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Dynamic Conformational Equilibria in the Physiological Function of the *Bombyx mori* Pheromone-Binding Protein

Erich Michel[1], Fred F. Damberger[1], Yuko Ishida[2], Francesco Fiorito[1], Donghan Lee[1], Walter S. Leal[2] and Kurt Wüthrich[1,3]*

[1]*Institute of Molecular Biology and Biophysics, ETH Zurich, 8093 Zurich, Switzerland*
[2]*Honorary Maeda–Duffey Laboratory, Department of Entomology, University of California, Davis, CA 95616, USA*
[3]*Department of Molecular Biology and Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA 92037, USA*

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The *Bombyx mori* pheromone-binding protein (BmorPBP) undergoes a pH-dependent conformational transition from a form at basic pH, which contains an open cavity suitable for ligand binding (BmorPBP^{B}), to a form at pH 4.5, where this cavity is occupied by an additional helix (BmorPBP^{A}). This helix α7 is formed by the C-terminal dodecapeptide 131–142, which is flexibly disordered on the protein surface in BmorPBP^{B} and in its complex with the pheromone bombykol. Previous work showed that the ligand-binding cavity cannot accommodate both bombykol and helix α7. Here we further investigated mechanistic aspects of the physiologically crucial ejection of the ligand at lower pH values by solution NMR studies of the variant protein BmorPBP(1–128), where the C-terminal helix-forming tetradecapeptide is removed. The NMR structure of the truncated protein at pH 6.5 corresponds closely to BmorPBP^{B}. At pH 4.5, BmorPBP(1–128) maintains a B-type structure that is in a slow equilibrium, on the NMR chemical shift timescale, with a low-pH conformation for which a discrete set of ^{15}N–^{1}H correlation peaks is NMR unobservable. The full NMR spectrum was recovered upon readjusting the pH of the protein solution to 6.5. These data reveal dual roles for the C-terminal tetradecapeptide of BmorPBP in the mechanism of reversible pheromone binding and transport, where it governs dynamic equilibria between two locally different protein conformations at acidic pH and competes with the ligand for binding to the interior cavity.

*Corresponding author. Institute of Molecular Biology and Biophysics, ETH Zurich, 8093 Zurich, Switzerland.
E-mail address: kw@mol.biol.ethz.ch.

Present addresses: Y. Ishida, Graduate School of Science, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan; D. Lee, Max-Planck-Institut für Biophysikalische Chemie, Abteilung NMR-Basierte Strukturbiologie, Am Faßberg 11, 37077 Göttingen, Germany.

Abbreviations used: BmorPBP, *Bombyx mori* pheromone-binding protein; NOE, nuclear Overhauser enhancement; 3D, three-dimensional; NOESY, NOE spectroscopy; HSQC, heteronuclear single-quantum coherence; 2D, two-dimensional.

Introduction

The *Bombyx mori* pheromone-binding protein (BmorPBP; sometimes also referred to as BmorPBP1)[1,2] occurs at high concentrations in the pheromone-detecting sensilla of male silkworm moths, where it binds and transports the pheromone component bombykol (emitted by the females) across the aqueous sensillar lymph to the membrane-standing olfactory receptor.[3,4] A crystal structure determination of the BmorPBP–bombykol complex showed that the ligand is bound in a large cavity in the core of the protein.[5] Intriguingly, a second conformation was observed at pH 4.5, BmorPBP^A (low-pH form of BmorPBP), where the ligand-binding site identified in the crystal structure at near-neutral pH is filled with a regular α-helix.[6] This helix α7 is formed by the C-terminal dodeca-peptide segment of residues 131–142, which is present as a flexibly disordered random coil on the protein surface in the high-pH form of the protein, BmorPBP^B, and in the bombykol–BmorPBP complex.[5,7] Formation of the isoform BmorPBP^A provides a rationale for the absence of pheromone binding at low pH, and structural data suggest that as BmorPBP approaches the dendritic membrane, where local pH is reduced,[8,9] the protein ejects the ligand to the membrane-standing olfactory receptor.[6,10–12] In line with these structure-based conclusions, binding assays showed that the C-terminally truncated variant of BmorPBP, BmorPBP (1–128), binds pheromone with the same affinity as the full-length protein at pH 7; however, in contrast to the wild-type protein, it retains binding affinity at pH 5.[13] In order to further investigate the role of the C-terminal residues 129–142 in pH-dependent conformational transition and in the physiological function of BmorPBP, we present an NMR structure determination of BmorPBP(1–128) at pH 6.5 and NMR investigations of the pH dependence of the solution structure of this protein in the pH range 4.5–6.5.

Results

NMR structure determination of BmorPBP(1–128)

Data collection for NMR structure determination was performed with a 1 mM solution of BmorPBP (1–128) in NMR buffer [50 mM potassium phosphate (pH 6.5), 0.2% NaN_3, and 95% H_2O/5% ^2H_2O]. In view of previous reports on fatty acid binding to moth pheromone-binding proteins,[14–18] we conducted the present study with delipidated protein samples (see Materials and Methods). The sequence-specific resonance assignments for BmorPBP(1–128) have previously been reported.[19] For structure determination, we used the automated ATNOS/CANDID/DYANA procedure.[20–22] This approach resulted in the assignment of 1127, 446, and 3259 nuclear Overhauser enhancement (NOE) cross-peaks in the three-dimensional (3D) ^{15}N-resolved ^{1}H,^{1}H NOE spectroscopy (NOESY) spectrum, the 3D aromatic ^{13}C-resolved ^{1}H,^{1}H NOESY spectrum, and the 3D aliphatic ^{13}C-resolved ^{1}H,^{1}H NOESY spectrum, respectively, yielding 2368 meaningful NOE upper-distance limits for the final input for DYANA, which was supplemented with 560 backbone and side-chain torsion-angle constraints. After energy minimization of the DYANA conformers in a water shell with the program OPALp[23,24] using the AMBER[25] force field, a well-defined structure for BmorPBP(1–128) was obtained, with global RMSDs of 0.47 Å and 0.84 Å calculated for backbone atoms and all heavy atoms, respectively (Table 1, Fig. 1a).

Table 1. Input for the structure calculation and characterization of the energy-minimized NMR structures of BmorPBP(1–128)

Parameter	Value[a]
NOE upper-distance limits[b]	2368
Intraresidual	668
Short range	526
Medium range	581
Long range	593
Dihedral-angle constraints (ϕ, ψ, χ^1, and χ^2)	560
Residual target function value (Å^2)	2.13 ± 0.27
Residual distance constraint violations	
Number ≥ 0.1 Å	26 ± 4
Maximum (Å)	0.17 ± 0.09
Residual dihedral-angle constraint violations	
Number ≥ 2.5°	1 ± 1
Maximum (°)	2.2 ± 1.0
AMBER energies (kcal/mol)	
Total	−5378 ± 103
Van der Waals	−428 ± 12
Electrostatic	−5962 ± 97
RMSD from ideal geometry	
Bond lengths (Å)	0.0074 ± 0.0001
Bond angles (°)	1.90 ± 0.05
RMSD to the mean coordinates (Å)	
bb (1–128)[c]	0.47 ± 0.05
All heavy atoms (1–128)[c]	0.84 ± 0.06
Ramachandran plot statistics[d]	
Most favored regions (%)	86.2
Additionally allowed regions (%)	13.1
Generously allowed regions (%)	0.4
Disallowed regions (%)	0.3

[a] Except for the top six entries, the data characterize a group of 20 conformers used to represent the NMR structure. The means and standard deviations are given.

[b] The input also included nine upper-distance constraints and nine lower-distance constraints to enforce the disulfide bonds 19–54, 50–108, and 97–117.

[c] bb indicates the backbone atoms N, C^α, and C′. The numbers in parentheses indicate the residues for which the RMSD was calculated.

[d] As determined by PROCHECK.[26]