PS04.03b.10 CRYSTALLIZATION OF THE α-HEMOLYSIN MONOMER. G.-Q. Huang*, A. Villa*, S. Cheley#, C. Shustak#, H. Bayley#, and J.E. Gouaux*. #Worcester Found. for Biomedical Research, Shrewsbury MA 01545, *Department of Biochem. & Mol. Biol., University of Chicago, Chicago IL 60637.

α-Hemolysin (aHL) is secreted from *Staph. aureus* as a water-soluble monomer of 33.4 kDa that binds to erythrocyte cell membranes and creates a heptameric transmembrane pore[1]. Here we present crystallization and crystallographic data on a single amino acid mutant that binds to cell membranes but does not form heptamer (H35->W)[2]. We have obtained 20 crystal forms in the presence of various phospholipids; in the absence of phospholipids, crystal growth has been unsuccessful.

PEG and DiC_7PC produce at least seven different crystal forms. One of the crystal forms (I) diffracts to 3.0 Å resolution and has the space group $P4_22_12$ (a=b =219.7 Å, c=191.3 Å). A related form (II) grows using sodium phosphate and different phospholipids, such as DiC_5PC, $MonoC_{14}PC$, $MonoC_8PC$, or mixtures of DiC_5P+$MonoC_{14}P$ or DiC_5P+$MonoC_8P$. I and II have the same space group and similar *a* cell dimensions. However, the *c* dimension of form II is about 4% smaller than the *c* dimension of form I. We estimate that there are 8-10 monomers in the asymmetric unit of form I on the basis of measurement of crystal volume combined with quantitative amino acid analysis[3]. A series of strong reflections along *00l* is indicative of an axis of noncrystallographic symmetry. These data, combined with analysis of self-rotation functions in which there is a strong peak at κ=45° parallel to c*, implies that there may be an 8-fold axis of rotational or screw-axis noncrystallographic symmetry oriented along the *c* axis. There are additional weaker peaks in the self- rotation function maps at κ = 120° and κ =180°. αHL obtained from dissolved crystals migrates as a monomer as judged by gel filtration in buffer containing DiC_7PC. Since these crystals are grown in the presence of phospholipids, the structure of the protein in these crystal forms may serve as a good model for a membrane-bound conformation of αHL.

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[2]Walker, B. And Bayley, H. (1995) *Prot. Engineer.* 8 491-5.
[3]Kwong, P., Pound, A. And Hendrickson, W.A. (1994) *J. Appl. Cryst.* 27 504-9.

PS04.03b.11 X-RAY CRYSTALLOGRAPHIC STUDIES ON SPECIFIC INHIBITORS OF MITOCHONDRIAL BC1 COMPLEX. Hoeon Kim, Di Xia, Johann Deisenhofer[1], Chang-An Yu, Anatoly Kachurin and Linda Yu[2], HHMI and Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas, TX 75235[1], Oklahoma State University, Stillwater, OK 74078[2]

Mitochondrial or prokaryotic bc1 complexes contain four redox centers, whose electron flow are affected by various specific inhibitors. These inhibitors usually interact with the complex with high affinity and specificity, and they can be classified into several groups based on points of action in the electron transfer pathway and chemical structures.

A series of co-crystals of the bc1 complex with different types of inhibitors were grown and analyzed by the difference Fourier technique, using available phase information of the native system. In difference maps calculated with experimental MIR phases up to 3.5 Å, electron density for inhibitors were identified with high confidence in at least two co-crystal systems; antimycin-A and UHDBT (undecylhydroxydioxobenzothiazole).

In the case of antimycin-A, the difference map showed a single density peak exceeding the 20 sigma level. This strong density is probably due to the high binding constant of the inhibitor to the complex. The density for the inhibitor is located very close to the putative high-potential heme of cytochrome b. Moreover, there is also a significant negative density that could be a ubiquinone molecule present in the native structure but displaced upon antimycin-A binding. Displacement of ubiquinone would explain the inhibitory function of antimycin-A.

In the case of UHDBT, which blocks electron transfer between ubiquinone and iron-sulfur center, the density of the highest peak (10 sigma level) overlaps partially with that of the putative iron-sulfur cluster.

The density of these inhibitors became significantly better after combination of experimental phases with model phases. This allowed to confidently build inhibitor structures into the density.

PS04.03b.12 X-RAY CRYSTAL STRUCTURE OF MOUSE PROSTAGLANDIN H_2 SYNTHASE-2. Ravi G. Kurumbail, William C. Stallings, Anna M. Stevens, Jina Pak, Daniel A. Gildehaus, Roderick A. Stegeman, James Gierse, Karen Seibert, Peter C. Isakson. Monsanto, Mail Zone BB4K, 700 Chesterfield Pkwy, St. Louis, Missouri 63198

The integral membrane protein Prostaglandin H_2 Synthase or cyclooxygenase (COX) is the pharmacological target of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) such as aspirin and ibuprofen. COX is a bifunctional enzyme that catalyzes the conversion of arachidonic acid to prostaglandin H_2 through a cyclooxygenase reaction followed by a peroxidase activity. Two isoforms of COX have been identified: COX-1 and COX-2. COX-1 is constitutively expressed in most tissues and organs and is essential for stomach mucosal membrane integrity, normal renal function and hemostasis. By contrast, COX-2 is primarily localized in inflammatory cells and tissues and is induced in response to cytokines, mitogens or hormones. It has been shown that COX-2 is responsible for the elevated levels of prostaglandin production during inflammation. Selective inhibition of COX-2 could lead to the production of superior anti-inflammatory drugs without the side effects associated with common NSAIDs.

We have determined the three-dimensional structure of mouse COX-2 cocrystallized with flurbiprofen at 2.5 Å resolution by molecular replacement methods using the structure of sheep seminal vesicles COX-1. The crystals belong to the space group $P2_12_12$ with two dimers of COX-2 in the asymmetric unit. The crystals were frozen to near liquid nitrogen temperature to facilitate data collection from a single crystal. The amino acid sequence of the two isoforms are 60% identical but COX-2 has an insertion of 18 amino acids near the carboxy terminus. The structure is being refined by X-plor and the current R-factor is 24%. The overall structure of the two COX isoforms are well conserved, particularly near the cyclooxygenase active site. However, the NSAID binding site in COX-2 is considerably enlarged due to subtle changes in the binding pocket. Additional electron density is observed for COX-2 near the carboxy terminus.