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HA Fusion Peptide, but Not Two Biologically Inactive Mutants, Lowers Activation Barrier of the Pore Formation Step during PEG-mediated Fusion

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Poly(ethylene glycol)- (PEG-) mediated fusion of 25 nm vesicles was examined in the presence of hemagglutinin (HA) fusion peptide and its biologically inactive mutants (G1V, G1S and W14A) at different temperatures between 26°C and 43°C. Lipid composition was dioleoylphosphatidylcholine (DOPC), dioleoylphosphatidylethanolamine (DOPE), bovine brain sphingomyelin (SM) and Cholesterol (CH) in a ratio of 35:30:15:20. Lipid mixing (LM), content mixing (CM) and content leakage (L) time courses were fitted globally to 3-state or 4-state sequential models (*Biophys. J.*, 2007, 92; 4012). From this we obtained estimates of rate constants for conversion between states as well as probabilities of the occurrence of LM, CM, or L in each state. Non-linear Arrhenius plots implied that the nature of the barrier between states changed with temperature such that activation enthalpy and entropy for all systems varied with temperature. Wild type (WT) fusion peptide enhanced the rate of pore formation by lowering the activation free energy (increased favorable activation entropy) as well as increased the extent of content mixing. G1S and W14A mutants decreased the rate of pore formation and extent of content mixing. A G1V mutant enhanced the rate of pore formation even more than did wild type HA peptide but made the vesicles so leaky that content mixing could not be observed. For both control vesicles and in the presence of WT peptide, the probability of CM increased for states early in the process, such that fusion became 3-state model at higher temperatures with WT. Fluorescence probes revealed that W14A increased the acyl chain packing at all the temperatures and G1V and W14A reduce bilayer packing at the water-membrane interface while WT peptide had little effect. Supported by NIGMS grant 32707 to BRL.

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The Role Of Class Ii And Iii Viral Protein Transmembrane Domains (tmds) In Voltage-dependent Fusion

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We have found that fusion mediated by class II (e.g. Semliki Forest Virus E1) and class III (e.g., Vesicular Stomatitis Virus G) viral proteins are promoted by a trans-negative voltage and prevented by a trans-positive potential across the target membrane. Endosomes, from which these viruses normally fuse, naturally have a trans-negative potential and so voltage dependence may have important biological relevance. We have shown that in contrast to class II and III proteins, fusion induced by class I viral proteins (influenza HA, HIV Env, and ASLV Env) is independent of the target membrane voltage. We are currently seeking to identify the voltage sensor. Because the sensor must lie within the membrane to respond to the electric field, the fusion peptides and TMDs are sensor candidates. We therefore prepared viral protein chimeras that consist of the class I protein influenza HA as ectodomain, and the TMD and/or the cytoplasmic tail (CT) of VSV G. All chimeras fuse well to red blood cells, in accord with previous demonstrations that the precise TMD and CT of HA were of relatively little importance in fusion to red cells. But surprisingly, all chimeras were less efficient that WT HA in fusing to nucleated cells. We have therefore produced fluorescently-labeled pseudovirus using WT and chimeras as the envelope protein and are now monitoring fusion of these pseudovirions to voltage-clamped target cells. Supported by Public Health Service grants R01 GM27367 and GM057454.

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The Hydrophobic Surfactant Proteins Induce Cubic-Phase Formation in a Hii Forming Phospholipid

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The hydrophobic surfactant proteins, SP-B and SP-C, which represent ~1.5% (w:w) of pulmonary surfactant, promote the rapid adsorption of the surfactant phospholipids into the alveolar air/water interface. Available evidence suggests that they facilitate adsorption by stabilizing a tightly curved rate-limiting structure that bridges the gap between the adsorbing vesicle and the nascent interfacial film. To determine if the surfactant proteins can affect the curvature of phospholipid leaflets, we used small-angle X-ray diffraction to determine how the physiological mixture of SP-B and SP-C affected the structure of 1-palmitoyl-2-oleoyl phosphatidylethanolamine, which forms the inverted hexagonal (Hii) phase at high temperatures. Measurements from 5-95°C at 5-10°C intervals on the phospholipid alone were consistent with previously-described transitions between two lamellar phases at 24°C, with formation of the Hii phase at ~72°C. Addition of 0.1-2.0% (w:w) protein had no effect on the lamellar phases, but induced formation of two cubic phases, Pm3n and Im3m, beginning at temperatures as low as 50°C. The intensity of cubic relative to lamellar peaks increased with larger amounts of the proteins and temperature. At 55°C, for instance, progressive increases in protein shifted the samples from strictly lamellar structures to lamellar-cubic coexistence to strictly cubic phases. Similarly, in samples with 0.1% protein, increasing temperature shifted lamellar structures through a coexistence region (60-70°C) to exclusively cubic diffraction at higher temperatures. These results indicate that with a phospholipid that can form curved structures, the hydrophobic surfactant proteins can significantly alter its polymorphisms. These findings support the model in which the proteins achieve their kinetic effects by stabilizing a curved structure.
Portions of this research were carried out at the Stanford Synchrotron Radiation Laboratory, a national user facility operated by Stanford University on behalf of the U.S. Department of Energy.

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Fusion Activities Of Human (h3n2) And Avian (h5n2) Influenza Viruses With Lipid Vesicles Containing Different Gangliosides

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We have studied the fusion of human (H3N2) and avian (H5N2) influenza viruses with lipid vesicles containing various gangliosides using spectroscopic fusion assay. The lipid vesicles used in these experiments were of DOPC and DOPE (2:1 molar ratio) containing 10% molar ratio of total lipids of three different types of gangliosides , GD_{1a}, GD_{1b}, GM_3. Fluorescence fusion assay used involved the NBD/Rhodamine fluorescence resonance energy transfer. The fusion as a result of the interaction of virus and the ganglidside was measured as an increase in NBD signals. The experiments were carried out at temperatures ranging from 37 to 20 °C and fusion activity measured at different concentrations of the target membranes. Using the same amount virus based on the hemagglutinin activity, the fusion activity of the human influenza virus was significantly higher (50% higher) than that of the avian influenza virus with all three types of gangliosides in all temperature ranges tested.
To further characterize the interaction between these viruses (human and avian) and its receptors, experiments are in progress to determine the role of membrane proteins and various gangliosides.

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Kinetic and Thermodynamic Basis of Guanine Nucleotide Exchange on the Rab Ypt1p by the Membrane Tethering Complex TRAPPI

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TRAPPI is a multiprotein tethering complex involved in mediating the initial interaction of endoplasmic reticulum (ER)-derived vesicles at the cis-Golgi, that serves as a guanine nucleotide exchange factor (GEF) for the Rab GTPase, Ypt1p. Activation of Ypt1p by TRAPPI is required for membrane fusion, and thus the mechanism of nucleotide exchange by TRAPPI is a crucial step of regulation in vesicular transport. A minimal complex of five proteins (Bet5p, Trs23p, Trs31p, and 2 copies of Bet3p) is required for GEF activity, suggesting a complex interaction between TRAPPI and Ypt1p.
The large majority of GEFs accelerate nucleotide exchange from G-proteins by inserting a glutamate finger wedge into the nucleotide binding pocket. We recently solved the crystal structure of nucleotide-free Ypt1p in complex with TRAPPI, which revealed the absence of the canonical glutamate finger and suggested a novel mechanism of GEF activity by TRAPPI.
In this study, we measured the rate and equilibrium constants for nucleotide and TRAPPI binding to Ypt1p. Nucleotide binding is accelerated ~30-fold and dissociation more than three orders of magnitude, suggesting that the nucleotide binding site is more accessible in the presence of TRAPPI. Analysis of various mutant TRAPPI complexes, a Ypt1 mutant defective in nucleotide binding, and the Mg2+-dependence of exchange do not support a glutamate finger mechanism, and favor nucleotide exchange through a novel mechanism in which TRAPPI accelerates nucleotide dissociation by opening the nucleotide binding site through interactions with switch I and II. Thermodynamic linkage analysis indicates that TRAPPI binds nucleotide-free Ypt1 with greater affinity than Ypt1 with bound nucleotide. However, this increased stability cannot account for the >1000-fold increase in the rate of nucleotide exchange, indicating that GEF activity of TRAPPI is under kinetic control.