Structure
Article

Bombyx mori Pheromone-Binding Protein Binding Nonpheromone Ligands: Implications for Pheromone Recognition

Catherine Lautenschlager,[1] Walter S. Leal,[2] and Jon Clardy[1,*]
[1]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA
[2]Department of Entomology, University of California, Davis, Davis, CA 95616, USA
*Correspondence: jon_clardy@hms.harvard.edu
DOI 10.1016/j.str.2007.07.013

SUMMARY

Insect pheromone-binding proteins (PBPs) transport sex pheromones through the aqueous layer surrounding G protein-coupled receptors that initiate signaling events leading to mating. This PBP-receptor system strongly discriminates between ligands with subtle structural differences, but it has proved difficult to distinguish the degree of discrimination of the PBP from that of the G protein-coupled receptor. The three-dimensional structures of the PBP of *Bombyx mori*, the silkworm moth, both with and without its cognate ligand bombykol ([*E*,*Z*]-10,12-hexadecadienol), have been determined by X-ray crystallography and NMR. In this paper, the structures of the same binding protein with bound iodohexadecane and bell pepper odorant were determined at 1.9 and 2.0 Å, respectively. These structures illustrate the remarkable plasticity in the ligand binding site of the PBP, but suggest the protein might still act as a filter during pheromone signal processing.

INTRODUCTION

A female moth sends out a chemical message that, upon being encountered by an appropriate male, is processed to generate a correct response within milliseconds. The sex pheromone of *Bombyx mori*, bombykol ([*E*,*Z*]-10,12-hexadecadienol; Figure 1), is synthesized by the female and detected in the highly branched antennae of male moths. Pheromone is adsorbed into specialized male moth olfactory hairs, or sensilla, which cover the antennae. The pheromone diffuses through pore tubules into the aqueous sensillar lymph, where it is bound to the pheromone-binding protein and transported to the G protein-coupled receptor on the neural cell. Using this signaling system, the male follows the pheromone plume to its mate. Transporting the hydrophobic pheromone through the aqueous sensillar lymph could delay the rapid response needed to follow the pheromone plume Leal et al., 2005c. The lymph of pheromone-sensitive olfactory hairs in *Bombyx mori* antennae contains a high concentration of pheromone-binding proteins (PBPs), 10–20 mM or about 160 mg/ml (Vogt, 1987). PBPs are members of the encapsulins (Leal, 2003), a family of proteins that solubilize hydrophobic compounds in an aqueous environment. The subclass of pheromone-binding proteins mediates the delivery of the sex pheromone to its receptor in the dendritic membrane.

Pheromone reception in male moths is both highly sensitive—responses can be seen with only a few molecules—and selective—small structural changes typically lead to loss of activity by several orders of magnitude. This ligand selectivity is essential given the degree of similarity of moth sex pheromones, which are typically 12- to 18-carbon partially unsaturated aliphatic chains (Ando et al., 2004). These molecules differ only by carbon chain length, placement of double bonds, and terminal functional group (aldehyde, alcohol, or acetate). Early studies indicated that simply switching either the *cis* or *trans* double bond in bombykol rendered the molecule ineffective as an attractant (Butenandt, 1963). It has been suggested that PBPs play a role in discriminating among potential molecular signals (Pelosi, 1994; Prestwich and Du, 1997; Leal, 2003, 2005) and in speeding up the pheromone signaling process (Syed et al., 2006; Leal et al., 2005c).

There is evidence both supporting and contradicting the idea that PBPs are involved in pheromone recognition. Analysis of the primary structure of PBPs from different moth species shows limited diversity among the proteins. The sequences are about 70% identical and 85% similar to each other, and searching for amino acid residues that might be involved in specificity has yielded little information, although sequence and structural data suggest that serine residues interact with alcohol groups in 14- to 16-carbon chain pheromones and that asparagine residues might specifically interface with acetate groups (Sandler et al., 2000; Mohanty et al., 2004). However, for most moth species, the pheromone signal consists of several molecules and several PBPs. It has been demonstrated through binding studies for *Antheraea polyphemus* and *A. pernyi* moths, each of which has three pheromone-binding proteins and a three-component pheromone blend, that each PBP preferentially binds a specific component of the blend (Maida et al., 2003). All three

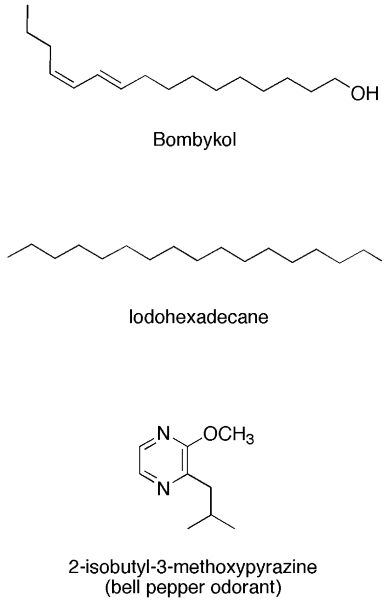

Figure 1. Ligands Used for Cocrystallization with BmorPBP

constituents of the sex pheromone of the wild silkmoth, *A. polyphemus*, bound to the major PBP from this species, ApolPBP1, with apparent high affinity, but in competitive assays ApolPBP1 showed considerable preference for the major constituent of the sex pheromone.

We felt that structural studies with ligands very different from pheromones might offer some insight into PBP specificity and plasticity. To explore the specificity of *B. mori* PBP (BmorPBP), the protein was crystallized in complex with nonpheromone ligands (Figure 1) and structures were determined by X-ray crystallography. To investigate limitations that would be imposed by stringent specificity, iodohexadecane was added to form the IHD-BmorPBP complex. Iodohexadecane (IHD) has a chain length slightly longer than that of bombykol, lacks the conformational restraint provided by the two double bonds in the pheromone, replaces the alcohol functional group with an iodine atom, and has no detectable pheromonal activity. To explore a potential ligand with quite different geometry than that of a typical moth sex pheromone, bell pepper odorant (2-isobutyl-3-methoxypyrazine) was added to form the BPO-BmorPBP complex. The bell pepper odorant molecule lacks the long-chain character of bombykol but retains the hydrophobic nature of sex pheromones, seems to be a promiscuous binder of the closely related odorant-binding protein family, and has no reported pheromone role. Remarkably, both crystal structures revealed electron density in the binding pocket of the protein that precisely fit the geometry of each added nonpheromone ligand. Surprisingly, the structures also provide solid experimental information that suggests the binding pocket of the protein is adapted for ligands with hook-shaped geometries, such as that of bombykol.

RESULTS

Overall Structures

All structures were solved by molecular replacement using the crystal structure of *B. mori* PBP bound to bombykol at pH 8 (Figure 2A) (Sandler et al., 2000). Bombykol was removed from the model for molecular replacement. In the bombykol complex, the protein is comprised of six α helices. Four of these helices converge to form a hydrophobic binding pocket for bombykol, with three disulfide bonds stabilizing the structure of this small extracellular protein. A loop region between helices α3 and α4 is believed to provide entrance for the ligand by becoming destabilized upon protonation of one or all of three histidine residues at low pH (Sandler et al., 2000). The bound bombykol has a roughly planar, hook-shaped conformation within the binding pocket. The hydroxyl group of bombykol forms a hydrogen bond with the side chain of Ser56, and one set of double bonds in bombykol is sandwiched between the Phe12 and Phe118 aromatic rings.

The protein has also been shown to exist in an empty structure (Figure 2B) (Lautenschlager et al., 2005). The bombykol-bound protein and empty protein differ structurally in three major ways: the bombykol-bound protein has a disordered C terminus whereas the empty protein has a disordered N terminus; the disordered looping region between helices α3 and α4 is more extended in the empty protein; and the C terminus of the empty protein forms a seventh α helix that fills the binding pocket of the protein.

Both the IHD-BmorPBP and BPO-BmorPBP structures (Figures 3A and 3B) show an overall protein conformation similar to that of the bombykol-bound structure. The C-terminal region of the protein exists as a disordered loop, and the N-terminal region is an ordered helix. All three disulfide bonds (Leal et al., 1999; Scaloni et al., 1999) are present. In both nonpheromone complexes, the protein crystallized with one molecule in the asymmetric unit, rather than as two molecules per asymmetric unit as seen in the bombykol-bound structure, suggesting a subtle change in molecular shape. Previous work using flow injection analysis of the protein followed by mass spectrometry indicated the protein forms a dimer at pH $>\sim$5.5 and a monomer at lower pH (Leal, 2000), though the NMR structure of BmorPBP at physiological pH also shows the protein as a monomer (Lee et al., 2002). The electron density in the pockets of these complexes clearly fits the modeled ligands. Backbone alignments of the proteins were performed using LSQMAN. The root-mean-square deviation of the Cα atoms between the iodohexadecane and bombykol complexes was 0.767 Å, and between the bell pepper odorant and bombykol complexes was 0.785 Å, indicating nearly identical backbone conformations among the complexed PBPs (data not shown). The positioning of side chains within the binding pocket is also consistent in all three complexes (Figure 4). Binding assays indicated that iodohexadecane binds to BmorPBP with significantly lower affinity than the sex pheromone of the silkmoth, bombykol, whereas no