protein, called E in flaviviruses. The present work focuses on the dependence of fusion on membrane composition for the flavivirus Dengue (DEN). Recently it has been reported that full fusion only occurs with membranes containing anionic lipids, i.e. late endosomes. The origin of this strong dependence on lipid composition is currently unknown. Regarding cholesterol, different effects have been reported. One group reported that cholesterol is not absolutely required but increases the efficiency of fusion of the flavivirus tick-borne encephalitis (TBE) with liposomes. Another group reported that an excess of cholesterol blocks fusion of DEN in mammalian cells. Others reported that fusion of DEN in insect cells was independent of cholesterol. To provide insight into the membrane dependence of fusion for DEN we used several biophysical techniques (neutron reflectivity, quartz crystal microbalance, density gradient centrifugation and liposome coflotation analysis) to study the depth of insertion of E into lipid membranes, the influence of E binding on membrane curvature, and the anchoring energy of E in the membrane as a function of membrane composition. Important effects were discovered for both negatively-charged lipids and cholesterol.


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The Viral Restriction Factor IFITM3 Promotes Hemifusion but Blocks Full Fusion of Influenza Virus

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Interferon-induced transmembrane proteins (IFITMs) are up-regulated as part of a cell’s defense against viral challenges. These small proteins inhibit entry of diverse viruses, such as influenza A (IAV), West Nile and dengue virus. The mechanism by which IFITMs block viral entry is not understood. Recent reports suggest that IFITMs inhibit viral hemifusion (merger of proximal leaflets of the viral and cellular membranes), presumably by disrupting cholesterol trafficking and causing aberrant cholesterol accumulation in late endosomes. Here we employed time-resolved single IAV imaging to identify fusion step(s) affected by IFITM3 protein ectopically expressed in lung epithelial cells. These experiments revealed that, contrary to previous reports, lipid mixing between IAV and endosome was, in fact, promoted upon IFITM3 expression. In contrast, virus-cell fusion assays monitoring the release of the viral content showed marked inhibition of fusion pore formation by IFITM3. This effect was not due to excessive cholesterol accumulation in endosomes or to reduced endosome acidity in IFITM3-expressing cells. Furthermore, conditions that induced the accumulation of cholesterol in late endosomes/lysosomes did not restrict IAV fusion. To conclude, IFITM3 blocks IAV fusion by disfavoring the formation of fusion pores, and this phenomenon is independent of cholesterol levels. We propose that IFITM3 can block viral fusion at a post-hemifusion stage either directly, by inserting into and stabilizing the cytosolic leaflet of endosomal membranes, or indirectly, by altering the lipid composition and thus disfavoring the formation of fusion pores. This work was partially supported by the NIH R01 GM054787 (to GBM) and IR01A019716 (to ALB) grants.

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New Broad-Spectrum Viral Fusion Inhibitors Act by Deleterious Effect on the Viral Membrane through the Production Singlet Oxygen Molecules

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Targeting membranes of enveloped viruses represents an exciting new paradigm to explore regarding the development of broad-spectrum antivirals. Recently, broad-spectrum small-molecule antiviral drugs were described, which prevent enveloped virus entry at an intermediate step, after virus binding but before virus-cell fusion [1, 2]. These compounds, an aryl methylidene rhodanine derivative, named JL901, and oxazolidine-2,4-dione derivatives named JL103 and JL118, act deleteriously on the virus envelope but not at the cellular membrane level. The aim of the present work was to study the interactions of active compounds (JL001, JL103 and JL118) and JL025 (an inactive analog used as negative control) with biological membrane models, in order to clarify the mechanism of action of these new enveloped virus entry inhibitors. Fluorescence spectroscopy was used to quantify the partition and determine the location of the molecules on membranes. The ability of the compounds to produce reactive oxygen molecules in the membrane was tested by using 9,10-dimethyl-1

lanthracene, which reacts selectively with singlet oxygen. Changes on the lipid packing and fluidity of membranes were assessed by fluorescence anisotropy and generalized polarization measurements. Finally, the ability to inhibit membrane fusion was evaluated using FRET. Our results indicate that singlet oxygen production by JL001, JL103 and JL118 is able to induce several changes on membrane properties, specially related with a decrease on its fluidity, concomitant with an increase on the order on the polar head groups region, resulting in an inhibition of the membrane fusion necessary for cell infection.


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GP41 Ectodomain Dissociates and Forms a Stable Monomer on Phospholipid Vesicles and Detergent Micelles: Implication for the HIV-1 Mediated Membrane Fusion

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The first step of HIV infection involves the fusion of the viral and target cell membranes, a process mediated by the viral envelope glycoproteins, gp120 and gp41. The binding of gp120 to the cell surface receptors CD4 triggers a cascade of conformational changes that disrupt the gp41-gp120 interactions and allows the insertion of the N-terminal fusion peptide of gp41 into the host cell membrane. The gp41 trimer then rapidly folds into a 6-helix bundle that pulls together the fusion peptide, inserted in the host cell membrane and the transmembrane domain, located in the viral membrane. Very little structural information is known about the pre-fusion state of gp41 notwithstanding its crucial importance for the design of fusion inhibitors. To investigate the dynamics and structural properties of such metastable states, we designed a set of protein constructs mimicking the extracellular ectodomain of gp41. We found that the secondary structure of gp41 ectodomain is pronouncedly impacted by the presence of phospholipid vesicles or detergents containing phosphatidyl choline head groups. Our NMR and multi-angle light scattering data attribute these changes to the transition between a trimer in the absence of detergent to a monomer in the presence of a membrane-mimicking environment. The structure of gp41 in a monomeric state was determined by state-of-the-art NMR techniques, including measurements of NOE distance restraints and residual dipolar couplings in weakly aligned solutions. 15N relaxation data provided a detailed view at the backbone dynamics of both the trimeric and monomeric conformations. This stable monomeric state may represent a crucial structural intermediate facilitating both the gp41 conformational change during fusion and the local apposition of the viral and cellular membranes.

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Single-Molecule Manipulation of Gp41 Folding Involved in HIV Infection and Drug Resistance

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The HIV-1 envelope (Env) glycoprotein, a homotrimeric complex of gp41 and gp120, mediates attachment and fusion of the HIV-1 virion to its host CD4 cells, the very first and rate-limiting step of HIV infection. The complex holds gp41 in a largely unfolded high energy state like a loaded spring. The virion attachment triggers profound conformational rearrangements of Env protein that couple gp41 folding to membrane fusion by an unknown mechanism. In this process, gp41 folds from a pre-hairpin structure into a trimer-of-hairpins structure containing a six-helix bundle. Each hairpin contains an N- and a C-terminal helix linked by a loop. Here, we characterized the energetics and kinetics of gp41 folding at a single molecule level using high-resolution optical tweezers. Our data showed that single gp41 complexes fold in a two-state manner independent of the solution pH value, releasing extraordinary high energy competent for membrane fusion. As the sole antigen on the virion surface, HIV Env complexes are primary targets for anti-HIV drugs and vaccine development. Our work will provide important insights into the mechanism of HIV-1 fusion and drug resistance and help improve anti-HIV medicines.

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The Gp41 Sequence Connecting Mper and Tm Domains Constitutes a Distinct HIV-1 “Fusion Peptide” Targeted by Neutralizing Antibodies

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The HIV-1 virus makes use of a membrane fusion strategy to enter into the host cell. As such, this process constitutes a clinical target for inhibitor and