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Can pyrene probes be used to measure lateral pressure profiles of lipid membranes? Perspective through atomistic simulations

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ABSTRACT

The lateral pressure profile of lipid bilayers has gained a lot of attention, since changes in the pressure profile have been suggested to shift the membrane protein conformational equilibrium. This relation has been mostly studied with theoretical methods, especially with molecular dynamics simulations, since established methods to measure the lateral pressure profile experimentally have not been available. The only experiments that have attempted to gauge the lateral pressure profile have been done by using di-pyrenyl-phosphatidylcholine (di-pyr-PC) probes. In these experiments, the excimer/monomer fluorescence ratio has been assumed to represent the lateral pressure in the location of the pyrene moieties. Here, we consider the validity of this assumption through atomistic molecular dynamics simulations in a DOPC (dioleoylphosphatidylcholine) membrane, which hosts di-pyr-PC probes with different acyl chain lengths. Based on the simulations, we calculate the pyrene dimerization rate and the lateral pressure at the location of the pyrenes. The dimerization rates are compared with the results of di-pyr-PC probes simulated in vacuum. The comparison indicates that the lateral pressure is not the dominant determinant of the excimer/monomer fluorescence ratio. Thus, the results do not support the usage of di-pyr-PC molecules to measure the shape of the lateral pressure profile. We yet discuss how the probes could potentially be exploited to gain qualitative insight of the changes in pressure profile when lipid composition is altered.

1. Introduction

Membrane elasticity has gained a lot of attention in cell membrane biophysics, since it, for example, explains various vesicular morphologies [1] and plays a role in membrane protein functionality [2,3]. Membrane elasticity emerges from the interactions that take place within a membrane. More precisely, it has been shown how membrane elastic coefficients can be derived from the lateral pressure profile that describes how the pressure is distributed inside a lipid membrane [4,5]. Therefore, it is not surprising that the connection between membrane elasticity and membrane protein functionality has also been derived by the lateral pressure concept [6,7]. Yet, there is still room for further development, since for a mechanosensitive channel and similar proteins, the second order elasticity theory seems to be too simple to describe the dependence of functionality on membrane physical properties [8]. Further, there is still critical discussion taking place concerning the relation between the lateral pressure profile and the elastic properties of membranes [9,10].

To analyze how much the lateral pressure profile contributes to the free energy of protein activation (inducing a shift in its conformational state), two characteristics are needed [6–8]: the cross-sectional area of a membrane protein in its active and inactive states, and the lateral pressure profile in the membrane surrounding the protein. Both of these quantities are difficult to measure. As for the cross-sectional area, one needs to know the 3D structure of the membrane protein in both of its two states [8]. Though considerable progress has been made, the structure determination is still a major challenge [11]. Regarding the second case, there is no generally accepted experimental method to measure the lateral pressure profile (see below).

Given the above concerns, there are still fundamental issues to be solved before the importance of the lateral pressure profile in modulating membrane protein function can be assessed quantitatively.

Lateral pressure profiles have been calculated from various molecular dynamics simulation models with numerous lipid compositions [12,13]. On the experimental side, the situation is more difficult, since currently only one technique has been used to measure the transmembrane distribution of pressure inside lipid membranes. The technique [14] is based on the assumption that the measured excimer/monomer fluorescence ratio of di-pyrenyl-phospatidylcholine (di-pyr-PC) probes correlates with the magnitude of lateral pressure in the location of the pyrene moiety. The experiments have been used to extract two different kinds of information about the lateral pressure profile. First, for a fixed lipid bilayer system, one measures the excimer/monomer fluorescence

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ratio with several di-pyr-PC probes with different acyl chain lengths [14]. In the second setup, the excimer/monomer fluorescence ratio is determined for a di-pyr-PC with a certain chain length, but here the experiments are done in lipid bilayers with different lipid compositions [15–17,14,18–20]. In the first case, the objective is to measure the shape of the lateral pressure profile, while in the latter case the aim is to measure the changes in the lateral pressure profile due to changes in lipid composition. The appropriateness of the latter measurement is supported by the increasing excimer/monomer fluorescence ratio with increasing lateral pressure (and vice versa) in phosphatidylcholine vesicles [21]. On the other hand, the decreasing excimer/monomer fluorescence ratio with increasing hydrostatic pressure has been interpreted in terms of free volume and molecular conformations, instead of lateral pressure [22,17,16,23]. Thus, currently it is not really clear whether the pyrene-based approach is appropriate for the determination of the lateral pressure profile inside lipid membranes.

In this work, our objective is to clarify this issue. We use atomistic molecular dynamics simulations to estimate the relative excimer/monomer fluorescence ratios in lipid bilayers, where some of the lipids are probes with pyrene moieties attached to varying positions in the chains of the host lipids. These simulations are compared to the simulations of probes in vacuum to separate the effect of the internal molecular conformations of di-pyr-PC and the properties of the surrounding lipid bilayer to the estimated relative excimer/monomer fluorescence ratio. Also the lateral pressure profile determined by using the approach suggested in Ref. [14]. is compared to the lateral pressure profiles calculated directly from the simulations.

To measure the lateral pressure profile in fluorescence experiments, one has to assume that the lateral pressure dominates the excimer/monomer fluorescence ratio. However, our simulation results indicate that the differences in excimer/monomer fluorescence ratios with different acyl chain lengths of di-pyr-PC arise from the differences in internal molecular conformations of di-pyr-PC instead of the lateral pressure profile. This suggests that these probes are not suitable to measure the shape of the lateral pressure profile. However, the probes might still be appropriate to measure how the lateral pressure profile changes at a fixed region in a membrane, when the experiment is carried out in lipid bilayers with different lipid compositions and with a di-pyr-PC probe whose chain length is fixed [15–17,14,18–20].

2. Methods

2.1. Simulation details

We simulated a lipid bilayer composed of 128 DOPC (dioleoylphosphatidylcholine) molecules symmetrically divided into two leaflets. The membrane was fully hydrated by 3655 water molecules. Four randomly chosen DOPC molecules (two in each leaflet) were transformed into DPPC (dipalmitoylphosphatidylcholine) molecules by replacing the double bond region with a saturated one. The DPPC lipids were then used as a basis for pyrene: we attached the pyrene moiety to the 4th, 6th, 8th, or 10th carbon in both hydrocarbon chains of the DPPC molecules, thus creating di-pyr-PCs (see Fig. 1 for a molecular structure). The corresponding systems, in respective order, are denoted as PYR4, PYR6, PYR8, and PYR10. Simulations of the pure DOPC membrane system without pyrene (denoted here as 'DOPC') were also performed for comparison. The choice of these lipids for our simulations is based on the experimental study by Templer et al. [14], as the aim of this work is to study the potential of these probes to measure the lateral pressure profile as suggested by Templer et al. However, the concentration of pyrene-labeled lipids in our simulations (about 3 mol%) is quite a bit larger than that of the concentrations typically used in experiments (about 0.1 mol%). Simulations with a similar number of pyrene-labeled lipids at a concentration of 0.1 mol% would have required about 100 times larger computing resources compared to those used in this work (due to increasing system size). Since the concentration used in our simulations is still quite small, it does not significantly affect the bulk membrane properties [24].

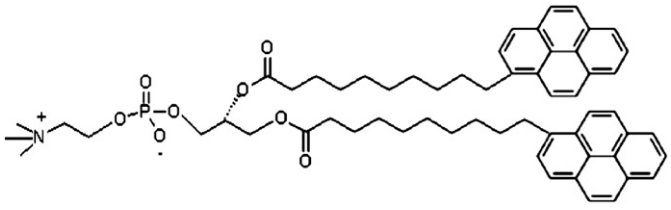

Fig. 1. Chemical structure of di-pyrenyl-phosphatidylcholine (di-pyr-PC) with pyrenes attached to the 10th acyl chain carbon (PYR10).

The parameters for lipids are based on the so-called Berger force field [25], except for the double bonds in DOPC hydrocarbon chains that were taken from Bachar et al. [26]. The general properties of lipid bilayers and acyl chain conformations are well described by the model, with good agreement with experimental data for, e.g., area per molecule and acyl chain order parameters [27,28]. The possible influence of the glycerol and headgroup parameters on our results is discussed in Section 4. For the pyrene moieties in question, we used force field parameters from Ref. [29]. The SPC (single point charge) model was used for water molecules [30]. The atomistic molecular dynamics (MD) simulations were carried out using the GROMACS 4 software package [31] in the NpT ensemble (constant particle number, pressure, and temperature). The temperature and pressure were set to 300 K and 1 bar to match the experimental settings used by Templer et al. [14]. Periodic boundary conditions were used in all three directions. The LINCS algorithm [32] was used to preserve all bond lengths. The time-step used in integrating the equations of motion was chosen to be 2 fs, and the data of the trajectory was saved every 10 ps.

All five systems were equilibrated for 20 ns with temperature and pressure controlled by the semi-isotropic Berendsen algorithm [33] using time constants of 0.1 and 1.0 ps, respectively. In the subsequent production simulations, each lasting for 500 ns, the pressure was controlled by the semi-isotropic Parrinello-Rahman barostat [34] and the temperature by the Nose–Hoover thermostat [35,36] (with no change in time constants). The last 400 ns was used for the analysis. Long-range electrostatic interactions were dealt with the particle mesh Ewald technique [37], with 1 nm real space cut-off. A plain cut-off with a radius of 1.0 nm was used for Lennard–Jones interactions.

We did also simulate di-pyr-PC molecules with each acyl chain length in vacuum. The length of these simulations was 4 μs and simulation parameters were exactly the same as in bilayer simulations, except that the simulation box size was constant and 2 nm plain cut-off was used for the electrostatics.

2.2. Analysis

An excimer is formed when an excited pyrene monomer forms a dimer with a non-excited pyrene monomer. Since this is a quantum-mechanical process, we cannot directly calculate the excimer/monomer fluorescence ratio from the classical simulations. However, we can observe dimers formed by non-excited dimers (see below). Here we analyze the dimer formation rates since in the lateral pressure profile measurement it was assumed that the excimer formation rate dominates the measured excimer/monomer fluorescence ratio [14]. We assume that the dependence of the non-excited dimer formation and the excimer formation rates on external conditions, like pressure and acyl chain length, is similar. In other words, we assume that the essential differences between the formation of a non-excited dimer and an excimer are related to the direct interactions between pyrenes in different states, not to the physical environment around them. Consequently, the dependence of the formation rates for non-excited dimers and excimers on pressure and acyl chain length should be similar, although the actual rates are different.