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Full Length Article

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 lengths, 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.

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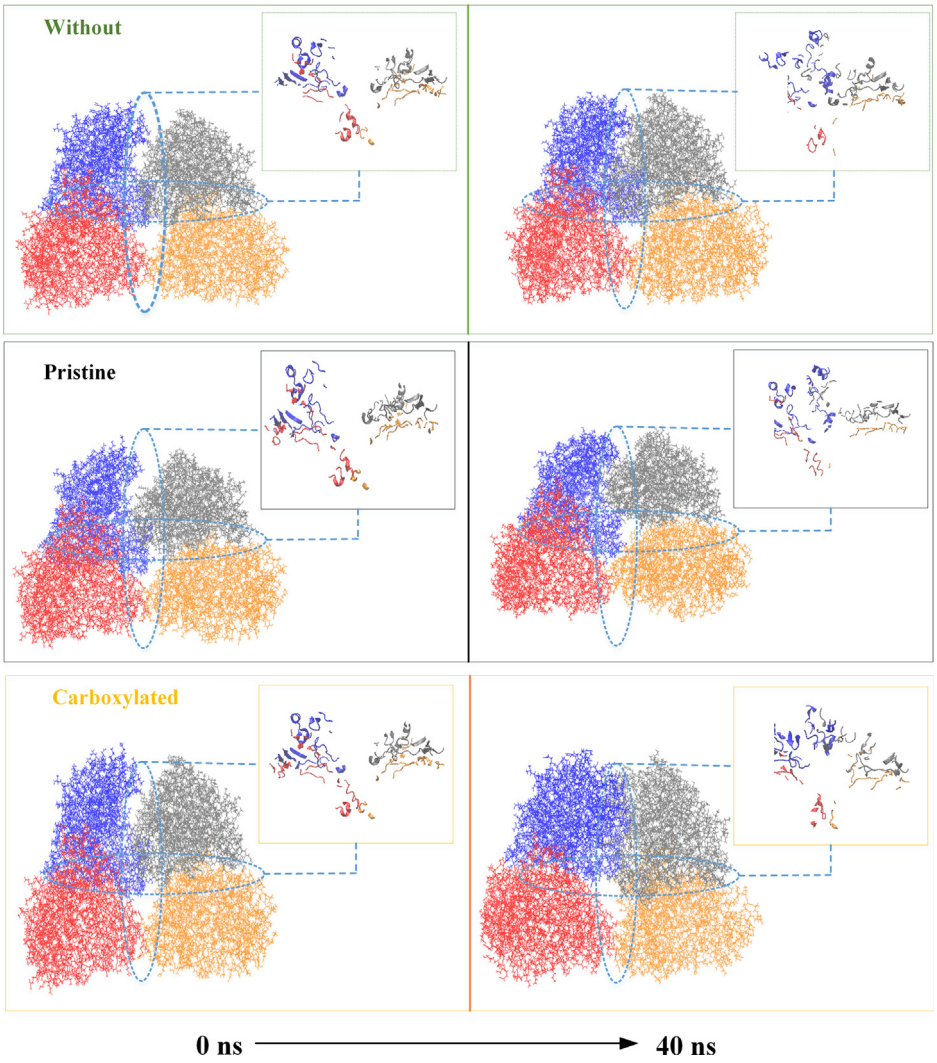

Fig. 1. Interactions between chains A, B, C and D with and without pristine or carboxylated (6,4)-SWCNTs at the begin and end of the simulations. The area within the ellipse refers to the binding area of four chains whose secondary structure is shown in the rectangle.

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:

the coordinates of the geometric center of chains A and C were calculated;
the coordinate of the midpoint between both geometric centers of chains A and C was calculated (a, b, c).
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.
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 405,560 and 124,708 for c-SWCNT-enz system, 405,560 and 124,708 for p-SWCNT-enz system and 405,673 and 124,847 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