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The influence of cholesterol on interactions and dynamics of ibuprofen in a lipid bilayer

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ABSTRACT

In this work, molecular dynamics (MD) simulations with atomistic details were performed to examine the influence of the cholesterol on the interactions and the partitioning of the hydrophobic drug ibuprofen in a fully hydrated 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) bilayer. Analysis of MD simulations indicated that ibuprofen molecules prefer to be located in the hydrophobic acyl chain region of DMPC/cholesterol bilayers. This distribution decreases the lateral motion of lipid molecules. The presence of ibuprofen molecules in the bilayers with 0 and 25 mol% cholesterol increases the ordering of hydrocarbon tails of lipids whereas for the bilayers with 50 mol% cholesterol, ibuprofen molecules perturb the flexible chains of DMPC lipids which leads to the reduction of the acyl chain order parameter. The potential of the mean force (PMF) method was used to calculate the free energy profile for the transferring of an ibuprofen molecule from the bulk water into the DMPC/cholesterol membranes. The PMF studies indicated that the presence of 50 mol% cholesterol in the bilayers increases the free energy barrier and slows down the permeation of the ibuprofen drug across the DMPC bilayer. This can be due to the condensing and ordering effects of the cholesterol on the bilayer.

1. Introduction

Molecular interactions between the drug molecules and lipid membranes have always been of interest for drug delivery and drug design investigations. Drug interactions with lipid are important because of the fact that many drugs are transported in the body by drug delivery systems based on lipoproteins and liposomes [1,2]. Drugs can exert their effects via interactions with the lipid bilayer membrane, by perturbing membrane integrity, increasing the bilayer permeability, binding to transmembrane protein targets, binding to lipid signaling molecules, or altering membrane protein conformation [3]. Therefore in the design of drug and drug delivery systems knowledge about the interactions of drug with lipids has been the subject of experimental and computational studies to optimize the drugs and drug delivery systems in the context of their abilities to reach intracellular targets [3].

Among the various computational approaches, molecular dynamics (MD) simulation has been frequently employed to study drug–membrane interactions because it can capture interaction details on the molecular scale, and also extract thermodynamic properties that are directly comparable to complementary analytical measurements [4–8]. MD simulations with the potential of mean force (PMF) calculations were particularly used for study partitioning and translocation of drugs from the water to the lipid membranes [9–12]. Umbrella sampling [13] which applies a biasing potential to obtain better sampling is a common method for the PMF calculations [11,12]. However MD simulations have been widely used to study drug–lipid membrane systems, there are only a few works on study the behavior of drug molecules in the lipid membranes containing cholesterol [14–16].

The study of interaction between drug molecules with lipid membranes containing cholesterol and their transport mechanism is very important and useful, with regard to the wide distribution of cholesterol in biological membranes and its crucial effects on functional, structural, and dynamical properties of the membranes [17–21]. Therefore over the past years extensive investigations were performed to study the influence of the cholesterol on the lipid membranes experimentally by using differential scanning calorimetry (DSC), spectroscopic methods and neutron- and X-ray scattering methods or computationally by Monte Carlo (MC) and molecular dynamics (MD) simulation techniques [22–27]. The results of these investigations indicated that the incorporation of cholesterol to a lipid bilayer strongly affects its mechanical and thermodynamic properties [20,28–30] by a) broadening and eventually eliminating the gel to liquid-crystalline phase transition; b) inducing new phase regions above the phase transition as the liquid-disordered, the liquid-ordered and the coexistence of liquid-ordered and liquid disordered phases; c) increasing the area per molecule in the gel phase, and reducing the area per molecule in a liquid-crystalline phase; d) affecting the orientational ordering of the hydrocarbon chains and e) decreasing the passive permeability of the bilayer above and increasing this property below the main transition temperature.

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Ibuprofen (Ibu) is one of the most widely nonsteroidal antiinflammatory (NSAID), analgesic, and antipyretic drugs used for treatment of rheumatic disorders, pain, and fever and can be considered as a potent drug against various cancers, Alzheimer's disease and heart disease [3]. It works by decreasing production of cyclo-oxygenase (COX)-1 and COX-2 derived prostanoids in the blood [31]. Based on the results obtained from molecular dynamics simulations the ibuprofen has a high partition coefficient in the DMPC bilayer from the water and the solubility-diffusion mechanism is the likely mechanism of the permeation of ibuprofen molecules in the neutral form [9].

The aim of this work is to investigate the behavior of ibuprofen as a hydrophobic drug inside the fully hydrated DMPC bilayer containing 0, 25 and 50 mol% cholesterol with atomistic detail by using molecular dynamics (MD) simulations. Several analyses such as the area per lipid, density distributions, deuterium order parameters, mean square displacement and tilt angle were performed to elucidate the role of cholesterol on the structural and dynamic properties of drug molecules and lipid bilayers and also to explore the localization of the drug and effects of its presence on bilayers. Moreover, translocation of the Ibu from the water to the DMPC bilayer with various cholesterol concentrations was determined by using the potential of the mean constrained force method. The main energy barrier in translocation of an Ibu molecule across the membrane was obtained from the free energy profile.

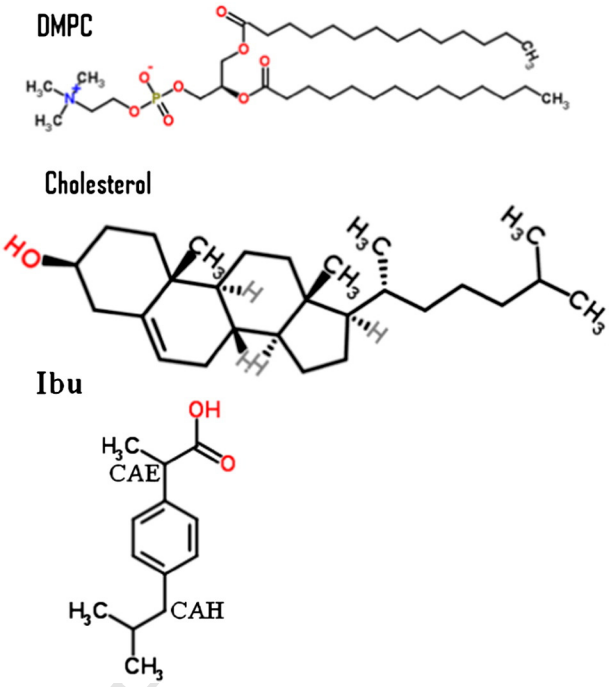

Fig. 1. Molecular structures of DMPC lipid, cholesterol and Ibu.

2. Simulation methods

In this work molecular dynamics (MD) simulations were performed on fully hydrated dimyristoylphosphatidylcholine (DMPC) lipid bilayers containing 0, 25 and 50% cholesterol (Chol) mol fractions. All preparation steps and simulations were carried out using the GROMACS v5.0.4 software package [32–34]. The pure lipid bilayer consists of 128 DMPC lipids which are arranged in a bilayer, 64 lipid molecules per leaflet, parallel to the x–y plane with three-dimensional periodic boundary conditions. Then the bilayers with the appropriate cholesterol concentrations were created by replacing the suitable number of lipids by cholesterol molecules. The number of lipid molecules and water in the simulation systems is shown in Table 1. The prepared bilayer was equilibrated by MD simulation with 20 ns time length before insertion of the drug molecules. Following insertion of 4 drug molecules (Ibu) in one side of the lipid membrane, the unit cell was filled with SPC [35] water. In all simulations, for the DMPC molecules, the partial charges and force field parameters of the Berger et al. [36] were used. The force field parameters and the topology of the cholesterol were based on the work of Holtje et al. [37]. The topology of the neutral Ibu molecule with the interaction parameters corresponding to the GROMOS 43A1 force field was obtained from the PRODRG server [38]. The atomic charges for the Ibu molecule were taken from Reference [9]. The structures of DMPC, cholesterol and Ibu molecules are shown in Fig. 1.

Each system was initially minimized using the steepest descent algorithm to remove any unfavorable contacts and interactions, followed by an equilibration simulation of 200 ps with the NVT ensemble. The 100 ns production simulation was run at constant pressure (P = 1 bar) and temperature (T = 323 K) with a 2 fs time step in the NPT ensemble and the leapfrog algorithm for integrating the equations of motion. By using the LINCS algorithm [39] all bond lengths and angles in the systems were constrained, allowing the 2 fs integration time step. The neighbor list for computing nonbonded pair forces was updated every 10 steps with a list cutoff of 1.2 nm. The Particle-Mesh-Ewald summation method [40] with a direct space cutoff of 1.2 nm, a grid spacing of 0.16 nm and an interpolation order of 4 was used to compute long-range electrostatic interactions. The system pressure was maintained at 1 bar by means of the Parrinello-Rahman barostat [41] with a coupling time constant of 1.0 ps. The temperature was maintained at 323 K, using the Nosé–Hoover thermostat scheme [42] with a coupling time constant of 0.5 ps. In this temperature DMPC bilayers with 0, 25, and 50 mol% cholesterol are in the liquid-disordered, the coexistence of liquid-ordered and liquid-disordered, and the liquid-ordered phases, respectively [43,44]. Snapshots from the production simulations of four Ibu molecules in membranes containing 0, 25 and 50 mol% cholesterol are depicted in Fig. 2.

The free energy profiles as the potential of mean force (PMF) for translocation of one Ibu molecule across the lipid bilayers were calculated using umbrella sampling [13] and the weighted histogram analysis method (WHAM) [45,46]. Starting configurations were obtained by positioning the center of mass (COM) of an Ibu molecule in the bulk water, and then pulling it into the bilayer center along the z-axis using the umbrella method. A harmonic spring with a force constant of 2000 kJ/(mol nm^2) and a pulling rate of 0.01 nm/ps was applied on COM of the drug molecule. These force and rate constants are sufficient for translocation of drug molecule across the lipid bilayers without disturbing the bilayer structure. From the obtained trajectory, 14 adjacent umbrella windows with a distance change of ~0.2 nm were selected which spanned the complete space between the bulk water and the center of bilayer. Each window was simulated for 8 ns where the *z* distance between the center of mass (COM) of the drug and DMPC bilayer (along the membrane normal direction) was constrained, and the drug Ibu was allowed to rotate and translate freely in the x–y plane. In several studies [9,12,47,48] it is shown that this simulation time for each window is sufficient for equilibration and accurate calculation of the free energy profiles. The PMF profile across the monolayer was calculated

Table 1
Volume of simulation box V (nm^3), number of water, DMPC and cholesterol in the simulation systems: system 1 (0 mol% Chol), system 2 (25 mol% Chol), and system 3 (50 mol% Chol).

	System 1	System 2	System 3
V (nm^3)	277.95	206.39	170.84
N_{H_2O}	4491	2725	2103
N_{DMPC}	128	96	64
N_{Chol}	0	32	64