Revealing the Conformational Change of SNAP-25 during Exocytosis using Electrochemical Detectors and TIR-FRET Imaging

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Abstract:

The direct relation of a conformational change in SNAP-25 to individual fusion events was investigated in bovine chromaffin cells over-expressing the SNARE Complex Reporter (SCORE). In the SCORE expressing cells we imaged the FRET change of SCORE localized to the plasma membrane by Total Internal Reflection - Fluorescence Resonance Energy Transfer (TIR-FRET) imaging and simultaneously recorded individual fusion events as amperometric spikes with a microfabricated electrochemical detector (ECD) array. Our study reveals the temporal correlation between the conformational change of SNAP-25 and membrane fusion.

Summary of Research:

The SNARE hypothesis postulates that the assembly of the Soluble N-ethylmaleimide-sensitive factor Attachment Protein Receptor (SNARE) complex is required to induce fusion pore opening and transmitter release. However, the direct relation between a conformational change in the SNARE complex and individual vesicle fusion pore openings, which is essential to understanding the molecular mechanism of exocytosis, has not been demonstrated.

To address this question, we performed experiments where a conformational change in the SNARE complex could be measured simultaneously with individual fusion events. SCORE was constructed by inserting as FRET donor Cyan Fluorescent Protein (CFP) and as FRET acceptor Venus (a variant of Yellow Fluorescent Protein) at the N-termini of the two SNARE motifs of SNAP-25, SN1 and SN2, respectively [1]. When Venus is excited, fluorescence resonance energy transfer (FRET) can occur if the SNAP-25 is in its folded state in the SNARE complex. In the SCORE expressing cells we imaged the FRET change of SCORE localized to the plasma membrane by TIR-FRET imaging and simultaneously recorded individual exocytic fusion events as amperometric spikes with a microfabricated electrochemical detector (ECD) array [2,3], (Figure 1).

The vesicle release location was determined with spatial resolution of ~300 nm based on the charge ratios of oxidation currents recorded by the four electrodes in the ECD array (2). And the accuracy of the position assignment...
was evaluated by the comparison of experimental recordings with simulated results, (Figure 2). We analyzed the fluorescence in a 2 × 2 pixel area (330 × 330 µm²) surrounding the release site as determined from the amperometric recording. For single release events the signal-to-noise ratio is insufficient to obtain a clear FRET signal. To improve the signal-to-noise ratio we averaged the fluorescence traces after aligning them to the starting time points (set to t = 0) of their respective amperometric spikes. By averaging 339 events, a FRET change associated with vesicle fusion became clearly evident (Figure 3).

The time resolution of fluorescence imaging (219 ms/frame) is much lower than that of amperometric spikes. The fluorescence intensities obtained from individual frames were therefore converted to the same time resolution as the amperometric recording, assigning the same fluorescence value obtained from a particular frame to all the points corresponding to that frame. Using a simulated data set assuming that the FRET change occurs stepwise at t = 0 a FRET trace is obtained which shows a linear increase over the time of one frame that has its half time at the time of the spike, except for a small shift that results from the inter-frame interval of 19 ms between the 200 ms exposures (Figure 3 dashed line). In Figure 3, the measured data (solid line) shows a similar increase but at a time preceding the amperometric spike by about 100 ms. This result shows for the first time a conformational change in SNAP-25 that is directly related to fusion pore opening.

References: