Nascent fusion pore opening monitored at single-SNAREpin resolution

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Vesicle fusion with a target membrane is a key event in cellulartrafficking and ensures cargo transport within the cell and between cells. The formation of a protein complex, called SNAREpin,provides the energy necessary for the fusion process. In a three-dimensional microfluidic chip, we monitored the fusion of small vesicles with a suspended asymmetric lipid bilayer. Adding ionchannels into the vesicles, our setup allows the observation of a single fusion event by electrophysiology with 10-µs precision. Intriguingly, we identified that small transient fusion pores of discrete sizes reversibly opened with a characteristic lifetime of~350 ms.The distribution of their apparent diameters displayed two peaks, at 0.4±0.1 nm and 0.8±0.2 nm. Varying the number of SNAREpins, we demonstrated that the first peak corresponds to fusion pores induced by a single SNAREpin and the second peak is associated with pores involving two SNAREpins acting simultaneously. The pore size fluctu-ations provide a direct estimate of the energy landscape of the pore.By extrapolation, the energy landscape for three SNAREpins does not exhibit any thermally significant energy barrier, showing that pores larger than 1.5 nm are spontaneously produced by three or moreSNAREpins acting simultaneously, and expand indefinitely. Our results quantitatively explain why one SNAREpin is sufficient to open a fusion pore and more than three SNAREpins are required for cargo release. Finally, they also explain why a machinery that synchronizes three SNAREpins, or more, is mandatory to ensure fast neurotransmitter release during synaptic transmission.

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