Abstract

Free divers can’t hold their breath as long as whales, but they train their bodies to maximize their oxygen ($O_2$) storing potential using the protein myoglobin. Myoglobin’s structure has been known for decades, but researchers are still trying to determine just how myoglobin functions. Found in muscle tissue, myoglobin stores $O_2$, a molecule needed to produce chemical energy. Toxic ligands, such as carbon monoxide (CO) and cyanide, also bind to myoglobin. When CO binds to a free heme, the heme’s binding affinity for $O_2$ is 20,000 times that for $O_2$. When heme is surrounded by myoglobin, that binding affinity ratio drops to only 25. The decrease was thought to be due to steric interactions which prevented CO from occupying the same space as $O_2$. Recent evidence suggests that electrostatic interactions and hydrogen bonds play a more important role. The CO is stabilized as opposed to the CO being pushed out. Several amino acids (His64, Val68, Arg75, and Lys29) seem to stabilize the ligand. With 3D printing technology, the Brown Deer SMART (Students Modeling a Research Topic) Team, funded by a grant from NIH-CTSA, created a model of myoglobin. If researchers can fully understand and discriminate by heme proteins, not only will divers be able to hold their breath longer, but we may be able to remedy conditions like hypoxia where there is a lack of $O_2$ in the blood.

3. Can the ligands fit?

Scientists originally thought that the drastic difference in binding affinities between $O_2$ and CO in a free heme compared to a heme protein on myoglobin was due to steric interactions. Amino acid residues in the heme proteins push CO, bending it and preventing it from bonding straight up. A protein residue in heme proteins close to the ligand called the distal histidine forces CO into a bent position. $O_2$, however, is not affected by the distal histidine’s force because it already binds in a bent position. The binding of CO prevents the ligand from occupying the same space as $O_2$. The ratio of $O_2$ to CO binding affinities decreases when bound to myoglobin compared to when bound to free heme.

4. How is Mb studied today?

According to current data, steric hindrance is not the only factor in determining ligand binding. Therefore, today’s research is focusing on electrostatic fields surrounding myoglobin molecules. Every molecule consists of a collection of electrical charges: positively charged nuclei and a negatively charged electron cloud. Every electrical charge creates an electric field that can exert a force on other charges. All the charges on a protein combine to form a electric field around and within myoglobin. This electrostatic field determines how a ligand binds to myoglobin. Electric fields are measured indirectly by measuring absorption spectra from myoglobin when bombarded with different wavelengths of light using hole burning spectroscopy.

5. What technology is used?

Scientists are studying the effect of myoglobin’s electric field on its active-site molecule, heme. Each amino acid in the myoglobin chain contributes its own small effect on heme. This electric field will also affect the interaction of myoglobin with potential ligands. In order to visualize the electric field, researchers use a hole burning spectroscopy table.

6. Why electric fields?

Hole burning spectroscopy is a relatively new way to study proteins, but scientists are hopeful it could lead to new breakthroughs in synthesizing blood substitutes, understanding industrial protein catalysts, and understanding how electrons are transported in photosynthesis. Many of the studies conducted with hole burning spectroscopy are not simply to find out more about myoglobin, but are also meant to test out the technique. Someday, hole burning spectroscopy might be used on more proteins, and electric fields may become a more important data set for each protein.

References

3. King, M. W., (2014). Thermodynamics of Protein Structure, Chapter 3, in。“The SMART Team Program is supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant Number 8UL1TR000055. Its http://themedicalbiochemistrypage.org/hemoglobin-myoglobin.php