A molecular dynamics study of interfaces: from pure liquids to biological membranes

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Chapter 5

Tetraether lipid membranes.*

5.1 Introduction

Over the three domains of life, the “new” Archaeal [103] domain differs from the Eukarya and Bacteria by, for example, most of its genes [104]. Furthermore, Archaeal organisms are delimited by a single membrane mainly made of isoprenoid etherlipids [105] (instead of the abundant esterlipids found elsewhere, see for example [106]). The ability of archaeal organisms to live under extreme physical conditions, as high salt solutions, acid or alkaline medium, and low, or nearly water-boiling temperature …, is directly related to the physical properties of the lipids of their membrane [107]. Archaeal membranes contain an isoprenoid hydrophobic core responsible for a low proton permeability which persists at high temperatures by an increase of the number of cyclopentane rings. The hydrophobic tails are linked to a glycerol group by an ether function, chemically more robust than an ester linkage.

Thermoplasma acidophilum is an archaeal organism with an optimal growth temperature of 55-59 °C and an optimal growth between pH 1 and 2 [108]. The lack of a cell wall supports the central role played by the membrane as a barrier against ion diffusion from low to high temperatures, maintaining physiological conditions (pH≈6) inside the cell while the organism lives in a strong acidic medium, i.e., a pH gradient of 5 pH units. The membrane of Thermoplasma acidophilum contains a rich variety of lipids, most of them (82%) being polar. The structure of the most abundant polar component, MPL (Main Polar Lipid, see Figure 5.1), has been recently identified [109]. It is a tetraether lipid with cyclopentane rings, and headgroups of phosphoglycerol and β-L-gulopyranose.

Figure 5.1: Molecular structure of the Main Polar Lipid (top) from Thermoplasmia acidophilum, and five analogues studied in this paper, from top to bottom: '0000', '0200', '0202', '1202', and '1212', where each digit gives the position of a ring, from one headgroup towards the mid-plane of the molecule, for the chain 2 and 1 successively. ('0' corresponds the absence of a ring, and only the two first positions along each half-chain are considered). On the gulose side, the cyclopentane rings are all cis, while on the phosphate side, they are either trans, or cis, or a mixture.
5.2 Molecular dynamics

Besides the work of Gabriel et al. [110] where for the first time different tetraether lipid membranes have been modelled, and the effect of cyclopentane rings on chain packing investigated, to the best of our knowledge, there has been no published molecular dynamics study of the structure and dynamics of tetraether lipid membranes. In this chapter, we study the structure of five membranes purely built from MPL analogues (see Figure 5.1). Furthermore, we investigate the influence of the cyclopentane rings and their stereochemistry on the membrane organization and the lateral pressure profile.

5.2 Molecular dynamics

Molecular dynamics computer simulations were carried out using the DL-POLY package [59]. An all-atom model was employed to describe molecules at an atomic scale using the potential energy parameter set PARM27 from the CHARMM package [55]. The TIP3P water model [56] was used in all simulations. Bonds involving hydrogen were held fixed with the SHAKE algorithm [18]. Electrostatic interactions were computed using the Smooth Particle Mesh Ewald method [57]. Our simulations were performed in the NVT ensemble [60], i.e., with constant temperature, volume, and number of particles. Simulations were run with periodic boundary conditions. All the simulations were performed using a cutoff radius of 12 Å for the van der Waals terms.

For each of the five lipids, we have used the same protocol to build up the corresponding hydrated membrane model. Initially, a single lipid molecule stretched along its longer axis was pre-equilibrated in vacuum. After, we have built our complete models by placing the lipids on a $6 \times 6$ grid, with carbohydrate headgroups forming one outer side of the membrane, and phosphate headgroups the other interface, mimicking the asymmetrical topology found in vivo [111, 112], both membrane surfaces being parallel to the $xy$ plane. The size of the grid is set such as to get a positive lateral pressure within the membrane, and the length of the simulation box along the $z$ axis such that the two membrane interfaces do not interact. The dry membrane has been equilibrated during a few hundred steps coupled to a gradual increase of the time step until 2 fs. Subsequently, the box is filled by adding water molecules. In such a way that the system contains 36 lipid molecules, and more than 2.200 water molecules, thus approximatively 17.000 atoms, and 30 water molecules per lipid headgroup, which corresponds to a fully hydrated membrane. The molecular area per lipid is 59.27, 58.57, 60, 62, and 62.73.
Å², for the '0000', '0202', '0200', '1202', and '1212' MPL lipid analogues, respectively.

The complete system has been equilibrated for 250,000 steps, with a time step of 2 fs at a temperature of 300 K. During equilibration, density profiles and energy convergence of the system have been monitored. After equilibration, we have recorded the dynamics of the system by accumulating coordinates at an interval of 0.1 ps during a period of 0.8 ns. An extension with 1.6 ns of molecular simulation has been achieved to get an accurate lateral pressure profile.

5.3 Results and discussion

5.3.1 Mass and electronic density profiles

Figure 5.2 reports the mass density profile and its components for the five membranes, and the electronic density profile of the '1212' lipid membrane. From the total mass and electronic density profiles of the '1212' lipid membrane, which is the model membrane which contains the larger number of cyclopentane rings, we observe two high and sharp peaks, each located at the headgroup-water interface. They correspond to the sum of the contributions of the glycerol group, and the phosphate or gulose moiety.

From the '1212' model to the '0000' model, the decrease of the number of cyclopentane rings yields a broadening and a diminishing of the headgroup density peaks. This is associated with a reduction of the ordering of the aliphatic chains. The overlap between density profile components is rather important in the '0000' membrane model, while almost nonexistent in the '1212' membrane model profile. The cyclopentane rings are responsible for a higher ordering of both the membrane core and each interface. The width of the aliphatic domain is reduced as the number of cyclopentane rings increases, as noticed from the first energy minimization study of a similar kind of membranes [110].

For all the model membranes, the shape of the electronic (only the '1212' model is shown) and mass density profile is nearly identical. Both density profiles show small features in the membrane phase, which suggest, considering the long recording time of the density profiles (0.8 ns), that the membranes are in a phase where motions are rather slow, as a "gel" phase (a fluid phase would have given a density profile with a smooth and almost flat density profile in the hydrophobic region). This observation does not depend on the degree of cyclization of the aliphatic chains and is consistent with previous results from molecular modelling [110].
Figure 5.2: Mass density profiles of the '0000' (top left), '0200' (top right), '0202' (centre left), '1202' (centre right), and '1212' (bottom left) lipid membranes. Electronic density profile of '1212' (bottom right) lipid membrane. The gulose is on the right interface, while the phosphate group is on the left one. The total density profile is represented by the dotted curve. From the centre of the membrane ($z \approx 0 \text{ Å}$) to the right, are reported one half of the chain 1 and one half of the chain 2 (on the saccharide side) (dashed curves), the glycerol linked to the gulose group and water (solid curves). From the centre of the membrane to the left, are reported one half of the chain 1 and one half of the chain 2 (on the phosphate side) (dashed curves), the glycerol linked to the phosphate group and water (solid curves).
Furthermore, while the aliphatic chains are identical in their connectivities on both sides of the membrane mid-plane, the density profiles show a slight asymmetry. Either the configurational isomerism of each aliphatic chain moiety (resulting from the different absolute configuration of the carbon stereocentres involved in cyclopentane rings) induces a peculiar molecular conformation which yields specific density profile features, or the two sides of the membrane simulated in the gel phase have been trapped in different conformations. These hypotheses will be discussed later in this chapter.

5.3.2 Molecular orientation

The orientation of parts of the lipid molecules, as the aliphatic chains or the headgroups, can be described by an order parameter. In the case of the aliphatic chains, we have defined two complementary parameters. First, we have computed the orientation of carbon chains from the vector defined by the coordinates of two carbons \( n \) and \( n + 2 \) belonging to the same chain. By skipping the carbon \( n + 1 \), the computed orientation of a chain segment is given without the contribution of the peculiar zigzag conformation of an aliphatic chain. Moreover, we have computed the C–H vector orientation, which can directly be compared to the scd chain parameter obtained from NMR spectroscopy. The order parameter reads:

\[
S(z) = \frac{1}{2} \langle 3\cos^2 \theta(z) - 1 \rangle, \tag{5.1}
\]

where \( \theta \) is the angle between the vector C–C or C–H and the interface normal. A value close to 1 corresponds to a distribution of vectors parallel to the normal of the interface, while a distribution of vectors parallel to the membrane plane yields an order parameter close to \(-0.5\). When the vector angles are randomly distributed, the order parameter will be equal to 0 (although an order parameter equal to 0 may also correspond to a phase perfectly ordered, with a tilt angle distribution centred on 54.7 degrees).

Figure 5.3 reports the C–C and C–H order parameters for each membrane. The numbering of atoms starts from the mid-plane of the molecule, and we assign to the vector the number/label of the carbon atom which defines the origin of the C–H vector, or the (skipped) \( n + 1 \) carbon of the \((n)–(n + 2)\) C–C vector. Only C–C vectors computed from the main carbon chain are shown. C–H vectors involved in cyclopentane rings are not reported.
5.3 Results and discussion

Figure 5.3: Order parameter of the hydrophobic chains for each lipid membranes: '0000' (top left), '0200' (top right), '0202' (centre left), '1202' (centre right), and '1212' (bottom left). The galactose is on the right interface, while the phosphate group is on the left one. For comparison, the order parameter of a DPPC lipid molecule embedded in a membrane is reported too (bottom right).
MPL lipid differs from the more classical DPPC (di-palmitoyl-phosphatidyl-choline) phospholipid by the spanning geometry of the lipid within the membrane, and the presence of methylene groups or cyclopentane rings on the aliphatic backbones, plus a few other features as the ether linkage. As we compare the two order parameters between a DPPC and a '0000' MPL membrane, we notice that the archaeal lipid membrane is on the whole much more ordered, with a maximum value of the order parameter located within the hydrophobic core of the membrane, close to the mid-plane of the membrane, while it is located on the headgroup side in the case of the DPPC membrane, and decreases slowly as the carbons get closer to the mid-plane. This major difference can be related to both the presence of methylene lateral groups, which act as spurs located on the chain backbone, and the transmembrane geometry of the backbone itself.

From Figure 5.4 which reports the angle distribution between the main axis of the aliphatic chains and the normal to the membrane plane, we cannot detect an effect of the number of rings. The orientation of the lipid molecules varies from 0 to 15 degrees. This is consistent with previous experimental results [112] obtained with bipolar lipid membranes at a water-water interface. Figure 5.3 shows an increase of the membrane order as soon as the lipid molecules contain one cyclopentane ring. However, the presence of additional rings does not improve the chain ordering. At the chain-ends, the aliphatic chain orientation is perturbed by the linkage to the glycerol, especially concerning the chain-end connected to the centre of the glycerol (via the ester linked to the central carbon of the glycerol). This suggests that small motions of both phosphate and gulose headgroups induce a slight disorder on the position of the outermost hydrophobic carbons.

The C–C bonds located at the hydrophobic mid-plane of the lipid molecules are in some cases less oriented compared to the other C–C bonds. This behaviour can be related to the length of the carbon chains which depends on both the number of cyclopentane rings per chain, and the conformation imposed by the stereochemistry of the carbons involved in the cyclopentane rings. Last, the C–C bond vector related to the cyclopentane rings (see the positions at ±8.5 and ±12.5 on the graphs) do not have a preferred orientation specific to a peculiar cis or trans ring substitution, but give an insight on the local conformation adopted by the rings.

Last, the cyclopentane rings, located in these membrane models on the two outermost methylene group positions, improve the order of the chain, counterbalancing the effect of headgroup motions observed for the 0000 model. This is consistent with the thin shape of the headgroup component peak of
5.3 Results and discussion

Figure 5.4: Orientation of the aliphatic chains relative to the membrane plane normal.

the density profiles, described above.

5.3.3 Pressure profiles

Figure 5.5 reports the pressure profile for the five kinds of membranes. As seen in the previous sections, when the number of cyclopentane rings increases, the ordering of the membrane both improves and differs from one side of the membrane to the other. We observe a similar trend in the pressure profiles. For the '0000' membrane model, the symmetry associated with a low ordering of the membrane yields a rather symmetrical pressure profile. But as the number of rings increases, the pressure profile is different on each side of the membrane. This is especially noticeable for membranes made of lipids containing more than 4 rings. Since the high pressure profiles are always located on the same side of the membrane, characterized by a specific configuration, this peculiar pressure profile shape is related to the absolute configuration of the carbon stereocentres (and not to a trapped conformation).
Figure 5.5: Pressure profile of the '0000' (top left), '0200' (top right), '0202' (centre left), '1202' (centre right), '1212' (bottom) lipid membranes.
5.4 Concluding remarks

The two interfacial headgroups are responsible for a negative or null contribution to the pressure profile. That means no stress is imposed by the two ends of the lipid. In addition, it is worth noticing that compared to phosphatidylcholine membranes, the pressure profile does not vanish in the mid-plane region, but only depends on the membrane conformation. This is a consequence of the spanning position of this peculiar kind of lipids.

5.4 Concluding remarks

The class of lipid, made of two spanning aliphatic chains linked to a phosphate and a sugar headgroup by two ether bonds and a glycerol group, presents properties dramatically different from the more familiar phospholipids. Chemically, they are much more stable, and form large and stable liposomes, being an ideal candidate as a drug delivery vector model for extreme media. Consequently, the synthesis of archaeal lipid analogues represents an interesting challenge for both a fundamental and an industrial point of view (see for example, [113-117]).

While the complete inventory of the lipid structures present in archaeal organisms is still in progress [105,109,118-120], being a difficult task mainly by the high variability of the sugar headgroups, very little information was available on the molecular shape and the membrane organization at an atomic scale [110,121-129].

To our knowledge, this work is the first report of a significant molecular dynamics study done on tetraether membrane models using simulation techniques. The atomic description of the membrane model allows a detailed analysis of the membrane organization. Thus, as suggested by experimental results [130], the membrane organization is determined by the hydrophobic core. We found from the analysis of the order parameters of the aliphatic chains that the cyclopentane rings increase the ordering of the membrane, which maintains the low permeability of the membrane when the temperature increases. But, the main finding of this work concerns the relation between the absolute configuration of stereocentres located on the aliphatic chains and the organization of the membrane in its hydrophobic domain.

Such peculiar properties of these lipids [107,131], correlated to the high dependence of the pressure profile on the chain conformation, suggests this class of molecules as an ideal tool in the study of the relation between the membrane pressure profile and the regulation of an ion-channel protein complex.