

AFM studies of SNAREs interactions and fusion

Midhat H. Abdulreda and Vincent T. Moy
University of Miami Miller School of Medicine, Physiology & Biophysics Dept.
1600 NW 10th Ave, RMSB Room 5064A
Miami, FL 33136

ABSTRACT

SNAREs are considered the minimal machinery for membrane fusion *in vitro*. Liposomes reconstituted with SNARE proteins have been used to investigate membrane fusion. We employed the atomic force microscope to measure fusion forces of two apposing lipid bilayers and rupture forces associated with the dissociation of v- and t-SNARE complexes during approach and retraction, respectively. Egg phosphatidylcholine bilayers containing v-SNAREs (synaptobrevin 2) or binary t-SNAREs (syntaxin 1 and SNAP 25) were formed by lipid vesicle adsorption onto opposite glass dishes and glass microbeads attached to cantilever tips. Fusion and rupture forces increased with loading rate (LR). The dynamic force spectrum (DFS) of the fusion process revealed a single linear loading regime in presence/absence of SNAREs. This indicates that membrane fusion is governed by one energy barrier under the current conditions. In the presence of SNAREs, the width of the energy barrier increased by ~3 fold compared to that in egg PC alone, whereas the activation potential was unchanged. This suggests that interaction of cognate v- and t-SNAREs facilitated membrane fusion by reducing the slope of the energy barrier. Alternatively, the DFS of the SNARE complex dissociation revealed two loading regimes, suggesting the presence of two energy barriers during dissociation of v-SNARE/t-SNARE complexes. The inner (high LRs) and outer (low LRs) barrier widths were 0.39Å and 2.9Å, and dissociation rate constants were 15.5 s⁻¹ and 0.3 s⁻¹, respectively. These energy barrier parameters suggest that under pulling force, dissociation of the v-SNARE/t-SNARE complex is effectively dominated by the outer barrier.