http://dx.doi.org/10.5012/bkcs.2013.34.3.946

Molecular Dynamics Simulation of Sorbitan Monooleate Bilayers

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Received November 5, 2012, Accepted December 5, 2012

Key Words : Molecular dynamics simulation, Sorbitan monooleate, Span 80, Niosome

Niosomes are vesicular liposomes prepared from nonionic surfactants such as Span, Tween, and Brij. With a good chemical stability and low cost of production, they were originally developed to substitute phospholipid vesicles in drug delivery. Due to a higher transdermal permeability and penetrability, niosomes have attracted much interest in the research field of topical drug delivery. There have been numerous reports on the application of niosomes in the transdermal delivery of various drugs.[1] Properties such as entrapment efficiency and release rate have also been studied extensively.[2] However, detailed structural studies are scarce and thus the physicochemical properties of niosomes are not readily explained at the atomic level. In this respect a theoretical modeling such as molecular dynamics (MD) simulation can provide a structural basis for the interpretation of experimental results.[3] To our knowledge this is the first report on MD simulation of a niosomal bilayer of any kind.

The structures and atom numbering of sorbitan monooleate (Span 80) and cholesterol are shown in Figure 1. The Gromos 43a1-s3 force field[4] was used in the calculation because it has been extended to include several phospholipids and cholesterol. Topology and parameters for the oleate and glycerol fragments of Span 80 were transferred from 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC). Parameters for sorbitan moiety were obtained from those of similar structures listed in the Gromos 43a1-s3 force filed. A monolayer was constructed by filling 144 Span 80 molecules into a 12 × 12 array of equally sized boxes. A 8 × 8 array (total 64 molecules) was used for DOPC. Random rotation of the molecule within a grid did not alter the final simulation suggesting that the results were independent of the initial structures. The layer was duplicated and inverted to make a bilayer. Packmol,[5] a free software useful for the construction of a solvated bilayer, was used to fill water molecules below and above the bilayer at a thickness of 1.5 nm and density of 10^3 kg/m^3. The resulting structure was subjected to an energy minimization and subsequently equilibrated for 100 ps at 1 bar and 298 K. A production MD simulation was carried out for 60 ns and the final 20 ns trace was used in the analysis of various physical properties. Particle mesh Ewald method[6] was used in the calculation of Coulomb interactions. Cutoff values for the Coulomb and van der Waals interactions were 1.0 and 1.6 nm, respectively. Bond lengths were constrained by using the LINCS algorithm[7] to allow a 2 fs integration. The temperature of

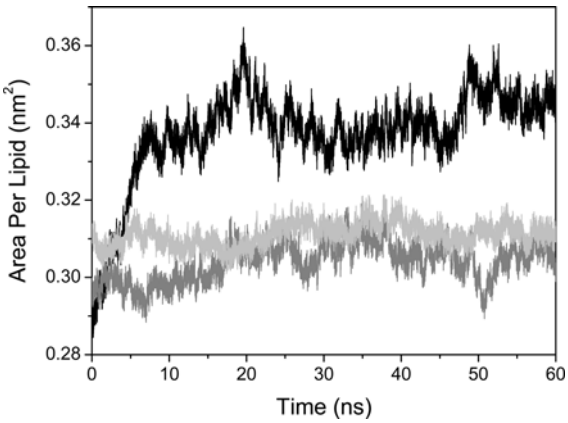

Figure 2. Time evolution of the area per lipid of Span 80 bilayer containing 0 mol % (dark), 25 mol % (gray), and 50 mol % cholesterol (light gray).

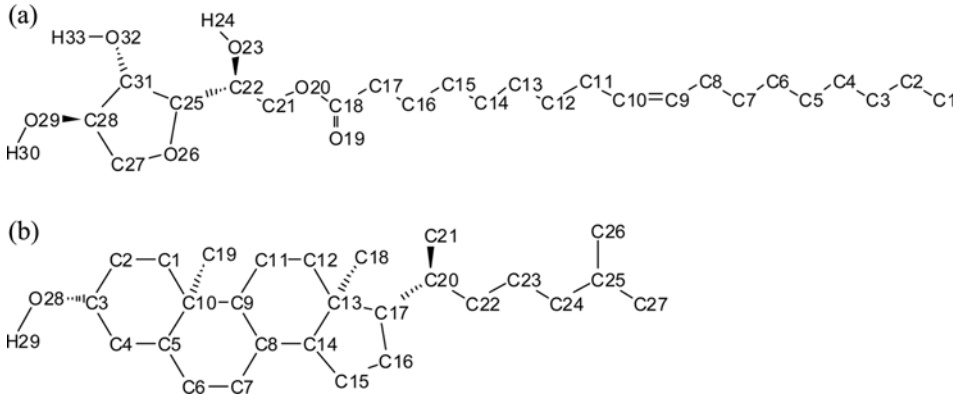

Figure 1. Structures and atom numbering of (a) Span 80 and (b) cholesterol.

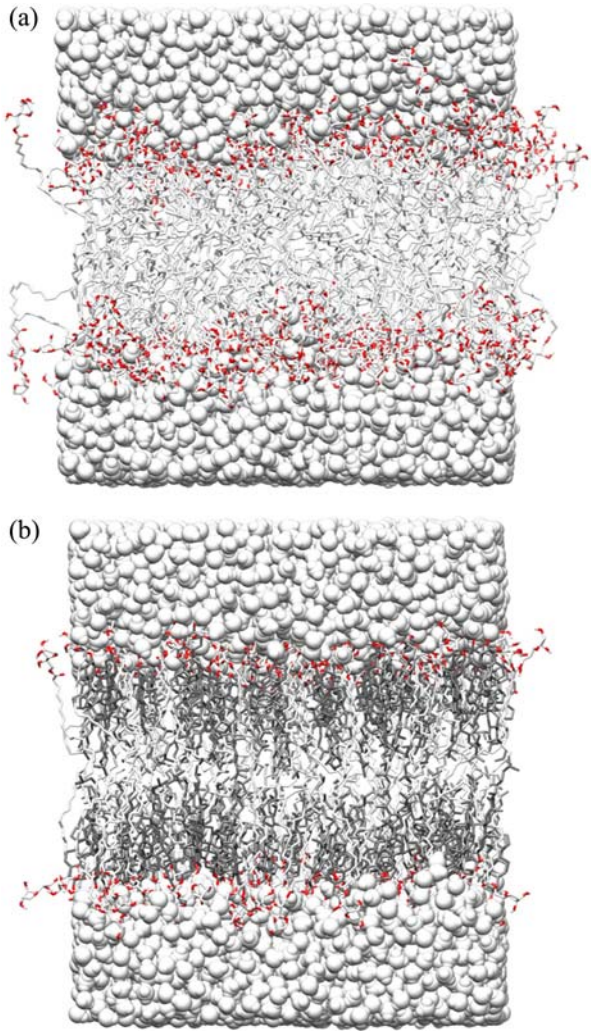

Figure 3. Snap shots at 60 ns of MD simulation of the Span 80 bilayer containing (a) 0 mol % and (b) 50 mol % cholesterol. Span 80 molecules are in light gray sticks and cholesterol in dark gray. Water molecules in space-filling model are shown below and above the bilayer.

lipids (Span 80 and cholesterol) and water were coupled separately using a V-rescale thermostat.[8] A Parrinello-Raman barostat[9] was used to couple the pressure semiisotropically.

Figure 2 shows the changes in the area per lipid (APL) as a function of simulation time. Fluctuations in APL were prominent in the bilayer composed of pure Span 80. There was a continual increase in APL during the first 10 ns and thereafter APL flattened with significant fluctuations. Inclusion of cholesterol reduced the time to reach equilibrium as well as the amplitude of fluctuations. This is consistent with the fact that cholesterol acts as a bilayer stabilizer in a liposome prepared from phospholipids or neutral surfactants.[10] Instability of Span 80 bilayer in the absence of cholesterol was consistent with a recent analysis of Span 80 vesicles.[11]

The structures of the bilayers after 60 ns of MD simulation are presented in Figure 3. In the absence of cholesterol (Fig. 3a) the bilayer was in a liquid crystalline phase with a large lateral diffusion constant (Table 1). This is expected as the phase transition temperature of Span 80 was determined to be 254 K,[11] which is much lower than the simulation temperature (298 K) of the present work. The APL was 0.343 nm^2, which was close to half the APL of DOPC (0.656 nm^2). This suggests that a major contribution to the APL was made by the area occupied by the fatty acid chains. The water density at the carbonyl carbon of the ester group was 0.053 g/cm^3, which was significantly smaller than the value for DOPC (0.150 g/cm^3). One molecule of Span 80 formed 3.11 hydrogen bonds with water and 0.87 with Span 80.

In the presence of 50 mol % cholesterol (Fig. 3(b)), a composition frequently used in the preparation of a niosome, the bilayer was thicker with a smaller average APL compared with the bilayer without cholesterol. The diffusion coefficient became smaller with the inclusion of cholesterol but it was still large enough to conclude that the membrane was in a liquid crystalline phase (see Table 1). Water density at the ester carbon increased slightly in both Span 80 and DOPC when cholesterol was added. However, the number of hydrogen bonds between the lipid and water increased from 3.11 to 3.97 in the Span 80 bilayer but decreased from 6.20 to 0.85 in DOPC. At this composition of cholesterol the Span 80 surface appeared to be more 'wet' than DOPC. This agrees with the results obtained from a fluorescence study.[12]

Table 1. Comparison of physical properties of Span 80, Span 60, and DOPC bilayers

Lipid	Span 80		DOPC	
mol % cholesterol	0	50	0	50
Area per lipid (nm^2)	0.343 ± 0.006	-	0.656 ± 0.010[a]	-
Thickness (nm)[b]	2.980	3.49	2.87	3.53
Water density at ester group[c]	0.053	0.079	0.150	0.190
Number of hydrogen bonds				
H_2O-Span 80 or DOPC[d]	3.11	3.97	6.20	0.85
H_2O-Cholesterol[d]	-	1.52	-	1.86
Order parameter, C2-C6	0.069 ± 0.014	0.154 ± 0.019	0.099 ± 0.022	0.215 ± 0.059
C13-C17	0.206 ± 0.019	0.354 ± 0.026	0.187 ± 0.017	0.349 ± 0.064
Diff. coefficient ($\mu m^2/s$)[e]	9.819 ± 0.002	4.831 ± 0.0023	5.663 ± 0.017[f]	2.452 ± 0.012

[a]Experimental value is 0.691 at 15°C.[14] [b]Taken from the peak-to-peak distance between the carbonyl carbons in a density profile. For DOPC the values for sn-1 and sn-2 are averaged. [c]Density of water at the peak position of carbonyl carbon in a density profile. [d]Values per lipid molecule. Hydrogen bonds were calculated for all possible combinations of donor and acceptor atoms. Default parameters of Gromacs were used in defining a hydrogen bond. [e]Diffusion coefficient for a Span 80 or DOPC molecule. [f]Experimental value is 8.25 at 25 °C.[15]

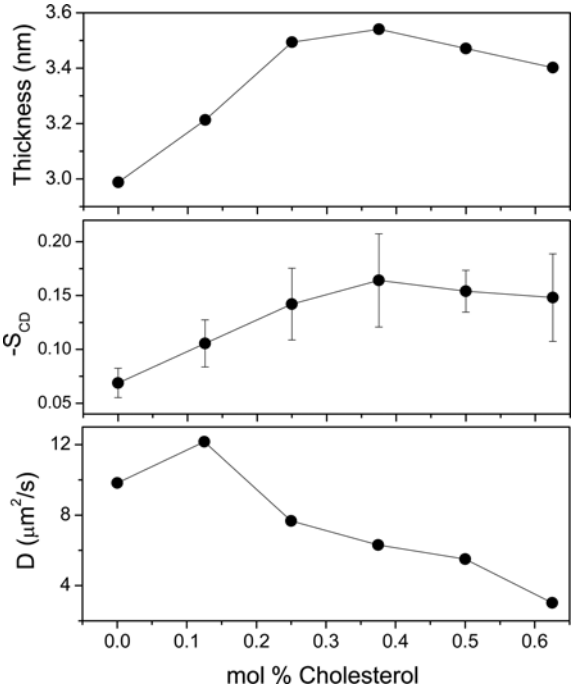

Figure 4. Changes in (a) the thickness, (b) order parameter, and (c) lateral diffusion coefficient as a function of mol % cholesterol. Thickness in (a) was taken from the distance between the carbonyl carbons (C18) of Span 80 and the average order parameter in (b) was calculated for C2-C6.

Majority of the cholesterol formed a hydrogen bond with the carbonyl oxygen of Span 80 and this structure was also proposed previously from the measurement of shear modulus of surface elasticity.[13]

In Figure 4, some important physical properties were summarized. The bilayer thickness estimated from the distance between the carbonyl carbon atoms increased with the mol % of cholesterol (Fig. 4(a)). It was 2.98 nm when cholesterol was absent and reached a maximum value of 3.54 nm at 37.5 mol % cholesterol. Parallel calculations on DOPC bilayers showed that the thickness of pure DOPC bilayer was 2.87 nm, which is not much different from that of Span 80. A similar result was obtained experimentally for a POPC bilayer.[11] Increase in the thickness by cholesterol was more dramatic in DOPC bilayers than Span 80 (see Table 1). It can be concluded from this result that the Span 80 bilayer was not excessively swollen.[11]

Order parameter ($-S_{CD}$) is often cited as an index of membrane fluidity. In Figure 4(b), the average order parameter of C2-C6 is plotted as a function of cholesterol mol %. Like the bilayer thickness, the order parameter also maximized at 37.5 mol % cholesterol. In reasonable agreement with our theoretical prediction, Nasseri[13] reported a maximum rigidity at 47.5 mol % cholesterol. Without cholesterol the order parameter of Span 80 bilayer (0.069) was slightly smaller than that of DOPC (0.099). At 50 mol % cholesterol, the values were 0.154 for Span 80 and 0.215 for DOPC (see Table 1). This suggests that a Span 80 bilayer has a larger fluidity than DOPC in the absence and presence of cholesterol. In contrast, the average order parameter for C13-C17 (close to the headgroup) was larger for Span 80 than DOPC (see Table 1). This suggests that, compared with DOPC, the central part of Span 80 bilayer was less ordered whereas the region near the headgroup was more ordered.

Diffusion coefficient of a Span 80 molecule was calculated from the mean-square deviation of the atom O29 in Span 80 ($<r^2> = 4Dt$). Without cholesterol the diffusion coefficient for Span 80 was 9.82 mm^2/s, which was much larger than 5.663, the corresponding value for DOPC (see Fig. 4(c) and Table 1). This value is reasonable considering that the experimental value for DOPC is 8.25 at 25 °C.[14] Interestingly the diffusion coefficient was maximized at 12.5 mol % cholesterol and thereafter continually decreased with the cholesterol concentration. At 50 mol % cholesterol the diffusion coefficient decreased to 4.831 but it was still larger than 2.452 of DOPC. This demonstrates that lateral diffusion of a lipid molecule was faster in a Span 80 bilayer than in a DOPC bilayer over the whole range of cholesterol concentration. In addition the value of Span 80 at 50 mol % cholesterol was large enough to conclude that the Span 80 bilayer was in a liquid crystalline phase.

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