Hot Macromolecular Structures

PS04.07.01 STRUCTURE OF THE MOSQUITOCIDAL TOXIN FROM *BACILLUS SPHAERICUS*. J. P. Allen[1], C. K. Chiou[1], E. Davidson[2], T. Thanabalu[3], A. Porter[3]. [1]Department of Chemistry and Biochemistry, and [2]Department of Zoology, Arizona State University, Tempe, AZ 85287-1604 USA, [3]Institute of Molecular and Cell Biology, National University of Singapore, Singapore

Bacillus sphaericus produces insecticidal proteins during sporulation that are highly toxic to larvae of certain mosquitoes, including vectors of certain encephalitis and malaria, but are not toxic to any other insects or mammals (Porter et *al.* (1994) Microbiol. Rev. 57:833). Two proteins, with molecular weights of 51 and 42 kDa, bind tightly together as a complex and cause toxicity by a mechanism that is not well understood. It has been suggested that one protein acts as a specific protease on the other or that one protein serves as a chaperone to bring the other into the cell. Alternatively, each protein may exert a different effect on the host that is insufficient to cause host death when acting independently.

To understand these interactions at a molecular level we have begun a study of the three dimensional structure of the individual proteins and the complex using X-ray diffraction. The proteins are separately purified using an E. *Coli* expression system. Tetragonal crystals ($P4_32_12$) of the 51 kDa protein have been obtained that are up to 0.2 mm in size with cell constants of a=b=133.6 Å and c= 69.7 Å. These crystals diffract to a resolution of 2.6 Å. Phasing with five derivatives followed by solvent flattening yielded an interpretable electron density map. Tracing of the polypeptide backbone is nearly complete. The structure of the toxin will be presented in its current state.

PS04.07.02 CRYSTAL STRUCTURE OF BPI, THE HUMAN BACTERICIDAL/PERMEABILITY-INCREASING PROTEIN Lesa J. Beamer, Stephen F. Carroll*, David Eisenberg, Molecular Biology Institute UCLA, Los Angeles, CA 90095 and *XOMA Corporation, 2910 7th Street, Berkeley, CA 94710.

Sepsis is a major source of mortality in the U.S., partly because of the biological properties of lipopolysaccharides (LPS or endotoxin) on or released from Gram-negative bacteria. Recently, two mammalian proteins which bind LPS and influence its toxic effects have been identified. One protein, BPI, is found in polymorphonuclear neutrophils, is bactericidal and can neutralize the inflammatory properties of LPS. These properties have been localized to N-terminal fragments of BPI, and a recombinant human N-terminal BPI protein is currently under clinical investigation for the treatment of complications due to Gram-negative bacteria. In contrast, the second protein (LPS binding protein or LBP) enhances the inflammatory properties of LPS. Amino acid sequence comparisons suggest that BPI and LBP are related to each other and to the cholesteryl ester and phospholipid transfer proteins. None of these proteins exhibits significant sequence homology with any protein of known 3D structure. Full-length human BPI (456 amino acids) has been crystallized in space group C2 and its structure determined by multiple isomorphous replacement to 2.8 Å. BPI consists of two domains with pseudo two-fold symmetry and each domain is a barrel composed of a ß-sheet and 2 a-helices arranged in a novel protein fold. Analysis of this structure should help further elucidate the structure/function properties of BPI. Based upon homology, the BPI structure should also serve as a useful template to model other members of this protein family.

PS04.07.03 X-RAY STRUCTURE OF VIPOXIN, A COMPLEX BETWEEN A TOXIC PHOSPHOLIPASE A2 AND ITS NATURAL INHIBITOR. Ch. Betzel*, M. Perbandt*, T. S. Singh**, N. Genov***, *Institute of Physiological Chemistry c/o DESY, Notkestrasse 85, 22603 Hamburg, Germany, **Department of Biophysics, All India Institute of Medical Sciences, New Delhi 110020, India, ***Institute of Organic Chemistry, Bulgarian Academy of Sciences, Sofia 1040, Bulgaria

The toxin Vipoxin is the first complex found between a basic toxic phospholipase A_2 and an acidic non-toxic protein inhibitor. It is found in the venom of the Bulgarian viper *(Vipera ammodytes ammodytes)*, the most toxic snake in Europe. The two polypeptide chains each consist of 122 residues and are highly homologous (62%). The Vipoxin complex is also the first reported and intriguing example of high structural homology between an enzyme and its natural inhibitor. Several homologous toxic phospholipases A_2 have been characterized, however except the PLA_2 of Vipoxin none of them form a complex with a natural inhibitor and all represent toxins with a presynaptic action. In contrast Vipoxin is a neurotoxin with postsynaptic action and also little is known about snake venom inhibitors so far. The three-dimensional structure of Vipoxin sheds light on the detailed relationship between the PLA_2 and its inhibitor. X-ray data were collected using synchrotron radiation; the structure was solved by molecular replacement. Details about the structure solution and refinement as well as the structure function relationship will be presented. The three-dimensional structure of Vipoxin allows a detailed description of the active site of the toxic PLA_2 and the means of its inhibitor.

PS04.07.04 CRYSTALLIZATION AND PRELIMINARY CHARACTERIZATION OF THE TETRAMERIZATION DOMAIN FROM THE POTASSIUM CHANNEL Kv3.1. Bixby, K. A., #Pfaffinger, P. J., *Stevens, C. F., Choe, S. Structural Biology and *Molecular Neurobiology Laboratories, The Salk Institute, La Jolla, CA 92037, #Baylor College of Medicine, Houston, TX 77030

Formation of a functional voltage-gated potassium channel requires the assembly of subunits into a tetramer. Our goal is to understand the structural basis of subfamily specific subunit specificity. The molecular basis for tetramerization among the Kv1 (Shaker-type) and Kv3 (Shaw-type) subfamilies of voltage-gated potassium channels has been localized to a N-terminal cytoplasmic domain called T1 (Shen and Pfaffinger, 1995). The T1 domain of the Kv3.1 potassium channel from *Aplysia* has been overexpressed in *Escherichia coli* using a pET16b (Qiagen) variant construct. The T1 domain with a 6 histidine tag was purified using metal-chelated affinity chromatography and gel filtration.

The purified T1 is monodisperse with a molecular weight of 81 kD measured by light scattering. The apparent molecular weight of the monomer is 17 kD on SDS-PAGE. The protein sample crystallizes by vapor diffusion and crystals grow in a flat, semicircular shape up to 0.2 mm and crystals diffract up to 3.5 Å at room temperature. Crystallographic characterization indicates that the crystals belong to I_{422} tetragonal space group with unit cells of approximately 68 x 68 x 145 Å. Diffraction data has been collected using Macscience image plates and processed using DENZO and Scalepack. Structure determination is underway using multiple isomorphous replacement method utilizing a metal binding site on the protein and also by molecular replacement using the model of the T1 domain of the Shaker channel.

Shen, N. V., & Pfaffinger, P. J. (1995) *Neuron* **14**, 625-633.