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Impact of the Carbon Allotropes on Cholesterol Domain: MD Simulation

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1. Introduction

In recent years, immense advancement has been made in the field of nanotechnology. This emerging field will indefinitely become a critical facet of many areas including chemistry, biology, electronics and optics, and will provide unique opportunities for researchers to innovate in unimaginable ways. Nanomaterials, because of their unique mechanical, thermal, optical and electronic properties, have reshaped many segments of modern science and engineering and are increasingly impacting our society, health care, and the environment. Specifically, nanotechnology has great potential in biomedical applications, as mammal/human biology is essentially a very complex system of nano-machines. Nowadays big changes are coming from the marriage of medicine and nanotechnology - the new branch of science called nanomedicine or molecular medicine. A field of utilizing molecular assemblies at the nano-scale of about 100 nm or less for novel and alternative diagnostics and therapeutics, in incredible selectivity and accuracy not achievable through conventional means. One would hope that with the future development of nanomedicine, we will be able to think of today's incurable diseases as curable tomorrow, by looking at a problem at its molecular or even atomic levels and apply medical intervention at the molecular scale. Presently nanomedicine involves detection of particles (nanobiosensors), drug delivery systems, emulsions, and carriers for delivering vaccines and biomaterials with unusual properties and improved biocompatibility.

Recently, within the realm of nano-scale, carbon nanotubes (CNT) are being tested as medical devices at an increasing rate. Just to mention a few examples: CNTs have been utilized as scaffolds for neuronal and ligamentous tissue growth for regenerative interventions of the central nervous system and orthopaedic sites (Hu et al., 2004), substrates for detecting antibodies associated with human autoimmune diseases with high specificity (Wang et al., 2004), carriers of contrast agent for enhanced magnetic resonance imaging (Sitharaman et al., 2005). When coated with nucleic acids (DNA or RNA), vaccines, and proteins, CNTs have been shown as effective substrates for gene sequencing and as gene and drug delivery vectors to challenge conventional viral and particulate delivery systems (Pantarotto et al., 2004; Kam et al., 2004; Liu et al., 2005; Lu et al., 2004). Carbon nanotube has been also probed as a vehicle for drug delivery into selected cells (Liu et al., 2008) and as bio-sensor (Wisitsoraat et al., 2010). In this chapter we consider and test the idea that carbon nanotubes might be utilized to remove unwanted molecular aggregates, particularly excess cholesterol, from a living tissues.
Cholesterol is a major sterol component of mammalian cell membranes, it plays important role in maintaining physical and mechanical properties of the membrane. There is a large literature of cholesterol in biomembranes (Róg et al., 2009). Its abundance influences such diverse membrane processes as signal transduction, protein stabilization, protein and lipid sorting, and membrane fusion. 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. Cholesterol can be also found in extracellular medium (lymphatic fluid) of the body. 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 subendothelial accumulations of cholesterol-engorged macrophages, called “foam cells”, later on leading to the formation of the plaque deposition in atherosclerosis disease (Lusis, 2000). Eventual build up of plaques, cells of inflammation, and blood clotting can block the normal blood flow in the coronary arteries. This is a catastrophic event that stops the flow of nutrients and oxygen to the heart muscle, leading to heart attack (myocardial infarction).

The study of the influence of carbon nanotube on cholesterol is in its infancy. In this report we present our molecular dynamics (MD) investigation of the influence of the carbon allotropes (nanotube, graphene) on the cholesterol molecules: a) embedded in a cell membrane, b) forming a lodgment around selected extracellular domain proteins.

2. Toward the extraction of cholesterol lodgement by carbon allotrope manipulation

The search for new methods for removing of excess cholesterol molecules, precursors of plaque deposition in an early phase of atherosclerosis disease, is a vital subject of molecular medicine. Our recent simulations (Raczyński et al., 2006a, 2007; Gburski et al., 2010) and the material presented here are related to this issue. In this context, we have chosen the carbon allotropes since they are known to be hydrophobic. This is an important feature when it comes to intervention in the biosystem, where water is the inherent component. We made a step towards the investigation of influence of carbon allotropes on cholesterol-contained systems of potential interest in nanomedicine. Particularly, these studies might be related to the quest for future, molecular level treatment of an embryonic phase of atherosclerosis, cerebral hemorrhage, lung diseases, ...etc.

2.1 Impact of carbon nanotube on cholesterol in a cell membrane

First we study the influence of carbon nanotube on mammalian cell membrane. The cell membrane separates the intracellular components from the extracellular environment, its architecture is that of phospholipid bilayer. Indispensable component of cell’s membrane is cholesterol, one of its primary functions is to guarantee a proper elasticity/stiffness of membrane. Cholesterol molecules are embedded between phospholipids, there is approximately one cholesterol per ten phospholipid molecules in the bilayer (see Fig.1). If we want to use CNT as a medical nanodevice, first we have to know what is the effect of CNT on just mentioned “membrane” cholesterol, i.e. those cholesterol molecules which reside within phospholipid bilayer.

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Fig. 1. Fragment of the simulated mammals’ cell membrane. The cholesterol molecules (green) are embedded between DMPC phospholipid bilayer (grey)

We consider the cholesterol molecules located between nonpolar hydrophobic fatty acid tails of neighbouring phospholipide molecules which form phospholipide bilayer sheet. The outer side of the bilayer form the polar hydrophilic phosphate heads of phospholipides. We have done MD simulations for this system (without nanotube), for the reference purpose. Next we have placed the carbon nanotube close to the “sea” of phosphate heads and repeated the simulation again. The essential simulation details are given at the end of this section. The dynamical observable of cholesterol molecule has been calculated, namely the mean square displacement $<|\Delta r(t)|^2>$ of the center of mass of a molecule

$$<|\Delta r(t)|^2> = <|r(t) - r(0)|^2>$$  \hspace{1cm} (1)

where $r$ is the position of the center of mass of a molecule of interest. The comparison of the mean square displacement of the center of mass of cholesterol molecule at $T = 309$ K with and without the presence of nanotube, is given in Fig. 2. In the absence of nanotube the cholesterol molecule - tighten between phospholipids - can’t walk further then 1.1 Å, whereas in the presence of nanotube cholesterol’s $<|\Delta r(t)|^2>$ reaches the saturation at 1.3 Å. We see that the cholesterol molecule somehow “feels” the nanotube, namely it is slightly attracted by nanotube surface because we observe the increase of the maximum distance it can move. However, the plot of $<|\Delta r(t)|^2>$ function (Fig. 2) tells that cholesterols always remain inside phospholipide bilayer and the nanotube can not pull them out of the cell membrane.

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Fig. 2. The mean square displacement of the centre of mass of cholesterol molecule embedded between phospholipids in the presence (solid line) and absence (dashed line) of carbon nanotube.

The slope of the mean square displacement is connected with the translational diffusion coefficient via Einstein relation

\[ \langle |\Delta r(t)|^2 \rangle \equiv 6Dt \]  

The plot of \( \langle |\Delta r(t)|^2 \rangle \) (see Fig. 2) indicates that the system is in the liquid phase, the slope of the linear part of mean square displacement of cholesterol is \( 1.4 \times 10^{-1} \, \text{Å}^2/\text{ps} \). Hence the value of diffusion coefficient \( D \), calculated from the linear part of \( \langle |\Delta r(t)|^2 \rangle \) using eq. (2), is \( D = 2.4 \times 10^{-6} \, \text{cm}^2/\text{s} \).

Summarizing so far presented results we conclude, that the carbon nanotube only very slightly influences the dynamics of those cholesterols which are located in a cell membrane, between phospholipides. Particularly, what is very important, the carbon nanotube can’t pull the cholesterol molecules out of the phospholipide layer.

Essential technical details of MD simulations analysed above.

We have used the standard Lennard-Jones (LJ) interaction potential \( V(r_{ij}) \) between carbon atoms of the armchair (10,10) nanotube and the atoms (sites) of rigid-body cholesterol \( \text{C}_{27}\text{H}_{45}\text{OH} \) and phospholipid. 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, \( \varepsilon \) is the minimum of potential at the distance \( 2^{1/6} \sigma \). The cholesterol and phospholipid molecules include lots of atomic sites, but in line with the common procedure for large molecules we treat CH, CH\(_2\) and CH\(_3\) atomic groups as a supersites (pseudoatoms). The LJ parameters for these groups and other atoms involved are taken from (Daura et al., 1988; Kuznetsova & Kvamme, 2002). Moreover, we have included the dipole moments of cholesterol and phospholipid (OH bonds) by putting the charge -0.376 \( e \) on oxygen and 0.376 \( e \) on hydrogen atoms of OH bonds (Phelps & Dalby,
The L-J potentials parameters between unlike atoms and pseudoatoms were calculated by the Lorentz-Berthelot rules (Allen & Tildesley, 1989)

\[ \sigma_{A-B} = \frac{(\sigma_A + \sigma_B)}{2}, \quad \varepsilon_{A-B} = \sqrt{\varepsilon_A \varepsilon_B}, \]  

where A, B are C, O, N, S, H, CH, CH\(_2\) and CH\(_3\) atoms or pseudoatoms. The classical equations of motion were integrated by predictor-corrector Adams-Moulton algorithm (Rapaport, 1995). The integration time step was 0.3 fs which ensured total energy conservation within 0.01%. The total simulation time was 1.5 ns.

2.2 Influence of carbon nanotube on cholesterol domain localized on a protein surface

Our next step is to simulate another ensemble, composed of a protein and cholesterols. We have chosen 1KF9 as an example of human extracellular domain protein (Shiffer et al., 2002) since the data required for MD simulation are available for this protein (Protein Data Bank, 2010). This protein was covered by 40 cholesterol molecules. During a preliminary MD simulation we have observed that the cholesterols gather together near protein surface, forming the cholesterol lodgment. Then, we have placed the carbon nanotube near this cholesterol cluster (see Fig. 3).

Fig. 3. The snapshot of an equilibrium configuration of the simulated ensemble, showing a fragment of 1KF9 protein and a cholesterol lodgment in the presence of carbon nanotube.
Holding the nanotube immobile in its place we have run simulation again, collecting MD data over 1.5 ns. The radial distribution function \( g(r) \) of the centers of mass of cholesterol in the lodgment without nanotube shows two peaks (see Fig. 4). The first one, quite sharp indicates the average near neighbors distance \( r = 6.1 \) Å while the second, very broad peak around \( r \approx 12.2 \) Å comes from the farther distance cholesterol neighbors (second shell). There are no other pronounced peaks and for larger intermolecular distance \( g(r) \) gradually tends toward zero. The highest peak of \( g(r) \) for the lodgment with nanotube (Fig. 4) appears at larger distance \( r = 7.1 \) Å comparing to \( r = 6.1 \) Å for the lodgment without nanotube. It must be associated with the average centers of mass distance between cholesterol covering nanotube. Note, that in (Raczyński, 2006b) we have calculated \( g(r) \) for the single layer of cholesterol surrounding carbon nanotube and the maximum of \( g(r) \) was located at \( r = 7.2 \) Å. A little peak at \( r = 5 \) Å comes from the small number of cholesterol confined between protein and nanotube. The value of \( g(r) \) reaches zero around \( r \approx 40 \) Å in both cases, indicating that way the diameter of the cholesterol lodgment.

![Fig. 4. The radial distribution function \( g(r) \) of the centre of mass of cholesterol molecule in the lodgment in the absence (solid line) and presence (dashed line) of the carbon nanotube.](image)

The mean square displacement of the center of mass of cholesterol molecule is shown in Fig. 5. The difference between \( <|\Delta r(t)|^2> \) with and without nanotube is distinct. Without nanotube, the plot of \( <|\Delta r(t)|^2> \) is somehow similar to dense media, the slope of \( <|\Delta r(t)|^2> \) is low hence the translational mobility of cholesterol within the lodgment is very weak. The value of \( <|\Delta r(t)|^2> \) and its slope spectacularly increases when the nanotube is nearby the cholesterol lodgment (cholesterols get mobile). The increase of \( <|\Delta r(t)|^2> \) while nanotube is nearby lodgment reflects a simple fact that the nanotube pulls cholesterol out of the lodgment. The pulled out cholesterol spread all over the nanotube surface forming thin layer covering carbon nanotube. Therefore, removing the nanotube, covered by the intercepted cholesterol, substantially diminishes the number of cholesterol molecules which remain within the lodgment. The process of extraction of the cholesterol lodgment is quite efficient. Our nanotube of 80.5 Å length has pulled out twenty three of the total number of forty cholesterols, reaching 57 % of efficiency (for the snapshot see Fig. 6).
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Fig. 5. The mean square displacement of the centre of mass of cholesterol molecule in the lodgment in the absence (dashed line) and presence (solid line) of carbon nanotube.

Fig. 6. The snapshot of an equilibrium configuration after pulling the carbon nanotube out of the cholesterol lodgment located over 1KF9 protein.
the molecular system discussed above doesn’t include water, which is an essential component of living organism. As a next step, we have turned to the study of a more realistic, albeit more complicated fragment of biosystem. Particularly, we made a reconnaissance study, via computer simulation, of the influence of the carbon nanotube on the dynamics of cholesterol molecules forming a domain around a selected extracellular protein in a water environment. As an example, 1LQV endothelial extracellular protein was chosen (Protein Data Bank, 2010), see Fig. 7.

Fig. 7. The endothelial 1LQV protein; ribbon representation (left), atomistic representation (right)

1LQV protein appears in the thin layer of cells named endothelium, this layer forms an interface between circulating blood and the rest of the vessel wall. That is why we selected the human endothelial protein that resides in the innermost layer of a blood artery (see Fig. 8.).

Fig. 8. Structure of a large blood artery
All molecules in the system were modeled on the full atomistic level (simulation details are given at the end of this subsection).

First, the mean square displacement of the center of mass of cholesterol was calculated. From the relation between $\langle |\Delta r(t) |^2 \rangle$ and the translational diffusion coefficient $D$ (eq. 2), one knows that a nonzero slope of $\langle |\Delta r(t) |^2 \rangle$ is an indicator of mobility (translational diffusion) of molecules. Fig. 9 shows $\langle |\Delta r(t) |^2 \rangle$ plots both in the presence and absence of the nanotube. It indicates that in both cases the cholesterol domain is not in the solid state. The diffusion coefficient $D$ of cholesterol, estimated from the linear part of the slope of $\langle |\Delta r(t) |^2 \rangle$ at the physiological temperature $T \approx 309$ K, is $D = 0.37 \times 10^{-6}$ cm$^2$/s (with nanotube) and is $D = 0.39 \times 10^{-6}$ cm$^2$/s (without nanotube). Note, that these values of $D$ are related to the short time translational dynamics of cholesterol molecule.

If there is no nanotube, the mean square displacement function shows saturation around 35 Å$^2$. Therefore, although the cholesterols have some mobility, their displacements are restricted by the interaction with protein surface. One can see that saturation of $\langle |\Delta r(t) |^2 \rangle$ vanishes when the carbon nanotube is placed near the domain. The cholesterol molecules now can migrate farther, the restriction on their translational dynamics, imposed by the protein surface, is overcome by the competitive interaction with the carbon atoms of the nanotube. Some cholesterols move from the domain and build up a thin layer around the carbon nanotube.
To visualize even more the migration of cholesterol molecules from the domain to nanotube we have calculated the radial distribution function of cholesterols with respect to the main axis of the carbon nanotube (denoted $g_{\text{CN-chol}}(r)$ in Fig. 10). Calculations were performed for both initial and final stage of simulation of system with CNT (averaged over 0.2 ns). The radius of nanotube studied is 6.8 Å. Apparently at the final stage some cholesterol molecules approach close to nanotube surface, the first maximum of radial distribution is higher and located nearer the nanotube.

![Fig. 10. Radial distribution function of the centers of mass of cholesterols with respect to the main axis of carbon nanotube, calculated at the beginning (open triangles) and the end of simulation run (filled squares).](image)

To show more directly the ability of CNT to remove cholesterol from the domain, steered molecular dynamics simulation (Philips et al., 2005) was carried out. In this simulation external forces were applied to pull out CNT from the domain (see Fig. 11). The process of removing cholesterols by carbon nanotube is efficient. The nanotube of 60 Å length has pulled out 17 of the total number of 21 cholesterols, reaching 80% efficiency. The repeating of this action, using clean nanotube, practically removes those remaining cholesterols which have survived the first intervention of the nanotube. The reported ability of the nanotube to extract the cholesterol lodgment at physiological temperature is quite appealing. One would say, this is a kind of nanosurgery (extraction of cholesterol lodgment) made in a computer laboratory.
Fig. 11. Final configuration of the simulated ensemble after pulling out the carbon nanotube. Carbon nanotube has removed the majority of cholesterols from the domain originally spread over the surface of 1LQV protein. Water molecules are not shown for the clarity of the picture.

Essential simulation details.
MD simulation of ensemble with 1K9F protein was performed with Lennard-Jones potential between carbon atoms of the armchair (10, 10) nanotube and the atoms (sites) of rigid-body cholesterol C_{27}H_{45}OH and the protein. The equations of motion were integrated by predictor-corrector Adams-Moulton algorithm. The integration time step was 0.3 fs which ensured sufficient total energy conservation. The total simulation time was 1.5 ns.

In case of ensemble with 1LQV protein more sophisticated force field was applied. The molecular dynamics (MD) simulations were performed using the NAMD 2.6 program (Philips et al., 2005) with the all atom CHARMM force field (MacKerell et al., 1998) in NVT (constant number of particles, constant volume and constant temperature) ensemble at the physiological temperature T = 309 K.

The CHARMM force field includes intramolecular harmonic stretching $V_{\text{bond}}$, harmonic bending $V_{\text{angle}}$, torsional $V_{\text{dihedral}}$, Van der Waals and Coulombic terms:

$$ V_{\text{total}} = V_{\text{bond}} + V_{\text{angle}} + V_{\text{dihedral}} + V_{\text{vdW}} + V_{\text{Coulomb}} $$

The standard NAMD integration algorithm (Brünger-Brooks-Karplus) was used with timestep of 0.5 fs. The ensemble consisting of protein, cholesterol and water molecules was equilibrated for $3 \times 10^5$ time steps with periodic boundary conditions (Rapaport, 1995). After equilibration, the system was simulated for $5 \times 10^5$ time steps (2.5 ns).
2.3 Removing of cholesterol lodgement by graphene sheet

The aim of our next computer experiment was to test whether the graphene, a sheet of carbon just one atom thick, could substantially influence the distribution of cholesterol molecules spread over the protein’s surface. For that reason, the graphene sheet (720 carbon atoms) was placed at a distance 2.3 nm from the center of cholesterol lodgment covered 1LQV endothelial protein. At this initial configuration the graphene sheet was separated from the direct interaction with cholesterol molecules by water (see Fig. 12). For the reference purpose we have also done MD simulations for the ensemble without graphene sheet.

Fig. 12. Snapshot of the initial configuration of the ensemble of 1LQV protein, cholesterol molecules and graphene wall solvated in water, at T = 309 K. Water molecules are not shown to improve the clarity of the picture.

When the graphene is placed nearby the cholesterol domain, the translational mobility of cholesterol molecules significantly increases, reflecting the migration of cholesterols towards the graphene sheet. To visualize the migration of cholesterols, we have also calculated a time evolution of the mean distance between the center of mass of cholesterol lodgment and graphene wall (Fig. 13). It declines with time, clearly showing the process of displacement of a large number of cholesterols from the domain to the graphene sheet.

The snapshot of the instantaneous configuration of the system studied, after completing the migration process, is shown in Fig. 14. Apparently, the large amount of cholesterol molecules was removed from the cluster surrounding the protein.
Fig. 13. Mean distance between the center of mass of cholesterols and graphene sheet.

Fig. 14. Snapshot of the final configuration of the ensemble of 1LQV protein, cholesterol molecules and graphene wall solvated in water, at $T = 309$ K. Water molecules are not shown to improve the clarity of the picture.
At the physiological temperature $T = 309$ K the graphene sheet substantially influences the dynamics of cholesterol molecules forming a lodgment around a chosen example of endothelial protein (1LQV). In the presence of graphene some cholesterol molecules migrate from the lodgment and settle down on the graphene surface. As a result, the cholesterol lodgment strongly diminishes.

Essential simulation details.

All MD simulations were carried out using NAMD program (Philips et al., 2005) with the all atom CHARMM27 force field (MacKerell et al., 1998; Feller & MacKerell, 2000). The net atomic charges borne by cholesterol were determined in (Hénin & Chipot, 2006). Initially, 21 cholesterol molecules were located near the surface of 1LQV protein, and the system was equilibrated over $10^6$ time steps $dt$ ($dt = 0.5$ fs). Next, as usual procedure in case of biological samples, water was added ($9 \times 10^3$ H$_2$O molecules, TIP3P model (Jorgensen et al., 1983)). All simulations were carried out in the NVT ensemble at the physiological temperature $T = 309$ K. The results presented here were obtained from a 5 ns production trajectory ($10^7$ time steps, $dt = 0.5$ fs) with the periodic boundary conditions.

3. Conclusion

Our MD simulations show that the carbon nanotube can not pull out those cholesterol molecules which are embedded in a cell membrane (phospholipid bilayer). That is exactly what one would appreciate. It means that the presence of nanotube even quite close to the cell membrane doesn’t destroy the structure and natural balance of cholesterol – phospholipide system. In other words, the presence of nanotube nearby a cell membrane is neutral for the dynamics of “membrane” cholesterol. Nevertheless, our simulations show that a carbon nanotube can diminish the unwanted cholesterol lodgment assembled on the protein surface. This happens, because the attraction of cholesterols by nanotube prevails cholesterols’ tendency to gather together over protein surface (setting up a lodgment). The cholesterol molecules tired out of the lodgment spread all over the nanotube surface, forming thin layer. Quite similar or even stronger removal effect can be achieved by applying graphene sheet instead of carbon nanotube. What we have done could be treated as a kind of nanosurgery made in a computer laboratory. Our simulations suggest that the reported ability of extraction of the cholesterol lodgment by carbon allotropes (nanotube, graphene) might be taken into account while searching for the future medical devices suitable for treatment of the cholesterol lodgment, a precursor of plaque deposition in an early phase of atherosclerosis disease. Note, that both carbon nanotube and graphene are hydrophobic – the very favorable feature in the discussed context. Naturally, more computer simulations and, very importantly, some real life experiments with carbon nanotubes, graphene and cholesterol domains covering selected components of mammals’ tissues, are required to farther test the reported observations.

4. Acknowledgment

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5. References


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Carbon Nanotubes are among the strongest, toughest, and most stiff materials found on earth. Moreover, they have remarkable electrical and thermal properties, which make them suitable for many applications including nanocomposites, electronics, and chemical detection devices. This book is the effort of many scientists and researchers all over the world to bring an anthology of recent developments in the field of nanotechnology and more specifically CNTs. In this book you will find:
- Recent developments in the growth of CNTs
- Methods to modify the surfaces of CNTs and decorate their surfaces for specific applications
- Applications of CNTs in biocomposites such as in orthopedic bone cement
- Application of CNTs as chemical sensors
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