Expression, Purification, and Crystallization of Recombinant Hemoglobin Mutant Protein alpha E7E11F/beta wildtype for X-ray Diffraction Studies

E. Carranza[1], J. Soman[2], J. S. Olson[2]

[1] University of Houston, College of Technology, Houston, Texas 77024
[2] BioSciences at Rice University, Houston, Texas 77251

The human body has approximately five liter of blood that is composed of components, such as plasma, platelets, red blood cells, and white blood cells, that aid in a specific function. For example, red blood cells contain hemoglobin which is responsible for oxygen transportation throughout the body. The hemoglobin structure is a tetramer composed of two alpha and two beta chains. Each chain contains a heme cofactor that binds oxygen to its iron atom. According to the Red Cross blood products are being distributed to hospitals faster than donations are coming in. This issue has led to the interest in synthetic blood substitutes. In order to create a substitute, we first must understand how hemoglobin functions. A hemoglobin protein with two mutations in its alpha chains and an unmodified beta chains was used to conduct this experiment. As part of a larger study on hemoglobin folding and assembly, preliminary results from hemoglobin mutant alpha His58Leu, Val68Phe/ beta wildtype suggest that it is very resistant to unfolding. In an effort to create a blood substitute we must discover a protein that is both stable and function as an oxygen carrier outside of the red blood cell. Hence we crystallized this mutant protein and used X-ray diffraction to solve the structure to correlate any structural changes due to the mutations and its stability.

The hemoglobin mutant was grown in JM109 *Escherichia coli* cells using the pHE2 plasmid and sequential flask method. Expression of the protein was induced by Isopropyl β-D-1-thiogalactopyranoside and the culture was supplemented with heme. Both affinity and ion-exchange chromatography were used to purify the protein. First, a chelating Zinc column removed *Escherichia coli* cell debris and misfolded hemoglobin. Second, a Q Sepharose column removed residual *Escherichia coli* proteins and modified hemoglobin. Third, a Source 15S column further refined the removal of misfolded and modified hemoglobin proteins. Once protein was obtained and purity confirmed using a spectrophotometer the batch method was used to crystallize it using 2.5M Sodium/Potassium Phosphate pH 6.4 as the precipitant. Once crystals formed they were imaged using an in-house X-ray generator and detector. Rotating the crystal through a large angle and collecting several diffraction patterns will aid in producing a 3D structure of the protein. Obtaining the 3D image will help us understand how the mutations aid in the stability of the protein. Future imaging of different mutant hemoglobin proteins will allow us to determine what mutations are needed to produce an effective blood substitute.