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Computer Modeling of Small Ligands Diffusion in *Drosophila Melanogaster* Hemoglobin

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The monomeric, intracellular hemoglobin from the fruit fly *Drosophila Melanogaster* (DmHb) has been discovered in 2005. It came out that the oxygen supply system in insects is more complex than previously thought. Details on diffusion of gases, ligands discrimination and hexa- to pentacoordination changes remain unclear. Here we present the results of molecular modeling and molecular dynamics (MD) simulations of gaseous ligands (O_2 , CO, NO) transport inside the DmHb matrix. In addition to a classical MD trajectory an approximate Locally Enhanced Sampling method (LES) and Implicit Ligand Sampling (ILS) have been employed. The structural and thermodynamics features of hexacoordinated and pentacoordinated DmHb were examined and compared to our previous results obtained for human neuroglobin and cytoglobin which display similar heme coordination. Several connected cavities and diffusion pathways, based on 3D ILS free-energy maps, have been indicated. Residues that are critical for kinetics of small gaseous ligands diffusion in primitive hemoglobin were discovered. These data may help do understand the impact of evolutionary pressure on proteins architecture.

1 Introduction

Recently, *Drosophila melanogaster* Hemoglobin (DmHb), a new member of invertebrate heme globin family, has been discovered¹. It belongs to the class of hexacoordinated globins. The sixth coordination position of heme iron ion is occupied by an external ligand (i.e. O_2 , CO) or the distal HisE7. *D. melanogaster* has the open circulatory system with fluid called hemolymph. In hemolymph there are no hemoglobin-like proteins. Tracheal system supports passive diffusion of oxygen to the tissues and for many years researchers thought that oxygen carriers are unnecessary in this taxon. DmHb expresses in fat body and pharynx muscle, so it is an intracellular protein. Its role is unclear and is a matter of the debate. Our goal is to find cavities network and diffusions pathways for small gaseous ligands. These data may help to understand the physiological role of DmHb and bring new information on the architecture and function of this ancient protein.

2 Methods

The structure of DmHb (2G3H) was obtained from Protein Data Bank. All MD simulations were carried out with the NAMD 2.6 code and CHARMM27 force field². In the DmHb structure heme group coordinates cyanide ligand. To perform MD simulations with dioxygen, the cyanide has been removed and dioxygen ligand has been docked. This initial structure was placed in an equilibrated TIP3 water box ($63 \times 54 \times 45 \text{ \AA}^3$). The relaxation, heating to 300 K, and equilibration of the system (about 17 000 atoms) were completed during a 500 ps run. A standard MD trajectory 15 ns long was produced. Additionally, the

Locally Enhanced Sampling (LES) method was used to facilitate O₂ diffusion³. The three 3 ns long LES trajectories with 5, 10 and 15 copies of O₂ for DmHb structures were generated (named LES5, LES10 and LES15, respectively). These simulations were run with the periodic boundary conditions. The Ewald mesh summation for long range electrostatic interactions was used. The integration timestep was 1 fs for bonded and non-bonded interactions and 2 fs for electrostatics. The cutoffs for Van der Waals and electrostatic interactions were 12 Å. Langevin dynamics with dumping factor 5 ps⁻¹ was used. The Potential of Mean Force by Implicit Ligand Sampling (PMF/ILS) calculation was carried out with the VMD code⁴. This method allows for indication of all low free-energy profile within a protein matrix. The analysis was performed using the VMD code⁵.

3 Results and Discussion

The inspection of root-mean-square (rms) distances from the DmHb model shows that all trajectories are reasonable stable (data not shown, average Cα rmsd < 1.5 Å). In a static crystal structure DmHb no entry channels from the solvent to the heme active site can be found¹. Moreover only three cavities are observed. However, when fluctuations of amino acids side chains at 300K were added, the transient routes leading from the solution to the binding site are presented. Using the PMF/ILS method such paths were determined for 3 small gaseous ligands: NO, CO and O₂. The complex network of cavities and channels in DmHb are determined by tracking dioxygen collisions with residues (LES trajectories) and by inspection of the PMF isosurface for selected ligands. Detailed data about amino acids involved in formation main cavities may be found in Fig. 1. Using the VMD soft-

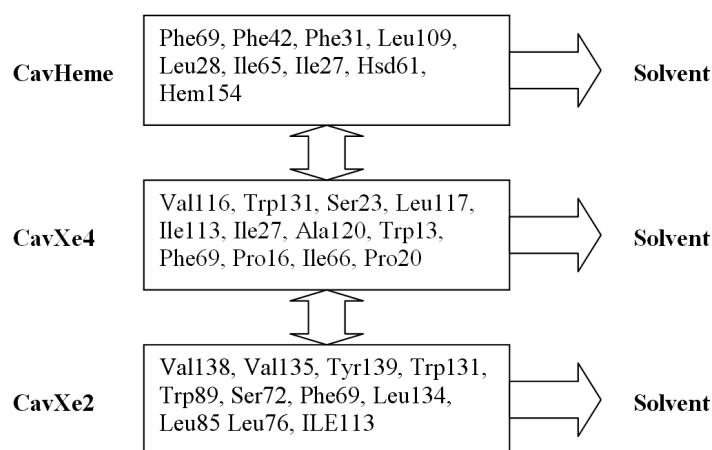


Figure 1. Diagram of the cavities in DmHb.

ware, traces of every O₂ ligand were registered in all calculated LES trajectories. Out of 5+10+15 ligands observed only 12 copies left the protein on a 3 ns timescale. Cavities DP, Xe4 and Xe2 exhibit exits path to the solvent. PMF isosurface is shown in the Fig. 2.

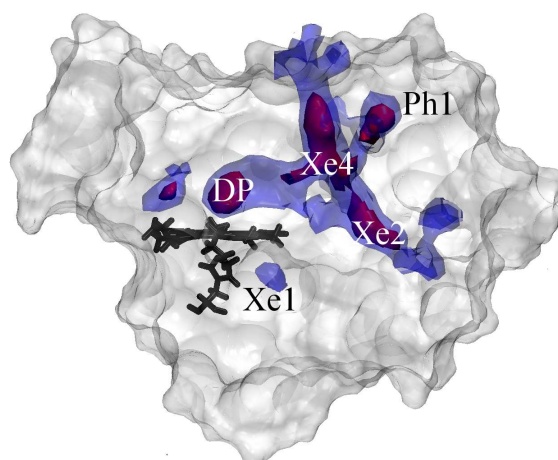


Figure 2. Free energy map for O₂ migration inside DmHb. Two free energy isosurfaces represent PMF/ ILS values of -1.5 kcal/mol (opaque black) and 1.6 kcal/mol (transparent dark gray). The DmHb surface is displayed in light gray and the heme group is represented in dark gray .

Five cavities have been found. Three of them are involved in building a big, hydrophobic channel (DP, Xe4 and Xe2). Cavities Xe1 and Ph1 are not connected with the channel thus their influence on the ligand diffusion may be neglected. The Ph1 pocket has connection only with the surface of the protein. These data are different from the standard myoglobin picture and are close to our previous study for the neuroglobin, cytoglobin and mini-hemoglobin^{6,7}. Maybe hexacoordination provide a completely different mechanism for controlling ligand affinity and tune ligand diffusion rate. Within the hydrophobic channel at least 4 different exits are located. From the distal pocket a gaseous ligand can move to Xe4 cavity or directly to the solvent passing HisE7 residues. From Xe4 cavities ligand travels either to Xe2 or escape to the solvent using rather big exit placed between AB turn and E helix. Another exit path from Xe2 pocket is located between G and H helices. Ligand trapped in Xe2 pocket can move to solution between EF turn and H helix. This is another large tunnel leading to exterior.

4 Conclusions

Our dynamical data provide new information in contrast to the static analysis of one X-ray structure. The thermodynamic PMF/ILS calculations confirmed the existence of multiple diffusion paths in DmHb. These paths are easily accessible to small gaseous ligands. Despite of well conserved tertiary structure, globins have very different pathways for ligand diffusion. Composition of the amino acids may be more important in this case than the tertiary structure. Present data should contribute to evolutionary studies of oxygen transport mechanism in biological systems.

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