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Interdigitation of long-chain sphingomyelin induces coupling of membrane leaflets in a cholesterol dependent manner

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ABSTRACT

It has been a long-standing question how the two leaflets in a lipid bilayer modulate each others' physical properties. In this paper, we discuss how this interaction may take place through interdigitation. We use atomistic molecular dynamics simulations to consider asymmetric lipid membrane models whose compositions are based on the lipidomics data determined for exosomes released by PC-3 prostate cancer cells. The simulations show interdigitation to be exceptionally strong for long-chain sphingomyelin (SM) molecules. In asymmetric membranes the amide-linked chain of SM is observed to extend deep into the opposing membrane leaflet. Interestingly, we find that the conformational order of the amide-linked SM chain increases the deeper it penetrates to the opposing leaflet. Analysis of this finding reveals that the amide-linked SM chain interacts favorably with the lipid chains in the opposite leaflet, and that cholesterol modulates the effect of SM interdigitation by influencing the conformational order of lipid hydrocarbon chains in the opposing (cytosolic) leaflet.

1. Introduction

Membrane proteins perform a variety of functions in cells. However, instead of functioning just on their own, a growing body of evidence has shown that membrane proteins carry out their functions together with lipids, whose purpose is to provide the proteins with a physical environment that renders them functional, promotes the formation of protein complexes, or even modulates protein conformations in terms of direct lipid–protein interactions [1–3]. Here, the organization of lipids in the vicinity of proteins plays a decisive role, since cell membranes are highly heterogeneous both in the membrane plane and across the membrane [4]: the compositions of nanoscopic lipid clusters/domains can be quite different along the membrane plane, and the distinctly different compositions and concentrations of lipids in the extracellular and cytosolic leaflets increase the complexity of membranes even further.

Nanoscale lipid clusters/domains have been studied quite substantially and shown to be biologically relevant [5,6]. Meanwhile, the causes and consequences of the asymmetric transmembrane lipid distribution [4] are much less understood, yet clearly the asymmetric nature of lipid distribution is also biologically important given that cells spend considerable resources to maintain it.

Recent studies have suggested that transmembrane asymmetricity may be coupled to in-plane domain organization in a manner where lipid domains (in either the liquid-disordered (Ld) or liquid-ordered (Lo) phase) in the extracellular and cytosolic leaflets may potentially be coupled [7–10]. However, the mechanisms driving this coupling are not well understood. A great deal of the discussion has focused on the possibility of phase symmetry or asymmetry (for discussion, see e.g. [10]), meaning that the phases of membrane domains in the two leaflets would be similar (either Lo or Ld phases in both) or different (Lo and Ld domains directly opposite to each other in the two membrane monolayers). Here, too, it is largely unclear which lipid compositions lead to phase symmetry/asymmetry, and what are the mechanisms that give rise to it.

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Experimentally, there are many studies that have focused on clarifying these questions [11–17]. However, as experiments have usually been done with fluorescent dyes, their influence on membrane behavior and the observed findings remains unclear. Perhaps more important, however, is the fact that experiments for asymmetrically distributed lipid membranes are typically carried out under non-equilibrium conditions promptly after the formation of the asymmetric membranes, to avoid flip-flops that in the course of time render the membranes symmetric. For these reasons, the interpretation of experimental data is not straightforward.

Here we consider the coupling of membrane leaflets from atomistic point of view. Already decades ago it was found that lipids may have a tendency to extend their hydrocarbon chains to the opposing leaflet (see e.g., [18,19]). While the biological as well as physical implications of this phenomenon known as interdigitation have remained unclear, intuitively it is reasonable to assume that increasing interdigitation would increase the (shear) viscosity between membrane leaflets, and atomistic simulation results support this view [20]. Theoretical work based on phenomenological models also suggests that lipid chain interdigitation contributes to the coupling between the two leaflets in lipid bilayers [7]. Given the possibility that interdigitation has an important role to play in membrane domain coupling, there is a need to consider this matter through detailed and realistic models in the nanoscale, without undesired effects due to membrane perturbing dyes and non-equilibrium effects.

We use molecular dynamics (MD) simulations to assess how the extracellular and cytosolic leaflets of cell membranes interact through interdigitation. To this end, we consider model membrane systems that are as realistic as possible. In particular, we have studied membranes based on the composition of exosomes as revealed by lipidomic analyses of PC-3 cell-derived exosomes [21,22]. We aim to identify lipid compositions that give rise to interdigitation, and to clarify in atomistic details the role of interdigitation in the coupling of membrane domains in the two leaflets. The simulation results show that there is one particular lipid type, long-chain sphingomyelin (SM) in the extracellular leaflet, whose interdigitation is exceptionally strong and extends deep into the opposing leaflet. Cholesterol (CHOL) is found to modulate the effect of SM by influencing the conformation order of lipid hydrocarbon chains in the opposing leaflet. The findings suggest that specific lipids can contribute significantly to the registration of nanoscale membrane domains in the opposing leaflets and hence locally stabilize the lipid composition around membrane inclusions such as membrane proteins.

2. Models and methods

2.1. Simulation models

The compositions of the lipid membrane systems chosen for atomistic simulations carried out in this work were based on recently published quantitative lipidomics analyses of 280 lipid species in exosomes released by PC-3 prostate cancer cells [21]. Interestingly, exosomes were enriched in CHOL, sphingolipids (both glycosphingolipids and SM), and phosphatidylserine (PS) compared to mother cells. Furthermore, some particular species of these lipids were enriched in exosomes. In particular, a high enrichment was observed in particular for glycosphingolipid species although their total concentration was low. Also, PS 18:0/18:1 was enriched compared to other PS; phosphatidylethanolamine (PE) with the 18:1 *sn*-2 chain was enriched compared to other PE lipids; and a slight increase of SM with the 24:0 chain and a slight decrease of SM with the 16:0 chain were also identified.

For the construction of the simulation models, we used the above lipidomics data as a basis. Furthermore, we made use of the well-known fact that SM resides mainly in the outer (extracellular) leaflet, while PS and PE are almost fully in the inner (cytosolic) monolayer [23,24]. As to the transmembrane distribution of CHOL, it is generally considered that CHOL resides mainly in the outer leaflet due to its strong interactions with sphingolipids, though a different opinion has also been suggested [25]. Here we favored the view of having more CHOL in the outer monolayer compared to the inner one, but for completeness we studied a number of scenarios through models where the relative concentration of CHOL in the outer and inner leaflets was varied. Finally, phosphatidylcholines (PCs) were distributed in the two leaflets in such a manner that the resulting lipid compositions resulted in flat bilayers.

Based on this reasoning, we considered 14 different lipid bilayer models with varying lipid compositions, with an asymmetric lipid distribution between the two leaflets (Table 1). Each of the systems was studied through atomistic 200 ns MD simulations. In three of the 14 models (M1, M2, M3), the inner leaflet mimicking the composition of the cytosolic leaflet of a eukaryotic plasma membrane was composed of PS (36 mol%), PE (19 mol%), PC (9 mol%), and CHOL (36 mol%). Meanwhile, the outer monolayer mimicking the composition of the extracellular leaflet was composed of SM (30 mol%), PC (24 mol%), and CHOL (46 mol%). What differentiated models M1–M3 was the type of SM: it was d18:1/16:0 in M1, d18:1/24:0 in M3, and a 1:1 mixture of d18:1/16:0 and d18:1/24:0 in M2.

The next two models (M4, M5, Table 1) had a quite similar composition of phospholipids as in model M3 but the transmembrane distribution of CHOL was reverted between the two leaflets. The remaining nine models (M6–M14; Table 1) were also asymmetric, but they were chosen to be less complex. In these cases the individual leaflets were composed of only 1–2 different lipid components to better understand the importance of each lipid type in exosomes.

In this paper, we have used the convention [A]/[B] to describe the contents of the lipid bilayers, where A is the lipid composition of the inner monolayer, and B is the lipid composition of the outer membrane leaflet.

2.2. Preparation of asymmetric bilayers

To create the asymmetric lipid bilayer models (with regard to distribution of lipids in the two leaflets), we first constructed symmetric models (Table S1), reflecting the compositions of all individual leaflets used in the asymmetric lipid bilayer models (Table 1). Altogether, 14 symmetric models (S1, S2, …, S14) were constructed and simulated. The initial structures of the symmetric bilayers were obtained by modifying the structures of lipids in models described in our previous papers [26,27]. Modifications were performed gradually by adding or removing one heavy atom in the lipids, one atom at a time, and performing a cycle of structure optimization with 500–1000 steps by the steepest descend algorithm and a short (1 ns) molecular dynamics simulation run for equilibration. For each of the symmetric models, we performed 50–100 ns molecular dynamics simulation to equilibrate the surface area of each symmetric bilayer, the objective being to find the number of lipids with a given lipid composition to result in a desired membrane area. As the areas of these constructed models did not match one another to form a flat asymmetric bilayer, we created asymmetric membranes by removing excess lipids such that the areas of the two leaflets in a given asymmetric bilayer were equal. Having done this, we equilibrated the models again. Through this procedure, for each lipid composition in Table S1 we determined the number of lipids in a monolayer that generated the same membrane area, such that we were able to join monolayer leaflets together to create flat asymmetric lipid bilayers (whose radius of curvature was expected to be infinite).

The constructed models were composed of ~265 lipid molecules and 8000 water molecules. When needed, Na^+ counter-ions were added to neutralize the charges of the systems.

2.3. Simulation methods

For lipids and ions, we used the all-atom OPLS (Optimized Parameters for Liquid Simulations) force field [28] using additional parameters