Purification and Crystallization of Carbon Monoxide-bound Dwarf Sperm Whale Myoglobin

O. Adeliyi[1], J. Soman[2], G.N. Phillips, Jr.[2], J.S. Olson[2]

[1]Program in Neuroscience, Middlebury College, Middlebury, USA.
[2]Department of BioSciences, Rice University, Houston, USA.

Developing stable blood substitutes can help to provide temporary but suitable alternatives for patients who need an immediate blood transfusion. Successful blood transfusions are often limited by donated blood's short shelf life, risk of disease transmission, and blood matching. Developing a cell-free substitute, such as a recombinant hemoglobin-based oxygen carrier (rHbOC) that would have a long shelf life is the overarching goal of this research. Longer shelf life of hemoglobin is determined by both globin stability and rate of auto-oxidation of the bound heme from the ferrous to ferric state. Hemoglobin stability studies in general are complicated because of hemoglobin's tetrameric nature. Instead, the monomeric mammalian myoglobin, which is structurally homologous to a subunit of hemoglobin, is a simpler model and therefore is often used to study globin stability. This research focused on crystallizing wild-type dwarf sperm whale myoglobin (DSW Mb) a highly stable form of myoglobin (Scott et al 2000; Samuel et al 2015) with the bound heme in the ferrous state. Much of this project focused on expressing the protein through the Escherichia coli (E. coli) expression system, and purifying the protein through multiple chromatography methods. We were then able to set up carbon monoxide-bound DSW Mb for crystallization using one promising condition of ninety-six crystal conditions previously determined for ferric state of DSW Mb.