turn content. DPH and TMA-DPH anisotropy measurements revealed that the FP-TMD complex reduced membrane order in both the interface and interior regions of the bilayer, whereas FP alone increased order in both regions. The complex also increased water penetration into the bilayer (lifetime ratio of TMA-DPH in H2O and D2O) much more than for the individual peptides. While the FP decreased membrane free volume (from partitioning of C6NBDPC from micellar to bilayer phase), the presence of TMD enhanced this effect enormously. The time courses of lipid mixing (LM), content mixing (CM) and content leakage (L) were fitted globally to 3-state or 4-state sequential models (Biophys. J., 2007, 92; 4012), providing estimates of rate constants for inter-conversion between states as well as probabilities of the occurrence of LM, CM or L in each state. The FP-TMD complex inhibited the rates of initial intermediate but especially pore formation. The effect on pore formation was predominantly due to a large increase in the activation enthalpy not matched by as large an increase activation entropy. Supported by NIGMS grant 32707 to BRL.

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Both Fusion Peptide and Trans-Membrane Domain of HIV gp41 Individually Reduce the Activation Barriers for the Fusion Process

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Poly(ethylene glycol)- (PEG-) mediated fusion of 25 nm vesicles was examined in the presence of the HIV gp41 fusion peptide (FP) and trans-membrane domain (TMD) at temperatures between $17°C$ and $37°C$. Membrane lipid composition was Dioleoylphosphatidylcholine (DOPC), dioleoylphosphatidylethanolamine (DOPE), bovine brain sphingomyelin (SM) and Cholesterol (CH) (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), yielding 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 in control and peptide-containing vesicles implied that the nature of the barrier between states for all systems changed with temperature (i.e., activation enthalpy and entropy varied with temperature). In control vesicles, CM occurred earlier in the process at higher temperatures such that fusion shifts from a 4-state to a 3-state model above ($\geq 27°C$). Mainly FP but also TMD enhanced the rate of initial intermediate formation. Above about $22°C$, this resulted from a large increase in activation entropy overcoming an unfavorable large increase in activation enthalpy, suggesting that the peptides reduced exposure of water to hydrocarbon in the transition state relative to control vesicles. Both peptides enhanced the rate of final pore formation to such an extent that the fusion process followed a 3-state (single intermediate) model even at low temperature (170C). This effect was greatest for TMD and was also largely an entropic effect. Supported by NIGMS grant 32707 to BRL.

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The Trans-Membrane Domain of the SNARE Fusion Protein Syntaxin (SX) Enhances the Rate of Intermediate Formation

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During neurotransmitter release, synaptic vesicles fuse with the pre-synaptic plasma membrane, leading to the merger of two lipid bilayers and the release of neurotransmitters. This process requires formation of a complex between SNARE proteins in both membranes. One of the SNARE proteins required for membrane fusion is Syntaxin (SX), a membrane anchored protein in the pre-synaptic membrane. Since viral fusion peptides and trans-membrane domains (TMDs), which also insert into membranes, can affect rates of fusion, we hypothesized that the SX TMD may also affect fusion kinetics. Polyethylene glycol (PEG)-triggered fusion of highly curved 25 nm vesicles (SUVs) was examined in the presence of the TMD of SX (1:900 protein:lipid ratio) at temperatures between $17°C$ and $42°C$. These SUVs were composed of a mixture of DOPC/DOPE/sphingomeylin/DOPS/cholesterol (32/25/15/8/20), which closely models the lipid composition of the natural membranes. Lipid mixing (LM), contents mixing (CM) and leakage (L) time courses were fitted globally to a 3 state sequential model (Weinreb, Biophys. J., 2007), from which we obtained estimates of rate constants for conversion between states as well as probabilities of LM, CM and L for each state. Results show that the SX TMD enhanced somewhat the rate of initial intermediate formation but had an even effect on the rate of pore formation. In addition, the probability of pore formation increased in the initial intermediate relative to the final fusion pore state. Since we have shown that uncomplexed SNARE proteins enhance PEG-mediated fusion roughly as effectively as does the complex, it may be that the SX TMD helps lower the activation barrier for fusion of closely juxtaposed bilayers in vivo. Supported by GM32707 to BRL and NIGMS grants GM000678 to UNC.

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Role of Anionic Lipids on Peg-Mediated Model Membrane Fusion

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Poly(ethylene glycol)- (PEG) mediated fusion of 25 nm vesicles composed of dioleoylphosphatidylcholine (DOPC), dioleoylphosphatidylethanolamine (DOPE), bovine brain sphingomyelin (SM), cholesterol (CH) and phosphatidylserine (PS) was examined in order to investigate the effects of phosphatidylserine (PS) on the fusion mechanism. Lipid mixing (LM), content mixing (CM) and content leakage (L) measurements were carried out with vesicles containing from 0 to 10 mol% PS and similar amounts of phosphatidylglycerol (PG) as controls. Fitting these time courses globally to a 3-state (aggregate, intermediate, pore) sequential model established the rate constants for each step as well as probabilities for the occurrence of LM, CM, or L in each state. Charged lipids inhibited rates of intermediate and pore formation, with inhibition of intermediate formation being directly proportional to negative surface potential for PG but greater for PS. Inhibition of pore formation was limited up to 4% PG or PS but increased dramatically above that. Even low PG content inhibited the rapid rate of PEG-induced aggregation (detected by turbidity) and led to smaller aggregates (detected by DLS), while a slower component of turbidity increase roughly tracked the dependence on PG content of the rate of pore formation. PS or PG content above 6% also inhibited lipid mixing in the initial intermediate. We conclude that, aside from an expected effect on the rate and extent of PEG-induced aggregation, PS at physiological membrane contents alters the nature of the initial intermediate such that lipid mixing between joined monolayers is considerably reduced prior to formation of a stable fusion pore. Supported by NIH grant GM32707 to BRL.

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Snare-Mediated Fusion Between Highly Curved and Un-Curved Membranes

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During neurotransmitter release, curved synaptic vesicles fuse with the uncurved pre-synaptic plasma membrane, leading to the merger of two lipid bilayers and the release of neurotransmitters, a process that requires SNARE proteins in both membranes. Our previous modeling of this system employed two populations of highly curved vesicles (SUV), while others have used two populations of vesicles having ill-defined but likely not high curvature. In our studies, the v-SNARE synaptobrevin (SB), the t-SNARE syntaxin (SX), and SNAP-25 linked vesicles via a SNARE complex, but were unable to promote fusion without poly ethylene glycol (PEG) to force close membrane contact. We hypothesized that the geometry of the membranes may contribute to native synaptic vesicle fusion. Here we reconstitute SB into SUVs and SX into relatively uncurved (LUV) vesicles, whose composition, DOPC/DOPE/sphingomeylin/DOPS/cholesterol (32/25/15/8/20), models that of the native membranes. Lipid mixing (LM), contents mixing (CM) and leakage (L) time courses were fitted globally to 3- or 4-state sequential models, from which we obtained estimates of rate constants for conversion between states as well as probabilities of LM, CM and L for each state (Biophys. J., 2007, 92; 4012). In the absence of SNAREs, the mismatched curvature of the LUV-SUV system promotes more efficient and productive fusion events than fusion between SUVs (Biophys. J., 2010, 98, S1; 674a). LUVs containing SX (1:2250 P/L) and SUVs containing SB (1:950 P/L) still did not fuse in the absence of PEG. However with 6% PEG, the probability of CM was greatly shifted to the first step in the fusion process. The results suggest that with mismatched curvature, SNAREs may enhance rapid transient pore formation that precedes fully LM and final pore formation.

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Interfacial Protein-Lipid Interactions II

3441-Pos Board B546

Using Tyrosine to Anchor a Transmembrane Peptide

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Due to the complexities of membrane proteins, synthetic model membrane peptides have proven useful for determining fundamental peptide-lipid interactions. A frequently employed peptide design has involved a hydrophobic core of Leu-Ala residues along with polar or aromatic amino acids flanking each side to "anchor" the transmembrane orientation. For example, WALP family peptides (acetyl-GWW(LA)nLWWA-[ethanol]amide), anchored by four Trp residues, have received particular attention from both experimental