Molecular modeling of dynamic properties of the carrier protein from the yellow mealworm *Tenebrio molitor*

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**Abstract.** Steroids play an important role in the life of insects. However, transport of steroids and other hydrophobic molecules through hydrophilic media requires a specialized transport system. In the yellow mealworm *Tenebrio molitor* that function is implemented by the carrier protein THP12. This work focuses on study of dynamic properties of apo-state protein and its complex with an ergosterol using molecular modelling. Analyses of calculated molecular dynamics trajectories for both free and ligand-bound THP12 revealed a conformational shift arising from a ligand binding. Further free binding energy and dissociation constant of a complex were determined.

**1. Introduction**

THP12 is a small 12.4 kDa hydrophilic protein. This protein was expressed from hemolymph of mealworm beetle *Tenebrio molitor*. THP12 was originally classified as an antifreeze protein because mealworm larvae can survive at low temperature. However, following studies show that recombinant protein does not have antifreeze activity. Then hemolymph was fractionated by gel exclusion chromatography. As a result it was found that antifreeze activity corresponds to two other proteins (8.4 kDa and 9.6 kDa).

THP12 has significant sequence similarity to three other proteins (two B proteins and sericotropin). B proteins represent one of the main protein groups secreted from the tubular accessory glands of the adult male mealworm beetle. Sericotropin also is a secretory protein that was identified in wax moth larvae (*Galleria mellonella*). A role as a carrier for small hydrophobic ligands has been suggested for B proteins and sericotropin on the basis of their weak sequence homology to a group of pheromone-binding proteins (PBPs) and odorant-binding proteins (OBPs). OBPs and PBPs belong to the lipocalin-type superfamily. Lipocalines are responsible for the transport of such hydrophobic molecules as steroids, retinoids and lipids [1,2].

Steroids play an important role in the life of insects. For example, 20-hydroxyecdysone is a molting hormone. 20-hydroxyecdysone is synthesized from cholesterol. In turn, cholesterol, has a strong influence on the development of a mealworm beetle.

However, insects are unable to synthesize steroids and are therefore dependent upon a dietary source. After absorption in the intestine, steroids are transported through the hemolymph to organs and tissues. Because of its hydrophobicity, the delivery of steroids through hydrophilic media must be carried out through a specialized transport system. It is assumed that special transport proteins are involved in transporting steroids, as well as other hydrophobic molecules. A 3CZ1 protein was found in the PDB database, which has a significant similarity in structure with 1C3Y. The protein found is also a carrier
of various ligands, including hydrophobic ligands. Analysis of amino acid sequences of these proteins using the Smith-Waterman algorithm revealed several homologous regions [3,4]. The first three-dimensional structure of THP12 has been determined by multidimensional NMR spectroscopy. The structure can be summarized as a nonbundle α-helical structure consisting of six α helices with loops between the helices (figure 1) and a disordered N terminus. Position of the spiral relationship is supported by two salt bridges and two disulfide bridges Cys14-Cys45 and Cys85-Cys102. The hydrophobic core is formed by four spirals, three of which are parallel to each other, and the fourth is perpendicular to them. Nonpolar residues of phenylalanine are grouped in the hydrophobic protein core: Phe52, Phe68, Phe99 and Phe100 [1].

Figure 1. NMR structure of THP12 from the protein database (PDB) [5].

As two potential ligands ergosterol and 4-methylnonanol were found by size-exclusion chromatography and mass spectrometry. In this paper, to further investigate the interaction between THP12 and the ligand, ergosterol was chosen, since its structure is closest to steroids, for example, cholesterol (figure 2).

Figure 2. Chemical structure for ergosterol and cholesterol.

2. Computational details

2.1. Simulation protocol
The initial structure of the protein was taken from the protein database (PDB ID 1C3Y) [5]. The structure of 1C3Y was obtained by NMR and includes 23 conformers. According to the experimental
data, the structure consists of 6 α-helices loops between them, and disordered N-terminal region. We chose conformation in which the hydrophobic core is most available for binding ligand because of the preferred location of N terminus in solution. THP12 and its complex with ergosterol were solvated in a box of TIP3 water molecules. The distance from the surface of the protein to the cell boundary was not less than 15 Å. To neutralize the total charge of the protein, two sodium ions were added to the system.

Calculations of molecular dynamics (MD) trajectories for a total length of 100 ns were performed using program NAMD2.10 [6] with potentials of the force field CHARMM36 [7]. Calculations were carried out in the canonical ensemble NPT with a temperature of 298 K and a pressure of 1 atm, the integration step being 1 fs.

2.2. Binding free energy

One of the most important characteristics of the complexes of carrier proteins and ligands is the free binding energy and the dissociation constant. For the pheromone-binding protein described in [4], dissociation constants with different ligands were determined experimentally. For example, the first dissociation constants of the complexes of this protein with 9-keto-2(E)-decenoic acid and n-butylbenzenesulfonamide are 8.5±3 nM and 7.0±2 nM, and the second dissociation constants are 5.0±µM and 6.5±1.5 µM, respectively [8]. To determine binding parameters of ergosterol with THP12, the umbrella sampling technique (Umbrella Sampling - US) was used. In the US, the reaction path is divided into a set of system states, for each of which the MD trajectories are calculated in parallel. As the coordinate of the reaction, the distance between the position of the ligand in the binding center and the final position in the solution was taken. A limiting harmonic potential with a known constant of elasticity is imposed on the value of the reaction coordinate. The free energy profiles for each segment are combined by the weighted histogram analysis method (figure 3). Thus, the calculated free binding energy of ergosterol $\Delta G_{\text{bind}}$ is -9.03 kJ/mol and the dissociation constant of the $K_{\text{diss}}$ complex is 0.235 µM. The difference in the experimental and calculated dissociation constants can be explained by the different hydrophilicity of the ligands. The practical significance of the data obtained is the ability to validate the used modeling method.

![Figure 3. Binding free energy profile.](image)
3. Results and discussion
Analysis of molecular dynamic trajectories of the free protein system showed that it has a dynamically stable hydrophobic cavity consisting of 11 hydrophobic amino acids - Ile46, Leu47, Ala50, Phe52, Ile64, Phe68, Val78, Leu81, Phe99, Phe100, Val103. Molecular docking using the AutoDock 4.2 program [9] confirmed that the potential ergosterol binding site is located in this cavity (figure 4).

In solution THP12 exists predominantly in open and closed conformations. Open conformation corresponds to N-terminal region of the protein in solution, whereas closed conformation is characterized by hydrophobic cavity covered by N-terminal loop. The addition of ergosterol to the system shifts the equilibrium between the open and closed conformations towards the closed form, since the N-terminal region covers the ligand-binding cavity forming hydrophobic interactions with ergosterol and Leu7 and Val103 residues of THP12. In a closed conformation a stable salt bridge between Arg4 and Glu73 is formed.

| Line                  | Average distance in the free protein, Å | Average distance in the THP12-ergosterol complex, Å |
|-----------------------|-----------------------------------------|--------------------------------------------------|
| 2 α-helix – 5 α-helix | 11.32±0.01                              | 10.06±0.01                                       |
| 1 loop – 1 α-helix    | 15.05±0.04                              | 16.84±0.01                                       |
| 1 loop – 5 α-helix    | 12.64±0.02                              | 10.85±0.01                                       |

These conformational shifts are caused by the formation of a number of stable salt bridges in the protein bound to the ligand: Glu58-Lys88 and Glu33-Lys101. Such changes in the structure make the hydrophobic cavity more tighter, which is thermodynamically favored for ligand binding.
4. References

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Acknowledgments
The work was supported by Mendeleev University of Chemical Technology of Russia. Project Number 003-2018. Authors are grateful to the M.V. Lomonosov State University Research Supercomputer Center. Authors express special gratitude to prof. Alexander Nemukhin and prof. Vladimir Tsirelson.