**PS04.18.05 CRYSTAL STRUCTURE DETERMINATION OF THE SECA TRANSLOCATION ATPASE.** John F. Hunt, Sevil Weinkauf, Lisa Henry, Donald Oliver†, Johann Deisenhofer, HHMI and Department of Biochemistry, The University of Texas Southwestern Medical Center at Dallas, and †Department of Molecular Biology and Biochemistry, Wesleyan University

The SecA protein is believed to be the engine that powers the ATP-driven translocation of exported proteins through the bacterial inner membrane during the process of protein secretion. *In vivo*, this protein exists in two states, a fully watersoluble state and an integral membrane state; the insertion of the protein into the membrane is dependent on the ATPase activity of SecA which is also directly coupled to the physical translocation of the substrate polypeptide through the membrane. SecA has significant sequence homology to the transport ATPase family of transmembrane transport proteins. In the soluble state, SecA auto-regulates its own translation by interaction with the ribosomal initiation complex and a specific RNA site upstream of its translation initiation codon. The crystal structure determination of the soluble form of the SecA protein is in progress. Our crystals grow in space group $P3_112$ with unit cell constants of 130 X 130 X 151 Å at 130 Kelvin. We have obtained good experimental phases from multiple isomorphous replacement to a resolution of approximately 5 Å. Density modification techniques have been used to extend the resolution of the experimental phase set, and we are in the process of building a polyalanine model into the resulting electron density map. The current status of this project will be reported.

**PS04.18.06 TETRANECTIN - A PLASMINOGEN BINDING PROTEIN.** I. Kjøller Larsen, B. B. Nielsen, H. Rasmussen, J. S. Kastrup, Dept. of Medicinal Chemistry, Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen, Denmark

Tetranectin (TN) is a plasma protein, which binds specifically to the kringle 4 (K4) domain of plasminogen (1). The fibrinolytic proteins of the plasminogen activator/plasmin system are known to be involved in extracellular proteolysis, and is believed to be involved in the spread of cancer by invasion and metastasis. The concentration of TN is increased in cells with high metabolic activity, and is present in the extracellular matrix during tissue remodelling in contrast to normal tissues (2).

TN is a trimeric protein with three identical polypeptide chains each of 181 amino acid residues. TN has been shown to consist of three domains: TN1 (residues 116), TN2 (residues 17-49), which is the K4 binding domain, and TN3 (residues 50181). Sequence identity has been found between TN3 and other proteins containing a $Ca^{2+}$ dependent C- type lectin domain, *e.g.* the mannose binding protein (MBP). TN and the individual domains, as well as combinations of domains, have been expressed in *E. coli* (3), and X-ray structure determinations are in progress.

The structure of TN3 (2.7 Å resolution) has been solved by the MR method using the C-lectin domain of MBP as search model. A full data set of TN has been collected to 3.5 Å resolution, and an MR solution has been obtained, also with MBP as search model. TN exists in the crystal as a trimer in contrast to TN3, which is a monomer both in crystal and in solution. Crystals diffracting to 7 Å have been obtained of TN2,3.

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2. Høgdall, C. K, Christensen, L. & Clemmensen, I. (1993) *Cancer* 72, 2415-2422

3. Holtet, T. L, Etzerodt, M. & Thøgersen, H. C. Laboratory of Gene Expression, Århus University, Gustav Wieds vej 10, DK-8000 Århus, Denmark. To be published

**PS04.18.07 STRUCTURAL STUDIES ON MAIZE LIPID TRANSFER PROTEIN AT 1.2 Å RESOLUTION AND ITS OLEATE COMPLEX AT 1.3 Å RESOLUTION.** Jae Young Lee, Dong Hae Shin, and Se Won Suh, Department of Chemistry and Center for Molecular Catalysis, Seoul National University, Seoul 151742, Korea.

The three-dimensional structures of maize lipid transfer protein (MLTP) and its complex with *cis*-$\Delta^9$-octadecenoate (oleate) have been refined with Shelxl-93 using synchrotron data The structure of uncomplexed MLTP has been refined to an R-value of 15.3 % for 19,147 reflections between 8.0 and 1.2 Å resolution and the structure of complex with oleate has been refined to an R-value 14.1% for 14,082 reflections between 8.0 and 1.3 Å resolution. The final model for uncomplexed MLTP contains all of the 93 amino acid residues, 103 water molecules, and 3 formate ions. The model for MLTP-oleate complex includes 121 water molecules, 3 formate ions, and one oleate molecule. The refined structure of MLTP at 1.2 Å resolution provides a wealth of structural details with far greater accuracy than the previous report at 1.9 Å resolution [Shin *et al.* (1995) Structure 3, 189-199, pdb ID code: lmzl]. A structure comparison of uncomplexed MLTP with its oleate complex demonstrated that these MLTPs show no great differences in the backbone structure, but the side chains like Asn37, Val77, and Ile83 in the portal region show above 1 Å differences. In the MLTP-oleate complex structure, the 14 carbon atoms in the tail of the oleate are buried inside the cavity and the other carbon atoms and the carboxyl group of oleate are exposed. - These atomic resolution crystal structures will provide an accurate model for protein-lipid interaction and lipid transfer mechanism.

**PS04.18.08 CRYSTAL STRUCTURE OF ACTIVE SERPIN FAMILY:IMPLICATION FROM *MANDUCA SEXTA* SERPIN K AT 2.2Å RESOLUTION.** Jinping Li, Haobo Jiang*, Michael R. Kanost*, & Elizabeth J. Goldsmith, Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75235-9050, *Department of Biochemistry, Kansas State University, Manhattan, KS 66506

*Manduca sexta* serpin K is a member of the serine protease inhibitor superfamily that inhibit the activity of chymotrypsin. Serpins are important protease inhibitors that are widely distributed not only in vertebrate blood plasma but also in invertebrate body fluid. Sequence alignment based on the crystal structure of cleaved form of α1-antitrypsin, indicates that the serpins share a common fold structure(Huber, R. and Carrell, R. W.). On cleavage of the reactive center peptide bond, they undergo a remarkable conformational change with the newly generated C-terminal moving 70Å apart to the opposite pole of the molecule. The structures of the cleaved form and the two intact forms (antithrombin and antichymotrypsin) are available, but many important aspects of the conformation of the active serpin and particularly that of their reactive center is still not clear. In this report, we have determined the structure of recombinant active serpin K from *Manduca sexta* by molecular replacement to 2.2 Å. The space group of this molecule is monoclinic C2. The high resolution structure of the active serpin K shows that its structure is similar to ovalbumin and reveals the possible interaction of serpin with their target proteases. The other significant feature of the *Manduca Sexta* serpins is that eleven variants of serpins which are encoded from the same gene by alternative pre-mRNA splicing have already been found and each of them has the identical sequence except C-terminal 40-45 amino acids including the reactive center loop. The different reactive center loops generate different inhibitor activities. This structure also implicates how the conformation of the reactive center determines its specificity.