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INFLUENCE OF CARBON NANOTUBE ON CHOLESTEROL LODGMENT: MOLECULAR DYNAMICS SIMULATION

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Abstract. The dynamics of cholesterol molecules embedded between phospholipids in a cell membrane as well as those forming an ultrathin layer (lodgment) around an extracellular domain protein has been studied *via* molecular dynamics (MD) simulations. We have also investigated the impact of an armchair (10, 10) carbon nanotube on the nanosystems studied. We have shown that the presence of a nanotube even quite close to the cell membrane does not destroy the cholesterol – phospholipid system structure, *i.e.* it is neutral for the membrane functioning. On the other hand, the carbon nanotube influences the motion of cholesterol molecules forming a layer developed over the surface of a protein.

1. INTRODUCTION

Cholesterol is a major constituent of the eukaryotic cell membrane. Its abundance influences such diverse membrane processes as signal transduction, protein stabilization, protein and lipid sorting, and membrane fusion [1–4]. Independent of its permanent presence in a cell membrane, cholesterol is transported through the blood as a component of water-soluble carrier aggregates known as lipoproteins. A lipoprotein aggregate is composed of an outer shell of phospholipids which renders the particle soluble in water; a core of fats called lipid, including cholesterol and a surface apoprotein molecule that allows some tissues to recognize and take up the aggregate core content. Although cholesterol is essential for the proper functioning of cell membranes, excess cholesterol levels could prove detrimental. Particularly, excess cholesterol may precipitate in forming cholesterol lodgments (domains) in the inner lining of blood vessels. This triggers the formation of plaque deposition in the atherosclerosis disease [5,6]. In this work we have conducted, *via* simulation, a reconnaissance study of the influence of a carbon nanotube on the dynamics of cholesterol molecules; a) embedded between phospholipids in a cell membrane, and b) forming a lodgment around a selected extracellular domain protein.

2. SIMULATION DETAILS

We have used the standard Lennard-Jones (LJ) interaction potential $V(r_{ij})$ between carbon atoms of an armchair (10, 10) nanotube with a diameter of 13.6 Å [7] and the atoms (sites) of a rigid-body cholesterol $C_{27}H_{45}OH$, phospholipid and protein. Namely, $V(r_{ij}) = 4\varepsilon[(\sigma/r_{ij})^{12} - (\sigma/r_{ij})^{6}]$, where r_{ij} is the distance between the atoms *i-th* and *j-th*, ε is the potential minimum at a distance of $2^{1/6}\sigma$. Cholesterol and phospholipid molecules include lots of atomic sites, however, the CH, CH_2, and CH_3 atomic groups are treated as supersites (pseudoatoms) in line with the common procedure for large molecules [8]. The L-J parameters for these groups and other atoms involved are taken from [9–11]. Moreover, we have

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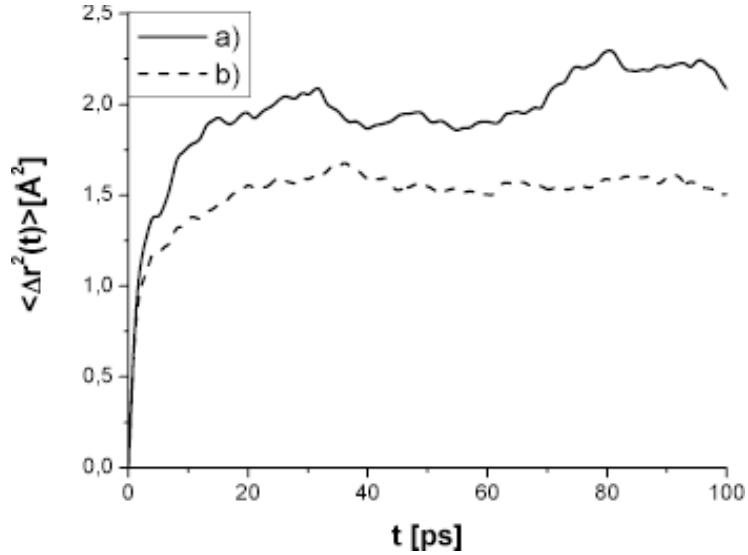

Fig. 1. The mean square displacement of the center of mass of a cholesterol molecule embedded between phospholipids in the presence (a) and absence (b) of a carbon nanotube.

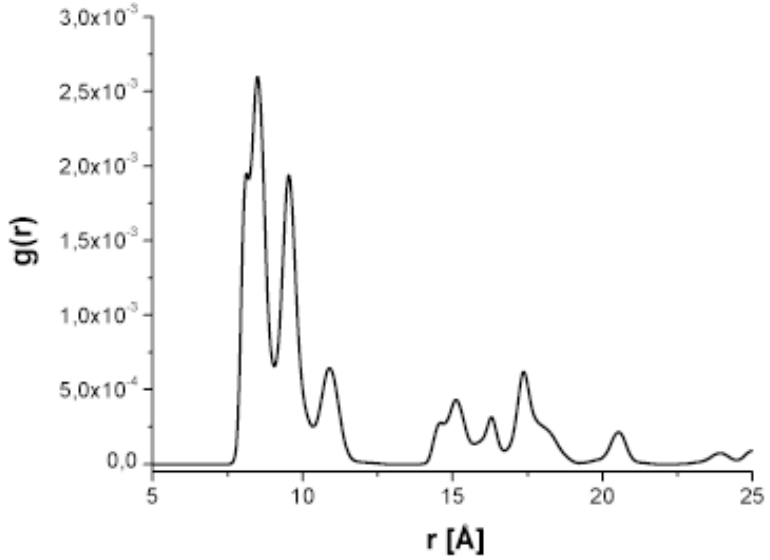

Fig. 2. The radial distribution function of the center of mass of a cholesterol molecule embedded between phospholipids in the presence of a nanotube.

included the cholesterol and phospholipid (OH bonds) dipole moments by putting a charge of 0.376 *e* and 0.376 *e* on oxygen and hydrogen atoms of OH bonds [12], respectively and the total of the Coulombic interaction between those charges has been calculated for different molecules. The parameters of L-J potentials between unlike atoms and pseudoatoms have been calculated by the Lorentz-Berthelot rules: $\sigma_{A\text{-}B} = (\sigma_A + \sigma_B)/2$ and $\varepsilon_{A-B} = \sqrt{\varepsilon_A \varepsilon_B}$ [13], where A, B are C, O, N, S, H, CH, CH_2, and CH_3 atoms or pseudoatoms. We have chosen 1L8J as an example of a human extracellular domain protein [14] (see also Protein Data Bank [15]). This protein appears in a thin layer of cells (called endothelium) that line the interior surface of blood vessels, forming an interface between the blood circulating in the lumen and the rest of the vessel wall. The initial distribution of molecules was generated by the Monte-Carlo (MC) algorithm of potential energy minimization [13]. The classical equations of motion were integrated up to 1 ns by the predictor-corrector Adams-Moulton algorithm [16]. The integration time step was 0.4 fs which ensured total energy conservation within 0.01%.

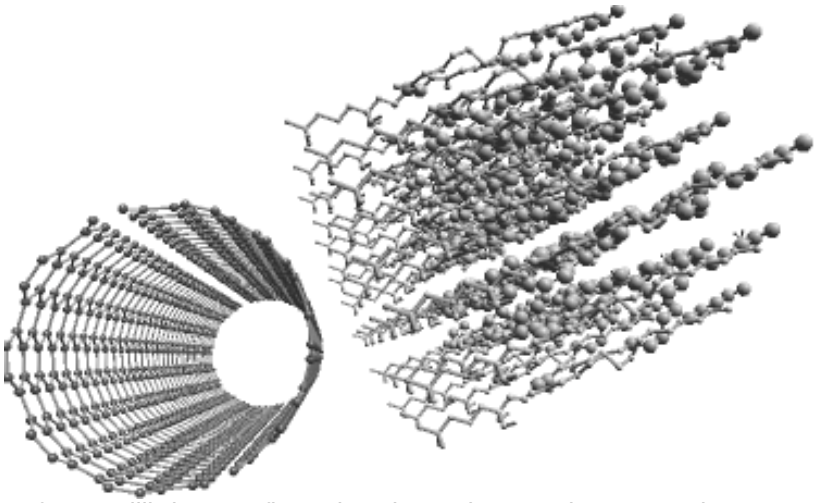

Fig. 3. A snapshot of an equilibrium configuration shows that a carbon nanotube cannot pull cholesterols out of a phospholipid bilayer.

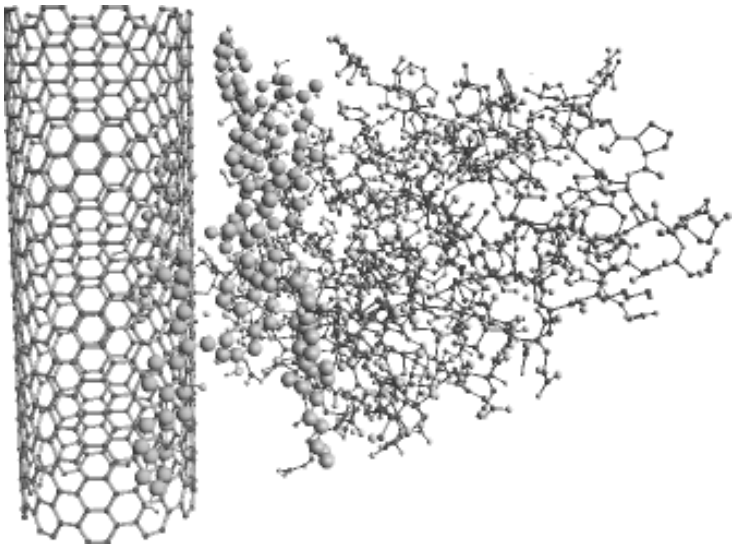

Fig. 4. A snapshot of an equilibrium configuration of a system composed of a 1L8J protein and cholesterols in the presence of a nanotube.

3. RESULTS

Cholesterol molecules located between nonpolar hydrophobic fatty acid tails of neighboring phospholipid molecules which form a phospholipid bilayer sheet are considered at first. The bilayer outer side forms polar hydrophilic phosphate heads of phospholipids. MD simulations have been performed for this system (without a nanotube) for reference purposes. Next, a carbon nanotube is placed close to the "sea" of phosphate heads and the simulation is repeated. Two dynamical observables of a cholesterol molecule have been calculated: the radial distribution function and the mean square displacement of the center of mass. The mean square displacement $\left\langle |\Delta \boldsymbol{r}(t)|^2 \right\rangle = \left\langle |\boldsymbol{r}(t) - \boldsymbol{r}(0)^2 \right\rangle$ (the three dimensional vector $\boldsymbol{r}(t)$ is the position of the center of mass of a single molecule) of the center of mass of cholesterol at T=309K with and without the presence of a nanotube is compared in Fig. 1. The cholesterol molecule set tight between phospholipids cannot walk farther than 0.47 Å in the absence of a nanotube, whereas the cholesterol's $\left\langle |\Delta \boldsymbol{r}(t)|^2 \right\rangle$ reaches saturation at 1.4 Å in the presence of a nanotube. It can be seen that the cholesterol somehow "feels" the nanotube, namely it is slightly attracted by the nanotube surface, hence, an increase in the maximum distance that it can "walk" can is observed. However, the $\left\langle |\Delta \boldsymbol{r}(t)|^2 \right\rangle$ plot (Fig. 1)

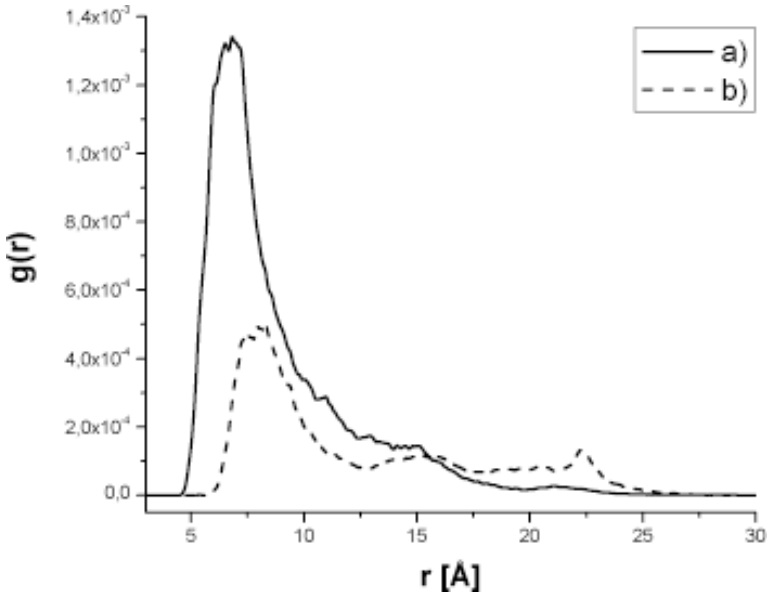

Fig. 5. The radial distribution function of the center of mass of a cholesterol molecule in a lodgment in the absence (a) and presence (b) of a carbon nanotube.

shows that cholesterol remains inside the phospholipid bilayer all the time and the nanotube cannot pull it out of the cell membrane Slight waving of $\langle |\Delta \boldsymbol{r}(t)|^2 \rangle$ might reflect the complicated translational dynamics of cholesterol excited by the nanotube and at the same time kept quite firmly overall by the phospholipids. The slope of $\langle |\Delta \boldsymbol{r}(t)|^2 \rangle$ is connected with the translational diffusion coefficient *via* Einstein relation $\langle |\Delta \boldsymbol{r}(t)|^2 \rangle \cong 6Dt$. The plot of $\langle |\Delta \boldsymbol{r}(t)|^2 \rangle$ indicates that the system is in the liquid phase, the slope of the linear part of cholesterol mean square displacement is 1.4 * 10^{-1} Å^2/ps. The diffusion coefficient value, calculated from the linear part of $\langle |\Delta \boldsymbol{r}(t)|^2 \rangle$ is D=2.4* 10^{-6} cm^2/s. Several groups of peaks can be distinguished in the plot of $g(r)$ (Fig. 2). The first group of three peaks in the range between 8 – 12 Å is associated with the near neighbors of a cholesterol molecule. The second group of four peaks in the range between 15 – 21 Å corresponds to the cholesterol neighbors occurring at a longer distance (second shell), and so forth. This means that, on average, the locations of cholesterols embedded between phospholipids are not affected by the nanotube. Summarizing the above presented results it can be concluded that the dynamics of the cholesterols which are located in a cell membrane between phospholipids is only slightly affected by the carbon nanotube. Particularly, which is very important, the carbon nanotube cannot pull cholesterol molecules out of the phospholipid layer – this is visualized on the snapshot of an equilibrium configuration (Fig. 3).

Our next step is to simulate the system composed of a 1L8J protein plus, let us say, seven cholesterols. We have observed that cholesterols gather together near the protein surface, forming a cholesterol lodgment. Then, we have placed a carbon nanotube near the cholesterol domain (see Fig. 4). Holding the nanotube immobile in its place, we run a simulation again collecting MD data. The radial distribution function $g(r)$ of the centers of mass of cholesterols in the lodgment (without a nanotube, see Fig. 5) shows one peak which indicates the average near neighbors distance r = 6.8 Å. The highest peak of $g(r)$ for a lodgment with a nanotube (Fig. 5) appears at a larger distance of r = 8.3 Å comparing to r = 6.8 Å for a lodgment without a nanotube. It must be associated with the average centers of mass distance between cholesterols covering the nanotube. Two little peaks at r = 15 Å and r = 22.2 Å come from farther neighbors. The $g(r)$ value reaches zero around $r \approx 20$ Å for the lodgment without a nanotube, and around $r \approx 27$ Å in case with a nanotube, indicating thus the cholesterol lodgment diameters.

The mean square displacement $\langle |\Delta \boldsymbol{r}(t)|^2 \rangle$ of the center of mass of a cholesterol molecule is shown in Fig. 6. The difference between $\langle |\Delta \boldsymbol{r}(t)|^2 \rangle$ with

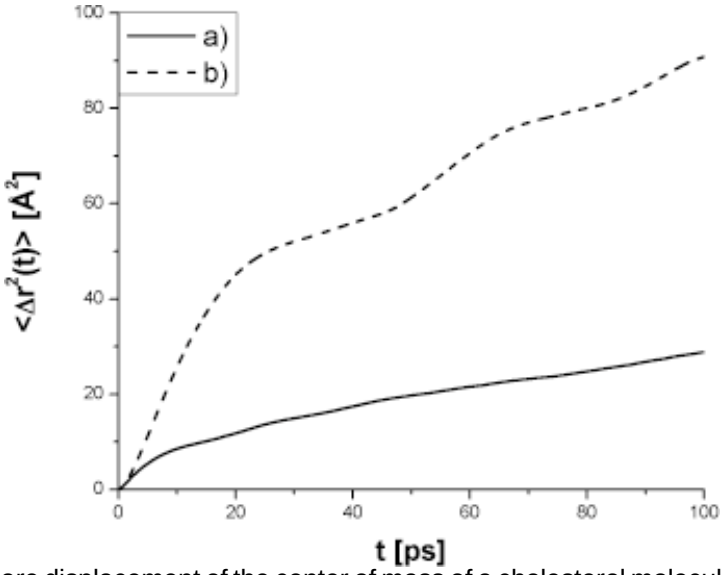

Fig. 6. The mean square displacement of the center of mass of a cholesterol molecule in a lodgment in the absence (a) and presence (b) of a carbon nanotube.

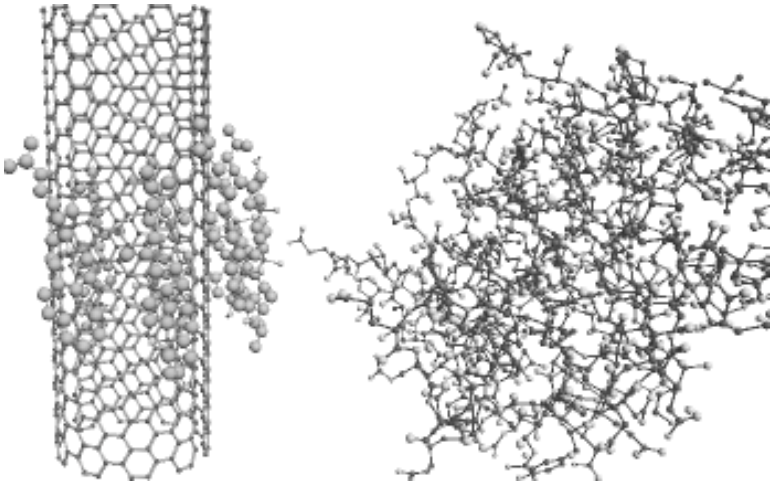

Fig. 7. A snapshot of an equilibrium configuration after pulling a carbon nanotube out of a cholesterol lodgment placed over a 1L8J protein.

and without a nanotube is distinct. The plot of $\left\langle |\, \Delta \boldsymbol{r}(t)\, |^2 \right\rangle$ without a nanotube is somehow similar to the dense media, the slope of $\left\langle |\, \Delta \boldsymbol{r}(t)\, |^2 \right\rangle$ is low, hence the translational mobility of cholesterols within the lodgment is very weak. The value of $\left\langle |\, \Delta \boldsymbol{r}(t)\, |^2 \right\rangle$ and its slope increases spectacularly when the nanotube is near the cholesterol lodgment (cholesterols get mobile). An increase in $\left\langle |\, \Delta \boldsymbol{r}(t)\, |^2 \right\rangle$ in the nanotube's presence reflects the simple fact that the nanotube is pulling cholesterols out of the lodgment. The pulled-out cholesterols spread all over the nanotube surface forming a thin layer covering the carbon nanotube. Therefore, removing the covered nanotube substantially diminishes the number of cholesterols remaining within the lodgment. The process of cholesterol lodgment extraction by a carbon nanotube is quite efficient. Our nanotube has pulled out all cholesterols (see Fig. 7 for a snapshot). The extraction process efficiency might not be 100 percent in case of a larger lodgment. It would be required to repeat this procedure (nanotubes return and departure) to remove all cholesterols surviving the first nanotube intervention. The reported ability of the nanotube to extract a cholesterol lodgment at a physiological temperature is quite appealing.

4. CONCLUSIONS

In conclusion, our MD calculations show that a carbon nanotube cannot pull out those cholesterol molecules which are embedded between phospholipids building a cell membrane (phospholipid bilayer). This is exactly what one would hope to happen. It means that the presence of a nanotube, even quite close to the cell membrane, does not destroy the cholesterol–phospholipid system's structure and natural balance. Nevertheless, our simulations show that a carbon nanotube can diminish the cholesterol lodgment placed on a protein. This happens as the attraction of cholesterols by a nanotube prevails over the cholesterols' tendency to gather together (setting up a lodgment). Being tired of their lodgment, cholesterol molecules spread out all over the nanotube surface, forming a thin layer [17]. Our simulations suggest that the reported ability of carbon nanotubes might be considered in designing future medical treatment of atherosclerosis diseases.

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