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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N chemical shift assignment of the honeybee pheromone carrier protein ASP1

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Biological context

In insects, the pheromone-binding proteins (PBP) are involved in the transport of the hydrophobic pheromones through the sensillum lymph towards their neuronal receptors. PBP have been extensively studied in the moth specialist system for sex-pheromone binding (Steinbrecht, 1998). They are small acidic proteins (13–17 kDa), highly concentrated in the sensilla lymph, and, in contrast to vertebrate olfactory binding proteins, they do not show homology with any member of the lipocalin family (Flower, 1996). This was recently supported by the determination of the 3D structure of the silkworm moth (*Bombyx mori*) PBP, shown to exhibit a novel protein fold (Sandler et al., 2000). In honeybee (*Apis mellifera* L.), the queen pheromone blend acts as a sex attractant for drones and also controls numerous activities of workers to maintain colony cohesion and stability. ASP1, which belongs to a subclass of honeybee antennal specific proteins (Danty et al., 1998), was identified as a PBP because of its higher abundance in drone, its location in sensilla placodea and ability to bind the most active components of the queen pheromone blend, 9-keto-2(*E*)-decenoic acid and 9-hydroxy-2(*E*)-decenoic acid (Danty et al., 1999). ASP1, recently cloned and heterogeneously expressed in the yeast *Pichia pastoris*, was shown to be a protein of 13180 Da (119 amino acids), deprived of post-translational modifications other than peptide signal removal and formation of three disulfide bridges (Danty et al., 1999). Its physico-chemical properties ascertain that it is a member of the insect PBP family, although sharing only little sequence homology with the silkworm moth PBP. Here we report the resonance assignments of ^{13}C and ^{15}N labeled ASP1 produced by the yeast system as a recombinant protein whose features, controlled by Edman sequencing and mass spectrometry, were indistinguishable from the native form. This assignment is an obvious prerequisite for investigating the solution structure of this protein and its interactions with ligands.

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Methods and experiments

Enriched proteins were produced using transformed *Pichia pastoris* cultured in buffered minimum medium containing ^{15}N ammonium sulfate (Euriso-top – CEA) and ^{13}C glucose (Martek Bioscience) for biomass production. ASP1 induction was achieved by ^{13}C methanol (Euriso-top – CEA) addition. ASP1 was purified by reverse phase HPLC. NMR samples (unlabeled, U-^{15}N and U-^{13}C,^{15}N) contained up to 1.5 mM of protein in H_2O:D_2O (95:5), 0.1 mM NaN_3 (pH = 5.6, adjusted through the addition of NaOH). Spectra were recorded at 22 °C on a Bruker DRX-800 spectrometer, equipped with a 5 mm TXI triple resonance, triple-axis gradient probe. Some experiments were performed on a Bruker AVANCE DRX-500 spectrometer using a 5 mm TXI triple resonance, z-axis gradient cryoprobe. External TSP was used for calibration of ^{1}H and ^{13}C chemical shifts while indirect referencing according to the absolute frequency values was used for ^{15}N (Wishart et al., 1995). Data were processed and analyzed via the XWIN-NMR 2.6 (Bruker) and Felix 98 (MSI) software packages.

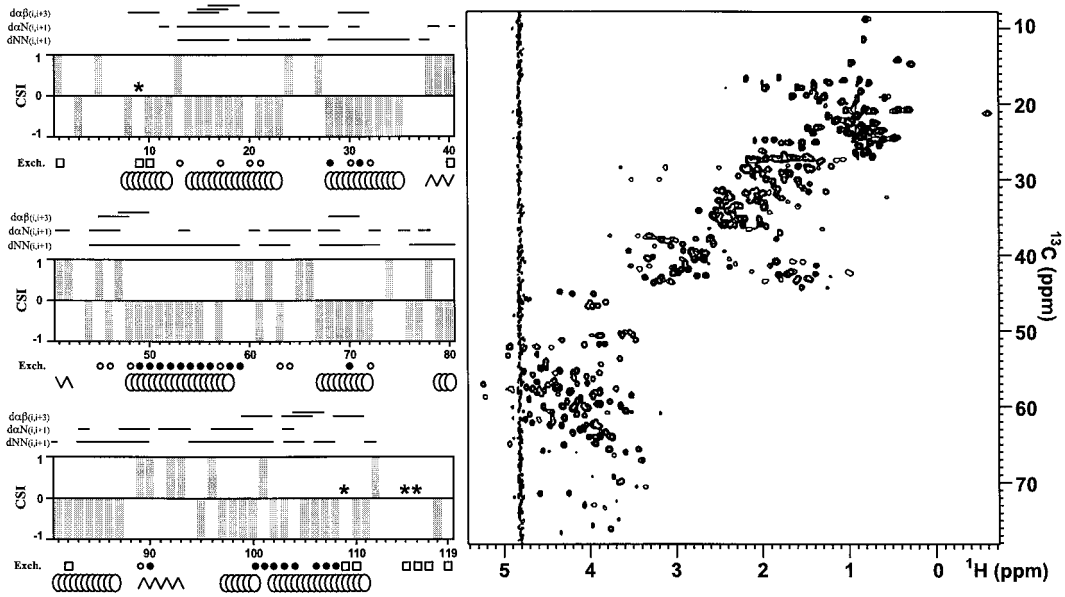

Figure 1. Left: Summary of the secondary structure elements based on NOE connectivities and chemical shift index data. Open and filled circles indicate the slowly exchangeable amide protons, visible 1 and 30 days, respectively, after dissolving lyophilised ASP1 in D_2O. Squares mark the residues with amide resonances absent in the ^{1}H-^{15}N HSQC spectrum. The 800 MHz ^{1}H-^{13}C HSQC spectrum of ASP1 in H_2O is shown on the right.

Backbone assignment was obtained from the analysis of a set of 3D triple resonance experiments (Sattler et al., 1999): HNCA/HN(CO)CA, HNCO/HN(CA)CO. Side-chains were assigned from HNCACB/CBCA(CO)NH, HBHA(CBCACO)NH, HCCH-TOCSY, H(CCCO)NH experiments. The assignment was confirmed and extended using 3D ^{15}N- and ^{13}C-resolved NOESY experiments (τ_m = 80 ms). The preliminary analysis of some characteristic medium-range NOEs together with the Cα, CO and Hα chemical shift index data (Wishart et al., 1994) permitted the identification of the secondary structure elements (Figure 1, left). Comparison with the structures obtained for other PBPs (Sandler et al., 2000) indicates a rather similar global folding pattern for ASP1.

Extent of assignments and data deposition

With the exception of the four residues marked with an asterisk in Figure 1, essentially complete backbone sequential assignment was obtained for ASP1. Moreover, a great number of side-chain resonances was attributed and a table of ^{1}H, ^{13}C and ^{15}N chemical shifts has been deposited in the BioMagResBank under accession number BMRB-4940.

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