Hot Macromolecular Structures

PS04.07.01 STRUCTURE OF THE MOSQUITOCIDAL TOXIN FROM BACILLUS SPHAERICUS. J. P. Allen, C. K. Chiou1, T. Davidson2, T. Thanabal4, A. Porter3. 1Department of Chemistry and Biochemistry, and 2Department of Zoology, Arizona State University, Tempe, AZ 85287-1604 USA, 3Institute 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 encephalitides and malaria, but are not toxic to any other insects or mammals (Porter et al. (1994) Microbiol. Rev. 57:835). 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 (P41212) 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 currently under clinical investigation. X-ray data were collected using synchrotron radiation; the structure was solved by multiple isomorphous replacement using the model PS04.07.02 CRYSTAL STRUCTURE OF BPI, THE HUMAN BACTERICIDAL/PERMEABILITY-INCREASING PROTEIN. Lesa J. Beamer, Stephen F. Carroll1, 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 (436 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 8-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. Betzel1, N. Pflaum2, T. S. Singh3, N. Genov**. 1Institute of Physiological Chemistry c/o DESY, Notkestrasse 85, 22603 Hamburg, Germany, 2Department 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 A2 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 A2 have been characterized, however except the PLA2 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 PLA2 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 PLA2 and the means of its inhibitor.