MS04.03b.06 CRYSTAL STRUCTURE OF BEEF HEART MITOCHONDRIAL CYTOCHROME BC1 COMPLEX. Di Xia, Hoeon Kim, Johann Deisenhofer[1], Chang-An Yu, Jia-Zhi Xia, Linda Yu[2], HHMI and Department of Biochemistry, University of Texas Sourthwestern Medical Center at Dallas TX 75235[1], Oklahoma State University, Stillwater, OK 74078[2]

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 crytallization has been reported by several laboratories. Our crystals, grown in the presence of glycerol, diffract X-ray to better than 3 Å resolution under cryogenic conditions. They have the symmetry of the space group $I4_122$ with unit cell dimensions of a=b=153.5 Å and c=597.7 Å, and one bcl 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 transmemebrane region with thirteen transmembrane helices. Four high peaks in the electron density and in maps calculated using anomalous scattering data measumd 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 irons of cytochromes b_{562} and b_{566}. 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. The distances between the symmetry-related irons of b_{562}, b_{566}, FeS and c1 are 33 Å, 21 Å, 63 Å and 53 Å, respectively.

PS04.03b.07 CRYSTALLIZATION OF THE REACTION CENTER OF PHOTOSYSTEM II. Noam Adir, Faculty of Chemistry, The Technion, Technion City, Haifa 32000 Israel

The reaction center of Photosystem II (RCII), a ~250 kDa membrane bound protein/pigment complex has been crystallized. RCII catalyzes the photochemically driven transfer of electrons from water, resulting in the formation of reduced and protonated quinones and the evolution of molecular oxygen. It is the source of linear electron flow utilized by all oxygenic photosynthetic organisms for both the reduction of NADP and the formation of the proton gradient needed for ATP synthesis. It is highly conserved in all species at all levels of structure and function. Isolation procedures have been developed for RCII from a variety of photosynthetic organisms with an emphasis not only on purity, but homogeneity and stability as well. Isolated RCII has been crystallized by the vapor diffusion method. Two crystal forms of isolated spinach RCII have been obtained: hexagonal rods and rectangular rods (with dimensions of 0.3 x 0.3 x 1.0 mm). These crystals diffract to a maximum resolution of 7 Å, using synchrotron radiation (SSRL, Stanford). The crystals were however quite mosaic, apparently due to their sensitivity to physical stress. Both crystal forms were obtained in the presence of mixtures of two non-ionic detergents and heptane-triol. In addition to these conditions, more than 15 additional conditions have been found to promote the growth of crystals with various needle morphologies from spinach, pea, squash, maize and Chlamydomonas reinhardtii RCII. Preliminary work on the crystallization of RCII was performed in the laboratory of Profs. George Feher and Mel Okamura, of the Dept. of Physics, University of California, San Diego

1) Adir et al. (1992) in Research in Photosynthesis, Vol II (N. Murata ed.) pp. 195-8. Kluwer Academic Publishers, Dordrect. Supported by the Womenís Division of the American Technion Society

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

Diphtheria toxin is a 535 residue proenzyme 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 diphtheria toxin mutants were prepared in the laboratory of R. John Collier. A mutant diphtheria 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 diphtheria 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. Hobaugh[1], Langzhou Song[1], Christopher Shustak[2], Steven Cheley[2], Hagan Bayley[2], and J.Eric Gouaux[1]. Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 60637[1], Worcester Foundation for Biomedical Research,Shrewsbury, MA 01545[2].

α-Hemolysin (αHL), a primary virulence factor in *Staphylococcus aureus* infection, forms a heptameric transmembrane pore (α_7HL) on susceptible mammalian cells leading to cell lysis. This activity is inhibited by mM concentrations of divalent cations (M^{2+})[1]. In addition, assembly of α_7HL from the water-soluble monomer is inhibited by M^{2+} and mutants have been engineered which contain a M^{2+} actuated switch whose inhibition may be relieved by addition of EDTA[2].

We have solved the structure of the native α_7HL crystallized from ß-OG, ammonium sulfate, cacodylate, and PEG by MIR[3]. α_7HL is predominantly ß-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 (Glu 111) protrude into the channel at the base of the stem where it joins the head of the mushroom.

Because the divalent polycation uranyl binds at this position in the structure of our uranyl derivative we believe that this position may be the site of binding for M^{2+}. Crystals utilizing a variety of M^{2+}, 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°, ß=89.5°, γ=95° 11'). Data collection on these crystal is ongoing and the structure will be solved by molecular replacement.

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[2]B.Walker, J.Kasianowicz, M.Krishnasastry, H.Bayley,*Prot. Engin.* 7:655-662 (1994).
[3]Manuscript in preparation.