Chemistry and Physics of Lipids 177 (2014) 19–25

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

How to link pyrene to its host lipid to minimize the extent of membrane perturbations and to optimize pyrene dimer formation

Miroslava Dékány Fraňová [a], Jarmila Repáková [b], Juha M. Holopainen [c], Ilpo Vattulainen [b,d,*]

[a] Department of Chemical Physics and Optics, Faculty of Mathematics and Physics, Charles University, Ke Karlovu 3, Prague 2 CZ-12116, Czech Republic
[b] Department of Physics, Tampere University of Technology, P.O. Box 692, FI-33101 Tampere, Finland
[c] Helsinki Eye Lab, Department of Ophthalmology, University of Helsinki, Helsinki, Finland
[d] MEMPHYS – Center for Biomembrane Physics, University of Southern Denmark, Odense, Denmark

ARTICLE INFO

Article history:
Received 5 July 2013
Received in revised form 8 October 2013
Accepted 11 October 2013
Available online 8 November 2013

Keywords:
Fluorescent probe
Pyrene
Bis-pyrene
Molecular dynamics simulation
Lipid bilayer
Lipid membrane

ABSTRACT

We study how lipid probes based on pyrene-labeling could be designed to minimize perturbations in lipid bilayers, and how the same design principles could be exploited to develop probes which gauge lipid dynamics primarily within a single lipid monolayer or between them. To this end, we use atomistic molecular dynamics simulations to consider membranes where pyrene moieties are attached to lipid acyl chains in varying positions. We find that in a DOPC bilayer the conformational ordering of lipids around di-pyrenyl-PC probes is altered to a largely similar extent regardless of where the pyrene moiety is attached to the hydrocarbon chain. This is in contrast to saturated membranes, where pyrene-induced perturbations have been observed to be more prominent. Meanwhile, the formation of pyrene dimers depends on the linkage point between pyrene and its host lipid. Membrane-spanning dimers between lipids in different membrane leaflets are observed only if the pyrene moiety is attached to the latter half of the acyl chain. A seemingly minor change to link pyrene to an acyl chain that is two carbons shorter leads to a situation where membrane-spanning dimers are no longer observed. Further, simulations suggest that formation of dimers is a slow process, where the rate is limited by both lateral diffusion and the dimerization process once the two probes are neighbors to one another. Typical lifetimes of pyrene dimers turn out be of the order of nanoseconds. The results are expected to pave the way for designing ways to consider experimentally topics such as intraleaflet lateral diffusion, motion of lipids within and between membrane domains, and membrane domain registration across bilayers.

1. Introduction

Imaging has become one of the most useful and illustrative means to study lipid membrane behavior (Somerharju, 2002; Wustner, 2007; Groves et al., 2008; de Almeida et al., 2009). Over the years it has been particularly helpful in visualization of large-scale phenomena such as formation of membrane domains, molecular traffic, and partitioning of proteins to different membrane environments. However, recent progress in the development of imaging techniques and probe molecules has also paved the way for very detailed descriptions of membrane structure and dynamics, as super-resolution imaging techniques have rendered it possible to investigate membranes over scales as small as tens of nanometers (Eggeling et al., 2009).

For imaging, one needs probes. Numerous fluorescent markers have thus been developed to consider a variety of different lipid bilayer properties (Somerharju, 2002; Wustner, 2007; Chattopadhyay and London, 1987; Epand et al., 1996; Lakowicz, 1983; Lentz, 1989, 1993; Maier et al., 2002; Holtta-Vuori et al., 2008; Sezgin et al., 2012). Free probes (not linked to lipids or other molecules) allow one to explore how different membrane regions are formed, as even seemingly minor changes in the structure of probes affect their partitioning to domains with different physical properties. Meanwhile, the probes linked to lipids can provide one with highly useful insight of, e.g., lipid dynamics. However, how useful and reliable insight is given by imaging depends quite critically on the probes' ability to mimic native membrane behavior. Since free probes are usually not natural, and since the lipids linked to probes have quite certainly properties that are different from those of the corresponding native lipids, some perturbations in membrane behavior are inevitable (Lentz, 1989, 1993; Koivusalo et al., 2004; Leonard-Latour et al., 1996; Parente and Lentz, 1985; Somerharju et al., 1985; Yashar et al., 1987; Repáková et al., 2005).

* Corresponding author at: Department of Physics, Tampere University of Technology, P.O. Box 692, FI-33101 Tampere, Finland.
E-mail address: Ilpo.Vattulainen@tut.fi (I. Vattulainen).

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http://dx.doi.org/10.1016/j.chemphyslip.2013.10.004

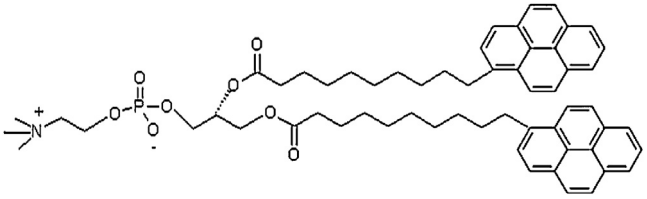

Fig. 1. Schematic structure of a pyrene-linked DPPC lipid considered in this study, the case PYR10 serving here as an example.

Detailed atomistic simulation studies have yet shown that the perturbations are typically local, taking place only in the right vicinity of the probe (usually within a range of a few nanometers from the marker) (Holtta-Vuori et al., 2008; Repáková et al., 2005, 2004; Repakova et al., 2006; Curdova et al., 2007; Franova et al., 2010).

The above problems can partially be avoided by using probes which mimic the behavior of native molecules as closely as possible, or which overall alter membrane behavior as little as possible. One of the commonly used probes matching these requirements quite well is pyrene. It is often used in acyl chains of the host lipid that is labeled with pyrene (see Fig. 1), the aim being to gain information of membrane structure and lipid dynamics under varying conditions. For instance, one of the typical experiments exploiting pyrene-labeled lipids is to consider formation of excited pyrene dimers, where an excited pyrene moiety, together with a non-excited pyrene, forms an excited-state dimer (excimer) whose fluorescence (emission) spectrum is different from the one of pyrene monomers. The formation rate of dimers is considered a useful indicator of, e.g., the lateral diffusion of lipids.

Nonetheless, as pyrene is a foreign molecule, it certainly alters membrane behavior. This raises a question about how to minimize the extent of membrane perturbations. Given that there is quite little freedom to design lipid probes using pyrene, one is tempted to consider whether it would be possible to regulate the extent of perturbations by adjusting the location of pyrene in its host lipid in some appropriate way. Furthermore, could the same idea be used to develop pyrene-labeled lipid probes that would specifically investigate lipid dynamics in some well defined region only, such as within a single membrane leaflet, or between the two opposing leaflets.

In this paper, the objective is to shed light on these questions. To this end, we carry out atomistic simulations for a DOPC (dioleoylphosphatidylcholine) membrane with pyrene-labeled DPPC (dipalmitoylphosphatidylcholine) probes (Templer et al., 1998). We use the same approach as in many experiments, that is, we link pyrenes to the hydrocarbon chains of its host lipid. However, instead of considering just a single case, we explore systematically how the linkage point between pyrene and an acyl chain affects the probe's properties. Due to the nanoscale nature of atomistic simulations, we expect the simulations discussed here to provide added value to complement experimental work in the same field. The same simulation approach has been used successfully in quite a few recent studies (Holtta-Vuori et al., 2008; Repáková et al., 2005, 2004; Repakova et al., 2006; Curdova et al., 2007; Franova et al., 2010; Loura and Ramalho, 2011, 2007; Loura et al., 2008; Skaug et al., 2011, 2009).

We find that the conformational ordering of lipids around pyrene is altered to a largely similar extent regardless of where the pyrene moiety is attached to the hydrocarbon chain. This is perhaps quite unexpected but stems largely from the unsaturated DOPC environment where probe-induced perturbations are more limited than in saturated bilayers (Repakova et al., 2006). However, what is more appealing is that when we consider interactions between pyrene moieties whose host lipids are in opposing membrane leaflets, the formation rate of membrane-spanning pyrene dimers depends very strongly on the position of the carbon attached to pyrene. If pyrene is linked to carbons C4–C8 in the hydrocarbon chain of DPPC (small numbers describing the carbons close to the headgroup, and C8 being the carbon in the middle of the chain), then there is practically no dimer formation at all. However, if the pyrene moiety is attached to the carbon C10 in the chain, the formation rate of membrane-spanning pyrene dimers increases abruptly to a significant value.

The simulation results reported here are expected to help in designing setups for experimental studies to consider topics such as intraleaflet lateral diffusion, motion of lipids within and between membrane domains, and membrane domain registration across bilayers.

2. Methods

2.1. Models

We considered a lipid bilayer composed of 128 DOPC 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 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 6th, 8th, or 10th carbon in both hydrocarbon chains of the DPPC molecules, thus creating bis-pyrene PCs (see Fig. 1 for a molecular structure). The corresponding systems, in respective order, are denoted as 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. (1998), as one of the aims of this work is to compare our simulation data with experiments reported in Templer et al. (1998). For the same purpose, and to increase sampling due to simulation times that are always considerably shorter than measurement times in experiments, we used concentrations of pyrene-labeled lipids (about 3 mol%) that are quite a bit larger than those 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 100–1000 larger computing resources compared to those used in this work (due to increasing system size and requirements for sufficient sampling of pyrene dimer formation through diffusion).

The parameters for lipids are based on the so-called Berger force field (Berger et al., 1997), except for the double bonds in DOPC hydrocarbon chains that were taken from Bachar et al. (2004). The model performs well, as comparison of simulation results with experiments indicates quite good agreement (see below). Also, a previous simulation study based on the same model (except for having POPC instead of DOPC) compared very well against experiments (Ollila et al., 2007). The SPC (single point charge) model was used for water molecules (Berendsen et al., 1981). For the pyrene moiety, we used force field parameters available and validated in Repakova et al. (2006).

The atomistic molecular dynamics (MD) simulations were carried out using the GROMACS software package (Hess et al., 2008) 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. (1998). Periodic boundary conditions were used in all three directions. The LINCS algorithm (Hess et al., 1997) 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.

First, all five systems were equilibrated for 20 ns with temperature and pressure controlled by the Berendsen algorithm