HIV gp41 Trans-Membrane Domain Promotes both Stalk and Fusion Pore Formation in Poly(Ethylene-) Glycol Mediated Membrane Fusion

ARTICLE *in* BIOPHYSICAL JOURNAL · JANUARY 2012

Impact Factor: 3.97 · DOI: 10.1016/j.bpj.2011.11.2735

4 AUTHORS, INCLUDING:

Hirak Chakraborty

Sambalpur University

38 PUBLICATIONS **359** CITATIONS

SEE PROFILE

David Klapper

University of North Carolina at Chapel Hill

99 PUBLICATIONS **3,000** CITATIONS

SEE PROFILE

Barry R Lentz

University of North Carolina at Chapel Hill

167 PUBLICATIONS **6,404** CITATIONS

SEE PROFILE

membrane. Simulations show that a minimally-sized cluster ring expands outward, driven principally by electrostatic and steric repulsions between the SNARE complexes. Ring expansion thereby pulls the curved vesicle surface toward the target membrane. Addition of new complexes to the cluster further expands the ring, thus reducing the membrane separation and elevating the pressure between the membranes. We find a cluster of ~5-10 complexes docking a 50-nm vesicle exerts local pressures of tens of atmospheres, similar to the threshold pressures required for fusion measured in planar bilayer systems [Wong et al, Biophys J., 1999]. Thus, the SNARE cluster-generated pressure may be sufficient for fusion. This is consistent with the recently reported 5-11 complex requirement. In addition, our model makes the testable prediction that fusion of smaller (higher curvature) vesicles requires fewer SNAREs.

2540-Pos Board B310

Direct Observation of Dual Pathways of Yeast Minimal-Machinery-SNARE Driven Vesicle Fusion

Je-Kyung Ryu[1,2], Jun Hyuk Moon[3], Malgi Choi[4], Kipom Kim[3,5], Yeon-Kyun Shin[6], Mahn Won Kim[3,5], Tae-Young Yoon[3,5].
[1]Center for Single-Molecule Systems Biology, National Creative Research Initiative, KAIST, Daejeon, Korea, Republic of, [2]Department of Physics, KAIST, Kaist, Korea, Republic of, [3]Department of Physics, KAIST, Daejeon, Korea, Republic of, [4]Postech, Pohang, Korea, Republic of, [5]Center for Single-Molecule Systems Biology, National Creative Research Initiative, KAIST, Daejeon, Korea, Republic of, [6]Iowa State university, Ames, IA, USA.

SNAREs (soluble N-ethyl maleimide sensitive factor attachment protein receptors) are well-known as membrane fusion machinery in eukaryotic cells. The vesicle-associated v-SNARE engages with its partner t-SNAREs on the target membrane to form 4-helix bundle that bridges two membranes and facilitates fusion. However, during the fusion process the geometric information of vesicles is unveiled due to the resolution limit of conventional light microscopy. Cryo-TEM (Cryogenic Transmission Electron Microscopy) directly shows the geometric information during the membrane fusion, overcoming the resolution limit. Since double vesicles which included a small vesicle inside outer vesicle are observed on the final stage of SNARE driven fusion process, we suggest that dual pathways of yeast minimal-machinery-SNARE driven vesicle fusion are available; 1) developing a single vesicle and 2) developing double vesicle as the final state. Furthemore, Cryo-TEM micrographs shows two kinds of intermediate states; double layers and one layer in the contact area. We guess that the final double vesicles might be developed by passing separate two bilayer contact state, while the final single vesicles might be developed by a fusion process with hemifusion state.

2541-Pos Board B311

Solution Single Vesicle Fusion Assay Reveals PIP_2 Mediated Sequential Actions of Synaptotagmin-1 onto SNAREs

Jea-Yeol Kim[1], Bong-Kyu Choi[1], Mal-Gi Choi[1], Sun-Ae Kim[2], Yeon-Kyun Shin[2], Nam Ki Lee[1].
[1]Postech, Pohang, Korea, Republic of, [2]Iowa State University, Ames, IA, USA.

Synaptotagmin-1 (Syt1) is a major Ca^{2+} sensor for fast synchronous neurotransmitter release, which requires vesicle fusion mediated by SNAREs. Syt1 is known to interact with target membrane (t-) SNARE, ternary SNARE complex, and anionic phospholipids. However, how Syt1 utilizes its diverse interactions to regulate vesicle fusion remains illusive. To dissect the functions of Syt1, we apply a single-molecule technique, alternating-laser excitation (ALEX), which is capable of sorting out all subpopulations of fusion intermediates in bulk solution, particularly the docking stage before lipid mixing. The results show that membrane-anchored Syt1 undergoes at least three distinct steps prior to lipid mixing. First, in the absence of Ca^{2+}, Syt1 mediates vesicle tethering by directly binding to t-SNARE, which requires PIP_2. Second, synaptobrevin-2 binding to t-SNARE to form the ternary complex displaces Syt1 from the SNARE complex. Third, in the presence of Ca^{2+}, Syt1 rebinds to the SNARE complex, which again requires PIP_2. Thus in the absence of Ca^{2+}, Syt1 may bring vesicles to the plasma membrane in proximity via binding to t-SNARE/PIP_2 to help ternary SNARE complex formation and then, upon Ca^{2+}-influx, it may rebind to the ternary complex with the aid of PIP_2, which may trigger fast synchronous fusion.

2542-Pos Board B312

Structural Model of the Juxtamembrane Region of the *Trans*-SNARE Complex with EPR

Xiaochu Lou.
Iowa State University, AMES, IA, USA.

Neuronal SNARE proteins play a central role in mediating the fusion of synaptic vesicles with the nerve cell plasma membrane, which is necessary for neurotransmitter release. Plasma membrane SNARE Syxtaxin 1A and vesicle SNARE Synaptobrevin 2, anchor to respective membranes and the juxtamembrane regions connect their SNARE motifs to the respective transmembrane domains. SNARE complex formation, *in trans*, brings vesicle and membrane closely together to the pre-fusion state. The zippering may continue to the linker regions and extend the helical structure all the way through the transmembrane domain (*cis*- SNARE complex). Although the *trans*-SNARE core complex and *cis*-SNARE complex structures are known, it is not known what the linker region structure should be act in the *trans*-complex, which is believed to be a force transducer that plays a role in membrane merging. Here, we investigated SNARE complexes containing linker region truncated version of Syxtaxin 1A or Synaptobrevin 2 to mimic the *trans*-complex using spin labeling electron paramagnetic resonance (EPR). We will present the new EPR results that might shed lights on the structure of the *trans*-SNARE complex.

2543-Pos Board B313

Single Vesicle Fusion System for Content Mixing and SNARE Complex Formstion

Woori Bae[1], Mal-Gi Choi[2], Tae-Young Yoon[1].
[1]KAIST, Daejeon, Korea, Republic of, [2]POSTECH, Pohang, Korea, Republic of.

SNARE proteins drive membrane fusion by SNARE complex formation. However, it is hard to study SNARE complex formation dynamics and fusion kinetics with single molecule resolution in realtime. We developed new single-molecule FRET analyze mechanism and adapted single vesicle content dequenching assay to observe content mixing and SNARE complex formation simultaneously. Our result reveals existence of two different kinetic components in SNARE complex formation which was inaccessible in previous studies.

2544-Pos Board B314

Insights into Membrane Fusion from Molecular Dynamics Simulations of SNARE Proteins

Alex Tek[1], Peter J. Bond[2], Mark S.P. Sansom[3], Marc Baaden[1].
[1]Institut de Biologie Physico-Chimique Laboratoire de Biochimie Théorique, CNRS UPR 9080, Paris, France, [2]Unilever Centre, Department of Chemistry, Lensfield Road, University of Cambridge, Cambridge CB2 1EW, United Kingdom, [3]Structural Bioinformatics & Computational Biochemistry Unit Department of Biochemistry, University of Oxford, Oxford, United Kingdom.

The SNARE proteins family affects numerous intra-cellular fusion processes. Synaptobrevin (Sb), Syntaxin-1 (Sx) and both SNAP25 (Sn) chains are involved during synaptic vesicular exocytosis. They form a bundle of four alpha-helices. Both Sb and Sx have a trans-membrane domain (TMD) used to anchor the bundle between the vesicle and cell membranes. It has been shown experimentally that these proteins trigger the fusion process in-vitro as well as in-vivo. Yet the molecular mechanisms of this process are not elucidated. Molecular dynamics simulation (MD) approaches offer very detailed insights into such systems and can describe the behavior of each component. We performed several simulations of a membrane-embedded SNARE complex between two mixed POPC/POPS membranes. Despite a simulation artefact initially moving the membranes away from each other, we observe strong deformations around the TMDs and a decreasing distance between them. If the link between the TMDs and the bundle is severed, both membranes go back to a flat state. This observation can be explained by very robust inter-helical interactions that prevent the bundle from breaking away. In addition, the TMD composition allows them to be strongly anchored to the membranes. Electrostatic interactions between the proteins and the membrane further seem to help accelerate this process.

2545-Pos Board B315

HIV gp41 Trans-Membrane Domain Promotes both Stalk and Fusion Pore Formation in Poly(Ethylene-) Glycol Mediated Membrane Fusion

Hirak Chakraborty[1], Sekaran Moses Dennison[2], David G. Klapper[1], **Barry R. Lentz**[1].
[1]UNC at Chapel Hill, Chapel Hill, NC, USA, [2]Duke University, Durham, NC, USA.

The trans-membrane domain (TMD) of gp41 is essential for efficient fusion between HIV-1 and its host cell in vivo. HIV virus with gp41 mutated by R696L is reported to be defective in infectivity and fusion (Helseth, J Vir, 1990, 6314), so we have examined both native and R696L gp41 TMDs' effects on PEG-mediated fusion of PC/PE/SM/CH (35/30/15/20) SUVs. Lipid mixing (LM), contents mixing (CM) and leakage (L) time courses were fitted globally to a 3-state sequential model (Weinreb & Lentz, BJ, 2007, 4012), from which we obtained estimates of rate constants for conversion between states as well as probabilities of LM, CM and L for each state. The WT peptide increased the rates of stalk (k1) and fusion pore (k3) formation in a cooperative fashion

(maximum effect seen at ~ 0.25mol% peptide or 6 peptides/vesicle). R696L peptide had no effect on k3 and a very minor effect on k1. At this concentration, CD spectroscopy showed the WT peptide to be ~ 39% helix, 39% unordered, and 22% β-sheet, but to increase in β- and decrease in α-content at high peptide/lipid ratios up to 1/50. Neither peptide affected the extent of content mixing, but the R696L mutant actually inhibited the extent of lipid mixing. The native but not the mutant peptide increased the probability of content mixing in the stalk intermediate. Studies with hexadecane as a space-filling agent showed that the TMD peptide was unlikely to promote fusion by this mechanism. Analysis of transition state thermodynamics suggests that the TMD may disrupt interfacial packing so as to promote penetration of lipid acyl chains into the inter-bilayer space as a means of catalyzing stalk formation. Supported by NIGMS grant 32707 to BRL.

2546-Pos Board B316

Synaptobrevin Trans-Membrane Domain forms a Complex that Enhances the Rate of "Stalk" and Pore Formation in PEG-Mediated Vesicle Fusion

Pradip K. Tarafdar, Barry R. Lentz.
Univ of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

Synaptobrevin (SB), a membrane anchored protein in the neuronal cell membrane, complexes with syntaxin (SX) and SNAP-25 to facilitate membrane fusion in neurotransmitter release. SB and SX promote Poly(ethylene glycol) (PEG)-mediated fusion whether or not they are assembled into a SNARE complex (Dennison et al., BJ, 2006, 1661). Viral fusion protein trans-membrane domains (TMDs) enhance the fusion. Thus, we hypothesized that the SB-TMD may also affect fusion kinetics. The kinetics of PEG-mediated fusion of DOPC/DOPE/sphingomyelin/cholesterol/DOPS (32/25/15/20/8) 25 nm vesicles (SUVs) was examined in the presence and absence of SB TMD. Lipid mixing (LM), contents mixing (CM) and leakage (L) time courses were fitted globally to a 3-state sequential model (Weinreb & Lentz, BJ, 2007, 4012), from which we obtained estimates of rate constants for conversion between states as well as probabilities of LM, CM and L for each state. SB TMD enhanced the rates of ''stalk'' and fusion pore (FP) formation in a cooperative fashion (maximum effect at 3 peptide/vesicle). TMD ordered the bilayer interior in a similarly cooperative fashion. The effects of hexadecane and TMD on fusion kinetics were quite distinct and not mutual. SB TMD increased the probability of both LM and CM in the initial intermediate, suggesting that it promoted formation of transient pores before the final pore state. Transition state thermodynamic changes indicate that the effects on ''stalk'' and FP formation involved different mechanism, although both showed that in presence of TMD the change in entropy is always greater than enthalpy. Supported by NIH grant GM32707 to BRL.

2547-Pos Board B317

Direct Visualization of Large and Protein-Free Hemifusion Diaphragms

Joerg Nikolaus[1], Jason M. Warner[2], Ben O'Shaughnessy[2], Andreas Herrmann[1].
[1]Humboldt University Berlin, Berlin, Germany, [2]Department of Chemical Engineering, Columbia University, New York, NY, USA.

Membrane fusion is ubiquitous in life requiring remodeling of two phospholipid bilayers. As supported by many experimental results and theoretical analyses, merging of membranes seems to proceed via similar sequential intermediates. Contacting membranes form a stalk between the proximal leaflets which expand radially into a hemifusion diaphragm (HD) and subsequently open to a fusion pore. Direct experimental verification of the HD is difficult due to its transient nature. Using confocal fluorescence microscopy we have investigated the fusion of giant unilamellar vesicles (GUVs) containing fluorescent membrane protein anchors and fluorescent lipid analogues in the presence of divalent cations. Time resolved imaging revealed that fusion was preceded by displacement of peptides and lipid analogues from the GUV-GUV contact region being of several Âμm in size. A detailed analysis showed that this structure is consistent with the formation of an HD. A quantitative model of the hemifusion equilibrium and kinetics of the growing HD was developed. Bilayer tension could be shown to drive HD expansion and interleaflet tension was found to act as a counterforce, because the outer leaflets are compressed upon HD growth. The model and its predictions fit nicely with observations above. In addition we are currently investigating the influence of membrane tension on the fusion pathway directly using the GUV system within a micromanipulation approach.

2548-Pos Board B318

Studying X31 Influenza Membrane Binding and Fusion using Stochastic Assays and Simulations

Donald Lee, Vikram Thapar, Deirdre Costello, Paulette Clancy, Gary Whittaker, Susan Daniel.
Cornell, Ithaca, NY, USA.

Stochastic kinetic data of binding and fusion of X31 influenza virus to a target membrane was obtained using individual-virion imaging techniques and compared to a stochastic simulation model to assess the rate limiting steps in viral fusion. Experiments were conducted inside a microfluidic channel where total internal reflection fluorescence microscopy was used to observe individual virions interacting with a supported lipid bilayer containing sialic acid receptors. The residence time of bound viruses was measured and used to determine the binding rate constants. Following the binding studies, an acidic solution was introduced into the microfluidic device to trigger viral fusion. Fusion events were detected through the dequenching of fluorescent membrane dye. Using standard procedures to analyze the fusion kinetic data, we obtain two fit parameters denoted as k and N. One common interpretation of the fit parameters is that k represents the rate constant of the slowest step in the fusion process while N represents the number of parallel steps required to initiate fusion. N is usually greater than 1 since viral fusion requires multiple fusion proteins to act in parallel to overcome the energy barrier of membrane fusion. However, we find that N approaches values closer to 1 if a more acidic solution is used to trigger fusion, suggesting that another interpretation of N might be appropriate. We discuss these possibilities here.

2549-Pos Board B319

A Quantitative Model for Formation of Protein-Mediated Protrusions, Based on Continuum Elasticity Theory

Rodion Molotkovsky[1], Joshua Zimmerberg[2], Fredric S. Cohen[3], Sergey Akimov[1].
[1]IPCE RAS, Moscow, Russian Federation, [2]Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD, USA, [3]Rush University Medical Center, Chicago, IL, USA.

The close opposition of membranes needed for fusion is initiated by the formation of local protrusions. In viral fusion, the protrusions should be generated by several fusion proteins acting cooperatively within a cluster. We start with two parallel planar membranes and show that membrane elasticity alone can spontaneously cause several fusion proteins to self-organize into a cylindrically symmetric cluster that consists of three to six proteins cluster, independent of specific short-range protein-protein interactions. In essence, fusion proteins induce membrane bending which then brings the proteins together, creating more bending – a positive feedback system. Calculations of energy minimization yield the following progression of protein arrangements: Three proteins initially arrange at the vertices of an equilateral triangle. Cluster formation continues by three additional proteins symmetrically arranging at the vertices of a more distal equilateral triangle that surrounds the three central proteins. These distal proteins move toward, and the central proteins away, from the center, yielding a cluster of six proteins arranged hexagonally on a circle. The energy needed to bend membranes into protrusions is supplied by the proteins in the cluster; continuum elasticity theory is used to calculate this energy. The total energy consists of the change in elastic energy of membrane deformation and the energy generated by an osmotic pressure difference that arises because the density of proteins outside the cluster is greater than the zero density inside cluster. The minimum energy is 75 kT for a cluster radius of 15 nm. The minimal energy per protein is 12 kT, which is a reasonable estimate.

2550-Pos Board B320

Tirfing Insulin-Stimulated GLUT4 Translocation with a Quench-Based Mehtod

Yu Chen, Jennifer Lippincott-Schwartz.
NIH, Bethesda, MD, USA.

Intrinsic to measuring insulin-stimulated GLUT4 translocation to the plasma membrane (PM) using total internal reflection fluorescence microscope (TIRFM) is the inability to differentiate intensity increment contributed by vesicles approaching the PM and GLUT4 molecules being inserted into the PM. Here, we combined IRAP-GFP probe, which exposes GFP to extracellular environment after GLUT4 vesicles fuse with the PM, with bromophenol blue (BPB), which quenches GFP fluorescence when in contact with it, trying to delineate the contribution of these two steps. It is found that insulin stimulation dramatically increased IRAP molecules localizing on the PM whereas GLUT4 vesicle density beneath the PM had little change through the translocation process. Therefore, we suggest that the fusion efficiency of GLUT4 vesicles at the PM is enhanced so much by insulin that GLUT4 vesicles barely accumulate underneath the PM.

2551-Pos Board B321

A Dynamic Model of Fusion Pores in Lipid Bilayers

Rolf Ryham[1], Fredric S. Cohen[2], Robert Eisenberg[2], Chun Liu[3].
[1]Fordham University, Bronx, NY, USA, [2]Rush University, Chicago, IL, USA, [3]The Pennsylvania State University, University Park, PA, USA.