**CRYSTALLOGRAPHY OF BIOLOGICAL MACROMOLECULES**

**MS04.03b.06 CRYSTAL STRUCTURE OF BEEF HEART MITOCHONDRIAL CYTOCHROME bc1 COMPLEX.** Di Xia, Hoeeon Kim, Johann Deisenhofer1, Chang-An Yu, Jia-Zhi Xia, Linda Yu2; HHMI and Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas TX 752351; Oklahoma State University, Stillwater, OK 74078

The cytochrome bc1 complex from bovine mitochondria plays a central role in cell respiration. It is a membrane protein complex that consists of ten protein subunits and four redox centers with a molecular weight of 240,000 Daltons. Its crystallization has been reported by several laboratories. Our crystals, grown in the presence of glycerol, diffused X-ray to better than 3 Å resolution under cryogenic conditions. They have the symmetry of the space group I4122 with unit cell dimensions of a=13.5 Å and c=597 Å, and one bc1 complex in the crystallographic asymmetric unit. Phases have been determined to 3.3 Å resolution by the MIR method with four heavy atom derivatives. The solvent flattened electron density clearly shows the transmembrane region with thirteen transmembrane helices. Four high peaks in the electron density and in maps calculated using anomalous scattering data measured near the iron absorption edge are interpreted as the redox centers of the bc1 complex. Two of these sites are 20 Å apart in the transmembrane region and most likely represent the heme iron of cytochromes b562 and b566. Another site near the membrane surface and 26 Å away from the nearest b-heme could be the iron-sulfur center; the fourth site, presumably the cytochrome c1 heme is 31 Å apart from this center. The majority of the molecular mass outside the membrane is located on the side of the membrane opposite from the redox centers, presumably the matrix side of the mitochondrial membrane. The electron density map also reveals a very tight association of two monomers related by a two-fold symmetry. The overall dimensions of the dimer are about 130 Å in diameter and 151 Å in height, with the heights for the inter-membrane space region, the transmembrane region and the matrix region 41 Å, 35 Å and 75 Å, respectively. Distances between the symmetry-related iron of b562, b566, FeS and c1 are 33 Å, 21 Å, 65 Å and 53 Å, respectively.

**PS04.03b.08 TOWARDS THE CRYSTALLIZATION OF DIPHTHERIA TOXIN TRANSLOCATION MUTANTS P345C, P345E, and P345G.** Melinda Balbinime, Ralf Landgraf, David Eisenberg, Department of Chemistry and Biochemistry, UCLA, 405 Hilgard Ave., LA, CA 90095

Diphteria toxin is a 535 residue protein that binds to and enters human cells, forms a pore across the endosomal membrane, and translocates a toxic domain into the cytoplasm which kills the cell. The translocation has been ascribed to a two-helical segment (helices 8 and 9). Proline 345 is located at the end of helix 8 and mutation at this position to cysteine, glutamate, or glycine abolishes translocation activity. The diphteria toxin mutants were prepared in the laboratory of R. John Collier. A mutant diphteria toxin with proline 345 replaced by cysteine has been expressed and purified. The aim of the present study is to develop an improved structure-based model of diphteria toxin membrane translocation. We present our current progress on this project.

**PS04.03b.09 THE STRUCTURE OF THE α-HEMOLYSIN TRANSMEMBRANE PORE IN NATIVE AND DIVALENT CATION INHIBITED FORMS.** Michael R. Hobbaugh1, Langzhou Song1, Christopher Slusallek1, Steven Cheley1, Hagan Bayley1, and J. Eric Gouaux1; Department of Chemistry and Molecular Biology, University of Chicago, Chicago, IL 606371, Worcester Foundation for Biomedical Research, Shrewsbury, MA 015452.

α-Hemolysin (αHL), a primary virulence factor in Staphylococcus aureus infection, forms a heptameric transmembrane pore (α7HL) on susceptible mammalian cells leading to cell lysis. This activity is inhibited by mM concentrations of divalent cations (M2+). In addition, assembly of αHL from the water-soluble monomer is inhibited by M2+ and mutants have been engineered which contain a M2+ actuated switch whose inhibition may be relieved by addition of EDTA.

We have solved the structure of the native αHL crystallized from 8-OG, ammonium sulfate, cacodylate, and PEG by MIR. αHL is predominantly β-sheet and forms a mushroom-like structure where the hydrophobic stem of the mushroom penetrates the host cell membrane. Examination of the interior of the pore reveals that the diameter ranges from a maximum of ~65 Å to a constriction of ~12 Å in which seven glutamic acids (Glu111) protrude into the channel at the base of the stem where it joins the head of the mushroom.

Because the divalent polyacrylamide uranyl binds at this position in the structure of our uranyl derivative we believe that this position may be the site of binding for M2+. Crystals utilizing a variety of M2+, buffers, and PEGs have been characterized. Their diffraction limit is ~2.8 Å using conventional sources and all occur in the same space group (P1) with the same unit cell dimensions (a=173.5 Å, b=172.5 Å, c=101.1 Å, α=90.5°, β=89.5°, γ=92° 11). Data collection on this crystal is ongoing and the structure will be solved by molecular replacement.

3Manuscript in preparation.