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Stability and softening of a lipid monolayer in the presence of a pain-killer drug

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ABSTRACT

The aim of this study is to investigate the interaction of a drug (Piroxicam, 4-hydroxy-2-methyl-N-(2-pyridinyl)-2H-1,2-benzothiazine-3-carboxamide 1,1 dioxide) with a lipid (DMPC) monolayer used as a membrane-mime in terms of drug-induced changes in stability and compressibility with variation in temperature, surface-pressure, drug-dose and ionic states of the monolayers. Drug-induced fluidization is noticed in the $\pi - A$ isotherms through increase in phase-transition pressure at constant temperature. The long-term dynamics of the lipid-monolayer is characterized by algebraic decays in surface-energy E with time t, $E \sim t^{-p_{1,2,3}}$, with an initial decay exponent p_1 that changes to p_2 after ∼1000 s, and, at high surface pressures and/or drug-dose, to a third exponent p_3 after ∼3500 s, suggesting structural reorganizations in the monolayer. With increasing drug–lipid ratio (D/L), p_1 shows a decrease ending at an almost constant value after 0.05, p_2 shows an almost negligible lowering while p_3 shows a monotonic and considerable increase. The reorganization is summarized by proposing two mechanisms: (a) 'charging–discharging' where drug-molecules sitting parallel to the interface increase headgroup separations and (b) 'discharging–charging' where drug-molecules sitting roughly perpendicular to the interface bring headgroups closer. Drug-induced softening of lipid-monolayers is characterized by the compressibilites of pure and mixed lipid monolayers. Compressibility-change (i.e., compressibility difference between drug/lipid and pure lipid monolayer) with pressure is maximum in the LE–LC transition zone and compressibility-change with drug-dose reveals an optimum dose of drug for maximum increase in compressibility. Molecular dynamics simulation shows that the ordering in the different parts of the lipid chains is changed to different extents in the presence of drugs with maximum change near the headgroups and again points to an optimum dose for maximum disorder.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs), the most common group of drugs used as anti-inflammatory, antipyretic and analgesic agents work by targeting and assembling with the enzyme cyclooxygenase-2 (COX-2) in the cell-membrane, thereby inhibiting the inflammatory function of the latter. This process involves extensive drug–membrane interaction and, since lipids constitute the major structural and active component of the cell-membrane, interactions between NSAIDs and phospholipids has become a focus of research. The complexity of this interaction becomes apparent if we consider the series of ionization states assumed by the drugs and the phospholipid-headgroups at the different pH-values under physiological (pH = 7.4) and pathological conditions (pH ≈ 5.0), ambient temperatures, interfacial pressures and drug/lipid-headgroup concentration ratios (i.e., drug-dose). The major question from a structural point of view that surely affects bio-functionality is regarding the position and orientation of drug-molecules relative to the lipid-headgroups, how this is affected by the above factors and what effects do the changes in position and orientation have on the mechanical properties of the cell-membrane. It is very important to gain detailed insight into the NSAID-membrane interaction because the therapeutic ability of the drugs is related to this complex event.

Langmuir monolayer (LM) has an advantage over other model membranes because of its easy tunability over packing density, lateral pressure, thermal and ionic conditions. Recently there have been few attempts to understand the drug-membrane interaction using LM as a model membrane [1–4]. Marlene et al. have reported NSAID-induced perturbations in the liquid-crystalline phase of DPPC monolayer. Kundu et al. [1] have found an anomalous dependence of NSAID/lipid-monolayer interaction on drug-dose. The

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drug-induced cooperativity of the phase-transition in DPPC monolayer is changed with the ionic states of drugs and lipids [3]. Thus the drug-molecules may change the physical properties of a lipid-monolayer. This modification is, again, decided by the position and orientation of drug-molecules with respect to the lipid-molecules, and in particular, to the phospholipid-headgroups, while its expression is, among others, in the mechanical properties of the lipid assembly. In our specific example of lipid-monolayers in presence of NSAIDs, the major effects of the drug are expected in the mechanical stability of the monolayer over long time scale and in its rigidity. Both these effects have important consequences for biofunctionality of the cell-membrane. The stability of a lipid-monolayer in presence of an NSAID under inflammatory and extreme pH conditions may indicate the long-term stability or otherwise of the membrane under similar conditions in vivo. On the other hand, as mentioned above, NSAID biofunction involves penetration of the cell-membrane and the penetrability of the drug depends on the softening or otherwise of the membrane due to its presence. The effect of NSAIDs on the compressibility of the lipid-monolayer serves as a membrane-mime in this case, too.

In this communication, we present the drug-monolayer interaction in terms of drug-induced stability and compressibility-change for varying temperatures, surface densities, drug-doses and ionic states of Piroxicam/DMPC monolayers. At the same time, we try to reveal the microscopic picture of this interaction from molecular ordering using united-atom molecular dynamics (MD) simulations.

2. Experimental details

DMPC (dimyristoylphosphatidylcholine), Piroxicam (4-hydroxy-2-methyl-N-(2-pyridinyl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide, $C_{15}H_{13}N_3O_4S$), Chloroform and methanol used are of quoted purity >99%. 115 μL of DMPC–Piroxicam solution in chloroform/methanol (9:1, v/v) of four different drug/lipid (D/L, w/w) ratios 0.000 (i.e., pure DMPC), 0.025, 0.050, 0.100 was spread in Langmuir trough on Milli-Q water (resistivity 18.2 MΩ) and compressed with a speed of 5 cm^2/min after solvent evaporation and equilibration. Surface-pressure was measured by a Whilhelmy plate during compression to obtain surface pressure-specific molecular area ($\pi - A$) isotherms. Data was collected at 15 °C, 20 °C, 25 °C, 30 °C, 35 °C and 40 °C by maintaining subphase temperature using Julabo Recirculating Cooler (FL300). Data was also collected for different subphase-pH's, low pH's (2.5, 3.0, 3.5 and 4.5) using hydrochloric acid (HCl) and high pH's (6.0, 6.5, 7.0 and 7.5) using sodium bicarbonate ($NaHCO_3$). Relaxation (area-fraction vs. time) curves were obtained by recording monolayer area with time at constant surface-pressures of π = 20 mN/m to 40 mN/m (at 5 mN/m intervals) and at 43 mN/m and pH = 2.5, 3.5, 4.5 and 5.5 for D/L = 0.000 and 0.025.

3. Experimental results

3.1. LE/LC phase-transition in presence of drug

DMPC monolayer undergoes a liquid-expanded (LE) to liquid-condensed (LC) phase transition (π_t) as the surface-pressure (π) is increased [5,6] which corresponds, respectively, to the more fluid 'gel' phase and the denser 'solid' phase of lipid membranes. Table 1a shows the values of π_t's obtained from surface pressure-specific molecular area ($\pi - A$) isotherms of pristine DMPC and Drug/DMPC monolayers at different temperatures. π_t of pure DMPC monolayers is increased as its temperature is raised. In the LE-phase the hydrocarbon chains of lipids are disordered between the *trans* and *gauche* conformations [7] while in the LC-phase they are all-*trans* in conformation. Thermal movements of lipids prevents the condensation in the monolayer resulting in increase in π_t with temperature. However, what is more interesting is that π_t is also increased due to the presence of drugs in DMPC monolayer for a constant temperature except at the high temperature of 40 °C. This indicates that the drug-molecules break the ordering in the lipids making the monolayer more fluid. Our studies of stability and softening of DMPC monolayer by Piroxicam are centred around π_t.

3.2. Stability of monolayers and effects of drug

3.2.1. Reorganization of the monolayers

Fig. 2a presents typical $\pi - A$ isotherms of a pure DMPC monolayer at 25 °C (ambient), on water with unadjusted pH (≈5.5) as the reference isotherm; mixture of DMPC with Piroxicam at drug/lipid (D/L) of 0.025 at ambient temperature and unadjusted pH ($R = D/L$); pure DMPC at 15 °C and unadjusted pH and pure DMPC at 25 °C and pH = 2.5. The area-fraction vs. time ($A_n - t$) curves at a particular π were obtained by maintaining the monolayer at that π-value while recording the monolayer area as a function of time (t) and finally normalizing the area-values with the initial area [8]. The $A_n - t$ curves were then converted to the surface-energy (E) vs. time (t) curves using the expression $E = \pi A_n$, π being the surface-pressure corresponding to each $A_n - t$ curve.

Fig. 2b and c shows, again as typical curves, the $\log E - \log t$ plots for DMPC monolayer at 25 °C and subphase pH = 2.5 for different π's, in absence and presence of Piroxicam (D/L = 0.025), respectively, while Fig. 1d shows the data of Fig. 1b in a $\log E - t$ curve. The effect of drug dosage is shown in Fig. 1e and f where the $\log E - \log t$ curves for D/L = 0.0, 0.025, 0.05 and 0.1 are shown for 25 °C and subphase pH = 2.5 at π = 30 mN/m and 40 mN/m, respectively. From these curves it is clear that, in contrast to DPPC [9] or polymer [10] monolayers, the DMPC monolayer does not show an exponential decay in surface-energy (or surface-area) with time but rather an algebraic decay given by $E \sim t^{-p}$ where the p-values extracted from Fig. 2b and c, and from Fig. 1e and f are given in Table 1b and c, respectively.

This algebraic nature of the decay strongly suggests that it entails neither desorption into the subphase nor nucleation in air [8]. This is confirmed from results of Brewster Angle Microscopy (BAM) carried out on the monolayer using an Imaging Ellipsometer (EP3, Accurion GmbH) and presented for the pristine monolayer at π = 40 mN/m after t = 0 s and 2 h in Fig. 2a and b, respectively, while the corresponding BAM images for the mixed-monolayer (D/L = 0.025) are shown in Fig. 2c and d, respectively. The small bright patches on otherwise uniformly dark background show no perceptible change in shape, size or number in any of BAM images. Thus they cannot be taken to signify any observable phase change or clustering. We feel that (a) these are either artefacts or surface structures formed as the lipid monolayer is spread on water surface and (b) both the LE and LC domains have sizes below the in-plane resolution of BAM.

The algebraic decay of surface energy with time is consistent with similar decays in two-dimensional systems, especially of complex liquids near the gelation transition [11]. Even in diffusion limited cluster aggregation of droplets in two-dimension the mean field rate equation leading to a sigmoidal growth becomes invalid and the mean cluster size grows algebraically with time [12]. In our system, on the other hand, the surface-area (or surface-energy) of the monolayer decays algebraically. In absence of structural data, we propose that surface-energy change with time corresponds to some structural relaxations whereby the monolayer assumes a closer packing.

Stability with surface pressure and drug dosage. It is expected that the ionization states of the lipid-headgroups and the drugs at different subphase-pH's will affect such structural relaxations. This is borne out from the changes in the p exponents in Table 1b