Interaction of tetramer protein with carbon nanotubes

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Abstract

Carbon nanotubes can interact with proteins or enzymes upon penetration into cell membranes of living organisms that may affect the protein-protein interactions *in vivo*. Here, three structural models composed of four-chain esterase from *Hungatella hathewayi* with and without pristine or carboxylated single-walled carbon nanotube (SWCNT) are constructed to investigate the changes in protein-protein interactions and SWCNT orientations by molecular dynamics simulations. The results show that the protein-protein interact very tightly to protect them from the separation by the carboxylated or pristine SWCNTs, as shown by the calculations of binding affinity and the distances between the centers of mass of different protein chains. Both pristine and carboxylated SWCNTs cause structural changes in the esterase. In addition, functionalization (e.g., carboxylation) can regulate the SWCNT orientation when positioned near the esterase.

1 Introduction

Since the discovery of carbon nanotubes (CNTs), they have been identified as the promising materials for environmental, industrial and commercial applications [1-5]. Extensive applications increase the possibility for their emissions to the natural environment [6-8]. Upon release, nanomaterials can touch the living organisms, enter the cells and organelles, and interact with the proteins or enzymes in the ecosystem [9]. Understanding their ecological risks becomes urgent for their safe applications [10-12].

There is more and more evidence that show carbon nanomaterials have negative impacts on living organisms [13]. CNTs can induce genotoxicity, such as mutagenesis and DNA damage [14]. The incorporation of 1000 and 2000 mg/L MWCNTs led to the decrease of shoot and root length, electrolyte leakage and cell death of multiple plant species, including lettuce, red spinach and cucumber [15]. Begum et al. [16] have shown graphene had toxic effects on tomato, cabbage and red spinach by oxidative stress necrosis and these toxic effects are related to exposure time, dose and species. Bennett et al. [17] investigated the toxicity of purified and raw SWCNTs to freshwater algae. They found that commercial CNTs brought the toxicity to freshwater algae by their photoactivity rather than by metal leaching.

Enzymes are the basic components of microorganisms, and are important to normal microbial functions [6, 18-20]. Previous studies showed that CNTs could affect the

protein/enzyme structures and further disrupt their functions, which may act as a new mechanism to induce the toxicity to proteins and corresponding biological systems [10, 21-23]. The binding interactions of CNTs with several proteins have been investigated, including bovine fibrinogen, gamma globulin, transferrin, bovine serum albumin, lysozyme, α-chymotrypsin, laccase, organophosphate hydrolase, tau protein, catalase, albumin, fibronectin, *etc* [24-30]. However, the effect of CNTs on the enzymes composed of four protein chains is still unclear until now at the molecular level. The situation for four protein chains becomes very complex, where protein-protein-protein-protein interactions will occur. These protein chains may act together to prevent them from the disturbance by CNTs. However, they also may not. What the true situation is still unknown. To solve this problem, a polymer-degrading esterase from *Hungatella hathewayi* was used, whose 3D structure is composed of four protein chains [31]. Notably, Sayes et al. [32] found that functionalization of CNTs could result in a change in cellular response to them. Thus, we designed two types of SWCNTs (carboxylated and pristine SWCNTs) in this study.

2 Materials and methods

The enzyme analyzed in this study is a polymer-degrading esterase from *Hungatella hathewayi*. Its 3D structure (ID 5A2G from PDB) is recently determined [31], being composed of four protein chains with 522 amino acids for each chain. The attached phosphate ion and water molecules were not kept in the latter study. The selected CNT in this study is a (6,4)-SWCNT in two forms: pristine and carboxylated. These

two forms of SWCNTs include 304 and 384 atoms, respectively. The tube radius and length of these two SWCNTs are 3.4 Å and 37.1 Å, respectively.

Three systems were established by VMD [33]: one with the enzyme and carboxylated SWCNT (c-SWCNT-enz), one with the enzyme and pristine SWCNT (p-SWCNT-enz) and one with only the enzyme (no-SWCNT-enz). The third system was considered as the reference group. The initial conformation of the enzyme is completely consistent in all these three systems. To make both of carboxylated and pristine SWCNTs have similar coordinate and orientation at the beginning of MD simulations, the following steps were performed:

Step 1: the coordinates of the geometric center of chains A and C were calculated;

Step 2: the coordinate of the midpoint between both geometric centers of chains A and C was calculated (a, b, c).

Step 3: for c-SWCNT-enz, this coordinate (a, b, c) was then modified to (a1, b1, c1) to make the carboxylated SWCNT almost perpendicular to the binding area of four chains. Saving the carboxylated SWCNT and the protein as two separate pdb files.

Step 4: for p-SWCNT-enz, moving the pristine SWCNT to (a1, b1, c1), and rotating slightly the pristine SWCNT to the orientation almost perpendicular to the binding area of four chains. Saving the pristine SWCNT and the protein as two separate pdb files.

These three systems were adopted as the starting points of the MD simulations using the software package GROMACS [34, 35]. The missing parameters of the carboxyl group at the c-SWCNT are from those for GLU. The topology files of both the enzyme and SWCNT were constructed on the basis of OPLS-AA force field [36]. The starting structures were soaked by the simple point charge (SPC) model [37] in a cubic box with a distance between the structures and the box edges is equal or more than 1.0 nm. The total atom number and water-molecules number is 405560 and 124708 for c-SWCNT-enz system, 405560 and 124708 for p-SWCNT-enz system and 405673 and 124847 for no-SWCNT-enz system after solvation and energy minimization. The 1-ns isothermal–isobaric (NPT) ensemble was performed by using LINCS algorithm [38] for bonds constrains and Particle Mesh Ewald [39] for long-range electrostatics interaction. Then, the 1-ns isothermal-isobaric (NPT) ensemble was run to achieve further equilibration, where the pressure was constant under periodic boundary conditions. Subsequently, 40-ns MD trajectories with a 2-fs time step were calculated for each system.

3 Results and discussion

3.1 Protein-protein-protein-protein interactions

It is evident that protein-protein interactions play an important role in metabolic activity of living organisms [9]. More generally, proteins often need to work together to achieve their functions. If these normal interactions are disrupted, their normal

functions may be prevented [40]. The presence of nanomaterial in the environment can cause conformational transitions in proteins that may interfere the protein-protein interactions in living organisms and produce the toxicity to them [22]. Therefore, the exploration of protein-protein interactions in the presence of nanomaterials is critical for understanding the impacts of nanomaterials on living organisms and to assess the potential risks. The conformational transitions in proteins can be tracked by MD simulations [41]. Several research groups have employed MD simulations to investigate nanomaterial-induced conformational changes in lignin-degrading enzymes (LiP, MnP and laccase) [10, 42], human immunovirus-1 integrase [40], lysozyme [43], DNA [44], *etc*. In this study, three MD simulations were carried out: (1) the system composed of pristine SWCNT and four-chain esterase; (2) the system composed of oxidized SWCNT and four-chain esterase; and (3) the system composed of only esterase. By comparing the trajectories of these systems, we can easily identify the effect of pristine SWCNT on the enzymatic conformations and how this effect would be changed after oxidization by adding the carboxyl groups at both ends of the SWCNT.

The protein-protein-protein-protein interactions in esterase in the presence and absence of SWCNTs were investigated by exploring the interactive modes, interaction energies, binding affinity, the number of intermolecular contacts, and the distance between the centers of mass (COMs) of two chains (*Distance*).

Figure 1 shows the interactions between chains A, B, C and D with and without pristine or carboxylated (6,4)-SWCNTs at the begin and end of the simulations. The binding areas between these four chains were highlighted. At 0 ns, the esterase exhibited a similar conformation. However, after 40 ns, the esterase conformation became different. The most direct evidence is from the interaction area between four chains, where their secondary structure transformed obviously. For example, a β sheet and some tube structures appeared in this area for chain A in the case of without the SWCNTs; the appearance of pristine SWCNT resulted in that tube structures were the only secondary structural forms in this area for chain A; interestingly, the carboxylated SWCNT led to the appearance of more secondary structural forms in this area for chain A, including β sheets, α helix and some tube structures. These observations suggest that not only the pristine SWCNT but also the carboxylated SWCNT change the conformation of free four-chain esterase. Ge et al. [25] also found structural changes in bovine fibrinogen, bovine serum albumin and gamma globulin where α-helices were reduced and β sheets were increased. Another study performed by Zeinabad et al. [24] suggested that the increase of β sheets in tau protein upon binding to CNTs led to the protein fold. The change in secondary structure of proteins is significantly related to the surface chemistry of CNTs. For example, α-helical content of bovine serum albumin was inconsistent upon adsorption onto CNTs modified by different functional groups [45]. Indeed, when the CNTs interact with the proteins, conformational change of proteins is a common phenomenon [20, 46, 47].

For example, Chen et al. [10] found that the SWCNTs changed the conformational dynamics of N- and C-terminus. In addition to CNTs, other nanomaterials such as gold nanoparticles [48] and silica nanoparticles [49], had a profound impact on the secondary structures of peptides or proteins.

Interaction energies between proteins and SWCNT were analyzed for both c-SWCNT-enz and p-SWCNT-enz, following the method from previous studies [2, 42, 50, 51]. The average interaction energy for c-SWCNT-enz was lower than that for p-SWCNT-enz (Figure 2), which suggests that, overall, the carboxylation increased the stability of the whole complex. However, the interaction energy of c-SWCNT-enz underwent more changes than that of p-SWCNT-enz. The difference in interaction energy between c-SWCNT-enz and p-SWCNT-enz was attributed to the presence of functional group (carboxyl) that contributed to the binding of protein to CNTs [45]. A study from Gu et al. [52] also showed that the adsorption ability of protein onto carbon nanomaterials was associated with the surface properties of carbon nanomaterials.

Binding affinity is a variable which is often adopted to explore the protein-protein interactions [53-56]. The binding affinity can be used to observe and predict the events of recognition, association and dysfunction in protein-protein complexes [56]. In this study, binding affinity between proteins was used to assess the impact of the SWCNTs on protein-protein-protein-protein interactions. If the absolute value of binding affinity between chains in the system with the SWCNT is smaller than that

without the SWCNT, that means that the SWCNT decreases the binding strength between chains. If the situation is just the opposite, the SWCNT will enhance their binding strength. The binding affinity is calculated using a recently developed program PRODIGY (http://milou.science.uu.nl/services/PRODIGY/) with a good accuracy and performance reported in the published paper [57]. The 3D structure of protein-protein-protein-protein complex for each snapshot at an interval of 4 ns was imported into this program to perform the binding affinity calculation. The binding affinity between chains A, B, C and D as a function of time is shown in Figure 3a. The average binding affinity is about -24.5, -21.6, and -23.7 kcal mol^{-1} for c-SWCNT-enz, p-SWCNT-enz, and no-SWCNT-enz, respectively. This shows that the carboxylated SWCNT can slightly enhance the protein-protein-protein-protein interaction, while the pristine SWCNT cannot. This is also supported by the results about the number of intermolecular contacts (NIC) (Figure 3b). NIC in c-SWCNT-enz is larger than that in no-SWCNT-enz in most of simulation time, whereas the NIC in p-SWCNT-enz is smaller than that in no-SWCNT-enz during the whole simulation. This can be expected since the carboxylated SWCNT contains more atoms and groups than the pristine one, which can bring additional interactions with the proteins. Thus, functionalization such as carboxylation may be used as a strategy to regulate or control the protein-protein interactions. Our results on protein-protein binding affinity will be helpful in understanding the energetics of protein-protein interactions and their function mechanism.

To further observe whether the presence of carboxylated or pristine SWCNTs would break the interactions between two chains (A and B; A and C; B and D; and C and D), we calculated the *Distance*. Our study showed that the *Distances* between A and B (3.93 ±0.03 nm for c-SWCNT-enz, 3.96 ±0.002 nm for p-SWCNT-enz, and 3.92 ±0.03 nm for no-SWCNT-enz) or A and C (4.65 ±0.09 nm for c-SWCNT-enz, 4.95 ±0.003 nm for p-SWCNT-enz, and 4.57 ±0.19 nm for no-SWCNT-enz) or B and D (7.28 ±0.05 nm for c-SWCNT-enz, 7.31 ±0.003 nm for p-SWCNT-enz, and 7.15 ±0.075 nm for no-SWCNT-enz) or C and D (3.93 ±0.03 nm for c-SWCNT-enz, 3.91 ±0.001 nm for p-SWCNT-enz, and 3.96 ±0.04 nm for no-SWCNT-enz) almost did not change with time, with slight fluctuations as shown by the standard deviations, regardless of the SWCNTs are present (Figure 4). Clearly, the *Distances* between A and B, between A and C, between B and D, or between C and D under three conditions (with carboxylated SWCNT, with pristine SWCNT and without SWCNT) are similar, respectively. Thus, both the carboxylated and pristine SWCNTs almost do not change the distances between chains.

These observations based on the binding affinity and *Distance* show that the overall stability of esterase is not broken by the pristine and functionalized SWCNTs. Four protein chains cooperate together to protect themselves from the separation between each other. Despite the stability, the changes in secondary structures of esterase may affect its folding and further its catalytic activity, because enzymatic activity is directly related to their proper folding [9].

3.2 Changes in SWCNT orientation by carboxylation

Pristine and carboxylated SWCNTs were put at the same position near the esterase with the similar orientations where these two SWCNTs can almost vertically insert into the binding hole formed by four chains (Figure 5a,b). In this section, we investigated the SWCNT orientation changes at time t relative to the initial orientation (t0) using an angle between the vectors from the SWCNT at times t and t0 ($Angle_{SWNCT}$). Thus, $Angle_{SWNCT}$ is 0 °for pristine or carboxylated SWCNT at 0 ns. The orientations for these two types of SWCNTs varied completely differently with time (Figure 5c). The $Angle_{SWCNT}$ for carboxylated SWCNT varied in a range of 0-66.2 °. The maximum $Angle_{SWCNT}$ for pristine SWCNT is 94.6 °. Pristine SWCNT has a larger orientation change than carboxylated one (average values of $Angle_{SWNCT}$: 72 ±13.4 °vs. 27 ±14.6 °). Figure 5d shows the probabilities of the $Angle_{SWCNT}$ during the whole simulation. 17.5 °and 77.5 °show the maximum probabilities for carboxylated (6%) and pristine SWCNTs (5.1%), respectively, during the simulations. In particular, the $Angle_{SWCNT}$ for carboxylated SWCNT mainly varied along a value smaller than 50 °, whereas that is opposite for pristine SWCNT which moved along an angle >50 °. These results showed that the carboxylation led to a large change in SWCNT orientations during the interactions with the four-chain esterase. This can be expected, since the orientation of protein towards CNTs was largely affected by surface properties of CNTs [27]. In addition, this means that functionalization such as carboxylation can be used to operate the orientations of nanomaterials towards the

proteins, producing the required protein-nanomaterial interactions that one wants. Orientation changes of CNTs upon functionalization when interacting with proteins were little investigated in previous studies where the researchers focused on the functionalization-caused variations in properties of CNTs and proteins [6, 58].

4 Conclusion

This study provides key insights into molecular interactions of CNTs with four-chain esterase from *Hungatella hathewayi*, with emphasis on the influence of SWCNTs on protein-protein-protein-protein interaction in esterase in an attempt to understand the molecular basis of nanotoxicity and functionalization-caused behaviour change of nanomaterials. The results show that the SWCNTs in pristine or carboxylated forms have a minor impact on the binding affinity and distances between four protein chains of esterase, but result in that secondary structures change with time. Carboxylated SWCNT exhibits a smaller orientation change than pristine one when they are positioned near this four protein chains.

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Figure legends

Figure 1. Interactions between chains A, B, C and D with and without pristine or carboxylated (6,4)-SWCNTs at the begin and end of the simulations. The area within the ellipse refers to the binding area of four chains whose secondary structure is shown in the rectangle.

Figure 2. Interaction energies for c-SWCNT-enz and p-SWCNT-enz.

Figure 3. The binding affinity (a) and the number of intermolecular contacts (b) between chains A, B, C and D as a function of time.

Figure 4. The distances between the centers of mass (COMs) of two chains (a) in cases of without SWCNT (b), with pristine SWCNT (c) and with carboxylated SWCNT (d).

Figure 5. SWCNT orientation towards the esterase. Pristine SWCNT orientation at 0 ns (a); carboxylated SWCNT orientation at 0 ns (b); evolution of $Angle_{SWNCT}$ with time (c); evolution of probabilities of the $Angle_{SWCNT}$ with time (d); pristine SWCNT orientation at 40 ns (e); carboxylated SWCNT orientation at 40 ns (f).