



Letter to the Editor: Sequence-specific ^1H , ^{13}C and ^{15}N resonance assignments of the major cherry allergen Pru a 1

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Biological context

In industrialized countries about 2–4% of the adult population suffer from IgE-mediated allergies against foodstuff (Vieths, 1997). Food hypersensitivity often co-occurs with birch pollinosis, and up to 70% of birch pollen allergic patients show allergic reactions after consumption of fresh fruit or vegetables. 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 and include from itching and swelling of lips, tongue and throat, to anaphylactic shock.

On the molecular level pollen-related food allergies can be explained by the cross-reaction of food allergens with pollen-specific IgE-antibodies, consistent with a high sequence similarity of food and pollen allergens. The major birch (*Betula verrucosa*) pollen allergen Bet v 1 and Pru a 1, the major cherry (*Prunus avium*) allergen, have a sequence identity of nearly 60%. Both are produced as 160-residue precursor proteins that are processed by cleavage of the NH₂-terminal methionine (Schöning et al., 1995). Although the physiological function of Bet v 1 and Pru a 1 is unknown, high sequence similarity to stress-induced proteins and pathogenesis-related proteins from parsley, potato, and soy bean suggests that both allergens are involved in stress response. In addition to the sequence similarity, biological data such as immunoblot inhibition experiments and histamine release in ba-

sophils have suggested common structural elements of Bet v 1 and food allergens (Scheurer et al., 1999).

The three-dimensional structure of the birch pollen allergen Bet v 1 is known (Faber et al., 1996; Gajhede et al., 1996; Schweimer et al., 1999), while there is no such information available yet for any of the related food allergens. Understanding of the molecular basis of the observed immune cross-reactivity on a structural level, however, requires comparison of the conformations of at least one pollen allergen and one food allergen. We thus assigned most of the ^1H , ^{13}C , and ^{15}N resonances of the major cherry allergen Pru a 1 and determined its secondary structure based on multidimensional heteronuclear NMR data. The results serve as a starting point for structural analysis of Pru a 1.

Methods and results

His-tagged recombinant Pru a 1 was overexpressed in *E. coli* grown on M9 minimal medium with $^{15}\text{NH}_4\text{Cl}$ and $^{13}\text{C}_6$ glucose and subsequently purified using immobilized metal affinity chromatography (IMAC) (Boehm and Rösch, 1997). After cleavage with cyanogen bromide a second IMAC yielded authentic protein, which was refolded by solubilization in 6 M urea and dialysis against decreasing urea concentrations. For NMR studies samples of 0.8–1.2 mM Pru a 1 and 10 mM potassium phosphate (pH 7.0) in H₂O/D₂O (9:1) were prepared.

All NMR spectra were acquired on a Bruker DRX 600 NMR spectrometer at a temperature of either 25 °C or 35 °C. The following 3D

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NMR spectra were recorded for the backbone and aliphatic side chain resonance assignment: HNCO, HNCA, HNCACB, CBCA(CO)NH, H(C)CH-COSY, (H)CCH-COSY, cp-HC(C)H-TOCSY, ^{15}N -TOCSY-HSQC, ^{15}N -NOESYHSQC, and HNHA (Bax and Grzesiek, 1993; Sattler et al., 1999). The assignment of aromatic proton resonances was performed using a 2D [^1H , ^1H] TOCSY of an unlabeled sample and a ^{15}N -filtered 2D [^1H , ^1H] NOESY of a uniformly ^{15}N -labeled sample. The NMR data was processed using in-house written software and analyzed with the program packages NMRView (B.A. Johnson, Merck, Whitehouse Station, NJ, U.S.A.) and NDEE (SpinUp Inc., Dortmund, Germany).

Analysis of the $\text{H}\alpha$, $\text{C}\alpha$, and CO chemical shifts together with an assessment of the medium-range amide proton NOE patterns confirms that the secondary structure elements of Pru a 1 and Bet v 1 are virtually identical. The protein consists of seven β -strands (one of them with a kink at D72 indicated by a medium to strong NOE between the amide protons of D72 and S73), two short α -helices and a long COOH-terminal α -helix.

Extent of assignments and data deposition

Analysis of the triple resonance spectra allowed identification and sequential assignment of 147 of the 150 backbone amide resonances (Figure 1). Exceptions were S62, Q63, and S109, which are supposed to be located in loop regions, possibly allowing rapid exchange with the solvent. Apart from P14, P15, S62, Q63, and P108 all of the $\text{H}\alpha$, $\text{C}\alpha$, and $\text{C}\beta$ chemical shifts could be assigned (97%). In spite of severe spectral overlap and the presence of some minor degradation products in the $^{13}\text{C}/^{15}\text{N}$ -labeled sample (cf. Figure 1) seriously hampering the assignment – especially that of aliphatic side chain resonances – 1589 ^1H , ^{13}C , and ^{15}N chemical shifts out of 2023 ones expected (79%) had been determined upon completion of the side chain resonance assignment process, 853 of which were proton chemical shifts (83% of the 1029 ones expected). In addition, we were able to measure 97 $^3J_{\text{HNH}\alpha}$ scalar coupling constants using the HNHA experiment. ^1H , ^{13}C , and ^{15}N chemical shifts and scalar coupling constants have been deposited with the BioMagResBank (access code: 4671).

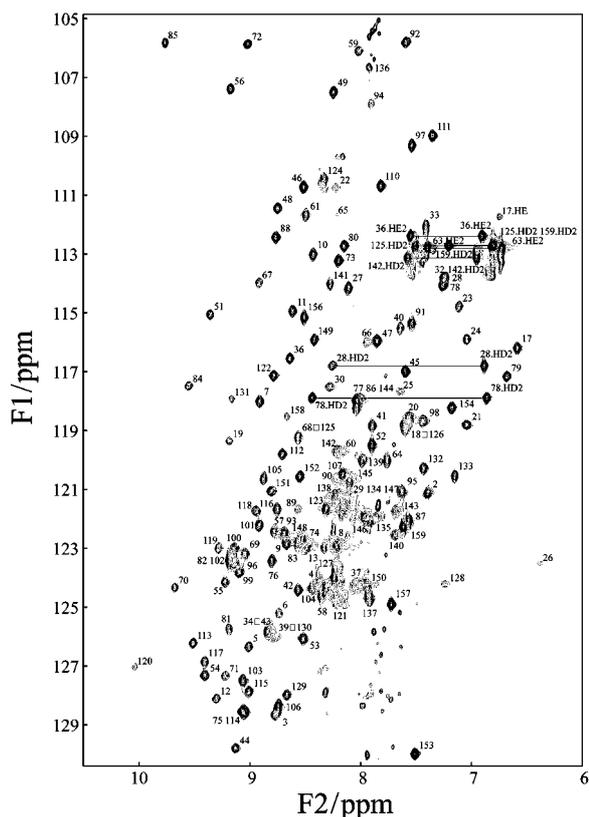


Figure 1. [^1H , ^{15}N] HSQC spectrum of uniformly $^{13}\text{C}/^{15}\text{N}$ -labeled Pru a 1, showing the well-dispersed amide proton resonances labeled according to their residue numbers. The HN resonances of residues 85, 72, 56, and 153, and the H ϵ resonance of arginine 17 are aliased in the indirect ^{15}N dimension F1. Sharp resonances in the lower part of the spectrum are from degradation products.

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