HI-IMI 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$_1$2$_1_2$ 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.

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 similar to ovalbumin and reveals the possible interaction of serpin K from Manduca sexta with their target proteases. The other significant feature of the structure of cleaved protein-lipid interaction and lipid transfer mechanism.

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.