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Structure-based design of carbon nanotubes as HIV-1 protease inhibitors: Atomistic and coarse-grained simulations

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ABSTRACT

Nanoparticles such as fullerenes and carbon nanotubes have been extensively studied for biomedical applications. In this paper, we report the design of carbon nanotubes as HIV-1 protease inhibitors. Docking and molecular dynamics calculations are performed using an atomistic model to explore the optimal interaction structure and free energy between the nanotube and HIV-1 protease. A coarse-grained model is then developed based on the atomistic model, allowing us to investigate the dynamic behaviors of the protease in the bound and unbound states. The dynamic process reveals that the carbon nanotube is able to bind to the active site of the protease and prevent the active flaps from opening up, thus blocking the function of the protease. This process is strongly influenced by the size of the nanotube. The binding of carbon nanotubes to an alternative binding site other than the active site is also explored. Therefore, carbon nanotube-based inhibitors have great potential for application as HIV-1 protease inhibitors.

1. Introduction

HIV-1 protease (HIV-PR) has been one of the major targets in anti-AIDS drug discovery. Although a number of potent and selective inhibitors have been developed as drugs for the treatment of HIV infection, great efforts are still needed to explore more stable and effective inhibitors.

HIV-PR belongs to a family of aspartic acid proteases. This enzyme is composed of two 99-amino acid monomers, named monomer *A* and monomer *B* in this paper. The structure of the protease features a pair of flaps and the active site, where ILE50A and ILE50B are defined as flap tips, as shown in Fig. 1. The flaps constantly sample different states, the most populated of which are the closed, semi-open, and open states. In the open state of the protease, the flap tip distance reaches up to 20–30 Å [1]. Experimental NMR data have shown that the large-scale flap motions occur on the microsecond–millisecond timescale [2]. The active site of this enzyme can be roughly described as an open-ended cylindrical tube that is lined almost exclusively by hydrophobic amino acids [2,3] and that cleaves polypeptides involved in the generation of the mature HIV virus. Once the polypeptides enter the active site through the opened flaps, the flaps close up to allow the protease to function. HIV-PR inhibitors function by controlling the flap dynamics and blocking the pathway of polypeptides entering the active site.

Among the different types of HIV-PR inhibitors, nanoparticles possess several advantages over conventional peptide-based inhibitors. First, compared to peptide-based inhibitors, nanoparticles are stable and do not easily react with other chemical compounds [3]. Second, nanoparticles such as C_{60} and carbon nanotubes (CNTs) are rigid and tend to retain their geometrical structure, and therefore, they are expected to effectively interact with non-polar chemical motifs [4]. The inhibition effect of fullerene derivatives has been investigated via computational and experimental approaches. For example, experimental studies have shown that fullerene C_{60}-derivatives can be very effective inhibitors, with an inhibition constant K_i ~100 nM [4–7]. Furthermore, fullerene drugs in human PBM, Vero or CEM cells at concentrations of up to ~100 μM are non-cytotoxic [8]. These results indicate that fullerene C_{60}-based inhibitors are promising to be utilized as drugs [3–10]. Enzyme inhibition by fullerene-based compounds has also been found for nitric oxide syntheses [10,11] and glutathione reductase [12]. Based on previous work, the assumptions of our present study are that CNT-based ligands can also serve as effective inhibitors and have advantages over conventional inhibitors owing to their geometrical and chemical properties. The geometry of the CNTs is expected to

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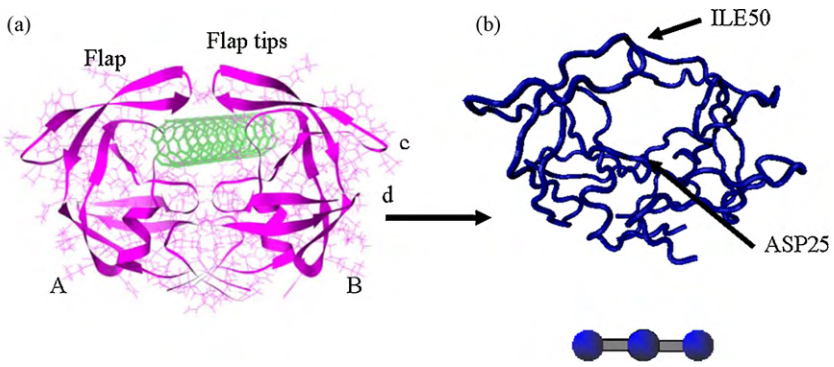

Fig. 1. (a) Configuration of the SWCNT–protease complex. (b) CG model for HIV-PR and SWCNT, respectively. The pictures were generated using the UCSF Chimera package [40] and VMD [41], respectively.

complement the active cavity of the protease even better than C_{60}.

All-atom models have been a powerful tool in the exploration of interactions between enzymes and their inhibitors [3,5,13,14]. For example, Friedman et al. [3] studied the inhibition of HIV-PR by fullerene derivatives, showing that functionalized C_{60} could serve as an effective, high affinity HIV-PR inhibitor through careful design.

However, the timescales of inhibitors entering the active site, as well as the dynamic behaviors of the protease, are not accessible to all-atom models. Therefore, the development of coarse-grained (CG) models is also crucial for understanding the dynamic behaviors of the enzyme. In the present work, an all-atom model was developed to determine the optimal interaction structure of the complex of a single-walled carbon nanotube (SWCNT) and the protease through docking. A molecular dynamics (MD) simulation was carried out to evaluate the binding free energy between CNT and the protease. A CG model of HIV-PR was also adopted for the simulation of CNT binding dynamics. The CG model was first developed by McCammon and co-worker [15]. The model has been successfully adopted to study the dynamic behaviors of HIV-PR and the binding pathways of ligands to the protease [16], which allows one to obtain the μs timescale to simulate the flap opening dynamics. We further incorporated the simplified model of the SWCNT into the CG system, with the CNT–protease interaction parameters determined based on the results of the all-atom model. Dynamic simulation results suggest that the CNT may enter the active site of the protease without flap opening and is able to prevent the flap from opening. Binding of the CNT to alternative target sites other than the active site was also observed, indicating the control of dynamic behaviors of the protease through allosteric effects.

By integrating the simulation results based on different molecular level resolutions, this paper proposes a general approach to designing nanoparticle-based inhibitors according to the geometrical properties of the enzyme.

2. Methods

2.1. Modeling the CNT–protease complex through docking

The docking algorithm was used to find the optimal configuration of the CNT inside the active site of the protease. The structure of the ligand-free HIV-PR was obtained from the protein data bank (entry: 1HHP [17]). A (5, 5) SWCNT with a diameter of 6.8 Å and a length of 24.0 Å was selected as a candidate for the inhibitor. Force field AMBER99 [18] was used throughout the simulation. The parameters for carbon atoms on CNTs were taken from type CA in the AMBER99 force field, and these parameters are designed for aromatic carbon atoms. The software package SYBYL 8.0 (Tripos Associates, Inc., St. Louis, USA) was used for modeling the molecular structure. Initially, the SWCNT was placed inside the active site of the protease, and the docking algorithm was carried out using the software package DOCK6 [19].

To model the optimal CNT–protease interaction configuration, molecular surface areas were determined from molecular surfaces generated by the program DMS (Conrad Huang, University of California, San Francisco, USA). The default van der Waals (VDW) radii and a probe sphere diameter of 1.4 Å were used. The 28 spheres surrounding the active site were generated. Using docking, the optimal orientations of a ligand inside the binding pocket could be found by scoring the energy based on the VDW contacts and complementary electrostatics. Therefore, the grid-based score was generated by calculating the non-bonded terms of the molecular mechanic force field, and the structure with the highest score was then adopted for MD simulation. A representative SWCNT–protease complex configuration is shown in Fig. 1a.

2.2. MD simulation

2.2.1. MD simulation in explicit solvent

Atomistic MD simulation was carried out using the software AMBER 8.0 [20]. To prepare the system for the binding free energy calculation in the next stage, MD simulations were performed on the CNT, the HIV-PR, and the CNT–protease complex, with each object solvated in a TIP3P water box. A layer of 8 Å of water molecules was applied in all three directions surrounding the solute, and the periodic boundary condition was used on all sides. For each system, 20,000 cycles of energy minimization were carried out, followed by 4 ns of MD simulation. The temperature of the system was heated from 100 K to 300 K during the first 200 ps using a constant volume and constant temperature (NVT) ensemble. Afterwards, the system was kept at room temperature of 300 K and a pressure of 1 bar using a constant pressure and constant temperature (NPT) ensemble [21]. The stability of the simulation was tracked, and the structure was prepared for the free energy calculation in the next stage.

2.2.2. Calculating the interaction free energy using the molecular mechanics-general Borned surface area (MM-GBSA) method

Water molecules from the previous equilibrated systems were removed, and the binding free energy between the protease and the SWCNT was calculated using the MM-GBSA method, where the GB model is the pairwise generalized Born model (GB^{HCT}) by Hawkins et al. [22,23], with parameters described by Tsui and Case [24]. The simulation was carried out for 500 ps for equilibrium and 500 ps for data collection. Here, the binding free energy was determined as the energy difference between the free energies of the complex in the implicit water solvent ($G^{solvate}_{complex}$) and the sum of the SWCNT