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Perspective Article

Molecular structure and dynamical properties of niosome bilayers with and without cholesterol incorporation: A molecular dynamics simulation study

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ABSTRACT

Niosomes are non-ionic surfactant vesicles having a bilayer structure formed by self-assembly of hydrated surfactants, usually with cholesterol incorporation. Stability and mechanical properties of niosomes strongly depend on type of non-ionic surfactants and compositions used. In this study we present the structural and dynamical properties of niosome bilayers composed of sorbitan monostearate (Span60) with 0% and 50% cholesterol compositions which are investigated by using molecular dynamics simulations. The simulations reveal that niosome bilayer without cholesterol prefer to form in the gel phase with a higher order structure, while in the presence of cholesterol the bilayer exhibits more fluidity having a less ordered structure. The niosome bilayer with 50% cholesterol inclusion shows an increase of area per lipid (~11%) and thickness (~39%) compared with the niosome bilayer without cholesterol. The Span60 tailgroup orientation of the niosome bilayers without cholesterol exhibits more tilt ($34.5^{o} \pm 0.5$) than that of the bilayer with 50% cholesterol ($15.4^{o} \pm 0.8$). Additionally, our results show that the addition of cholesterol to the bilayer causes the higher in lateral and transverse diffusion, as well as an increase in the hydrogen bond number between Span60 and water. Such characteristics not only enhance the niosome stability but also increase the fluidity, which are necessary for the niosomal drug delivery.

1. Introduction

Applications of nanotechnology in the field of nanomedicine for therapy and diagnosis have made rapid progress, particularly in drug delivery systems [1]. In the recent years, much more attention has been paid to vesicular drug delivery systems including liposomes and niosomes due to their special characteristics, such as the high entrapment and release rate, low toxicity and biodegradability [1,2]. As a result, synthesis and characterization of these carriers for drug delivery to specific targets through nano-encapsulation have been widely studied by experimental and theoretical methods [3–11]. These studies provide valuable information on mechanisms of vesicular formation, stability, molecular transport through the cell membrane, and molecular interaction between drug and carriers. However, liposomes suffer from the problem that they are composed of very reactive naturally occurring phospholipids that are not stable in air and rapidly undergo oxidation and hydrolysis, compromising long term stability and making formulation very costly [12]. Furthermore natural sources of phospholipids are impure and synthetic sources prohibitively expensive for production drug manufacture.

Niosomes are very similar to liposomes, but are composed of synthetic non-ionic surfactants, e.g. sorbitans (Spans) or polysorbates (Tweens), usually in combination with cholesterol [12]. This makes them more resistant to oxidation and hydrolysis, making formulation relative to liposomes much cheaper and simpler, and giving good long term stability [10]. The Span and Tween surfactants are also biocompatible, have very low toxicity and are cheaply available at high purity [1]. Due to their characteristics, they are widely used for the food and drug industry [13]. The Spans are often used as absorption enhancers [8,14] due to their surfactant properties. It is well known that the mechanism of niosome formation is closely related to the self-assembly of non-ionic surfactants

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on water surfaces, leading to formation of monolayer and bilayer structures [15].

In the past decades, many molecular simulations (MD) have extensively studied liposomes due to a lot of available experimental data to support models [11,16–22]. A few MD studies of niosomes have also been reported [4,5]. However there is a limited amount of experimental data available on Span60 monolayers or bilayers [23,24]. These previous works provide insight into the monolayer structure, stability and conditions of layer formation. The study of Nasseri [26] showed that niosomes prepared from the Span60 with cholesterol inclusion exhibits the most stable and condensed layers at both room and body temperatures. According to the study of Peltonen et al. [23], the Span60 showed the most stable monolayer film at the water–air interface over the temperature range (from 20 to 42 °C) comparing with other Span family. Recently, Han [5] has reported the MD simulation of the Span80 bilayers with and without cholesterol at 27 °C. His study provides detailed information on the bilayer structures and dynamical properties which are consistent with the experimental data for DOPC lipid bilayers. As far as we know, a molecular dynamics simulation of the Span60 bilayers with and without cholesterol inclusion was never studied before. It is important to understand the molecular level properties of the surfactant assembly, molecular structure, and dynamical behavior, as these give rise to the macromolecular properties of fluidity, elasticity, and shear strength that are critical for stability of nanoparticulate vesicles [7,9,15], their behavior within other fluids such as blood, and their transport across cell membranes [15]. In this study we focused on the bilayer structure and dynamical properties of the pure Span60 bilayer and the Span60 bilayer with 50 mol% cholesterol composition based on using molecular dynamics simulation technique. Bilayer properties including area per lipid, membrane thickness, molecular orientation, order parameters, lateral and transversal diffusion, and number and dynamics of hydrogen bonds for the Span60/water, Span60/Span60, Span60/cholesterol were extracted from the simulations at temperature 298 K and compared to previous studies [3–5,23]. The results obtained from these simulations not only provide structural and dynamical properties, but also give a clear picture of the dynamics of the bilayer formation at the molecular level.

2. Materials and methods

2.1. General system setting

Firstly, the structures of the Span60 and cholesterol molecules were geometrically optimized by using B3LYP/6-3G(d,p) calculations. Then these optimized structures (Fig. 1a) were employed to generate Span60 bilayers with and without cholesterol inclusion by using the CELL microcosmos 2.2 software [25]. To build the niosome bilayer formed by the 50:50 mol% of the Span60: cholesterol compositions (Span60/Chol bilayer), the Span60 and cholesterol molecules were placed randomly in a box area of 5.00 nm × 5.00 nm with the hydrophilic parts in each layer pointing away from each other while the hydrocarbon parts faced each other. The Span60/Chol bilayer consisted of 50 Span60 and 50 cholesterol molecules in each leaflet, leading to 100 Span60 and 100 cholesterol molecules in total. In order to make comparison, a bilayer consisting of the pure Span60 molecules (Span60 bilayer) was generated in the same manner. The Span60 bilayer contained 85 Span60 molecules in each leaflet, leading to 175 Span60 molecules in total. Experimental study has revealed that niosomes composing of the Span60 and cholesterol mixture with equal ratio have more stability than the other compositions [26]. As a result, we have selected this model for the simulation study.

To set up the Span60 bilayers with and without cholesterols in aqueous solution, a water slab consisting of 2447 water molecules in a rectangular box of 5.00 nm × 5.00 nm × 3.00 nm in x, y and z directions, respectively was simulated at the temperature of 298 K and a pressure of 1 bar until the system reached to equilibrium using the Gromacs program version 4.5.4 [27,28]. The equilibrated water slab was duplicated and inverted in order to make a bilayer, i.e. one capped at the top of Sapn60 layer and the other capped at the bottom of Span60 layer, leading to form the Span60 bilayer with and without cholesterol molecules in aqueous solution as displayed in Fig. 1b and c, respectively. Each bilayer system was filled in the simulation box of 5.00 nm × 5.00 nm × 12.50 nm which contained 4894 water molecules. In these bilayer structures the hydrophilic head groups are exposed to water phase while the hydrocarbon tails point toward to the center of the bilayers. The initial area per lipid of the Span60 and Span60/Chol bilayers was 29.41 Å^2 and 25.00 Å^2, respectively.

2.2. Simulation details

The simulations of the niosome bilayers were carried out by using Gromacs 4.5.4 package [27,28]. The force field parameters and electrostatic charge distributions of the Span60 and cholesterol molecules were obtained from our previous work [4] and from the MD study of Holtje et al. [29] respectively. These parameters were based on Gromos87 force field [30], except that atomic partial charges were implemented for the both molecules. The united atom description was applied to hydrocarbon sites, i.e., CH_1, CH_2, and CH_3. The SPC/E model was employed to describe water molecules. It should be noticed that such a methodology has been successfully employed for describing self-assemblies of porphyrin and Span60 at the water–air interface [4,31]. These studies indicate that the simulation results well reproduced experimental data.

To remove undesired overlap between neighboring atoms, the niosome bilayers with and without cholesterol were first subjected to energy minimization (EM) by using the steepest descent method. After that, the resultant configurations for each system were used to perform molecular dynamics at a temperature of 500 K for 100 ps in order to remove any undesired interaction between neighboring molecules. Then both systems were subjected to NTP molecular dynamics at constant temperature of 298 K and pressure of 1 bar. The choice of this temperature corresponds optimal conditions for the formation of niosome vesicles that show the highest stability [26]. To maintain a constant pressure, a Berendsen barostat [32] with semi-isotropic coupling in the lateral direction (in xy-plane) and the normal direction (in z direction) were used with a time constant (τ_P) of 0.5 and 0.5 ps and a compressibility of 4.5×10^{-5} bar^{-1} and 4.5×10^{-5} bar^{-1}, respectively. To maintain a constant temperature in the system, the Span60, cholesterol, and water were coupled separately to the v-rescale thermostat with a coupling constant $\tau_T = 0.1$ ps. The periodic boundary conditions were applied in all three directions. The bonds between atoms of the molecules were constrained by using the LINCS algorithm [33] with fourth order expansion. The equations of motion were integrated using the leap-frog algorithm with a time step (Δt) of 2 fs. The electrostatic interactions were calculated using the Fast Particle Mesh Ewald method (PME) method [34] and fourth order (cubic) interpolation was used with a grid spacing of 0.15 nm. The coulomb and van der Waal interactions were cut off after a distance of 1.5 nm. The neighbor list was updated every 10 time steps. Both two systems were simulated for 120 ns (60 ns initial equilibrium, 60 ns production run). Molecular structures and configurations of these systems were visualized by using the VMD program [35].

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