STRUCTURE AND FUNCTION OF PROTEINS INVOLVED IN REGULATED SECRETION: COMPLEXIN AND SYNAPTOTAGMINS

Timothy Kellogg Craig, Ph.D

The University of Texas Southwestern Medical Center at Dallas, 2009

Supervising Professor: Josep Rizo-Rey,Ph.D

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The release of neurotransmitter from neurons is a tightly regulated process. There are a number of proteins required for membrane fusion to occur, and then there are regulatory proteins that allow membrane fusion to proceed at incredible speed with the precise timing necessary for complex functions such as sight, motor control, and conscious thought. This study will explore the role of three such regulators through biophysical and structural methods.

There are a number of proteins that are essential for membrane fusion. The SNARE proteins are the plasma membrane protein Syntaxin, the vesicle membrane protein Synaptobrevin, and the plasma membrane associated protein SNAP25. These proteins form a tight complex called the SNARE complex that is required for neurotransmitter release. This complex bridges the vesicle and plasma membranes, bringing them into close proximity. Formation of this complex is thus an important point of regulation for the neurotransmitter release process. This SNARE complex serves not only to bridge two membranes, but also to become an anchoring point for a number of regulators of neurotransmitter release such as Complexin, and Synaptotagmin as well as other required proteins such as Munc13 and Munc18.

Complexin is a small soluble protein that binds to the SNARE complex with high affinity and regulates the formation of the SNARE complex. Synaptotagmin is the calcium sensor for fast release of neurotransmitter. Here I present data showing that the N-terminus of Complexin is involved in a critical interaction with the C-terminus of the SNARE complex that is responsible for the excitatory effect of complexin in neurotransmitter release.

Synaptotagmins work with Complexins to trigger rapid membrane fusion in response to calcium influx. Synaptotagmin VII is an important protein for the release of glucagon from islets of langerhans. The C2B domain of this protein is nearly 50% identical to the C2B domain of SytI, but when the C2B domains of SytVII and SytI are switched, the protein does not function correctly. In this study the structure of SytVII was determined by x-ray crystallography to 1.44Å resolution in order to determine if the C2B domain of SytVII is structurally different from other C2B domains. Additionally I crystallized and solved the structure of the C2A domain of Synaptotagmin IX in an effort to compare it to the C2 domains of the other members of the synaptotagmin family. This analysis resulted in the surprising conclusion that a high degree of structural similarity does not necessarily relate to interoperability of the domains.