−dimensional structure of a fruit allergen, a well−defined structure of the 17.5 kDa major cherry allergen Pru a 1 in solution. Comparison with previously determined high−resolution structures of Bet v 1 (crystalline as well as in solution) reveals a highly similar molecular structure as far as secondary structure, global fold and surface charge distribution are concerned. Therefore the observed immune cross−reactivity can be attributed to common B−cell binding epitopes due to similar local protein surface shapes and charges.

Introduction

In industrialized countries about 2 to 4 % of the adult population suffer from IgE−mediated allergies against foodstuff[1]. Food hypersensitivity often co−occurs with birch pollinosis. Almost 70 % of birch pollen allergic patients show allergic reactions after consumption of fresh fruit (like apples, plums, cherries and other stone fruit), hazelnuts or vegetables (carrots and celery, for instance)[2]. Allergic reactions against pollen lead to clinical syndromes like hay fever, asthma and dermatitis; after ingestion of foodstuff allergic reactions are most often located in the oropharynx, i. e. itching and swelling of lips, tongue and throat, but in rare cases even an anaphylactic shock is possible.

On the molecular level pollen−related food allergies can be explained by the cross−reaction of food allergens with pollen−specific IgE−antibodies. This is consistent with the high sequence similarity of food allergens and pollen allergens. One group of food allergens are proteins related to the major birch (*Betula verrucosa*) pollen allergen Bet v 1, a 17.4 kDa protein of 159 amino acids[3]. Pru a 1, the major cherry (*Prunus avium*) allergen, has a sequence identity of nearly 60 % with Bet v 1. Pru a 1 is produced as a precursor protein of 160 residues; cleavage of the NH_2−terminal methionine yields a protein with a molecular mass of 17.5 kDa[3].

The physiological function of both Bet v 1 and Pru a 1 is still unknown. A high sequence similarity to stress−induced proteins and pathogenesis−related proteins from parsley, potato and soy bean indicates that these allergens are involved in stress response.

Apart from sequence similarity only biological data including immunoblot inhibition experiments and histamine release in basophils have suggested common structural elements of Bet v 1 and several food allergens so far[4, 5, 6]. We were able to show that Pru a 1 and Bet v 1 have identical secondary and tertiary structures by using biophysical methods, namely Circular Dichroism (CD) and Nuclear Magnetic Resonance (NMR) Spectroscopy.

Methods and Results

His−tagged recombinant Pru a 1 was overexpressed in *E. coli* and purified using immobilized metal affinity chromatography (IMAC). After cleavage with cyanogen bromide a second IMAC yielded authentical protein, which was refolded by solubilization in 6 M urea and dialyzation against decreasing urea concentrations. For NMR samples lyophilized Pru a 1 was dissolved in 10 mM potassium phosphate buffer containing 10 % (v/v) D_2O for the field lock channel.

Far UV CD spectra are a suitable tool to estimate the secondary structure content of proteins[7]. The CD spectra of Pru a 1 and Bet v 1 shown in Fig. 1 are largely superimposable, indicating similar secondary structure elements with a high content of both α−helical regions and β−strands.

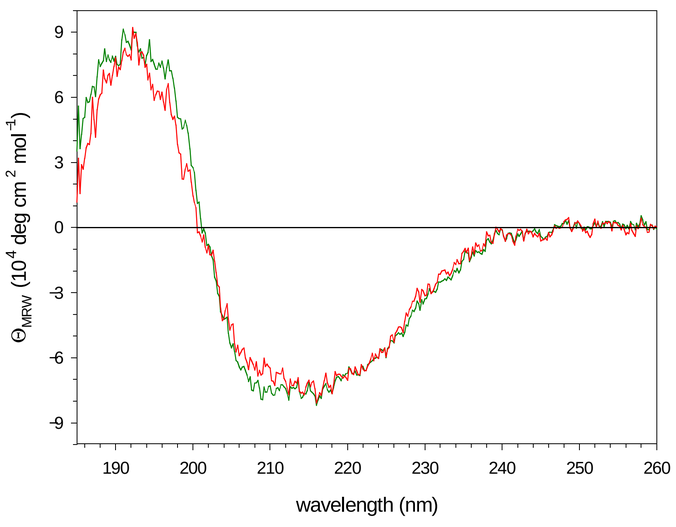

Fig. 1: CD spectra of Pru a 1 (red) and Bet v 1 (green), each recorded of 5 µM protein in 10 mM potassium phosphate, pH = 7.0. The almost identical spectra suggest similar secondary structures of the two proteins, both with a high content of α−helical regions and β−strands.

A series of mostly heteronuclear NMR experiments performed on both uniformly ^{15}N− and uniformly ^{13}C−^{15}N−labeled samples on a Bruker DRX 600 spectrometer equipped with a triple−resonance probe and pulsed field gradient capabilities were necessary for the structure determination. 147 of the 151 non−proline backbone amide resonances could be assigned (Fig. 2) in the backbone assignment process based mainly on triple−resonance experiments like HNCO, HNCA, HNCACB and CBCA(CO)NH. The sidechain assignment was completed using 3D−^1H,^{15}N−TOCSY−HSQC, H(C)CH−COSY, (H)CCH−COSY and HC(C)H−TOCSY experiments, resulting in about 80 % of all the proton, carbon and nitrogen chemical shifts.

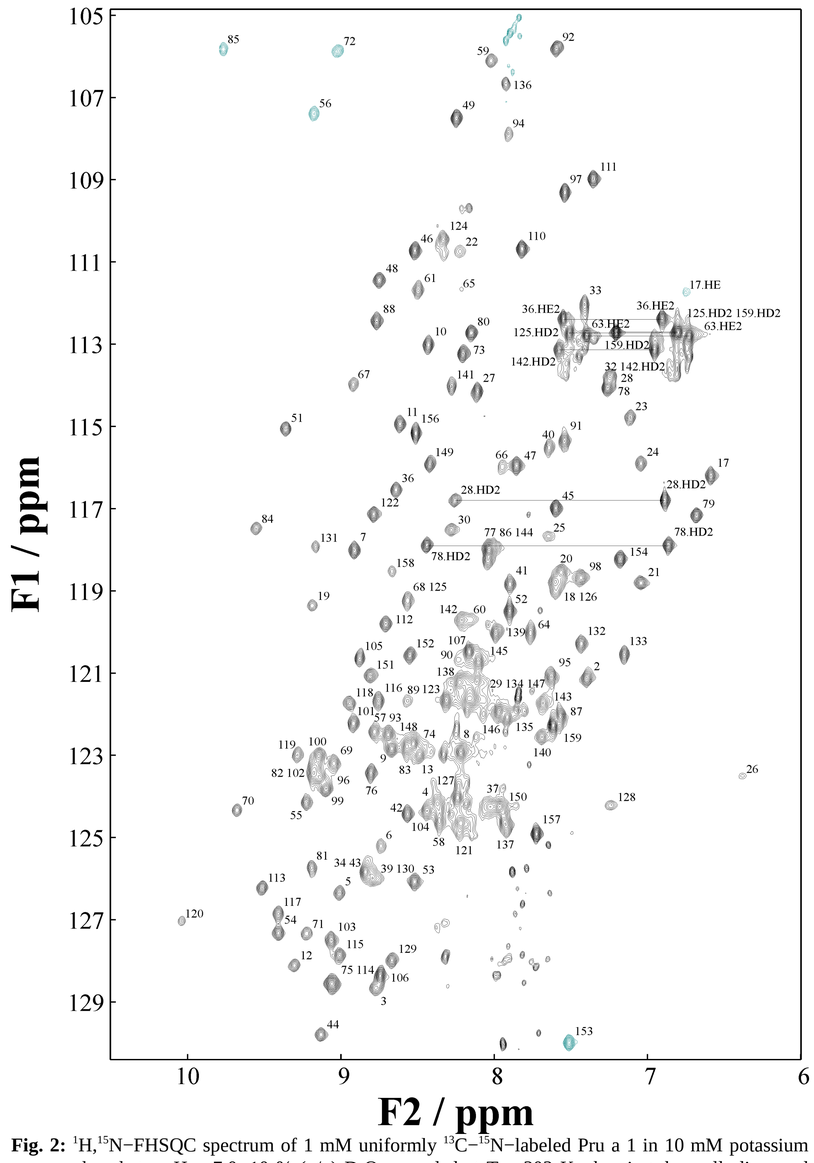

Fig. 2: ^1H,^{15}N−FHSQC spectrum of 1 mM uniformly ^{13}C−^{15}N−labeled Pru a 1 in 10 mM potassium phosphate, pH = 7.0, 10 % (v/v) D_2O, recorded at T = 303 K, showing the well−dispersed amide protons resonances labeled according to their residue numbers. Blue peaks are aliased in the indirect ^{15}N−dimension (F1).

Several ^{15}N− and ^{13}C−edited NOESY experiments provided 2299 NOE distance restraints; together with dihedral angle restraints from an HNHA spectrum and H−bonds identified as slowly exchanging amide protons a total of 2509 experimental restraints served as an input for the structure calculation with X−PLOR 3.851[8], summarized in Table 1. The 22 accepted structures out of 60 ones calculated are shown in Fig. 3.

Experimental Restraints Used for the Structure Calculation		
Intraresidual NOEs		658
Interresidual NOEs	sequential (| i − j | = 1)	729
	medium−range (1 < | i − j | < 5)	330
	long−range (| i − j | > 4)	582
Dihedral Angle Restraints		71
Hydrogen Bonds		34
Molecular Dynamics Statistics		
Average Energy / kcal/mol	total	244 ± 7
	bonds	7.1 ± 0.5
	angles	180 ± 3
	impropers	22.2 ± 0.7
	van−der−Waals repulsion	13.3 ± 1.2
	NOEs	22 ± 3
	dihedral restraints	0.037 ± 0.021
RMSDs from ideal distances / Å	bonds	0.00169 ± 0.00006
	NOEs	0.0135 ± 0.0010
RMSDs from ideal angles / °	angles	0.510 ± 0.005
	impropers	0.438 ± 0.004
	dihedral restraints	0.028 ± 0.008
Atomic RMSDs of the 22 Accepted Structures		
	backbone	heavy atoms
Overall[a]	0.60 ± 0.09	0.93 ± 0.09
Regular Secondary Structure[b]	0.41 ± 0.08	0.72 ± 0.06
Beta Sheets[c]	0.29 ± 0.06	0.61 ± 0.07
COOH−terminal Helix[d]	0.39 ± 0.12	0.85 ± 0.12
Comparison with Structures of Bet v 1		
Backbone Atomic RMSDs / Å	X−Ray[9]	1.94 ± 0.15
	NMR[10, e]	2.40 ± 0.12

Table 1: Summary of the structure calculation
a residues 1−159
b residues 2−58, 65−85, 97−104, 112−122, 130−153
c residues 2−11, 41−58, 65−85, 97−104, 112−122
d residues 130−153
e residues 1−154 of the lowest−energy structure (COOH−terminus is not well defined)

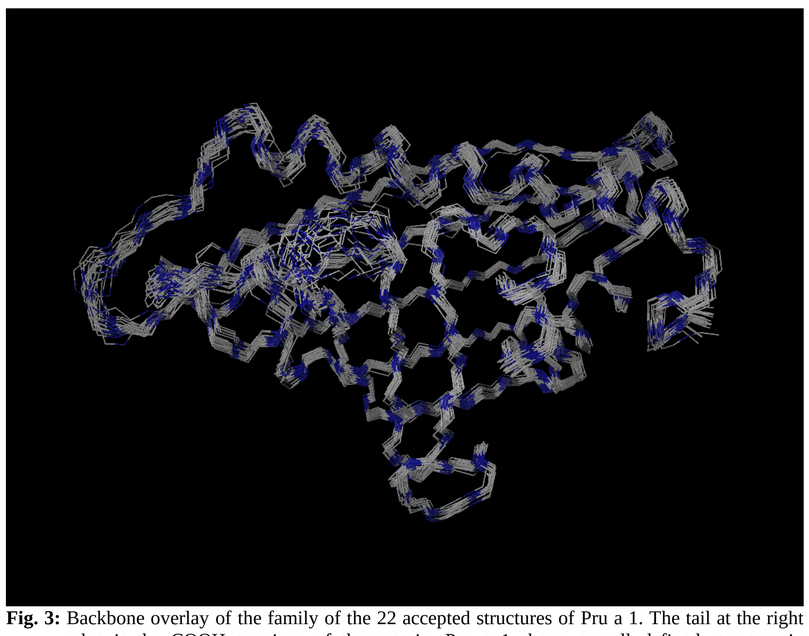

Fig. 3: Backbone overlay of the family of the 22 accepted structures of Pru a 1. The tail at the right edge is the COOH−terminus of the protein. Pru a 1 shows a well−defined structure in solution.

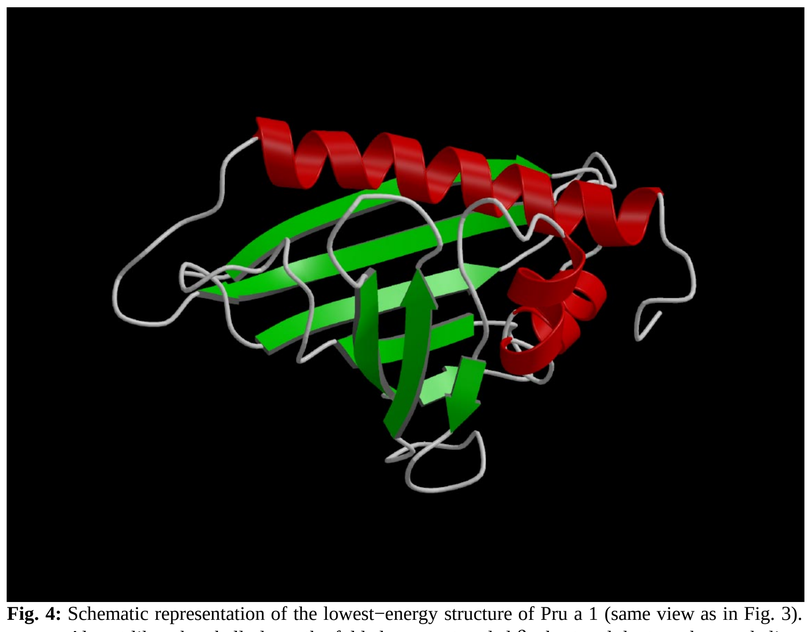

Fig. 4: Schematic representation of the lowest−energy structure of Pru a 1 (same view as in Fig. 3). Almost like a baseball glove, the folded seven−stranded β−sheet and the two short α−helices − arranged in a V−shaped manner − wrap around the long COOH−terminal α−helix. This type of tertiary fold creates a large cavity in the center, which is very unusual for proteins.

A comparison of the backbone topology of the solution structure of Pru a 1 with previously published structures of Bet v 1 yields atomic RMSDs of 1.94 Å for the crystal structure[9] and 2.40 Å for the solution structure[10]. Since this is of the order of the difference between the two Bet v 1 structures (which is 1.78 Å[10]) we can conclude that the tertiary fold of Pru a 1 and Bet v 1 is virtually identical, which can also be seen by inspection (cf. Fig. 5).

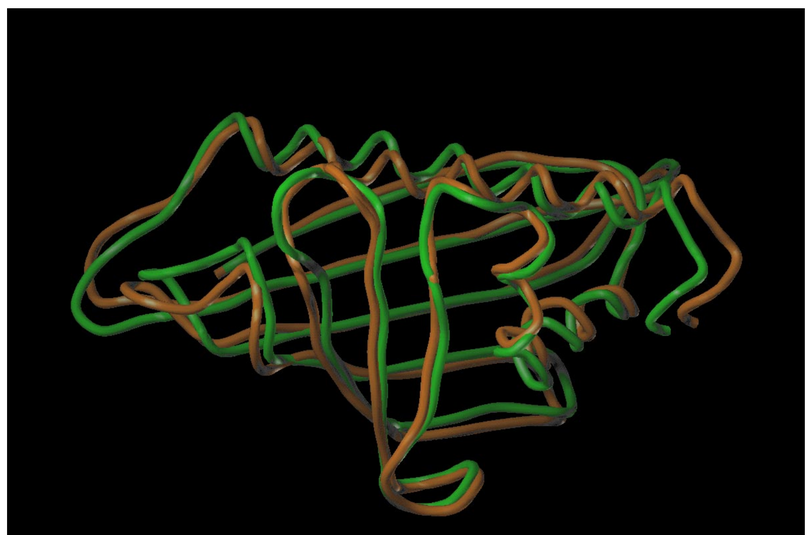

Fig. 5: Backbone overlay of the lowest−energy solution structure of Pru a 1 (green) and the crystal structure of Bet v 1[9] (orange) (same view as in Fig. 3 and 4). The tertiary fold of both proteins is almost identical.

In contrast to T−cell binding epitopes that are generated by proteolytic digestion of the molecule, B−cell binding epitopes are exclusively located on the protein surface[11]. The similar surface properties of Pru a 1 and Bet v 1 − resulting from the high sequence identity together with the conserved tertiary fold − are therefore supposed to be the molecular origin of the observed cross−reactivity of these allergens.

Summary and Future Directions

We were able to prove the high similarity of the structures of the major allergens from cherry and birch pollen, Pru a 1 and Bet v 1, respectively, by

1. CD−spectroscopic measurements and
2. a high−resolution structure determination of Pru a 1 in solution based on multidimensional heteronuclear NMR experiments, and a comparison with known structures of Bet v 1.

Since the same similarity can be expected for several other closely related allergens, e. g. Mal d 1 (apple), Api g 1 (celery) or Dau c 1 (carrot), the Pru a 1 structure presented here is anticipated to be a suitable starting point for homology modeling of these allergens. Similar surface properties like shape and charge distribution can explain the immune cross−reactivity, which is the reason for food hypersensitivity of birch pollen allergic patients. A knowledge of the molecular structures of allergens and allergen−antibody complexes will allow to selectively substitute individual amino acids in order to create molecules with altered effects on the immune system.

References

[1] S. Vieths in: J. P. F. D´Mello (ed.): Handbook of Plant and Fungal Toxicants, CRC Press, Boca Raton 1997, pp. 157−174

[2] G. Pauli, J. P. Oster, P. Deviller, S. Heiss, J. C. Bessot, M. Susani, F. Ferreira, D. Kraft and R. Valenta, J. Allergy Clin. Immun. **97**, 1100−1109 (1996)

[3] S. Schöning, S. Vieths, A. Petersen and W. Baltes, J. Sci. Food Agric. **67**, 431−440 (1995)

[4] T. Caballero and M. Martín−Esteban, Invest. Allergol. Clin. Immunol. **8**, 6−16 (1998)

[5] S. Scheurer, K. Metzner, D. Haustein and S. Vieths, Mol. Immunol. **34**, 619−629 (1997)

[6] S. Scheurer, D. Y. Son, M. Boehm, F. Karamloo, S. Franke, A. Hoffmann, D. Haustein and S. Vieths, Mol. Immunol., in press

[7] V. P. Saxena and D. B. Wetlaufer, Proc. Natl. Acad. Sci. USA **68**, 969−972 (1971)

[8] A. T. Brünger: X−PLOR Version 3.1, Howard Hughes Institute and Yale University, New Haven 1993

[9] M. Gajhede, P. Osmark, F. M. Poulsen, H. Ipsen, J. N. Larsen, R. J. J. van Neeren, C. Schou, H. Løwenstein and M. D. Spangfort, Nature Struct. Biol. **3**, 1040−1045 (1996)

[10] K. Schweimer, H. Sticht, J. Nerkamp, M. Boehm, M. Breitenbach, S. Vieths and P. Rösch, Appl. Magn. Reson. **17**, 449−464 (1999)

[11] D. C. Benjamin, J. A. Berzofsky, I. J. East, F. R. Gurd, C. Hannum, S. J. Leach, E. Margoliash, J. G. Michael, A. Miller, E. M. Prager, M. Reichlin, E. E. Sercarz, S. J. Smith−Gill, D. E. Todd and A. C. Wilson, Annu. Rev. Immunol. **2**, 529−535 (1984)