**PS04.03b.13 PREPARATION OF A PROTEASE RESISTANT DOMAIN OF THE α-HEMOLYSIN MONOMER FOR CRYSTALLIZATION.** Y.-D. Mo and J.E. Gouaux, Department of Biochemistry & Molecular Biology, University of Chicago, Chicago, IL

α-Hemolysin (αHL) is secreted as a water-soluble monomer of 33.4 kDa that self-assembles to form heptameric transmembrane pores. Molecular entities along this pathway are the water-soluble monomer (1), the membrane-bound monomer (2), the heptameric prepore (3) and the fully assembled heptameric channel (4)[1]. To elucidate molecular mechanisms for the assembly, we aim to determine high resolution structures of each species using x-ray diffraction techniques. The goal of this work is to determine the structure of the water-soluble monomer. However, the crystallization of wild-type αHL in a monomeric, water soluble form has proven difficult. Therefore, we have focused on partial proteolysis of the αHL monomer to obtain a species suitable for crystallization. Our assumption is that regions of the monomer that are easily removed by proteases under nondenaturing conditions represent flexible entities that are not portions of well-defined secondary or tertiary structure.

Screening and optimization of partial proteolysis conditions have been carried out using trypsin[2], proteinase K[3], elastase, pepsin, papain and subtilisin. In agreement with previous studies, limited proteolysis by trypsin, proteinase K and elastase produced one major protein band of about 17 kDa, indicating that cleavage occurs within the glycine-rich region near the middle of the primary structure. Further proteinase K treatment after cleavage by trypsin generated a major fragment with lower molecular weight than that produced by trypsin or proteinase K alone. The sequence and size of each proteolytic fragment will be determined by amino acid sequencing and high resolution electrospray mass spectrometry. Identification, characterization and purification of the major fragment of the trypsin-proteinase K digestion is in progress and further biochemical and crystallographic experiments will be reported.

[1]Walker, B., *et al.* (1992) *J. Biol. Chem.* 267 21782-86.
[2]Blomqvist, L *et al.* (1987) *FEBS Lett.* 211 127
[3]Tobkes, N., Wallace, B.A. and Bayley, H. (1985) *Biochemistry* 24 1915-20.

**PS04.03b.14 PROGRESS TOWARDS THE STRUCTURE OF A MEMBRANE PORE-FORMING TOXIN.** R.J. Morse, V. Ramalingam, and R.M. Stroud. Department of Biochemistry/Biophysics, University of California, San Francisco
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Progress on the solution of the atomic resolution structure of a pore-forming insecticidal toxin, CytA, is reported. CytA, the 28 kD delta endotoxin of Bacillus thuringiensis israelensis, is one of three diptera-specific insecticidal components produced in vivo as parasporal crystals. This protein is specific for mosquito larvae, including the malaria carrying Anopheles mosquito; toxins of this class are of importance to agriculture as biodegradable pesticides. The toxin forms cation-selective channels in the planar lipid-bilayers of epithelial cells leading to colloid osmotic lysis (FEBS Lett. **244**, 259-262 1989). Thus, the structure of this toxin should illustrate the mechanism of pore formation. The protein is very hydrophobic, and the diffraction-quality crystals resulting from the recrystallization of the parasporal crystals possess very low solvent content. Further, the observed unit cell is similar to that of the parasporal crystals (according to powder diffraction studies). The crystal structure solution is being pursued using MIRAS techniques and the synchrotron radiation source at SSRL. The search for isomorphous heavy atom derivatives was difficult, probably due to the low solvent content of the crystals, and the initial protein maps were challenging. The current state of our structure, based upon iterative cycles of model building, phase recombination, and density modification, is discussed.

**PS04.03b.15 CRYSTALLIZATION AND PRELIMINARY X-RAY STUDIES OF TYPE A INFLUENZA VIRUS MATRIX PROTEIN M1.** Bingdong Sha and Ming Luo, Center for Macromolecular Crystallography, Univ. of Alabama at Birmingham, Birmingham, AL 35294, USA

The matrix protein, M1, of influenza virus strain A/PR/8/34 has been purified from virions and crystallized. Influenza virus is a negative-strand RNA virus which is composed of eight single-stranded genomic segments coding for more than ten polypeptides. The matrix M1 protein(27Kd) is located as a bridge between the inner surface of the lipid bilayer of the virion envelope and the ribonucleocapsid protein(RNP) cores. The N-terminal of M1 protein has a hydrophobic domain which can be anchored in the virion lipid envelope to maintain the structural integrity of the virus particle. The C-terminal of M1 protein can be bound with RNP cores tightly to inhibit their transcription and replication. M1 can also determine the direction of RNP cores transport into or out of the nucleus. Upon entry of the virus into new host cell, M1 dissociates with RNP cores, allowing them to enter the nucleus. After transcription and replication, M1 induces the exit of RNP cores out of nucleus and prevents them from reentering the nucleus. M1 also plays a central role in virus assembly. The crystals consist of a stable C-terminal fragment(18Kd) of the M1 protein. We are also trying to get the crystals of N-terminal domain by subcloning. The typical crystal size is 0.05x0.05x0.2mm. The crystals diffracted X-ray to 2.35Å when X-rays from BNL synchrotron were used. X-ray diffraction studies indicated that the crystals have a space group of P3121 or P3221, with a=68.74Å, c=136.57Å. The suitable cryo-condition was also found in order to collet a whole data set from one single crystal. Vm calculations showed that there are two monomers in asymmetric unit. The successful crystallization of M1 protein will lead to the solution of the three-dimensional structure of the M1 protein.

**PS04.03b.16 FROM HOMOHEPTAMERS TO HETERO-HEPTAMERS: AN APPROACH TO THE STRUCTURE DETERMINATION OF HETEROMERIC TRANSMEMBRANE CHANNELS.** L. Song*, M.R. Hobaugh*, O. Braha#, B.J. Walker#, H. Bayley# and J.E. Gouaux*, *Dept. of Biochem. & Mol. Biol., University of Chicago, 920 E. 58th St., Chicago IL 60637, #Worcester Found. for Biomedical Research, 222 Maple Avenue, Shrewsbury MA 01545.

*Staphylococcus aureus* α-hemolysin (αHL) is a lytic toxin that forms transmembrane channels by assembling 7 identical, water-soluble subunits on the surface of erythrocytes or lipid bilayers. αHL is an ideal system for engineering channels and for understanding mechanisms of selectivity, gating and inhibition of ion channels. The mushroom-shaped heptamer structure has been determined in the Gouaux lab to a resolution of 1.9 Å. Defining the transmembrane channel is a 14-strand antiparallel ß-barrel that is approximately 20 Å in diameter and 50 Å long, measured from $C_\alpha$ to $C_\alpha$.

We aim to determine structures of heteroheptamers in which only one or a few amino acids on a single subunit have been changed. To do this, we must break the 7-fold axis of noncrystallographic symmetry. By examination of interheptamer contacts in the wild-type C2 crystal form[1], we found that S69 is located within a short section of interheptamer antiparallel ß-sheet close to the 2-fold crystallographic axis.

To test whether this site is suitable for introduction of a disulfide bond, coordinates for cysteine residues comprising a disulfide bond between 2 antiparallel strands were obtained from the 1.7 Å resolution crystal structure of restrictocin[2]. Perturbations