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Insights into the role of cyclic ladderane lipids in bacteria from computer simulations

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ABSTRACT

Ladderanes, which are multiple fused cyclobutane rings, are unique structures available only in nature. Anammox bacteria produce ladderane phospholipids during their life cycle, but the synthesis mechanism still remains a mystery. The function of ladderane lipids in the membrane is unclear as well. According to previous speculations, ladderane moieties of the bilayer might decrease permeability for certain molecules, which should not diffuse out of the compartment enclosed by the ladderane-containing membrane. We report the first atomistic-precision molecular dynamics simulations of bilayers containing ladderane lipids. The structural and thermodynamics differences among (1) pure ladderane containing bilayer, (2) POPC bilayer, and (3) their equimolar mixture are discussed. Potentials of mean force are reported for the translocation of a hydrazine molecule through all investigated bilayers. All bilayers offer a potential energy barrier to hydrazine. Contrary to expectations, the presence of the ladderane lipids somewhat lowers the barrier for translocation of hydrazine. We conclude that the presence of ladderane phospholipids in anammox bacteria does not serve as a barrier to hydrazine. It may serve as a barrier to larger and noxious intermediates in the anammox reaction, or, the true mission of ladderane lipids must be located in a different plane.

1. Introduction

Despite their geometric strain (Khoury et al., 2004), ladderane lipids (lipids containing multiple fused cyclobutane rings) are generated by nature (Damste et al., 2002). Furthermore, they are stable enough to be extracted (Rattray et al., 2008, 2010). Anammox bacteria produce ladderane lipids in the cellular compartment known as anammoxosome (Broda, 1977). Many methodologies have been, thus far, applied to obtaining ladderane structures in the laboratory (Nouri and Tantillo, 2006a,b). However, a biosynthetic pathway still remains unknown, being associated with an action of unidentified enzymes (Nouri and Tantillo, 2006a).

The life cycle of anammox bacteria (Fig. 1) requires efficient oxidation of ammonium under anoxic conditions, which occurs in anammoxosome (van Niftrik et al., 2004). The membrane of anammoxosome contains large concentrations of ladderane lipids, while the particular composition varies appreciably among genera. Naturally synthesized ladderane lipids possess three to five fused cyclobutane rings (Fig. 2) at the end of each hydrocarbon chain (Damste et al., 2002). These lipids include phospholipids with ethanolamine, choline and glycerol headgroups and ether- or ester-linked hydrocarbon chains (Boumann et al., 2006, 2009). Being approximately two times thicker than a linear aliphatic structure, fused cyclobutanes are responsible for the elevated local density of the ladderane containing membrane. According to earlier speculations, such a membrane is impermeable (or less permeable) for products of the anammox process, such as hydrazine and a proton.

The generalized chemical equation of the anammox process utilizes ammonium cation and nitrite anion as reactants, while molecular nitrogen and two water molecules are the products (van Niftrik et al., 2004). The reaction is believed to involve a number of intermediate stages, most of which are catalyzed by enzymes residing in the anammoxosome. On the outer side of anammoxosome membrane, ammonium and nitrite interact with protons to produce hydrazine and water, which are subsequently released to the inner side of the anammoxosome (Broda, 1977). Hydrazine is slowly oxidized up to molecular nitrogen under specific conditions. Anammoxosome chemistry is summarized in Fig. 1.

According to chemical considerations, it is important that all reactants and intermediate products do not leave the anammoxosome compartment, for instance due to transmembrane diffusion. For instance, the loss of just a single hydrazine molecule equals to the energy of 15 catabolic cycles of bacterium. One would correlate

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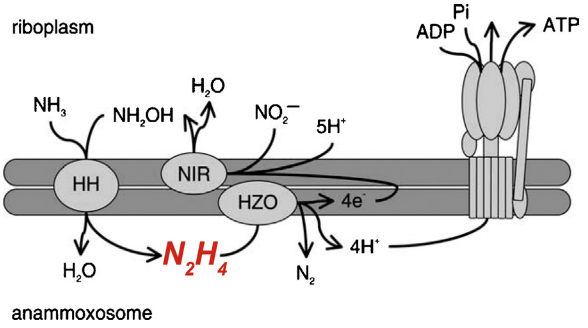

Fig. 1. The reactions occurring in the anammoxosome: HH – hydrazine hydrolase; the hydrazine-forming enzyme; HZO – hydrazine-oxidizing enzyme; NIR – nitrite-reducing enzyme.

Figure adopted from Ref. (van Niftrik et al., 2004).

an increased local density of the membrane, brought by ladderane lipids, with a necessity to keep inorganic molecules inside the anammoxosome (Broda, 1977). Physical properties of the ladderane phospholipids have been attended, thus far, only sporadically (Rattray et al., 2010; Boumann et al., 2006, 2009).

According to Rattray and coworkers (Rattray et al., 2008), all four investigated genera of anammox bacteria exhibit various compositions of the anammoxosome membrane. The fraction of ladderane lipids range from 22 to 63% (w/w) of the total lipid extract. The overall composition of the lipid extract is also very different among the genera. There are fatty acids, ladderane mono- and diethers, conventional linear saturated and linear unsaturated fatty acids, as well as branched fatty acids in the obtain extracts. Interestingly, certain percentage of triterpenoids, resulting from the combination of several isoprene units, has also been located in the anammoxosome membrane.

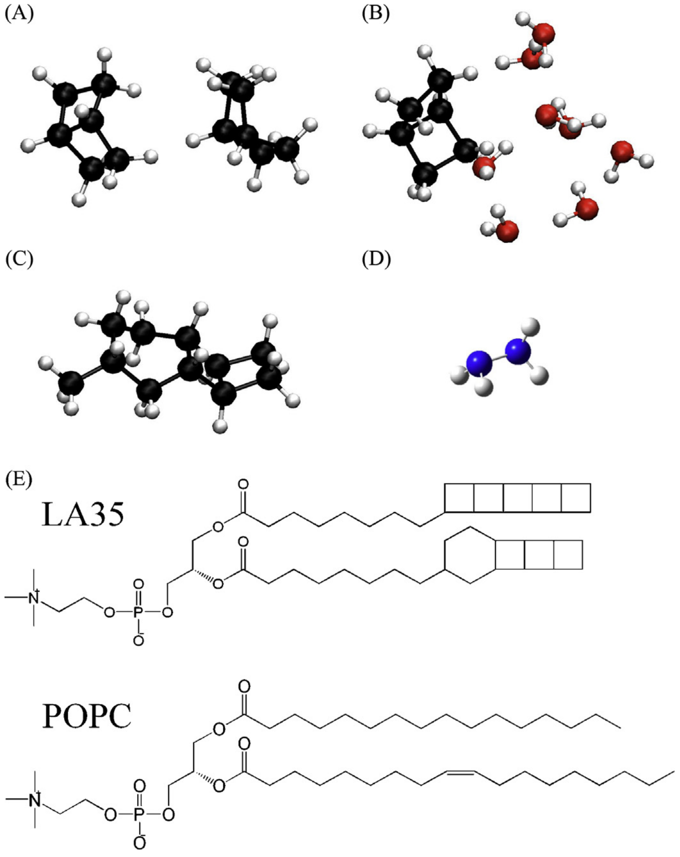

Fig. 2. The summary of chemical species considered in the present work: (a) interacting fused cyclobutane rings; (b) fused cyclobutane rings interacting with water cluster suggested by Hartree–Fock geometry optimization; (c) fused cyclobutane and cyclohexane rings; (d) hydrazine molecule; (e) structures of ladderane-3,5 lipid and POPC molecules. Geometries shown in (a), (b), and (c) are suggested by Hartree–Fock geometry optimization using aug-cc-pVTZ basis set.

As noted above, ladderane structures are energetically less stable than linear hydrocarbon structures (Khoury et al., 2004). However, a significant fraction of them is still present in natural systems. This fact engenders speculations that they must play an important role in the life cycle of the organism. The most intuitive role may be an increase of the membrane impermeability for a proton, hydrazine, and other nitrogen containing intermediates. The only published study (Damste et al., 2002) considers permeability of the ladderane containing membranes for various fluorophores. None of the fluorophores were able to spontaneously cross the anammoxosome membrane, while all of them penetrated conventional membranes. Although this research provided the first solid evidence of the elevated resistance of the ladderane containing membranes, it proposed no mechanism of such a process. Note that fluorophores are much larger than the reactants and products of the anammox process, and are also chemically very different (van Niftrik et al., 2004).

In the present work, we apply atomistic-precision molecular dynamics simulations to unveil the impact of ladderane lipids on the structure and permeability of phospholipid bilayers. A pure ladderane bilayer, an equimolar POPC/ladderane bilayer, and a pure POPC bilayer are considered in parallel. We develop a force field (FF) for the ladderane and ladderane-cyclohexane moieties. The force field excellently reproduces geometries of lipids, pairwise interaction energies, in particular ladderane–ladderane, ladderane–linear chain of POPC, and ladderane–water, versus the second order Möller–Plesset theory (MP2). The bonded parameters are compatible with the last revisions of the CHARMM force field (FF).

2. All-electron investigation and force-field development of ladderanes

MD simulations of ladderane structures have not been performed before. The CHARMM36 FF was successfully applied for the simulation of a variety of other phospholipids (Pastor and MacKerell, 2011). However, it does not have parameters for ladderane moieties. These parameters are introduced in the present work based on our Hartree–Fock and post Hartree–Fock ab initio calculations. No adjustment with respect to experimental properties of ladderane lipids was done, as no properties traditionally used to parameterize FF for lipids (area per lipid, thickness of the bilayer, specific density, etc.) are currently available. These data are presented here for the first time.

The structure of ladderane moiety (Fig. 2) was optimized using the Hartree–Fock (HF) self-consistent field method. The wavefunction was expanded employing basic Gaussian functions provided in the Dunning's triple-zeta correlation consistent basis set (Woon and Dunning, 1995), often referred to as aug-cc-pVTZ. Optimization of molecules using HF method is a common procedure, since geometries (equilibrium bonds, angles, dihedrals) are nearly insensitive to electron correlation. More sophisticated properties, such as binding energies and absolute energies of electron orbitals, however, often require more advanced methods. The convergence criterion for energy during geometry optimization was set to 10^{-8} Hartree. The optimized carbon–carbon bond length is the ladderane moiety is 0.1558 nm, while the optimized carbon-hydrogen bond length is 0.1083 nm. Each cyclobutane ring is essentially planar, while a dihedral between two neighboring rings equals to 115°. Planarity of the rings implies that all carbon–carbon–carbon angles are 90°. The fused cyclobutane–cyclohexane moiety (Fig. 2c)