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Flexibility of a loop in a pheromone binding protein from *Bombyx mori*: a molecular dynamics simulation

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Abstract

The three dimensional structure of a pheromone binding protein from *Bombyx mori* complexed with its substrate was determined recently [Sandler et al., *Chem. Biol.* 7, 143-151 (2000)]. The structure suggested that a loop formed by amino acid residues 60-69 could serve as a flexible lid into the pheromone binding pocket. To examine the above hypothesis, a molecular dynamics simulation was performed for a substrate free form of the protein starting from the crystal structure. In the simulation loop 60-69 took a conformation different from that in the substrate bound form. Also, the loop was the most flexible region of the protein. Thus, the simulation supports the hypothesis that the loop is a flexible lid.

Key Words: Pheromone, Binding Protein, Flexibility, Loop, Molecular Dynamics, *Bombyx mori*

Area of Interest: Molecular Computing

1. Introduction

The pheromone binding protein (PBP) is a key protein in the mating process of insects. The protein exists in high concentration (ca. 10mM) in the male sensilla lymph [1]. The insect sex pheromones are generally small, hydrophobic chemical substances secreted to attract the opposite sex. A single pheromone molecule is enough to trigger olfactory neurons in the insect antennae [2]. Hence, the molecular machinery to detect the sex pheromone and to transfer the signal is a very effective chemical communication system. When the hydrophobic pheromone molecules reach the male antenna, they are absorbed through micro openings in the cuticular wall, and are dissolved into the sensilla lymph with the assistance of PBP [3].

The PBP from the silkworm moth *Bombyx mori* (BmPBP), the subject of the current study, is the best characterized among the insect PBPs. BmPBP consists of 137 amino acid residues (Fig. 1). The three-dimensional structure of BmPBP has been determined by X-ray crystallography [4] (Fig. 2). In the crystal structure, BmPBP formed a dimer (Fig. 3 left), and a bombykol ((E,

Z)-10,12-hexadecadinen-1-ol) molecule, the pheromone, was deeply embedded in the hydrophobic cavity of each BmPBP monomer surrounded by α-helices stabilized by S-S bridges (Fig. 2). Each monomeric BmPBP consists of six α-helices; four anti-parallel α-helices form a binding pocket to contain the pheromone.

In the BmPBP structure, there is a long loop formed by amino acid residues 60-69. In the crystal structure of BmPBP/pheromone complex, the loop is located in a position covering the binding pocket (Fig. 2). This location of the loop has led us to hypothesize that the loop could serve as a flexible lid of the binding pocket. Namely, in the substrate-free state (Apo-BmPBP), the loop may not cover the binding pocket, and the pocket could be accessible from outside.

Nevertheless, no experimental data have been available concerning the flexibility of the loop in Apo-BmPBP. Therefore, we simulated the structure and dynamics of Apo-BmPBP by a Molecular Dynamics (MD) simulation. The conformation and flexibility of loop 60-69 was examined to test the hypothesis.

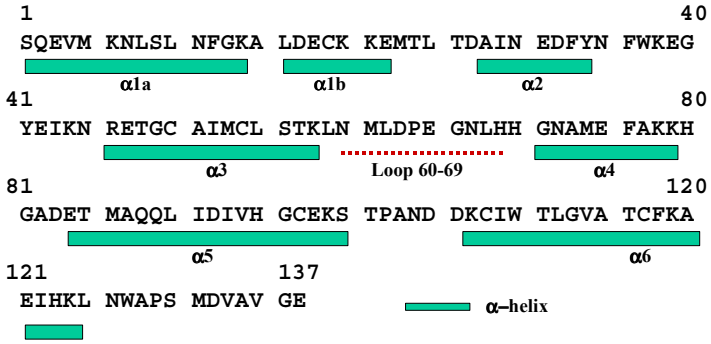

Fig. 1 Amino Acid sequence of BmPBP.

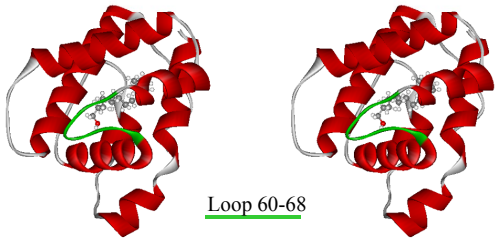

Fig. 2 Stereo view of BmPBP/Bombykol complex.

2. Method

A one ns MD trajectory of Apo-BmPBP was generated by a strategy shown in Fig. 3. A software package, "Program for Energetic Analysis of bioCHemical molecules (PEACH)" Ver. 3.0 A [5, 6], was used throughout the study. The crystal structure of the BmPBP/bombykol complex determined at 1.8 Å resolution (PDB entry 1DQE) [4] was used as the initial structure for the simulation. The asymmetric unit of the crystal contains two BmPBP monomers (Fig. 3 left), and the monomer colored in blue (Chain A) was selected. The bombykol was deleted, and then hydrogen atoms were added. The protein was immersed in a cubic box full of water molecules, and the water molecules within 2.8 Å of the protein were deleted. Na^+ ions sufficient to neutralize the systems were added by replacing them for some of the water molecules to minimize the protein-ion electrostatic interaction. The initial configuration thus prepared is represented in Fig. 4. The system size is summarized in Table 1. A steepest descent energy minimization was then performed for 50 steps, followed by 100 steps of quenched dynamics (5 K) with a time step of 0.02 fs and 1000 steps with a time step of 0.2 fs. Then the system was heated to 300 K within 9 ps, and the simulation was continued for 1 ns in a Nose-Hoover thermostat. The crystal and simulated structures were visualized either by WebLab Viewer Lite 4.0 [7] or by RASMOL 2.6 [8]. See Table 1 for further details of the simulation protocol.

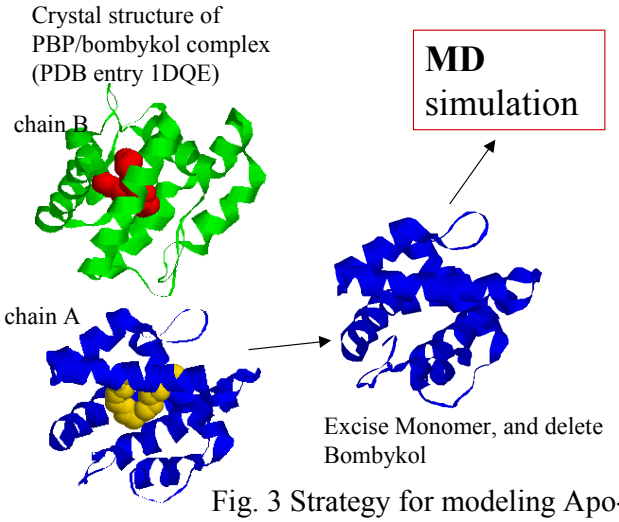

Fig. 3 Strategy for modeling Apo-PBP.

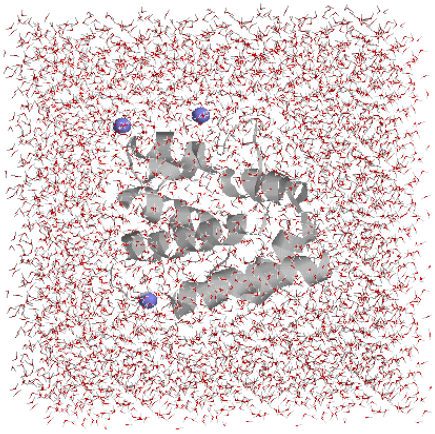

Fig. 4 The initial configuration for MD. The protein was represented as a ribbon, the ions as spheres, and the water molecules as bent sticks.

Table 1. Simulation protocol.

Software	PEACH ver. 3.0 A [5, 6]	
Hardware	DEC alphastation implemented with MD-GRAPE *	
Force Field	Protein and ions: AMBER94 [9]	
	Solvent: Flex SPC water [10]	
Coulomb interaction	Ewald summation (ε =3x10^{-5}, k_{max} = 7) *	
Ensemble	Nose-Hoover thermostat *	
	0-9 ps: heat 0-300 K	
	9 ps - 1 ns: 300 K	
Time integration	RESPA *	
Time steps	0.25 fs: bond, angle torsion	
	2.00 fs: VDW, Ewald-R	
	4.00 fs: Ewald-K	
Solvent	Periodic box (59 x 59 x 59 Å^3)	
Wall-Protein distance	11 Å	
Numbers of atoms	Protein	2,104
	Na^+ ions	3
	Solvent	17,070
	Total	19,177
Computation time	ca. 2 s/1fs MD	

* See [5, 6] for definitions and references for these methodological terms.

3. Results and Discussion

Averages and fluctuations of several energies were calculated from the 0.1 – 1 ns trajectory to test the overall stability of the simulation (Table 2). The Nose Hamiltonian, the conservative physical quantity in this ensemble, showed a fluctuation as small as 0.01 %, indicating that the time integration process was successful during the simulation. The total, kinetic, and potential energies showed fluctuations smaller than 1 %. Thus, the simulation trajectory was considered stable from an energetic viewpoint.

First, the time course of Root Mean Square Deviation (RMSD) from the initial structure was computed (Fig. 5a). The main chain RMSD gradually increased to ca. 1.7 Å within 0.2 ns and was almost stable during the simulation. This value of RMSD was small, considering that the MD simulation was performed without the bound bombykol.

Then, the time root mean square of RMSD for each residue was calculated to investigate which part of the protein were deviated the most from the initial structure (Fig. 5b). It was clearly shown that loop 60-69 was the most deviated from the crystal structure of the BmPBP/bombykol complex. These data suggested that the loop takes a different conformation in the apo state.

Next, Root Mean Square Fluctuation (RMSF), the fluctuation around the time averaged structure, was also computed for each residue to illustrate the fluctuation pattern within the protein (Fig. 5b). The overall fluctuation pattern was also illustrated by superimposing snapshots from the 0.2-1.0 ns trajectory (Fig. 6). The RMSF pattern showed that loop 60-69 was the most flexible region of this protein.

Thus, the flexibility of loop 60-69 was clearly shown from the MD trajectory of Apo-BmPBP. This result strongly supports the supposition that the loop has the potential to work as a flexible lid for the binding pocket in the pheromone-binding phenomenon.

Table 2. Averages and fluctuations of Energies (x 10^4 kcal/mol, 0.1-1 ns)

Nose Hamiltonian	-10.1080±0.0002 (0.01 %)
Total energy	-4.3095±0.0018 (0.4 %)
Potential energy	-6.0233±0.0014 (0.3 %)
Kinetic energy	1.7138±0.0011 (0.6 %)

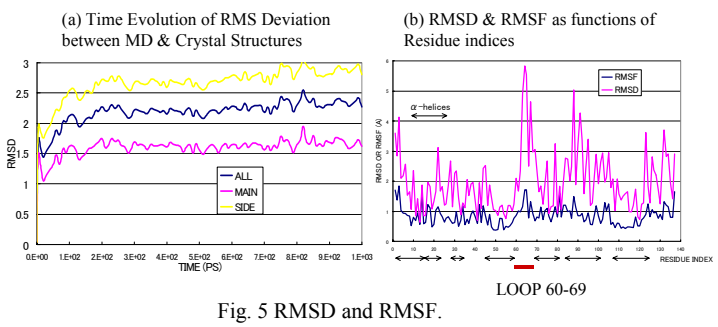

Fig. 5 RMSD and RMSF.

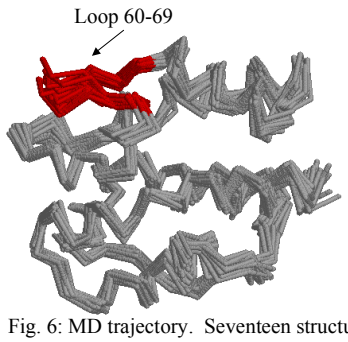

Fig. 6: MD trajectory. Seventeen structures were extracted from the 0.2-1.0 ns trajectory and superimposed.

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分子動力学法によるカイコフェロモン結合タンパク質のループの柔軟性の解析

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要旨

最近、カイコのフェロモン結合タンパク質とその基質との複合体の結晶構造が明らかになった [*Chem.Biol*. **7**, 143-151 (2000)]。その構造から、アミノ酸残基60-69からなるループは、結合ポケットの柔らかな蓋の役割を果たしていることが示唆された。この仮説を検証するために、複合体の結晶構造を基に、基質と結合していないアポタンパク質の分子動力学シミュレーションを行った。シミュレーションでは、問題のループは、結晶構造とは別のコンフォーメーションを取り、しかも、タンパク全体の中で一番柔軟性に富む部位であった。よって、このループが蓋の役割をしているという仮説の妥当性が、強く示唆された。

キーワード：フェロモン結合タンパク質、柔軟性、ループ、分子動力学、カイコ

領域区分：分子計算