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Characterization of thylakoid lipid membranes from cyanobacteria and higher plants by molecular dynamics simulations

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ABSTRACT

The thylakoid membrane is mainly composed of non-common lipids, so called galactolipids. Despite the importance of these lipids for the function of the photosynthetic reaction centers, the molecular organization of these membranes is largely unexplored. Here we use multiscale molecular dynamics simulations to characterize the thylakoid membrane of both cyanobacteria and higher plants. We consider mixtures of up to five different galactolipids plus phosphatidylglycerol to represent these complex membranes. We find that the different lipids generally mix well, although nanoscale heterogeneities are observed especially in case of the plant membrane. The fluidity of the cyanobacterial membrane is markedly reduced compared to the plant membrane, even considering elevated temperatures at which thermophilic cyanobacteria are found. We also find that the plant membrane more readily undergoes a phase transformation to an inverted hexagonal phase. We furthermore characterized the conformation and dynamics of the cofactors plastoquinone and plastoquinol, revealing of the fast flip-flop rates for the non-reduced form. Together, our results provide a molecular view on the dynamical organization of the thylakoid membrane.

1. Introduction

The thylakoid membrane is essential for most forms of life. It has the special capability to perform photosynthesis, the process in which solar energy is harvested and converted into biochemical energy. The thylakoid membrane is located inside chloroplasts and in the lumen of cyanobacteria. Only the light-dependent reactions of photosynthesis, in which ATP and NADPH are generated, take place in the thylakoid membrane. Photosynthesis is the result of a complex interplay between the proteins embedded in the thylakoid membrane, the lipids that make up the thylakoid membrane and a set of cofactors [1,2]. The most important photosynthetic proteins are photosystem II (PSII) and photosystem I (PSI) with the associated light-harvesting complexes (LHC), the cytochrome b_6/f complex and ATP synthase.

Four unique lipid classes make up the thylakoid membrane: phosphatidylglycerol (PG) (~13%), digalactosyldiacylglycerol (DGDG) (~32%), monogalactosyldiacylglycerol (MGDG) (~40%) and sulfoquinovosyldiacylglycerol (SQDG) (~15%) [2,3]. This composition is highly conserved in oxygenic organisms, but the type and stoichiometry of the lipid tails vary per species [2,4]. A growing number of studies indicate that the special lipid composition of the thylakoid membrane is mandatory for the proper functioning of the photosynthetic machinery [5].

In vivo the thylakoid lipids are organized as bilayers [6,7], but the overall composition gives the thylakoid membrane a high propensity to form non-bilayer phases [6]. Indeed a total lipid extract from the chloroplast membrane does not form bilayers in water [8]. PG, SQDG and DGDG are all bilayer forming lipids, but the most prominent component of the thylakoid membrane, MGDG, forms inverted hexagonal phases [9,10]. Next to the lamellar phase, a non-lamellar lipid phase of the thylakoid membrane might be required for photosynthesis [11]. It has been proposed that inverted hexagonal phases are important for the violaxanthin cycle, by facilitating the flip-flop of antheraxin. It is assumed that these inverted hexagonal phases are preferentially located in MGDG rich domains and that violaxanthin de-expoxidase especially binds in MGDG rich domains [12–14]. In the literature there is some uncertainty about the lateral heterogeneity in the thylakoid membrane. Initially it was thought that the various regions of the thylakoid membrane have different lipid compositions [15], but more recently it was shown that the bulk lipids of the thylakoid membrane do not display lateral heterogeneity [16]. The resolution of these techniques is, however, on a mesoscopic scale, and a detailed view of the nanoscale organization of thylakoid membranes is currently lacking.

In order to provide such a view and to obtain a more fundamental level understanding of the role of lipids in the thylakoid membrane,

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we resort to multiscale molecular dynamics (MD) simulations. MD offers excellent possibilities to study lipid systems, as it allows to measure time and length scales which are difficult to access with experimental techniques. Membrane simulations have become common practice [17,18] and simulations of glycolipids are gaining popularity [19]. Previous MD studies in the thylakoid field include the simulation of a mixed MGDG/phosphocholine membrane [20] and a short (1 ns) simulation of PSII solvated in a bilayer composed of 73 thylakoid lipids, but lacking SQDG [21]. Recently a 10 ns all-atom simulation of photosystem II embedded in the thylakoid membrane was performed [22].

Here we simulate the thylakoid membrane of both higher plants and cyanobacteria. Our model of the thylakoid membrane is based on the experimental characterization of the thylakoid membrane by Sakurai et al. [3]. The cyanobacterial membrane composition is from *Thermosynechococcus vulcanus*, a thermophilic cyanobacterium, which is isolated from Japanese hotsprings and grows optimally around 330 K [23]. Surviving in such an environment requires a membrane that is still in a liquid-crystalline phase and not too leaky for protons at these elevated temperatures [24,25]. Increasing the amount of fully saturated fatty acids is one way in which prokaryotes do this [26]. The plant membrane composition is taken from *Spinacia oleracea* (spinach). In contrast to the cyanobacterial membrane, the plant membrane is strongly enriched in polyunsaturated lipids [3] that keep the thylakoid membrane fluid at physiological temperatures.

We combine coarse-grain (CG) and all-atom (AA) MD simulations to characterize the thylakoid membranes. Using the CG Martini model [27,28] we study the long time scale properties of the membranes and we analyze structural properties such as lateral lipid mixing and lipid tail order, as well as dynamic properties including diffusion of lipids and plastoquinone and plastoquinol co-factors. The cyanobacterial and plant membranes are compared to each other, and to membranes composed of more common phospholipids. Backmapping of representative CG configurations to all-atom models finally provides a fully atomistic view on the lipid organization of the thylakoid membrane.

2. Methods

2.1. System composition

The cyanobacterial membrane was modeled to the membrane composition of *T. vulcanus* and the plant membrane to the thylakoid composition of *S. oleracea* (spinach). The actual compositions were based upon the experimental characterization of the thylakoid membrane by Sakurai et al. [3]. Both cyanobacterial and plant membranes contain four major classes of lipids: phosphatidylglycerol (PG), digalactosyldiacylglycerol (DGDG), monogalactosyldiacylglycerol (MGDG) and sulfoquinovosyldiacylglycerol (SQDG). The difference between cyanobacterial and plant membranes is found mainly in the percentage of poly-unsaturated tails, which is increased in case of the plant membrane. The structure of the lipid head groups and tails that we included in our model are shown in Fig. 1. The compositions determined by Sakurai et al. [3] and the composition of our *in silico* membrane model are compared in Table 1. For the simulated membranes, we used a slightly higher proportion of PG headgroups than was found experimentally. This choice was made to increase the statistics in future studies on interaction of PG with PSII, which is believed to be functionally relevant [3]. The percentage of saturated SQDG is somewhat increased in our *in silico* membrane (80% in comparison to 70% in the experimental extract) due to a misrepresented particle bead type in one of our input files. Since the overall percentage of SQDG is only 25%, the difference is unlikely to significantly affect any of the results presented. The composition of the lipid tails of the *in silico* membranes is adapted to the resolution of the Martini model and differs therefore somewhat from the experimentally determined composition, see Table 1. The lipid tail composition also differs between the two membranes. The cyanobacterial membrane has mainly palmitoyl and oleoyl tails, whereas the plant membrane does not contain oleoyl, but has α-linolenoyl tails instead. The decision of which tails belong to which headgroup was based upon the resolution achievable with the Martini model and the work of Sakurai et al. [3]. Of note, the positional distribution of the fatty acids at the *sn*-1 and *sn*-2 positions in the

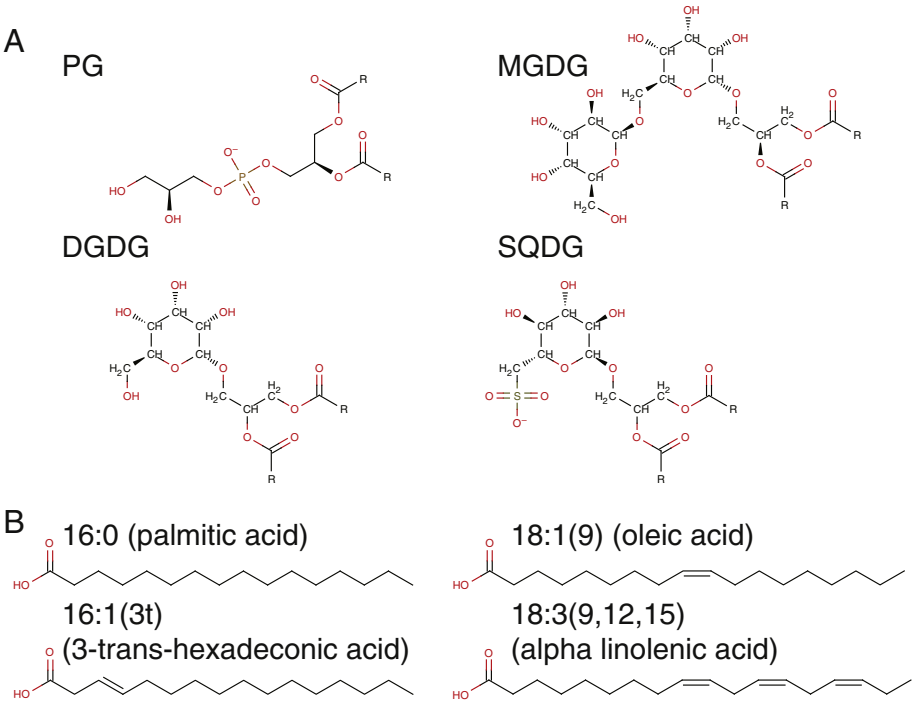

Fig. 1. Chemical structures of thylakoid lipid headgroups (A) and fatty acids (B).