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Behavior of pyrene as a polarity probe in palmitoylsphingomyelin and palmitoylsphingomyelin/cholesterol bilayers: A molecular dynamics simulation study

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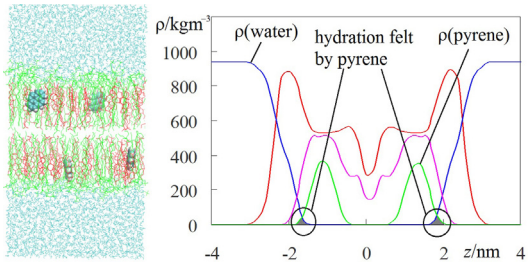
HIGHLIGHTS

- *N*-Palmitoylsphingomyelin/cholesterol bilayers are simulated in presence of pyrene.
- The presence of pyrene does not affect the host bilayer properties significantly.
- Pyrene dynamics are considerably slowed down by the addition of cholesterol.
- Pyrene location inside the bilayer hydrocarbon region is not affected by cholesterol.
- However, pyrene hydration is reduced upon increasing the cholesterol concentration.

GRAPHICAL ABSTRACT



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ABSTRACT

Pyrene is a polycyclic aromatic hydrocarbon noted for its remarkable optical spectroscopic properties. Among its uses as a fluorescent probe, measurement of lipid bilayer's equivalent polarity through the pyrene Ham effect stands out. To this effect, the ratio of the intensities of the first and third vibronic bands (I_1/I_3) in its emission spectrum of pyrene is measured. However, issues concerning the precise location of bilayer-inserted pyrene and the possibility of probe-induced perturbation of host bilayer properties are potential sources of concern in this regard. Atomistic molecular dynamics simulations constitute a useful method for the characterization of lipid membrane systems, and, in particular, to understand the behavior of fluorescence probes upon incorporation in lipid bilayers. In this report, we present a detailed characterization of the behavior of pyrene in fluid *N*-palmitoylsphingomyelin (PSM) and PSM/cholesterol membranes, with emphasis on the degree of proximity between the probe and water molecules inside bilayers, related to the use of pyrene to measure equivalent lipid bilayer polarity. It is concluded that pyrene exerts minor effects on bilayer properties, with slight local disordering being apparent for high

Abbreviations: ACF, autocorrelation function; Chol, cholesterol; DPPC, dipalmitoylphosphatidylcholine; MD, molecular dynamics; MSD, mean squared displacement; PC, phosphatidylcholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; PSM, *N*-palmitoylsphingomyelin; SM, sphingomyelin.

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cholesterol content. Whereas rotation and lateral diffusion of pyrene are greatly slowed in cholesterol-rich systems, its relative transverse location is not significantly affected. While hydration of PSM bilayers, as sensed by pyrene, is already low compared to that of fluid phosphatidylcholine, it becomes even smaller for high cholesterol mole fraction at the studied temperature.

1. Introduction

Phospholipid bilayers are the basic structural units of biological membranes, demarcating the interior and exterior of cells and their organelles. As models of biological membranes, lipid bilayers have been a major research topic in biophysical chemistry [1,2]. One of their most important properties is their hydration or polarity profile, which shapes the hydrophobic membrane barrier [3], and thereby constitutes an energetic determinant for insertion of peptides, proteins and amphiphilic molecules, as well as for transport of both polar and apolar solutes across the bilayer [4].

Several techniques based on fluorescence spectroscopy are available for the estimation of polarity in lipid bilayers, exploiting the environmental sensitivity of extrinsic probes, such as prodan, laurdan, dansyl, or anthroyl labeled probes (e.g. [5,6]). Among these methodologies, one that has been surprisingly seldom used in membrane systems is that based on the Ham effect of pyrene [7], the so-called *Py* scale of equivalent polarity [8,9]. Pyrene is a polycyclic aromatic hydrocarbon noted for its remarkable fluorescence properties, such as an unusually long excited-state lifetime (>100 ns in a variety of aerated solvents and micellar or model membrane systems), emission spectrum highly sensitive to solvent polarity and concentration-dependent and/or viscosity-influenced excimer formation [10]. The *Py* scale of polarity is based on the measurement of the ratio of the fluorescence intensities of the first and third vibronic bands (I_1/I_3) in the spectra of pyrene. Previously, we demonstrated the application of this scale to lipid bilayers composed of phosphatidylcholine (PC), both in presence and absence of cholesterol (Chol) [11].

However, there are two crucial issues regarding use of extrinsic membrane probes, and pyrene in particular: what is their precise location within the bilayer (and thus what region they report on), and whether they induce significant perturbation on the host bilayer properties. For this purpose, molecular dynamics (MD) simulations have been useful to characterize simultaneously membrane probe behavior and probe-induced perturbation with atomic detail, for several classes of fluorescent lipophilic probes (see [12,13] for reviews), including free pyrene [14,15]. Recently, we carried out MD simulations of pyrene in 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) bilayers, with varying amounts of Chol [16]. This work was valuable in the clarification of the use of the pyrene Ham Effect to effectively measure equivalent polarity in mixed lipid bilayers containing unsaturated PC and Chol.

In this report, we extend our previous MD study by applying this methodology to the study of pyrene inside *N*-palmitoyl-sphingomyelin (PSM)/Chol bilayers (structures are shown in Fig. 1, together with that of pyrene). Alongside PC and Chol, sphingosine-based sphingomyelin (SM) is a major component of mammalian plasma membranes, and is particularly relevant as (together with Chol) the main constituent of lipid rafts, specialized membrane domains implicated in processes including cellular signaling and trafficking [17–19]. SM chains typically display a larger degree of saturation and higher main transition temperature compared to other lipid classes [2]. For this reason, and because of their ability to form a network of intermolecular hydrogen bonds involving their amide and/or hydroxyl groups, SM-enriched membrane domains are highly ordered. This also applies to rafts, in which the ordering effects of Chol contribute to create a very tight molecular packing. To investigate if pyrene is well accommodated within SM/Chol bilayers, its precise location and effect on membrane properties, and to gain insights on the polarity sensed by the probe in these systems, we carried out extensive (300–400 ns) simulations of PSM/Chol bilayers (0 mol%, 5 mol%, 20 mol%, 40 mol%, 45 mol% and 50 mol% of the latter component). The determination of bilayer polarity corresponding to rafts enriched in SM/Chol is relevant for the understanding of the solvation properties of lipid bilayers, that may influence the structure and functioning of proteins associated to domain-limited biochemical reactions and processes. In bilayer hydration terms, it is expected that bilayer domains enriched in PSM, but with low Chol content ($\leq$5 mol%) are of significantly higher polarity than those domains containing upper Chol proportions (20 mol% $\leq$ Chol content $\leq$ 50 mol%). When changing the proportion of Chol in PSM bilayers, subtle changes in bilayer hydration are revealed, together with the effects of pyrene in the bilayer ordering and dynamics of the lipidic components, studied at the atomic resolution. Besides the intrinsic less fluid characteristics of bilayer domains enriched in PSM/Chol mixtures, there are unequivocal variations in bilayer hydration that must be taken into account when analyzing membrane phenomena involving the specialized raft domains.

2. Methods

MD simulations and analysis of trajectories were carried out using the GROMACS 4.6.3 package [20–22]. PSM united-atom structure and topology, as used by Niemelä et al. [23], were obtained through the Lipidbook web page [24]. Chol united atom structure and topology were adapted from those of Höltje et al. [25] (available for download at the GROMACS webpage [26]) by changing the molecule types from CH2/CH3 to LP2/LP3, to avoid overcondensation of the bilayer, as described and tested elsewhere [27]. Pyrene was parameterized as described by Hoff [14,28]. Bilayer models with varying numbers (shown in Table 1) of PSM and Chol molecules were assembled using GROMACS 4.6.3 tools, and fully hydrated with SPC water [29]. For the simulations with incorporated probe, initial structures with 2 or 4 pyrene molecules (one or two in each leaflet, respectively) were then obtained by randomly inserting probe molecules inside a PSM or PSM/Chol bilayer without replacement of lipids.

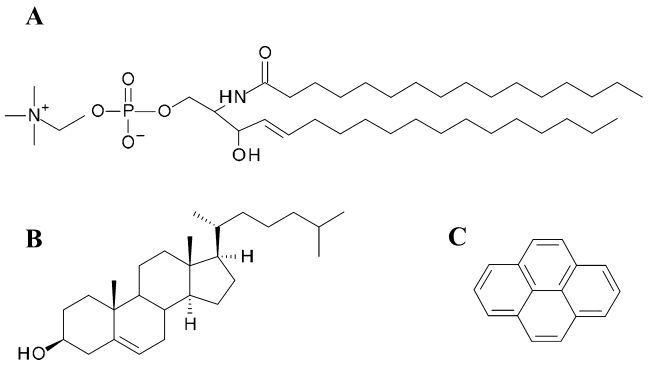

Fig. 1. Structures of PSM (A), Chol (B), and pyrene (C).