Molecular basis of coupling Ca2+-sensing to fast membrane fusion by Synaptotagmin-1 in neurotransmitter release

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Neuronal communication relies on rapid neurotransmitter release through Ca^{2+}-evoked synaptic vesicle exocytosis. Synaptotagmin-1 (Syt1) acts as the calcium sensor for fast, synchronous neurotransmitter release. However, the molecular mechanisms underlying Syt1 action and how Ca^{2+}-sensing is coupled to membrane fusion remain unknown. To address these questions, it is crucial to understand the cooperation between Syt1 and SNARE proteins, which drive membrane fusion by forming a tight four-helix bundle that brings the membranes together. In the primed state of synaptic vesicles, Syt1 binds to a partially assembled SNARE complex through a primary interface [described in Zhou et al. (2015) Nature 525, 7567], and to the plasma membrane through a polybasic region, inhibiting complete helical zippering and hence membrane fusion. The primary interface consists of two key regions involving interactions of an arginine cluster of Syt1 with a polyacidic patch on the SNARE complex (region II), and interactions of a tyrosine of Syt1 with another surface of the SNARE complex (region I). Using NMR spectroscopy, we show that mutation of the region II arginines completely abolishes Syt1-SNARE binding, whereas mutation of a key tyrosine abrogates binding at region I while region II remains intact. These data, together with fluorescence experiments, suggest a dissociation of the primary interface region II upon Ca^{2+}-binding, while the Syt1 C_2B domain remains persistently bound through the arginine cluster in region II. Our results lead us to propose a lever model for Syt1 action whereby a Ca^{2+}-induced re-orientation of Syt1 at the plasma membrane pulls the SNARE complex, enabling complete helical zippering that induces fast membrane fusion and subsequent neurotransmitter release.