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Erratum

Computational insight in the role of fusogenic lipopeptides at the onset of liposome fusion

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ABSTRACT

We performed an extensive computational study to obtain insight in the molecular mechanisms that take place prior to membrane fusion. We focused on membrane-anchored hybrid macromolecules (lipid–polymer–oligopeptide) that mimic biological SNARE proteins in terms of liposome fusion characteristics [H. Robson Marsden et al., 2009]; efficient micro-second simulation was enabled by combining validated MARTINI force fields for the molecular building blocks in coarse-grained molecular dynamics (CGMD). We find that individual peptide domains in the hybrid macromolecules bind and partially integrate parallel to the membrane surface, in agreement with experimental findings. By varying several experimental design parameters, we observe that peptide domains remain in the solvent phase only in two cases: (1) for solitary lipopeptides (low concentration), below a threshold area per lipid in the membrane, and (2) when the lipopeptide concentration is high enough for the peptide domains to self-assemble into tetrameric homo-complexes. The peptide-membrane binding is not affected by solvent-induced peptide unfolding, which we mimicked by relaxing the usual MARTINI helix constraints. Remarkably, in this case, a reverse transition to a helical secondary structure is observed after binding, highlighting the role of the membrane as a template (partitioning-folding coupling). Our findings undermine the current view of the initial stages towards fusion, in which membranes are thought to be kept in close apposition via dimerization of individual complementary peptides in the solvent phase. Although we did not study actual fusion, our simulations show that the formation of homomers, which is suppressed in experimental peptide pair design and therefore believed to be insignificant for fusion, by peptides anchored to the same membrane does play a key role in this locking mechanism and potentially also in membrane destabilization that precedes fusion.

1. Introduction

It is generally accepted that a specialized set of proteins plays a key role in overcoming energetic barriers that have been identified for lipid membrane fusion [1]. Understanding membrane fusion thus implies obtaining detailed molecular information on the role of the 'actors' in fusion, in particular on the membrane–protein interaction, protein/protein association, membrane destabilization and merging aspects of the fusion mechanism. One way to reduce the complexity of this task is to introduce a simplified model system that contains only necessary ingredients for biomimetic fusion. Recently Kros [2,3] introduced such a model system with desired fusion characteristics, both lipid and content mixing [3], that holds a promise for targeted drug delivery. This model system consists of liposomes that are decorated with fusogenic lipopeptides LPE or LPK, containing short peptide domains E: $(EIAALEK)_3$ and K: $(KIAALKE)_3$ that induce fusion via an unresolved mechanism that involves peptide self-assembly into E/K coiled-coils. Even for such a simplified fusion model, fundamental understanding is hampered by several unknowns: the protein secondary and quaternary transformations during self-assembly and membrane fusion, the protein interaction with the bilayer and the role of the solvent (e.g. Ca^{2+} ions) in fusion. One key issue in most experimental characterization techniques is that, by probing properties of ensembles of molecules, details at the molecular level remain elusive. X-ray crystallography faces the solubility problem of membrane-associating proteins and other imaging techniques lack appropriate resolution (e.g. NMR). As a result, the atomistic structures of both the membrane and protein complex, before and after assembly as well as during fusion, are difficult to determine.

Since molecular simulation methodology has in recent years matured into a reliable and increasingly efficient tool for studying molecular mechanisms, we believe that computational study can complement the experimental investigations by shedding light into the molecular mechanisms that trigger fusion. We therefore performed a detailed computational study of fusogenic lipopeptides LPE and LPK prior to

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fusion, concentrating on the overall lipopeptide binding behavior. We use the computational flexibility to assess several anticipated key factors independently, investigating their function in the general locking and membrane destabilization mechanisms. Our starting point is the lipopeptide anchored to a membrane, with a lipid/cholesterol composition that matches the experimental conditions [3].

Averaging over molecular degrees of freedom is unavoidable if one wants to realistically capture slow phenomena on a supramolecular scale. The size of the relevant molecular system, combined with the long (μs) simulation time, dictates the use of a coarse-grained (CG) simulation methodology. In the spirit of an earlier lipopeptide study [4], we employed validated coarse-grained MARTINI force fields for lipids [5], proteins [6] and polymers [7,8] and combined them into a unified force field for the hybrid lipopeptide. The coarse-grained MARTINI [5] force-field has been successfully used for simulating a variety of systems, including the longer-term dynamics of an antimicrobial lipopeptide [4] and other trans-membrane proteins [9,10], and, very recently, membrane associating proteins [11,12], near a lipid membrane. Moreover, MARTINI is known to accurately reproduce the structural and collective properties of a variety of lipids in the lamellar state [13]. We applied the standard MARTINI constraints to restrain the secondary structure of the two CG peptide domains to the resolved α-helix for the E/K dimer (by NMR), but also considered the effect of relaxing these constraints. Moreover, via simulations that exceed 500 μs total simulation time (see SI for a table), we have studied the significance of experimental factors such as the lipopeptide concentration, the average area per lipid in the membrane and the length of the polymeric spacer between the lipid and the peptide. Whenever possible, results have been compared to detailed measurements. Because of the good agreement between experiments and simulations, we conclude that this CGMD study provides the first detailed insight in the molecular factors that underlie the initial stages of membrane fusion.

2. Methods

All simulations were carried out with the MARTINI coarse-grained model [5–7], using the Gromacs MD package (version 4.5.3) [14], with the usual MARTINI coarse-grained molecular dynamics conditions: time step t = 20 fs, semi-isotropic pressure coupling P = 1 atm and Berendsen thermostat T = 293 K.

The lipopeptide [2,15] is a hybrid macromolecule that links a DOPE lipid with a peptide (E or K) at the C terminus, via a linker formed by one succinic anhydride and a PEG polymer, which can be of varying length. We select a length of 12 monomers, in agreement with the standard experimental setup, but consider other values in the Supporting Information (SI). Our MARTINI representation of the $LP_{12}K$ lipopeptide is shown in Fig. 1. It combines existing representations of the separate domains — lipid [5], protein [6] and polymer [7] — into a unified MARTINI hybrid force-field (see SI for more details). The α-helicity of the peptide domains is imposed through dihedral potentials, with the usual force constant $k = 400$ kJ mol^{-1}, along the backbone beads. The linkers between the domains were treated as covalent bonds with controlled rigidity. Our approach is equivalent to the one applied previously for PEGylated lipids [7] and a shorter lipopeptide [4], but it is for the first time that a three-component (lipid–polymer–peptide) hybrid is simulated.

The membrane consists of 192 DOPC and 96 DOPE lipids, and 96 cholesterol molecules, with relative fractions (2:1:1) chosen to agree with experimental conditions [15]. It was created from an equilibrated pure DOPC membrane [16], by changing a pre-defined fraction of DOPC into DOPE and cholesterol, keeping an equal distribution in the two leaflets. Technically, for DOPE, we changed the NC_3 into the NH_3 bead type and replaced the double bond from each lipid tail by a single bond. Cholesterol is generated by selecting only eight beads from a DOPC lipid and re-assigning their bead types, bonds and angles specific to cholesterol (see Fig. S1 in the SI). Consequently, the membrane is solvated by 10,000 coarse-grained water molecules, with 725 sodium cations and 725 chloride anions (see SI for more information on the salt representation). The setup contains approximately 16,500 coarse-grained particles. The membrane is equilibrated for 5 μs until the structural properties (area per lipid and bilayer thickness) assume constant values. The final area of the simulation box parallel with the standard membrane is 10 nm × 10 nm.

After membrane equilibration, a lipopeptide is incorporated into the membrane–water system, by replacing one of the membrane DOPE lipids with the DOPE domain in the lipopeptide, followed by a removal of overlapping water or ion molecules. A short run is performed, with the lipopeptide fixed, to allow for solvent rearrangement. Subsequently the run is continued with all constraints released and this part of the trajectory is used for analysis. An example of the whole system in the simulation box is shown in Fig. 2.

The sensitivity to initial lipopeptide conformations was considered by simulating ten different instances for each lipopeptide, unless mentioned otherwise; convergence to the same final state was identified in all cases. The starting structures for each peptide were generated by taking ten instances of the NMR solution structure of the heterodimeric coiled–coil complex E–K, PDB ID: 1U0I [17]. The lipopeptide is always initially anchored perpendicular to the membrane, with the polymer linker in an extended conformation.

Atomistic molecular dynamics studies have thus far focused on static and/or short-term dynamical properties, such as the role of stabilizing interactions, and the effect of local amino-acid replacement or

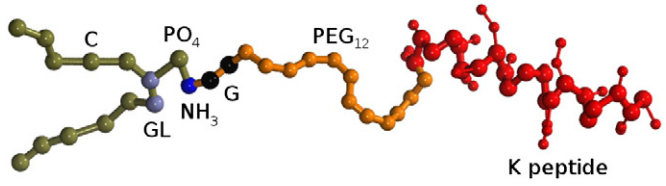

Fig. 1. The coarse-grained model of the lipo-peptide $LP_{12}K$. The lipid is depicted with tan tails and blue/tan/ice blue colored beads for the choline/phosphate/glycerol moieties. The PEG beads are orange and the succinic anhydride is black. The K peptide is represented with red beads, where the size indicates backbone (big) or side chain (small) particles. In the remainder, we use a blue bead representation for the E peptide.

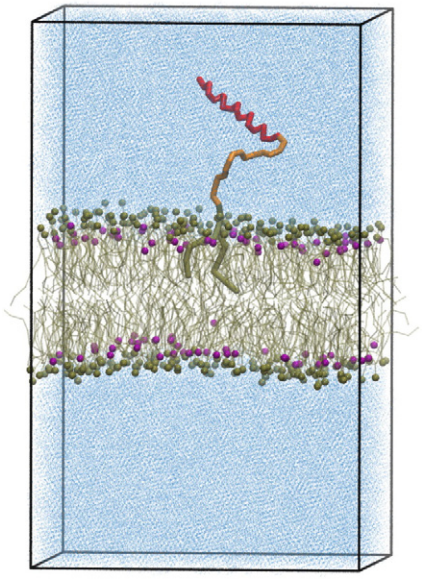

Fig. 2. An example of the simulation box consisting of membrane, $LP_{12}K$ lipo-peptide and solvent. Colors in the $LP_{12}K$ model are used to distinguish between: the peptide (red backbone), the polymer spacer (orange) and the DOPC lipid anchor (tan). In the membrane only the phosphate and the cholesterol ROH beads are displayed in tan and purple. Water and ion particles are shown as a blue haze.