v-SNARE-based protein-lipid interactions catalyze membrane fusion

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Cellular communication requires Ca^{2+}-triggered fusion of vesicles with the plasma membrane, enabling release of neurotransmitters. The fusion process involves a number of energetically complex steps that require both, protein-protein as well as protein-lipid interactions. Here we investigated the interplay between synaptobrevin2 (syb2) and phospholipids that seems crucial for Ca^{2+}-triggered neurotransmitter release. Using a combination of photolytic 'uncaging' of intracellular Ca^{2+} with membrane capacitance measurement in chromaffin cells, we found that reduced flexibility of the syb2 transmembrane domain (TMD) severely impairs exocytosis, whereas mutants which show enhanced TMD flexibility can fully rescue secretion. Analysis of single amperometric spikes revealed that reduced flexibility of the syb2-TMD slows the kinetics of neurotransmitter discharge from single vesicle and reduces the fusion pore dynamics. In contrast, mutants with higher TMD flexibility accelerate fusion pore expansion beyond the rate found for the wildtype protein.

Thus, our results demonstrate that SNARE TMDs play an active role in the fusion process that goes beyond simple anchoring of the protein. Specifically, we show that flexibility of TMD determines the magnitude of Ca^{2+} triggered exocytosis and kinetics of cargo discharge from single vesicles.