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Long-chain GM1 gangliosides alter transmembrane domain registration through interdigitation

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ABSTRACT

Extracellular and cytosolic leaflets in cellular membranes are distinctly different in lipid composition, yet they contribute together to signaling across the membranes. Here we consider a mechanism based on long-chain gangliosides for coupling the extracellular and cytosolic membrane leaflets together. Based on atomistic molecular dynamics simulations, we find that long-chain GM1 in the extracellular leaflet exhibits a strong tendency to protrude into the opposing bilayer leaflet. This interdigitation modulates the order in the cytosolic monolayer and thereby strengthens the interaction and coupling across a membrane. Coarse-grained simulations probing longer time scales in large membrane systems indicate that GM1 in the extracellular leaflet modulates the phase behavior in the cytosolic monolayer. While short-chain GM1 maintains phase-symmetric bilayers with a strong membrane registration effect, the situation is altered with long-chain GM1. Here, the significant interdigitation induced by long-chain GM1 modulates the behavior in the cytosolic GM1-free leaflet, weakening and slowing down the membrane registration process. The observed physical interaction mechanism provides a possible means to mediate or foster transmembrane communication associated with signal transduction.

1. Introduction

Cells invest substantial resources to synthesize a broad range of membrane lipids and to maintain their asymmetric inhomogeneous distributions in biological membranes [1,2]. In addition to roles in maintaining membrane stability and fluidity, awareness is growing that the headgroups of glycosphingolipids (GSLs) are of particular relevance for recognition processes, especially by tissue lectins [3–5]. Notably the presence of glycolipids and the mode of presentation are of critical significance to serve as functional counterreceptors [6]. In this respect, organization of lipids as in-plane membrane domains has been studied for decades [7–9], yet an understanding of domain structure and composition, and the biological function of domains overall, is still quite incomplete. The concept of lipid rafts [10–12] (functional nanoscale domains rich in cholesterol, saturated phospholipids, and bioactive lipids such as gangliosides) as well as different interpretations of research data [13] concur with the existence of functional membrane units comprised of proteins and lipids, where lipid-protein interactions modulate several aspects of protein activity [14]. This suggests that membrane-associated proteins can reorganize their membrane environment to be rich in lipids that modulate protein features dynamically. In comparison, under protein-free conditions the existence of membrane domains with different lipid compositions and physical properties is well established [15]. Studies on model membranes have unambiguously shown macroscopic phase separation into cholesterol-rich liquid-ordered (L_o) and cholesterol-poor liquid-disordered (L_d) domains in a manner [16,17], where the phase separation in the bilayer plane arises from lipid-dependent liquid-liquid immiscibility.

Due to their already noted asymmetry, the two leaflets of biological membranes are also different in composition [1,2]. The lipid mixture that is typical for the extracellular leaflet of eukaryotic plasma membranes contains sphingolipids with a high percentage of cholesterol. It tends to phase separate when reconstituted in model membranes. In contrast, such phase separation does not take place for the lipid mixture that represents the cytoplasmic leaflet enriched in, e.g., unsaturated lipids [18,19].

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The distinctly different transmembrane compositions of biological membranes can lead to phase asymmetry or antiregistration, meaning that L_o and L_d domains overlap each other in the two opposite leaflets [20,21]. Meanwhile, a domain in one leaflet can induce formation of a similar domain also in the opposite leaflet (i.e., both being in the L_o or L_d phase), leading to phase symmetry or registration [20–22]. Several mechanisms for protein-independent domain induction between membrane leaflets have been proposed, including interfacial energy minimization [23], electrostatic coupling [20], lipid and cholesterol flip-flop [20,24], composition-curvature coupling [25,26], and dynamic chain interdigitation [20,27,28]. However, despite considerable research efforts, there is no consensus on whether biological membranes are phase symmetric or asymmetric. Furthermore, the exact mechanism of induced domain formation remains undetermined.

There is evidence in favor of both bilayer registration and antiregistration [18,20,23,29] but the overall understanding of their causes is still unclear. This partly stems from difficulties to carry out robust and unambiguously interpretable experiments on domains in biological membranes, where, for instance, one applies methods such as detergent extraction that is unable to characterize the phases in individual bilayer leaflets. On the other hand, experiments on asymmetric model membranes are carried out under non-equilibrium conditions, right after the formation of an asymmetric membrane, since lipid flip-flops quite rapidly lead to transbilayer symmetry. Although these studies have significantly enhanced our current understanding of this topic, the interpretation of experimental data is admittedly not straightforward.

Theoretical and simulation studies provide another means to explore the coupling between membrane domains in different leaflets and to elucidate the physical principles that lead to domain registration and/or antiregistration [20,26,30–33]. These studies have suggested that the coupling of phase behavior between the leaflets is highly sensitive to lipid composition, yet it is not well understood why certain lipid mixtures induce domain registration whereas others do not.

Based on the concept of the sugar code that ascribes informational contents to glycans presented by scaffolds (sphingolipids, proteins) [34–36], giving special attention to GSLs is warranted. GSLs are integral components of ordered lipid domains [37,38], and their carbohydrate headgroup is linked to a ceramide moiety, exclusively sorted to the extracellular leaflet of plasma membranes, where they can engage in biorecognition [39,40]. We here focus on ganglioside GM1, a so-called true factotum of nature [41], because it is a counterreceptor for adhesion/growth-regulatory galectins, hereby involved for example in effector/regulatory T cell communication, lectin endogytosis, neuritogenesis, and neuroblastoma growth arrest [42–47]. To efficiently do so, positional aspects (density and presentation) appear crucial [48]. Notably, the interaction with the human bioeffector has also been analyzed in model membranes without proteins [49]. Looking at the structure of GSLs, a source of variability concerns the length of the acyl chain. While the majority of phospholipids have acyl chains with 16 to 18 carbons, GSLs can typically have considerably longer chains that consist of 24 carbons or more [50–52], allowing GSLs to pack tightly into ordered membrane domains. The long hydrocarbon chains may promote greater exposure of the carbohydrate headgroup and therefore enhance ligand binding of GSLs [51]. In fact, the routing signal for apical and axonal transport of glycoproteins by galectin-4, i.e. the sulfatide headgroup, is presented preferentially by ceramide with long (C24)-chain fatty acids [52–54]. As a further consequence, a long tail can extend into the opposite bilayer leaflet through interdigitation and thereby provide a potential means for signal transduction [50]. Given that contribution by both modes of placement of the long acyl chain can be of physiological relevance, consideration of the effect of GSL chain length on membrane registration is of interest.

To address this issue, we carried out a series of atomistic and coarse-grained molecular dynamics (MD) simulations on many-component membranes with GM1 to unravel how its acyl chain length (ranging from 16 to 30 carbons) contributes to membrane registration. We found that long-chain GM1 interdigitates to a substantial degree to the opposite membrane leaflet and alters its phase behavior.

2. Methods

2.1. Atomistic simulations of symmetric bilayers

We used all-atom MD simulations to consider eight types of model membranes that were composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (202 molecules), GM1 (14 molecules), and cholesterol (Chol) (72 molecules). These eight systems were also simulated in the absence of cholesterol. The molar concentrations of GM1 (POPC) were therefore 4.9 (70.1) and 6.5 (93.5) mol% in systems with and without cholesterol, respectively. Cholesterol concentration was 25 mol% in the three-component system (Table 1). The transmembrane lipid distributions were in all systems symmetric. The factor that differentiates the eight types of bilayers is the length of the fatty acid acyl chain of GM1: it ranged from palmitic acid (16:0, referred to as $GM1_{16}$) to melissic acid (30:0, referred to as $GM1_{30}$). Given the abundance of both POPC and cholesterol, the POPC/cholesterol/GM1 mixture provides an appropriate basis to consider the effects of GM1 in a system, whose phase behavior has been suggested to be rich, especially when it is extended with other lipid components (see, e.g., [55]). Fig. 1 shows the chemical structure of all lipid molecules involved in this study. Bilayers were extensively hydrated with 13,785 water molecules, and 14 Na^+ counterions were added to neutralize the excess charge of GM1.

Lipid molecules were described by the all-atom OPLS force field [56] with recent extensions for carbohydrates [57] and lipids [58–61]. Water was described by the TIP3P model [62] that is compatible with the OPLS-AA force field. Prior to MD simulations the systems were energy-minimized using the steepest-descend algorithm. For each of the 16 systems, an MD simulation of 400 ns was conducted using the GROMACS 4.6.6 software package [63]. The first 100 ns period of the trajectories was considered as equilibration and the last 300 ns of the trajectories were used for analyses.

Simulations were performed in the isobaric-isothermal (NpT) ensemble (310 K and 1 bar). The temperature of the system was maintained by the velocity-rescaling (v-rescale) thermostat with a time constant of 0.1 ps [64]. The temperatures of the solute and the solvent were controlled independently. The pressure of the system was maintained semiisotropically by the Parrinello–Rahman barostat with a 1 ps time constant [65]. The time step for integration of equations of motion was set to 2 fs. Periodic boundary conditions were imposed in all three dimensions. For the long-range electrostatic interactions, the particle-mesh Ewald (PME) method was used [66]. Lennard–Jones interactions were cutoff at a distance of 1.0 nm. The linear constraint solver (LINCS) algorithm was used to preserve covalent bond lengths [67].

2.2. Coarse-grained simulations of asymmetric bilayers

To study the effect of acyl chain length of GM1 on membrane phase behavior, we employed the coarse-grained Martini model [68–70]. A bilayer consisting of 1348 lipids (674 in each leaflet) was first constructed as a random arrangement of 30 mol% palmitoylsphingomyelin (PSM), 40 mol% dilinoleylphosphatidylcholine (DLiPC), and 30 mol% cholesterol (Fig. 1). This system was then used for preparing four systems with varying concentration and acyl chain length of GM1 (Table 1). First, either 1.5 or 6.0 mol% of GM1 was inserted into the upper leaflet by randomly replacing existing lipids from this leaflet with GM1 in a manner where the 30:40:30 ratio between PSM, DLiPC, and cholesterol, respectively, was maintained. Second, the fatty acid attached to these GM1 molecules was chosen to be either five (referred to as $GM1_{short}$) or eight beads (referred to as $GM1_{extended}$) long (Fig. 1). The "short" and "extended" cases correspond to GM1 molecules with an acyl chain of ~18 and ~30 carbons, respectively. The ternary DLiPC/PSM/cholesterol