THE KINETICS OF MOLECULAR OXYGEN MIGRATION IN THE ISOLATED α-CHAINS OF HEMOGLOBIN AS REVEALED BY MULTIPLE EXTENDED MOLECULAR DYNAMICS SIMULATIONS

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Hemoglobin is the paradigm of allosteric proteins. The functional form of human hemoglobin is a tetramer consisting of two identical heterodimers. Each dimer is made up of two different subunits called α and β. Each subunit carries one identical heme group to which one molecular oxygen (O_2) binds reversibly. In proteins, a key role in dynamics, ligand migration, and consequently in reaction mechanisms is played by internal cavities. Routes of substrate and product into and from the active site can be mapped by the identification of cavities within a protein matrix. A broad spectrum of protein activities is caused by a wide sequence variability that allows tuning of affinity and reactivity as well as by the presence of different cavity systems and tunnels involved in the access to the heme group and trapping of ligands within the protein matrix. The network of cavities in globins can be investigated by different methods. X-ray studies of protein structure, filled with xenon atoms under pressure, can reveal the presence of sites occupied with Xe. Recently [1], in the α subunits of hemoglobin, a constellation of sites hosting up to six Xe atoms have been detected (Fig.), mapping the presence of cavities that describe potential paths from the bulk to the heme iron. *In silico* molecular dynamics (MD) simulations can unveil the presence of transient cavities and describe pathways for ligand migration.

In the present work, the results of a computational investigation of the O_2 diffusion in the isolated α chain of hemoglobin are reported. Molecular modeling was carried out on a Linux workstation. MD simulations were performed with the Gromacs 4 software package [2] using the GROMOS96 force field, Ver. № G43a1. Starting coordinates employed for the simulation of isolated α chain were taken from the X-ray structure of O_2-bound ferrous human tetrameric hemoglobin at 2.1 Å resolution (PDB code 1HHO). The simulations were carried out within a fixed-volume rectangular box using periodic boundary conditions. The oxygenated α chain was solvated by generating a cubic box of simple point charge extended water molecules, such that the minimum distance between the protein and the edge of the periodic box would be 0.9 nm, resulting in a cubic box of 6.2 nm.

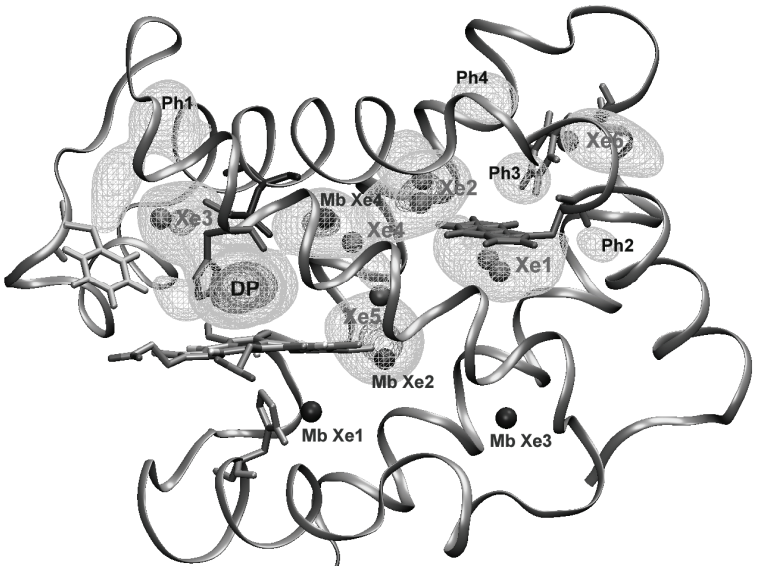

Figure – Density maps of O_2 migration within the isolated α chains of human hemoglobin. The O_2 probability density is expressed by isosurfaces. Xe atoms bound in human hemoglobin α subunits and sperm whale myoglobin (Mb) are shown for comparison. The position of bound Xe are represented by labeled spheres. The position of the distal heme pocket is labeled as DP

A total number of water molecules around the protein were ~ 8,000, a total number of atoms for the systems being ~ 25,000. The solvent was then relaxed by energy minimization using a steepest descent algorithm. Subsequently, the system temperature was brought to 293 K in a stepwise manner: 20-ps MD runs at 50, 100, 150, 200, 250 K, and 293 K. An integration time step of 1 fs was employed. After the initial equilibration, the bond between the heme iron and O_2 was broken for the simulation of O_2 migration within the deoxygenated protein matrix. Starting from different initial configurations, 140 independent up to 25-ns long MD simulations of photodissociated O_2 in the isolated α chain have been performed. Traces of every O_2 ligand were registered in all calculated trajectories. Regions with frequent presence of the O_2 ligand are interpreted as cavities in the α chains. In Fig.**,** the position and shape of the cavities in the structure of the α chain model are depicted in terms of density maps. The MD simulations show that there are at least ten secondary docking sites in which the ligand may remain for prolonged periods. Some computed cavities correspond well to the observed Xe sites in the α subunits within hemoglobin [1] as well as to the Xe4 and Xe2 sites in myoglobin. Additionally, at least four new cavities, phantom 1 (Ph1) to phantom 4 (Ph4), were detected (Fig.). All these cavities can potentially play an important role for the O_2 migration inside the protein, acting as temporary docking sites. Potential ligand escape routes within the α chains were found. Apart from a direct well known

exit from the heme distal pocket (DP) to the solvent, at least three additional major routes through the secondary Xe docking sites are possible. The first one leads directly from DP to the Xe3 cavity and, subsequently, to the phantom Ph1, from which the ligand can escape to the solvent. The second route goes from DP through the Xe4, Xe2 cavities, phantom Ph3, and Xe6 site, from where the ligand can leave the protein. In turn, the ligand can escape from the Xe2 cavity to the solvent near Trp^{14}, Gly^{15}, Gly^{18}, Asp^{64}, and Thr^{67}. Xe2 seems to be quite important because the O_2 ligand prefer to escape via this third channel. Xe6 may play some physiological role as well, because the second escape route goes through this region. One secondary docking site, pretty isolated from the exterior, is detected. It corresponds well to Mb Xe2 cavity, located close to DP, and can be populated only through the adjacent Xe4 site. Probably, a small gaseous ligand remains in this isolated space until reaction with the iron centre is possible. During all the simulations, no O_2 ligand was detected in cavities corresponding to the Mb Xe1 and Mb Xe3. Proximal sites are absent in the α chains. Moreover, our previous data of the O_2 rebinding [3], measured by laser flash-photolysis techniques, were reanalyzed using the Maximum Entropy Method. The time dependence of cavities occupancy, obtained by MD simulations, and the kinetics of O_2 rebinding allowed us to obtain the kinetics of the entire O_2 migration process within the nanosecond time range and construct an explicit kinetic model of the O_2 migration and rebinding process in the isolated α chains [4]. The amino acids that have the most pronounced effect on the ligand migration within the α chain matrix are predicted.

References

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