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The Mechanism of Vesicle Fusion as Revealed by Molecular Dynamics Simulations

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Membrane fusion lies at the heart of important biological processes. In vivo, membrane fusion is tightly regulated by proteins. The basic mechanism, however, is primarily determined by the physics of lipid−lipid interactions.[1] Here we describe molecular dynamics simulations used to elucidate the molecular details of the process of fusion for small lipid vesicles. The simulations are based on a coarse grained (CG) lipid model that accurately represents the lamellar state of a variety of phospholipids and enables us to observe intermediate stages during fusion at near atomic detail. The fusion intermediates found are in general agreement with the stalk−pore mechanism.[2] Transient pores sometimes form adjacent to the stalk, however, resulting in the mixing of lipids from the outer and inner monolayers. The speed of stalk formation and the opening of the fusion pore can be modulated by altering the lipid composition in qualitative agreement with experimental observations.

In CG models small groups of atoms are represented as single interaction centers. Recently CG models have been successfully used to study lipid membrane properties.[3] In the model used in this work, the headgroup consists of two polar particles for PC or PE, and two particles of intermediate polarity for the glycerol moiety. The tails are modeled by four non-polar particles.[4] Solvent is modeled explicitly, with four water molecules represented by a single CG particle. Details of the CG forcefield can be found elsewhere.[5] The lamellar state of the lipids simulated is represented very well with diverse properties such as the experimental area per lipid, atom density distributions along the membrane normal, the line tension, and the bending modulus reproduced.

In this study two unilamellar vesicles were placed in close proximity in a rectangular simulation box.[6] The vesicles were formed by spontaneous aggregation in solution,[5] and were therefore at thermodynamic equilibrium. The vesicles are small, consisting of close to 1000 lipids and measuring ∼15 nm across.[7] Various lipid compositions were studied including pure dipalmitoyl−PC (DPPC), pure palmitoyl−oleoyl−PE (POPE), and mixed systems (DPPC with 25% DPPE or 25% lysoPC). Fusion could be induced by constraining the vesicles to within a few nanometers of each other. For PE containing vesicles fusion is observed within tens of ns for distances up to ∼1.5 nm (measured from the position of the phosphate groups). Pure DPPC vesicles only fused spontaneously when placed closer together (<1 nm), the formation of the initial contact requiring more than 50 ns. Vesicles that contain lysoPC appear even more resistant to fusion. No contact formation was observed even for strongly dehydrated vesicles within 200 ns.

The fusion process is triggered by a fluctuation in one of the monolayers which results in some head groups merging with the opposing monolayer. This is a localized phenomenon involving only a few lipids (Figure 1). From this point onwards the interaction is attractive, and no constraints need to be applied. Due to the high curvature of the vesicles the fusion occurs very fast, on a nanosecond timescale. Fusion was found to proceed via two pathways. Pathway I is illustrated in Figure 2. This shows stages in the fusion of two small vesicles each consisting of 75% DPPC and 25% DPPE. The initial contact quickly expands radially (∼10 ns), forming a so-called stalk intermediate. The stalk intermediate is stable only for a short time (∼10 ns), before being replaced by a hemifusion diaphragm (HD) in which the inner monolayers have merged. Typically 5 to 15 ns after the HD forms a small fusion pore appears. Once this happens the bilayer ruptures, completing the fusion process.[8] The whole process is in line with the stalk−pore fusion mechanism.[2] As predicted theoretically,[9] the high energy of the stalk intermediate is solved by tilting of the tails, avoiding empty voids. However, in some simulations mixing of the outer and inner monolayers was observed, which is not accounted for in the stalk−pore model. Instead of a radial expansion of the initial contact into the stalk intermediate, as in pathway I, the contact appears to bend, forming a banana-shaped stalk (pathway II). The head groups "escape" from the stalk center by inducing a pore in one of the two vesicles (Figure 3). Such intermediates were predicted previously from lattice models and observed in Brownian dynamics simulations of lipid-like molecules.[10] They result from a reduced line tension in the vicinity of the bent stalk. This pore is transient, with a lifetime of less than 5 ns. After the pore seals, a HD structure forms as in pathway I. This is in contrast to the predictions of the simple models.[10] The time required to reach the HD state is significantly longer via pathway II (typically around 50 ns). There is also an important structural difference between the HD in the two pathways. Whereas in pathway I both vesicles contribute lipids to the HD, in pathway II almost all lipids involved

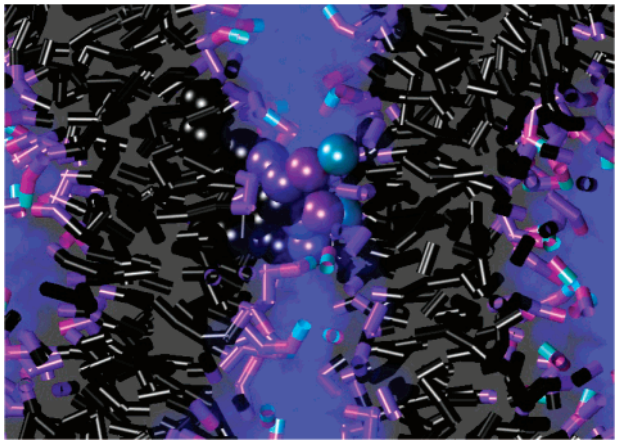

Figure 1. Initial contact of two fusing vesicles. The two lipids drawn in sphere representation trigger the fusion process.

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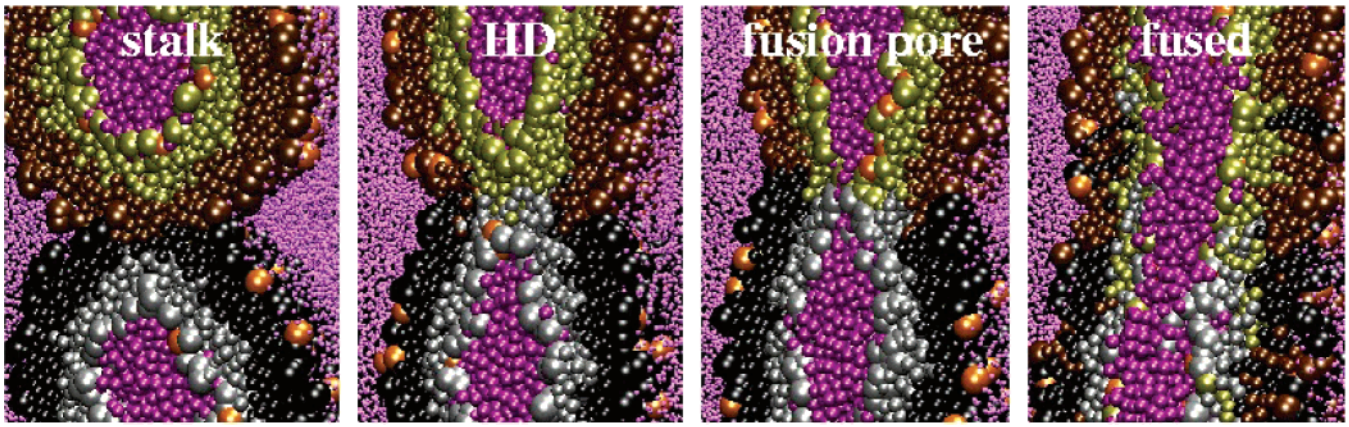

Figure 2. Intermediate structures during the fusion of two mixed PC/PE vesicles. Lipid headgroups are represented by large spheres, different colors distinguish between lipids in the inner and outer monolayer and between the two vesicles. Orange spheres denote the amine site of PE, purple spheres, water.

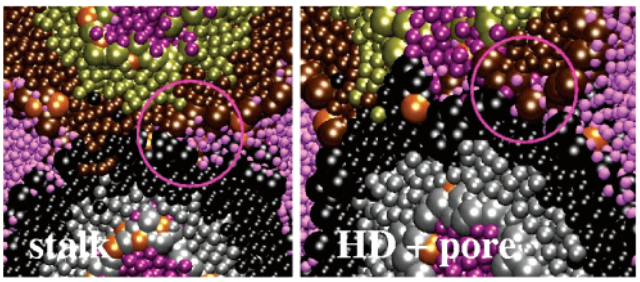

Figure 3. Close up of the stalk and HD formed in pathway II. Water and headgroups trapped in the stalk center induce a transient pore (encircled).

are from a single vesicle (Figure 3). The subsequent formation of the fusion pore and the swelling into a tubular vesicle is similar in both pathways. Due to the formation of this transient pore in pathway II, the inner monolayer of the fused vesicle is mixed and contains lipids originating from the outer monolayer of both vesicles. This modified stalk−pore mechanism can explain the mixing of monolayer content seen experimentally.[11a,b] The pore formation also makes the vesicles leaky during fusion, in accordance with experimental results.[11b,c] However, during pathway I an increased exchange of solvent was also observed during the transition from the stalk to the fusion pore.

Surprisingly, a given system can follow either pathway. For the mixed PC/PE system two out of six fusion events followed pathway I. For pure POPE vesicles pathway I occurred in two out of four attempts. Further study is required to understand pathway preference. In either pathway, there is a significant difference in the stability of the HD of the pure POPE system vs that of the mixed PC/PE system. Whereas a fusion pore appears quickly (5−15 ns) in the mixed case, the hemifused state was stable for at least 100 ns in three out of four simulations involving pure POPE. For pure DPPC vesicles, formation of a HD was never observed. Once the initial contact is formed, it quickly expands radially as in pathway I. The system remained trapped in the stalk state for the entire period simulated (200 ns). LysoPC, when present in the inner monolayer only, is found to accelerate fusion by strongly destabilizing the HD. The effect of composition on the ability of the vesicles to fuse is in qualitative accordance with the experimental observation[1a] that negatively curved lipids (such as PE) promote fusion when present in the contacting monolayers and inhibit fusion when present in the distal monolayers. Exactly the opposite is observed for positively curved lipids (such as lysoPC). Theoretical mean field calculations[1a] show that lipids with negative curvature lower the free energy of stalk formation and increase the free energy of pore formation in the HD. Mixed systems, especially systems enriched with lysoPC in the inner- and PE in the outer monolayers fuse most readily, in agreement with our simulations.

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Supporting Information Available: Details of the force field and additional pictures of the fusion process (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(4) The pairwise interactions between the CG atoms are modeled by Lennard-Jones (LJ) interactions. They range from weak (hydrophobic interactions) to strong (polar interactions) with three levels in between. A screened Coulombic interaction is used for the electrostatic interaction between the zwitterionic headgroups. Both LJ and Coulombic interactions are only short-ranged, using a shift-based cutoff of 1.2 nm. Soft springs between bonded pairs keep the molecule together. Angle potentials provide the adequate stiffness for the lipid tails.

(5) Marrink, S. J.; Mark, A. E. *J. Am. Chem. Soc.* **2003**. Manuscript submitted.

(6) The systems are simulated using the GROMACS software (Lindahl, E.; Hess, B.; Spoel, D. *J. Mol. Mod.* **2001**, *7*, 306) with a time step of t = 200 fs. The time scale in the CG model is scaled to reproduce the diffusion rate of lipids and water. Constant temperature (T = 300) and isotropic pressure (P = 1 atm) conditions were applied which keep the lipids in the biologically relevant fluid state.

(7) Experimentally, smallest vesicles obtained are ∼20 nm diameter. Cornell, B. A.; Fletcher, G. C.; Middlehurst, J.; Separovic, F. *Biochim. Biophys. Acta* **1982**, *690*, 15.

(8) The resulting vesicle slowly relaxes (∼100 ns) into a tubular structure, a process limited by the rate of adsorption of external water. Lateral diffusion of the lipids causes mixing of the lipids in both monolayers, but not between the inner and outer monolayers. Lipid flipflops are absent on the time scale of the simulations, preventing the tubular vesicle from becoming spherical.

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