Crystal structure of neuronal Sec1 from the squid Loligo pealei. A. Bracher*, A. Perrakis*, T. Dresbach*, H. Betz*, W. Weissenhorn*, European Molecular Biology Laboratory (EMBL), 6 rue Jules Horowitz, 38000 Grenoble, France; Max-Planck-Institut for Brain Research, Deutscher Breichstr. 46, 60528 Frankfurt/Main, Germany.

Keywords: neuronal Sec1, neurotransmitter release, X-ray structure.

The Sec1 family of proteins has been implicated in a variety of eukaryotic vesicle transport processes including neurotransmitter release by exocytosis. They regulate vesicle transport by binding to a t-SNARE from the syntaxin family. This process is thought to prevent SNARE complex formation, a protein complex required for membrane fusion. While Sec1 molecules are essential for neurotransmitter release and other secretory events, their interaction with syntaxin molecules seems to represent a negative regulatory step in secretion.

We present the X-ray crystal structure of a neuronal Sec1-homologue from the squid Loligo pealei, s-Sec1, at 2.4 Å resolution. Neuronal s-Sec1 is a modular protein that folds into a V-shaped three-domain assembly. Peptide and mutagenesis studies are discussed with respect to the mechanism of Sec1 regulation. Comparison of the structure of squid s-Sec1 with the complex structure of rat nSec1 and syntaxin indicates that conformational rearrangements induced by syntaxin binding are confined to a single domain of the protein. Local conformational changes might therefore be sufficient to release syntaxin from neuronal Sec1, a stimulated process that is thought to involve additional effector molecules.

Human cystatin C (HCC), a low-Mr protein of 120 residues with two disulfide bridges, is a potent inhibitor of cathepsin B and other human thiol proteases. It is responsible for the development of cerebral hemorrhage in patients with a hereditary form of amyloid angiopathy where a single-amino-acid mutant (L68Q) is deposited as amyloid fibrils in cerebral arteries. The crystal structure of an N-truncated chicken analog (62.5% sequence similarity) is known (Bode et al., 1988) and it shows a compact fold with close spatial proximity of the N-terminal region and two hairpin loops, all of which are implicated in enzyme binding. The N-terminal segment is particularly important as its proteolytic removal severely compromises the affinity for the target enzymes. Folding/refolding kinetic studies of the amyloidogenic variant of HCC showed that the protein undergoes irreversible dimerization before aggregating into insoluble deposits. Analogous phenomena have been observed for the wild-type protein, but at elevated temperatures. Solution NMR studies of HCC (Ekiel et al., 1997) confirm the general fold of the monomeric form derived from the chicken analog and indicate that the dimerization process involves the enzyme-binding elements without major structural changes. The NMR data could not distinguish, however, between this simple aggregation model and another one, leading to a symmetric dimer with domain exchange between the two subunits. Our crystallographic efforts have been concentrated on full-length HCC with the aim to characterize all three elements responsible for enzyme inhibition. The protein has been crystallized in several polymorphic forms with large unit cells suggesting oligomerization. For one of the crystal forms, I432, low-temperature synchrotron data to 3.2 Å resolution have been measured, and the structure has been solved by evolutionary programming molecular replacement using the chicken protein as a probe. Contrary to expectations, the asymmetric unit contains only one copy of the molecule with high Matthews volume (4.32 A³/Da). There are, however, very tight symmetric dimers formed across the crystallographic dyads. Those dimers have the same structural elements as the chicken protein model but connected in a different way, with a domain exchange occurring between the two components of the dimer. This crystal structure provides a fascinating example of 3D domain swapping during which two partly unfolded molecules reconstitute each other’s topology.