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Letter to the Editor: Sequence-specific ^{1}H, ^{13}C and ^{15}N resonance assignments of SAM22, an allergenic stress-induced protein from soy bean

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Biological context

Birch pollinosis is one of the prevailing allergic diseases in Northern and Central Europe and Northern America. The 17.4 kDa major birch pollen allergen Bet v 1 is responsible for IgE antibody binding in more than 95% of birch pollinotics (Breiteneder et al., 1989), and cross-reaction of Bet v 1 specific IgE antibodies with highly homologous proteins like Api g 1 from celery, Mal d 1 from apple, Pru av 1 (formerly Pru a 1) from cherry, Pyr c 1 from pear, and Cor a 1.0401 from hazelnut causes allergic reactions in up to 70% of these patients 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. The physiological function of these allergens is still unknown. They show high sequence similarity to the PR-10 family of pathogenesis-related and stress-induced proteins but seem to be expressed constitutively. Recent studies suggest phytosteroids and other lipids as putative ligands (Neudecker et al., 2001; Mogensen et al., 2002), and a potential ribonuclease activity was discussed.

The three-dimensional structure of Bet v 1 (Gajhede et al., 1996), Pru av 1 (Neudecker et al., 2001), and two closely related PR-10 proteins from yellow lupine (Biesiadka et al., 2002) is known. Recently, the stress-induced 16.6 kDa PR-10 protein SAM22 from soy bean (Crowell et al., 1992), whose 157 amino acids have a sequence identity of approximately 50% with Bet v 1, was observed to cause severe oropharyngeal and anaphylactic reactions in birch pollinotics (Kleine-Tebbe et al., 2002). Although soy-derived proteins are considered one of the most important nutrients of the legume family, detailed studies that may allow an allergic risk assessment of soy-containing dietary products have largely been restricted to other soy bean allergens and pediatric patients so far (Kleine-Tebbe et al., 2002), and the high-resolution three-dimensional structure of SAM22 is a prerequisite for a detailed understanding of the observed immune cross-reactivity on the molecular level. As a starting point to bridge the structural gap between the constitutively expressed Bet v 1 family of allergens and the stress-induced PR-10 family of pathogenesis-related proteins, we thus assigned the vast majority of the ^{1}H, ^{13}C, and ^{15}N resonances of SAM22 and determined its secondary structure based on multidimensional heteronuclear NMR data.

Methods and results

Recombinant SAM22 was overexpressed in *E. coli* grown on M9 minimal medium with $^{15}NH_4Cl$ and $^{13}C_6$ glucose and subsequently purified using anion exchange chromatography (Q-Sepharose Fast Flow Resin, Amersham Biosciences, Freiburg, Germany) and reversed phase chromatography (Delta-Pak^{TM}C18, Waters, U.S.A.). For NMR studies samples of 0.7–1.5 mM SAM22 and 10–50 mM potassium phosphate (pH 7.0) in H_2O/D_2O (9:1) were prepared.

All NMR spectra were acquired on Bruker DRX 600 and DMX 750 NMR spectrometers at a temperature of 25 °C. The following 3D NMR spectra were recorded for the backbone and aliphatic side chain resonance assignment: HNCO, HNCA, HNCACB, HBHA(CBCACO)NH, CBCA(CO)NH, H(CCO)NH, C(CO)NH, H(C)CH-COSY, (H)CCH-COSY, cp-HC(C)H-TOCSY, ^{15}N-TOCSYHSQC, ^{15}N-NOESYHSQC, $^{15}N/^{15}N$-HMQC-

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NOESYHSQC, and HNHA (Bax and Grzesiek, 1993; Sattler et al., 1999). The assignment of aromatic proton resonances was performed using a 2D [^{1}H, ^{1}H] TOCSY and a 2D [^{1}H, ^{1}H] NOESY of an unlabeled sample and 50 mM potassium phosphate (pH 7.0) in D_2O. The NMR data was processed using in-house written software and analyzed with the program packages NMRView (B.A. Johnson, Merck, Whitehouse Station, NJ) and NDEE (SpinUp Inc., Dortmund, Germany).

Analysis of the Hα, Cα, and CO chemical shifts together with an assessment of the medium-range amide proton NOE patterns confirms that the secondary structure elements of SAM22 and Bet v 1 are virtually identical. The protein consists of seven β-strands (one of them with a kink at E71 indicated by a medium to strong NOE between the amide protons of E71 and S72), two short α-helices and a long COOH-terminal α-helix. A homology model of SAM22 based on Pru av 1 and Bet v 1 (PDB access codes 1E09, 1B6F, 1FSK, and 1BV1) created with SwissModel followed by 100 steps of energy minimization after addition of protons in Sybyl 6.5 (Tripos Inc., St. Louis, MO) already fulfills the $^1D_{HN}$ dipolar coupling constants to a quality factor (Cornilescu et al., 1998) of $Q = 49.5\%$.

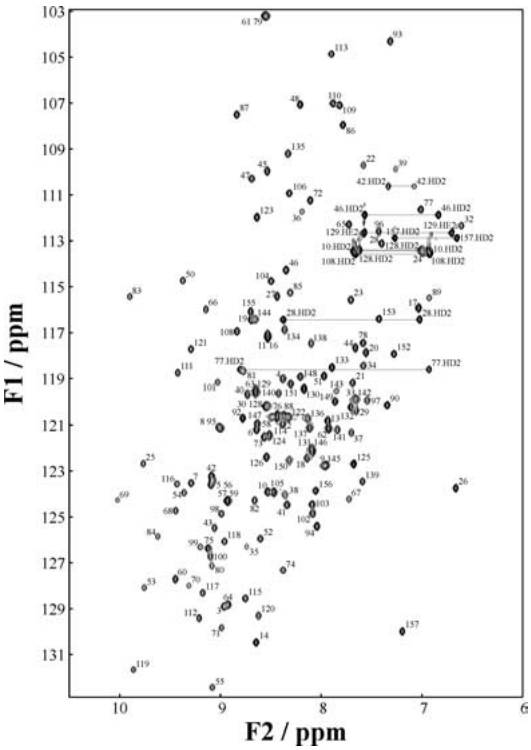

Figure 1. [^{1}H, ^{15}N] HSQC spectrum of uniformly ^{13}C/^{15}N-labeled SAM22, showing the well-dispersed amide proton resonances labeled according to their residue numbers.

Extent of assignment and data deposition

Analysis of the triple resonance spectra allowed identification and sequential assignment of all of the 148 backbone amide resonances (Figure 1) and all of the Hα, Cα, and Cβ chemical shifts. In spite of severe spectral overlap seriously hampering the assignment – especially that of aliphatic side chain resonances –, 1677 ^{1}H, ^{13}C, and ^{15}N chemical shifts out of 1877 ones expected (89%) had been determined upon completion of the side chain resonance assignment process, 890 of which were proton chemical shifts (94% of the 943 ones expected). In addition, we were able to measure 89 $^3J_{HNH\alpha}$ scalar coupling constants using the HNHA experiment and 118 $^1D_{HN}$ dipolar coupling constants of SAM22 weakly aligned by addition of 18 mg/ml Pf1 filamentous phage using IPAP experiments. ^{1}H, ^{13}C, and ^{15}N chemical shifts and scalar coupling constants have been deposited with the BioMagResBank (access code: 5605).

Note added in proof

On March 2nd, 2003, SAM22 has been assigned the allergen designation Gly m 4 by the International Union of Immunological Societies (IUIS) Allergen Nomenclature Sub-Committee.

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