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Research paper

A computer model of a polyunsaturated monogalactolipid bilayer

Krzysztof Baczynski [a], Michal Markiewicz [a], Marta Pasenkiewicz-Gierula [a, b, *]

[a] Department of Computational Biophysics and Bioinformatics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

[b] Malopolska Centre of Biotechnology, Jagiellonian University, Krakow, Poland

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ABSTRACT

1,2-di-O-acyl-3-O-β-D-galactopyranosyl-*sn*-glycerol (MGDG) is the main lipid component of thylakoid membranes of higher plants and algae. This monogalactolipid is thought of as a non-bilayer lipid but actually it can form both lamellar and nonlamellar phases. In this study, molecular dynamics (MD) simulations of the fully hydrated di-18:3 MGDG bilayer in the lamellar phase were carried out at 310 and 295 K for 200 and 450 ns, respectively, using the GROMACS 4 software package and OPLS-AA force field. At both temperatures, the lamellar phase of the systems was stable. The pure di-18:3 MGDG bilayer is the first step towards creating a computer model of the lipid matrix of the thylakoid membrane and the main aim of this study was to validate the computer model of di-18:3 MGDG in the bilayer and also to assess the properties of the bilayer. However, only a few of the predicted properties could be compared with those derived experimentally and in other MD simulations because of insufficient amount of such data. Thus, direct validation of the MGDG bilayer proved difficult. Therefore, in the validation process also an indirect approach was used, in which a computer model of the 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC) bilayer simulated at the same temperatures using the same force field as the MGDG bilayer was assessed. Successful validation of the DOPC bilayer parameterized in the OPLS-AA force field and similar properties of the MGDG molecules in the pure 18:3 MGDG and in binary 18:3 MGDG-PC bilayers indicate that the computer model of the MDGD molecule is faithful and the MGDG bilayer is representative on the time scales covered in these MD simulations.

1. Introduction

Photosynthesis is a physicochemical process by which organisms use light energy to drive the synthesis of organic compounds. In higher plants and green algae this process takes place in thylakoids – organelles that are surrounded by photosynthetic membranes of unusual lipid composition where galactolipids constitute more than 75% of the total lipid content [1]. Galactolipids are a class of glycoglycerolipids with one or more galactose moieties as the head group and the glycerol backbone. 1,2-di-O-acyl-3-O-β-D-galactopyranosyl-*sn*-glycerol (monogalactolipid, MGDG) whose head group consists of a single molecule of β-D-galactose, and 1,2-diacyl-3-O-(α-D-galactopyranosyl-(1 → 6)-O-β-D-galacto-pyranosyl)-*sn*-glycerol (digalactolipid, DGDG) with two galactoses are the most common galactolipids in the thylakoid membrane of chloroplasts. However, the chemical terms MGDG and DGDG are not precise since they describe a "family" of galactolipids with the same head group but various acyl chains. In the thylakoid membrane of higher plants, the acyl chains have a high degree of unsaturation, which accounts for its relatively high fluidity necessary for proper functioning of photosynthetic protein complexes [2,3]. DGDG that is synthesized by galactosylation of MGDG [4], is mainly involved in the overall organization and thermal stability of thylakoid membranes and also in stabilization of several complexes of the photosynthetic machinery [5]. MGDG plays an even more active role in the process of photosynthesis [6,7]; its proposed molecular functions are mediating protein–protein or protein–cofactor interactions and oligomerization of photosystem II subunits [8]. It has been demonstrated that *Arabidopsis thaliana* unable to synthesize MGDG exhibits significantly reduced amount of chlorophyll, defective chloroplast structure, a severe growth phenotype, and other deficiencies [9,10]. Thus, MGDG and DGDG are not only a

* Corresponding author. Department of Computational Biophysics and Bioinformatics, Faculty of Biochemistry, Biophysics, and Biotechnology, Jagiellonian University, ul. Gronostajowa 7, 30-387 Krakow, Poland..

E-mail address: marta.pasenkiewicz-gierula@uj.edu.pl (M. Pasenkiewicz-Gierula).

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passive "solvent" for membrane proteins but contribute directly to various photosynthesis-related processes, however, their individual contributions are distinct.

Different molecular shapes of MGDG and DGDG are responsible for their different abilities to form mesoscopic phases upon dispersion in water. DGDG has a cylindrical shape and spontaneously forms bilayers [11]. MGDG has a smaller head group than DGDG so the shape of a MGDG molecule (conical or cylindrical) and in the consequence, the mesoscopic phase of aggregates spontaneously formed by MGDG molecules in water (lamellar or non-lamellar), depend strongly on the type of the acyl chains (saturated, mono-unsaturated, or poly-unsaturated) attached to the glycerol backbone. Thus, MGDG can form a bilayer, an inverse hexagonal (H_{II}) phase, and other nonlamellar phases. An experimental study of Mannock et al. [12] showed that MGDG with both saturated acyl chains consisting of 10–20 carbon atoms forms thermotropic lamellar (L_α) phases at temperatures below 80 °C and nonlamellar phases above 80 °C. Also 16:0/16:1 MGDG forms a stable bilayer [13]. Stability of the lamellar phase of MGDG with two saturated acyl chains at 70 °C was confirm in molecular dynamics (MD) simulation studies [14,15].

The tendency of MGDG with polyunsaturated acyl chains to form nonlamellar structures when dispersed alone in water depends on the degree of unsaturation of the acyl chains [16]. However, experimental studies on the phase behavior of MGDG with polyunsaturated chains described in the literature have been carried out on mixtures of MGDG with acyl chains of various lengths and degrees of unsaturation [16–19]. The mixtures formed lamellar or nonlamellar phases depending on temperature and hydration. At full hydration and below −15 °C the phase was lamellar and above −15 °C up to +80 °C the phase was nonlamellar [17–19]. In contrast, dehydrated mixtures of MGDG with acyl chains of different length and unsaturation of which 64% were 18:3, formed the L_α phase between −15 °C and +85 °C and nonlamellar, mainly H_{II} phase, above 85 °C [20]. When polyunsaturated MGDG was mixed with lamellar lipids [17–19], particularly with 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) [21–23], the mixture formed bilayer structures.

In the present study, atomistic MD simulation in the OPLS-AA force field [24–26] was used to investigate the structural properties of the fully hydrated bilayer composed of MGDG with both α-linolenoyl (di-18:3, *cis*) acyl chains, at 22 and 37 °C (295 and 310 K, respectively). The α-linolenoyl acyl chains were chosen because they are the most commonly found in green plant thylakoids [27]. Such a bilayer has not been MD simulated so far, probably due to the lack of experimental evidence that this lamellar structure is stable. Nevertheless, based on the results of computer simulations of a bilayer composed of non-bilayer lipid 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) molecules, indicating that at higher hydration the initial lamellar phase of the bilayer was stable on a microsecond timescale [28,29], in this study, MGDG molecules were arranged in the form of a bilayer to test stability and to validate the computer model of the MGDG molecule in the bilayer. Construction and evaluation of the MGDG bilayer is essential for our larger project whose purpose is to create a reliable computer model of the lipid matrix of the thylakoid membrane comprising both MGDG and DGDG and also the MGDG H_{II} structure. Validation of di-18:3 MGDG is the first step towards achieving this purpose because it will practically imply validation of DGDG and also because the di-18:3 MGDG bilayer will serve as a reference system in the further study. Even though the MGDG bilayer is a simple model, its direct validation proved difficult due to limited appropriate experimental and computer simulation data. Molecular level experimental data are available for binary bilayers consisting of MGDG with various polyunsaturated chains and DMPC [21–23]; computer modeling data are available for di-18:3 MGDG mixed with DMPC [23] and for pure glycolipid bilayers with saturated [15], and saturated and unsaturated [14] chains. Thus, to validate di-18:3 MGDG also an indirect approach was applied, in which a 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC) bilayer was directly compared with experimental data. The reason for choosing DOPC in the validation process was two-fold, (1) DOPC has certain structural similarities to MGDG as it has the glycerol backbone and a double bond in each acyl chain, and (2) a DOPC bilayer is well-studied by a variety of methods both experimental and computational and a wealth of high quality data exist in the literature allowing direct validation of its computer model. However, even though DOPC bilayers have been extensively investigated, to our knowledge, there is only one report on MD simulation of a DOPC bilayer, where DOPC was parameterized in the OPLS-AA force field [30]. In the cited paper, the computer model of the DOPC bilayer was not fully validated thus, the results obtained in this study for the DOPC bilayer, are novel. The fact that DOPC is a bilayer-forming lipid is an additional argument to validate the MGDG molecule as a constituent of the bilayer. A similar approach of using a pure non-bilayer lipid DOPE bilayer was applied in an extensive comparative computer simulation study in Ref. [29] even though DOPE at full hydration was shown experimentally and computationally to spontaneously form the inverse hexagonal phase. In this study, both MGDG and DOPC bilayers consisted of 128 lipid molecules and were fully hydrated ($30H_2O$/MGDG, and $53H_2O$/DOPC); each was MD simulated for at least 200 ns under identical conditions (temperatures and pressure), using the same force field and the same software. In our indirect approach, we assume that positive validation of the computer model of the DOPC bilayer will be a strong supporting argument for positive validation of the MGDG bilayer. To facilitate comparison with experimental data obtained at various experimental conditions, simulations were carried out at two temperatures. The validation process (direct for DOPC and indirect for MGDG) was based on the assessment of the structural and mechanical properties of the bilayers. As comparison of the DOPC bilayer with the results of other studies published in the literature was favorable, in line with our assumption, we conclude that the computer model of the MGDG molecules and the MGDG bilayer presented in this study are faithful. An additional argument in favor of the di-18:3 MGDG bilayer model are similar properties of MGDG in the pure bilayer and in binary MGDG-PC bilayers referred to above.

2. Methods

2.1. Systems construction

The initial structures of DOPC, and di-18:3 MGDG molecules (Fig. 1) were created using PyMol program [31] from scratch. The initial structures of MGDG and DOPC bilayers, each consisting of $8 \times 8 \times 2$ (128) lipid molecules, were built in three steps, (1) each of the lipid molecules forming the first bilayer leaflet was rotated about the long axis by a random angle to reduce artificial long-range order and placed vertically to the plane constituting the bilayer surface to form a regular layer in a way to avoid van der Waals contacts; (2) the second leaflet was obtained from the first by 180° rotation and shifting to reduce the free volume; (3) the MGDG bilayer was hydrated with 3840 and the DOPC bilayer with 6747 water molecules by adding two layers of water stretching outwards from the average positions of the carbonyl oxygen atoms.

2.2. Simulation parameters and conditions

Parameters for the DOPC molecule as well as for the α-linolenic