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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N chemical shift assignment of the honeybee odorant-binding protein ASP2

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Biological context

In insects, odorant-binding proteins (OBP) are involved in the transport of the hydrophobic odorants through the sensillum lymph towards their neuronal receptors. They are small acidic soluble proteins (13–16 kDa), highly concentrated in the sensilla lymph, which, in contrast to vertebrate OBP, do not share homology with any member of the lipocalin family. The presence of six cysteines and their interval spacing are the most striking features shared by proteins belonging to this family (Pelosi, 1998). Although the physiological function of OBP is not yet well understood, their biological role has nevertheless been functionally demonstrated in the fruit fly (Kim et al., 1998). In honeybee (*Apis mellifera* L.), which is able to discriminate a wide range of odorants, several antennal specific proteins have been found, classified in three subclasses (Danty et al., 1998) and shown to be evolutionary divergent from the Lepidopteran OBP (Vogt et al., 1999). Based on sequence similarity, determined after cDNA cloning, and its tissue-specificity, one of these OBP, called ASP2, was indeed assigned to be a member of the insect OBP family. It is a protein of 13,695 Da, deprived of post-translational modifications other than peptide signal removal and formation of three disulfide bridges, which have recently been assigned (Briand et al., 2001a). This protein was indeed shown to bind several odorants, which are naturally perceived by honeybees (Briand et al., 2001a). Here we report the resonance assignment of ^{13}C and ^{15}N labeled ASP2, produced by the yeast *Pichia Pastoris* (Briand et al., 2001b) as a recombinant protein whose features, controlled by Edman sequencing and mass spectrometry, were indistinguishable from the native form. This assignment represents the first step for structural analysis and interaction studies with selected odorant molecules by NMR.

Methods and experiments

Enriched proteins were produced using transformed *Pichia Pastoris* cultured in buffered minimum medium containing ^{15}N ammonium sulfate (Eurisotop – CEA) and ^{13}C glucose (Martek Bioscience) for biomass production. ASP2 induction was achieved by ^{13}C methanol (Eurisotop – CEA) addition. ASP2 was purified by reverse phase HPLC (Briand et al., 2001b). NMR samples (unlabeled; U-^{15}N; U-^{13}C,^{15}N) contained up to 1.4 mM of protein in H_2O:D_2O (90:10 v/v), 100 mM phosphate buffer, 0.1 mM NaN_3, 0.1 mM TSP (pH = 5.7, adjusted through the addition of NaOH) and in 100% D_2O, 100 mM phosphate buffer, 0.1 mM NaN_3, 0.1 mM TSP (pH = 7.7). pH were measured with no correction with respect to deuterium isotopic effects. Spectra were recorded at 308K on a Bruker DRX-800 spectrometer, equipped with a 5 mm TXI triple resonance, triple-axis gradient probe. Internal TSP was used for calibration of ^{1}H chemical shifts while indirect referencing according to the absolute frequency values was used for ^{15}N and ^{13}C. Data were processed and analyzed via XWIN-NMR and Aurelia (Bruker) software packages.

We observed no global changes in ^{15}N-HSQC for pH varying from 5.7 to 7.7. ASP2 did not undergo any conformational change at neutral versus acidic pH. Number and shape of peaks are consistent with a major monomeric form of ASP2 under these conditions.

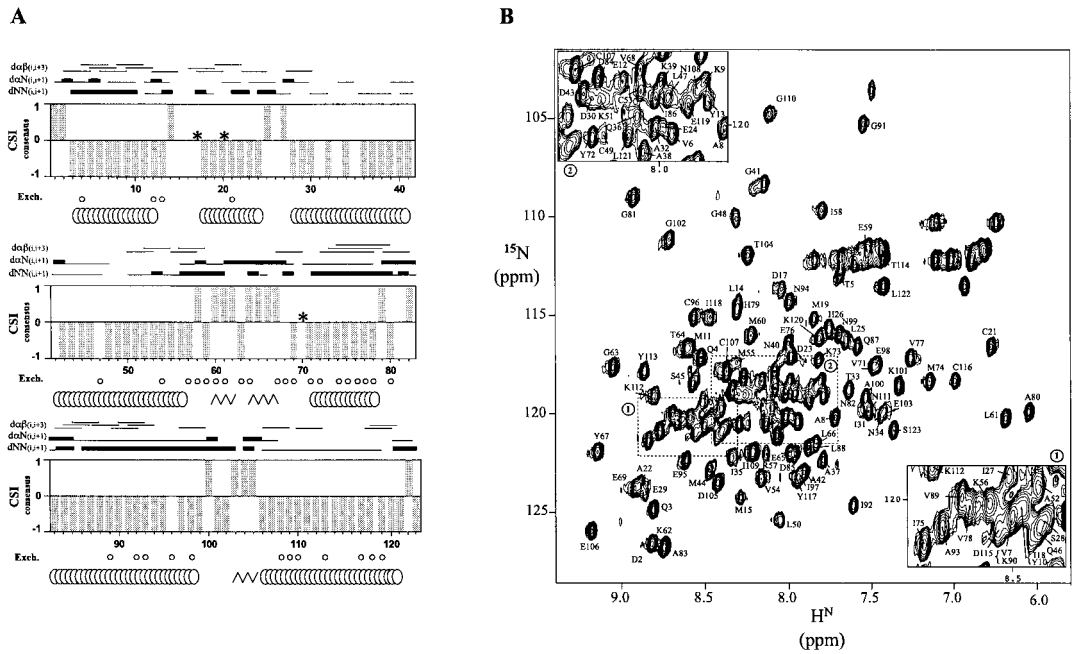

Figure 1. (A) Summary of the secondary structure elements based on nOe connectivities and chemical shift index data. Open cycles indicate the slowly exchangeable amide protons, visible 1 hour after dissolving lyophilized ASP2 in D_2O. Asterisks show the position of proline residues. (B) ^{1}H-^{15}N HSQC spectrum of ASP2 in 90% H_2O, 10% D_2O (v/v) at pH = 5.7 collected at 800 MHz with expanded views of two overlapped-peak regions.

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, ^{15}N-HMQC-NOESY-HMQC. Side-chains were assigned from HNCACB/CBCA(CO)NH, ^{15}N-TOCSY-HSQC (τ_m = 95 ms), HCCH-TOCSY (τ_m = 33 ms) experiments. The assignment was confirmed and extended using 3D ^{15}N- and ^{13}C-resolved NOESY experiments (τ_m = 150 ms, 120 ms resp.). ^{13}C-resolved experiments were carried on the D_2O sample. The preliminary analysis of some characteristic medium-range nOes together with the consensus chemical shift index obtained using C_α, C_β, CO and H_α chemical shift values (Wishart and Sykes, 1994), permitted the identification of the secondary structure elements (Figure 1A). It indicates that residues 3–13, 18–24, 28–57, 71–78, 83–99, and 106–121 are in α helices. Associated with disulfide bonds map (Briand et al., 2001a), ASP2 seems to present a global fold similar to that of *Bombyx Mori* PBP (Sandler et al., 2000).

Extent of assignments and data deposition

A complete backbone sequential assignment of $^{1}H^{N}$, $^{13}C_\alpha$, $^{13}C'$ and ^{15}N resonances, except for $^{1}H^{N}$ and ^{15}N of the N-terminal residue, was obtained for ASP2. Moreover, all $^{13}C_\beta$ and $^{1}H_\beta$ resonances except $^{13}C_\beta$ of residue I35, were assigned. A table of ^{1}H, ^{13}C and ^{15}N chemical shifts has been deposited in the BioMagResBank under the accession number BMRB-5030.

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