The Number of SNARE Complexes Changing Conformation in Vesicle Fusion Events

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Abstract:

The SNARE complex is a core component of the fusion nanomachine, and the zipping of SNARE complexes is thought to provide the force and energy to overcome the energy barrier for membrane fusion. Here, the SNARE Complex REporter2 (SCORE2), a FRET probe of SNAP25, was overexpressed to detect the SNARE complex conformation change during the membrane fusion. Time and location of individual fusion events were detected using microfabricated ElectroChemical Detector (ECD) arrays. Our studies show, under endogenous condition, there are approximately seven (26% of 26.4) endogenous SNAP25 molecules change the conformational during the membrane fusion.

Summary of Research:

In previous studies, we have detected a SNARE conformational change preceding the fusion pore opening [1] in SCORE2 overexpressing SNAP25 KO mouse chromaffin cells. *In vitro* studies [2] have shown that SCORE2 molecules can exhibit the highest FRET efficiency in the presence of excess syntaxin and synaptobrevin 2 by forming the SNARE core complex. In our *in vivo* experiments, the low FRET efficiency of SCORE2 was determined in SCORE2-alone overexpressing. To estimate the *in vivo* high SCORE2 FRET efficiency, SCORE2 were co-expressed with high level of syntaxin by introducing IRES in the transfection construct. By photobleaching the FRET acceptor Venus in both, the low and the high FRET states, the $FRET_{high}$ and $FRET_{low}$ efficiencies were determined as 0.38 (E_{high}) and 0.21(E_{low}), respectively.

The FRET ratio R can be expressed as a simplified function of FRET efficiency E [2].

$$R = A + B \times \frac{E}{1-E} \quad (1)$$

The measured FRET ratios in the low and high FRET states are R_{low} = 1.01 and R_{high} = 1.85, respectively. Based on the values of $E_{low,high}$ and $R_{low,high}$, the coefficients A and B are calculated as 0.37 and 2.42, respectively.

The pre- and post-fusion FRET ratios at the fusion sites R = 1.066 and R = 1.184 [1] correspond to pre- and post-fusion FRET efficiencies of 0.223 and 0.252, respectively, indicating a FRET efficiency increase preceding vesicle fusion within a in a 0.1 μm^2 area surrounding the fusion site. Assuming that the different apparent FRET efficiencies reflect different fractions of SCORE2 molecules in the two distinct FRET states, the fluorescence intensity ratio becomes

$$R = A + B \times \frac{E_{low}+\alpha\times(E_{high}-E_{low})}{1-[E_{low}+\alpha\times(E_{high}-E_{low})]} \quad (2)$$

where α is the fraction of molecules in the high FRET state. The fraction α can be calculated from the apparent FRET efficiency

$$E_{app} = E_{low} + \alpha \times (E_{high} - E_{low}),$$

$$a = \frac{E_{app}-E_{low}}{E_{high}-E_{low}} \quad (3)$$

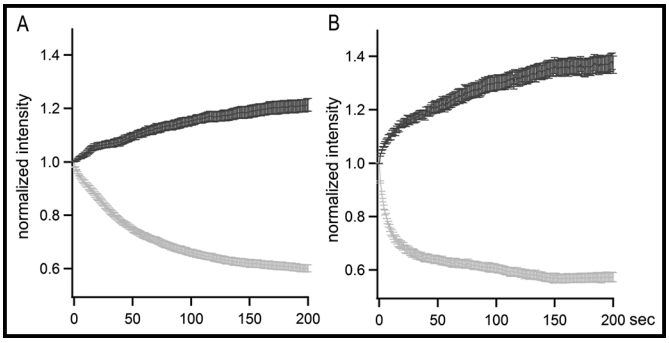

Figure 1: Determination of FRET efficiency by acceptor photobleaching. SCORE2 molecules alone (A) or with 10-fold higher Syntaxin (B) were overexpressed in SNAP25$^{-/-}$ mouse embryonal chromaffin cells and alternately excited for 100 ms at 442 nm in laser TIRF mode while images were acquired and for 1s with epifluorescence lamp excitation at 510/20 nm to bleach the acceptor. Intensities of Venus and mCerulean3 recorded from individual cells were normalized to the respective values before bleaching and the averaged increase in the mCeruleans3 channel indicates an average basal and high FRET efficiency of 21% and 38%, respectively.

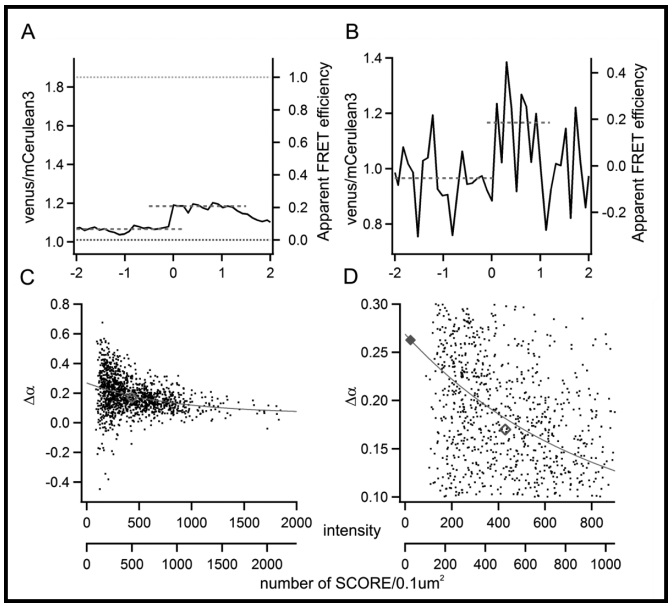

Figure 2: The fraction of SNAP25 molecules undergoing a conformational change as a function of expression level. FRET efficiency changes of all averaged fusion events (A) and of a single event (B), FRET ratio and corresponding apparent FRET efficiency before and after the fusion indicated by red dotted lines. (C-D) plots of Δα of individual events versus corresponding mCerulean3 intensities fitted by a single exponential (continuous red line). The open and filled red diamonds indicate the averaged Δα at average overexpression level and endogenous SNAP25 level, respectively.

According to equation (3), pre and post-function FRET efficiencies of 0.227 and 0.259 correspond to a value of α 0.076 and 0.247 (Figure 2A), suggesting that ~ 17% ($\Delta\alpha = E_{app,post} - E_{app,pre}$) of the SCORE2 molecules undergo a conformational transition from the low FRET state to the high FRET state just before fusion. With 500 SCORE2 molecules present in the fusion area at typical overexpression levels (data not shown), this corresponds to 85 molecules.

Immunostaining shows, however, that the overexpression level of SCORE2 molecules was ~ 19 fold higher than the amount of wild type SNAP25 molecules in untransfected wt cells, suggesting that only ~ 26 endogenous SNAP25 molecules are present at the vesicle fusion site.

To estimate the number of endogenous SNAP25 molecules undergoing conformational change during fusion, the change of α (Δα) was calculated for individual events (Figure 2B) and was plotted versus corresponding FRET donor (mCerulean3) intensities at releasing sites, indicating the expression level (Figure 2C).

Due to the low signal-to-noise ratio of individual events the points scatter widely (Figures 2C and 2D), but can be fitted well with a single exponential. In wild type cells we estimate ~26 SNAP25 molecules. Extrapolating the fit to this value, the Δα value of 0.26 is obtained, suggesting that on average, approximately seven endogenous SNAP25 molecules (26% of 26) undergo a conformational change before a fusion event.

References:

[1] Zhao Y., et al., SNARE conformational change precedes the fusion pore opening. CNF report 2017.

[2] An, S. J., and Almers, W. (2004) Tracking SNARE complex formation in live endocrine cells. Science 306, 1042-1046.

[3] Zhao, Y., Fang, Q., Herbst, A. D., Berberian, K. N., Almers, W., and Lindau, M. (2013) Rapid structural change in synaptosomal-associated protein 25 (SNAP25) precedes the fusion of single vesicles with the plasma membrane in live chromaffin cells. Proceedings of the National Academy of Sciences of the United States of America 110, 14249-14254.