Published in final edited form as:

Biomol NMR Assign. 2008 December ; 2(2): 105–106. doi:10.1007/s12104-008-9096-6.

¹H, ¹⁵N, and ¹³C Chemical Shift Assignments of the Navel Orange Worm Pheromone-binding Protein-1 (Atra-PBP1)

Xianzhong Xu, Yun Li, Josep Rayo, Yuko Ishida, Walter Leal, and James B. Ames*

Departments of Chemistry and Entomology, University of California, Davis, CA 95616.

Abstract

A pheromone-binding protein from navel orange worm, *Amyelois transitella* (Atra-PBP1) binds to non-polar pheromone molecules and facilitates the transport and delivery of pheromone to the membrane-bound pheromone receptors. We report complete NMR chemical shift assignments of Atra-PBP1 obtained at pH 4.5 and 25 °C (BMRB no. 15601).

Keywords

odorant-binding protein; pheromone signaling; olfaction; NMR

Biological Context

To find mates and successfully reproduce, male moths rely heavily on pheromone detection. Pheromone-binding proteins (PBPs) (Vogt and Riddiford, 1981) and odorant receptors (ORs) (Clyne et al., 1999, Vosshall et al., 1999) are essential for the uptake, delivery and detection of sex pheromones (Leal et al., 2005). Molecular interactions amongst pheromone molecules, PBPs and ORs lead to a remarkable selectivity and sensitivity of the olfactory system in insects. The pheromone-binding protein of the navel orange worm, *Amyelois transitella* (Atra-PBP1) binds tightly to pheromone molecules at neutral pH (but not at pH < 5). Atra-PBP1 undergoes a conformational transition at low pH (Damberger et al., 2007) to facilitate the transport and delivery of bound pheromone to the OR on the membrane surface, where the local pH is estimated to be low. Three-dimensional structures of a few PBPs are known (Damberger et al., 2007, Lautenschlager et al., 2007), but the atomic-level recognition of PBPs and ORs are not well understood. Three-dimensional structures of PBPs as a function of pH and bound ligand may unveil how ORs are activated by pheromones. We report here NMR assignments of Atra-PBP1 at pH 4.5, as a first step toward elucidating the atomic-level structural changes and regulatory mechanisms necessary for the activation of ORs by PBPs.

*To whom correspondence should be addressed: ames@chem.ucdavis.edu.

Methods and Experiments

Expression and Purification of Atra-PBP1.

Uniformly ^{15}N -labeled and ^{13}C , ^{15}N -labeled AtraPBP1 was expressed in *E. coli* and purified by ion-exchange and gel-filtration chromatography as described previously (Damberger et al., 2007). Typically, 5 mg of purified protein was obtained from a 1-liter culture. The identity and integrity of the final protein sample was confirmed by SDS-PAGE and LC-ESI/MS.

NMR Spectroscopy.

Samples for NMR analysis were prepared by dissolving $^{13}\text{C}/^{15}\text{N}$ -labeled Atra-PBP1 (0.5 mM) in 0.3 ml of a 95% H_2O /5% $[\text{D}_2]\text{H}_2\text{O}$ solution containing 10 mM $[\text{D}_3]\text{H}_3\text{PO}_4$ acetate at pH 4.5. All NMR experiments were performed at 25 °C on a Bruker Avance 600 MHz spectrometer equipped with a four-channel interface and triple-resonance cryogenic probe. The ^{15}N - ^1H HSQC spectra (Fig. 1) were recorded on samples of ^{15}N -labeled Atra-PBP1 (in 95% H_2O , 5% $^2\text{H}_2\text{O}$). The number of complex points and acquisition times were: 256, 180 ms (^{15}N (F_1)), and 512, 64 ms (^1H (F_2)). Assignment of backbone and side-chain resonances were obtained from the following spectra: HNCACB, HN(CO)CACB, HNCO, CBCACONH, HBHACONH, C(CO)NH-TOCSY, H(CCO)NH-TOCSY, HCCH-TOCSY, and H(CCH)-COSY. The NMR data were processed using NMRPipe and analyzed using NMRview.

Assignments and Data Deposition

Figure 1 presents $^1\text{H}/^{15}\text{N}$ HSQC spectrum of Atra-PBP1 to illustrate representative backbone resonance assignments. NMR assignments were based on 3D heteronuclear NMR experiments performed on $^{13}\text{C}/^{15}\text{N}$ -labeled Atra-PBP1 (residues 1–142). The protein sample in this study consists of 142 native residues and does not contain any affinity tags or extra residues. All 142 residues exhibited strong NMR signals with uniform intensities, indicative of a well-defined three-dimensional protein structure. More than 95% the backbone resonances (^1HN , ^{15}N , $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$, and ^{13}CO) and ~85% of aliphatic side chain resonances were assigned, including stereospecific assignment of valine and leucine methyl groups. The chemical shift assignments (^1H , ^{15}N , ^{13}C) of Atra-PBP1 have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu>) under accession number 15601.

Acknowledgements

We thank Jeff de Ropp for technical support and help with NMR experiments. Work supported by NIH grant (EY012347) to J.B.A., NSF (0234769), USDA-NRI (2003-35302-13648), the Almond Board of California, and UC Davis NMR facility.

References

- Clyne PJ, Warr CG, Freeman MR, Lessing D, Kim J and Carlson JR (1999) *Neuron*, 22, 327–338. [PubMed: 10069338]
- Damberger FF, Ishida Y, Leal WS and Wuthrich K (2007) *J. Mol. Biol.*, 373, 811–819. [PubMed: 17884092]
- Lautenschlager C, Leal WS and Clardy J (2007) *Structure*, 15, 1148–1154. [PubMed: 17850754]

- Leal WS, Chen AM, Ishida Y, Chiang VP, Erickson ML, Morgan TI and Tsuruda JM (2005) Proc. Natl. Acad. Sci. U S A, 102, 5386–5391. [PubMed: 15784736]
- Vogt RG and Riddiford LM (1981) Nature, 293, 161–163. [PubMed: 18074618]
- Vosshall LB, Amrein H, Morozov PS, Rzhetsky A and Axel R (1999) Cell, 96, 725–736. [PubMed: 10089887]

FIG. 1.

Two-dimensional ^{15}N - ^1H HSQC spectrum of Atra-PBP1 recorded at 600-MHz ^1H frequency. The protein sample was uniformly labeled with nitrogen-15. Side-chain amide proton resonances (NH_2) are connected by dotted lines.