Molecular dynamics exploration of the binding mechanism and properties of single-walled carbon nanotube to WT and mutant VP35 FBP region of Ebola virus

Yan-Jun Zhang¹ · Jing-Na Ding¹ · Hui Zhong¹ ·
Chang-Ping Sun^{1,2} · Ju-Guang Han¹

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Abstract VP35 of Ebola viruses (EBOVs) is an attractive potential target because of its multifunction. All-atom molecular dynamics (MD) simulations and Molecular Mechanics Generalized Born surface area (MM/GBSA) energy calculations are performed to investigate the single-walled carbon nanotube (SWCNT) as an inhibitor in wild-type (WT) VP35 as well as in three primary mutants (K248A, I295A, and K248A/I295A) through docking the SWCNT in the first basic patch (FBP) of VP35. The SWCNTs of all the four systems effectively bind to the FBP. Interestingly, the sites and orientations of the SWCNT binding to the I295A mutant and K248A/I295A double mutants change significantly to accommodate the variation of the VP35 conformation. Moreover, the VDW can provide the major forces for affinity binding in all four systems.

Keywords VP35 · SWCNT · MD simulations · MM/GBSA · EBOVs

1 Introduction

EBOVs are members of the Filoviridae family, which cause lethal viral hemorrhagic fever in humans and non-human primates [1]. The genus Ebola contain five species; Zaire Ebola virus (EBOV), Sudan virus (SUDV), Taï Forest virus (TAFV), Reston virus (RESTV), and Bundibugyo virus (BDBV) [2]. The infections with EBOV are highly lethal and generally have a 70–90% case fatality [3]. Following infection, disease onset is extremely rapid with death approximately 7–11 days after symptoms [4]. EBOV is considered to be a potential

✉ Ju-Guang Han
jghan@ustc.edu.cn

¹ National Synchrotron Radiation Laboratory, University of Science and Technology of China, Hefei 230029, People's Republic of China

² Department of Physics, Linyi University, Shandong 276000, People's Republic of China

bioweapon and is classified as a Category A bioterrorism agent, because of its pathogenicity, high mortality and human-to-human transmission [4]. Therefore, it is important to discover the determinants that contribute to EBOV virulence and pathogenesis in order to facilitate the development of countermeasures [5].

The EBOV genome consists of a single-stranded non-segmented negative-sense RNA molecule with a coding capacity for the surface glycoprotein (GP), an RNA-dependent RNA polymerase (RdRp), a nucleocapsid protein (NP), as well as viral protein 35 (VP35), VP30 and VP24 [6, 7]. VP35 is a multifunctional protein that is an innate immune antagonist, an essential cofactor of the viral RNA polymerase complexes, required for viral assembly and RNA silencing suppressor; hence it is considered an attractive potential target [8–10]. VP35 is an important multifunctional protein that can antagonize host immune responses, including IFN production initiated by retinoic-acid-inducible gene-I-like receptors (RLRs) and it functions as a cofactor in the viral polymerase complex [11]. VP35 consists in an N-terminal coiled-coil domain and a C-terminal domain termed the IFN inhibitory domain (IID) [12–14]. The C-terminal domain of VP35 includes two basic patches: the first basic patch (FBP) is important for interactions with EBOV nucleoprotein (NP) and VP35 polymerase cofactor function; the central basic patch (CBP) is important for VP35-dsRNA binding and IFN inhibition [14, 15]. In the absence of VP35, no reported gene expression has been detected, while in the presence of optimal concentration of VP35, maximal reported gene activation has been detected [16, 17]. A previous study found that GA017 binding within the FBP is important for interaction with the NP and replication complex formation through testing select compounds for the ability to inhibit VP35 IID-NP interactions *in vitro* as well as VP35 function in a mini genome assay and EBOV replication [11]. Moreover, both computational and experimental methods demonstrate that the mutated residues K248 and I295, which are located in the FBP, individually resulted in a near-complete loss of compound binding but residues outside the binding pocket had no impact on ligand binding [11, 18].

The carbon nanotube (CNT) is among the most interesting nanomaterials developed to date, and has attracted growing attention since its discovery [19]. It has been extensively studied, used, and commercialized during the past two decades and many unique properties and capabilities have been observed [20]. CNT tends to retain its geometrical properties and shape, making it a rigid structure, which helps with firm interactions with other non-polar biochemical motifs [21]. Researchers have investigated the inhibition effects of fullerenes both through computational and experimental means [22]. For example, C₆₀-derivatives have been found experimentally to be potent inhibitors with an inhibition constant K_i of ~100 nM [21, 23]. Zhu et al. used MD simulations and free-energy calculations to observe the ability of fullerene-based compounds to desolvate the cavity region, which leads to a strong hydrophobic interaction between the C₆₀ moiety and active site residues [24]. Hence, they suggested that fullerene-based derivatives could be used as an effective inhibitor of HIV. Moreover, the interactions of a single-walled carbon nanotube (SWCNT) with human serum proteins lead to a competitive binding with different adsorption capacities and packing modes [22]. Researchers found that fullerene-based inhibitors are non-cytotoxic in human CEM, PBM, H9, and Vero cells with a concentration up to ~100 μM [22].

In the present study, we examine SWCNTs as inhibitors in wild-type (WT) as well as in three primary mutants (K248A, I295A, and K248A/I295A) of VP35 by docking the SWCNT (mutant-SWCNT) in the FBP and then performing all-atom MD simulations for all complexes. The major objective for MD simulation is to investigate the similarity and distinction of the dynamic behaviors of the two WT (VP35-free, VP35-SWCNT) and mutant complexes. To quantitatively describe the influence of the mutant complexes, the MM-GBSA method was

used to calculate the absolute binding free energies, which were further decomposed to a per-residue basis. The primary objectives for the binding energy calculations are to gain an insight into the interaction mechanism between SWCNT and the FBP at an atomic level.

2 Materials and methods

2.1 Preparation of VP35-SWCNT complexes

The crystal structures of WT VP35 (PDB ID: 3FKE), which is a dipolymer that just contains the C-terminal domain of VP35, were obtained from the Protein Data Bank [15]. All of the crystal water molecules were deleted from the PDB file. In order to improve the computational efficiency, only chain A was reserved for the molecular dynamics simulation. The mutation complexes (K248A, I295A, and K248A/I295A) were implemented by PYMOL [25–27]. The structures and field parameters for SWCNT were obtained as follows. A SWCNT (3, 3) with a diameter of 4.335 Å and length 9.613 Å, which was generated by TubeGen [28], was selected as a candidate for the inhibitor. The carbon atom of the SWCNT was taken from type CA for aromatic carbon atoms in the ff99SB force field [29]. The PatchDock program [30] was used to position the SWCNT in the FBP of VP35, which was produced by calculating the non-bonded terms of the molecular mechanic force field. Partial charges and force field parameters for the SWCNT were generated automatically using the Antechamber program in AMBER12 and the AM1-BCC was used to calculate the charges of SWCNT. GAFF force field [31] parameters were used to produce parameters for the SWCNT. The missing hydrogen atoms were added using the LEaP module [32]. The standard amber force field (ff99SB) was used as the parameter for all of these complexes. Each complex was solvated in a truncated octahedron periodic box of TIP3P [33] water modes with a cut-off of 12 Å. An appropriate number of Cl[−] counterions were added to neutralize the positive charge of each system. A cutoff of 10 Å was used for Lennard–Jones interactions. The Particle Mesh Ewald (PME) method [34] was used to calculate long-range electrostatic interactions. The SHAKE method [35] was applied to constrain bonds involving hydrogen atoms.

2.2 Molecular dynamics (MD) simulation

All the MD simulations presented in this work were performed by using the AMBER12 package [32]. The entire system was minimized by two steps before MD simulations. In the first step, the system was minimized by giving restraints of 2.0 kcal/(mol·Å [2]) to all heavy atoms of protein and SWCNT and 2500 cycles of the steep descent method were performed followed by 2500 cycles of conjugate gradient minimization to resolve the clashes between the solute and the solvent molecules. In the second step, the system was gradually heated from 0 to 300 K over 250 ps with a 2-fs time step at a constant pressure of 1 atm for another 250 ps. The whole system was minimized again for 500 ps at the NPT ensemble. In MD simulations, periodic boundary conditions were applied to all dimensions. The temperature of the MD was set to 300.0 K. The time step for a MD production run was 2 fs and the coordinates were saved at intervals of 2 ps for analyses. All the MD simulations were performed with AMBER12 on the PMEMD module and analyzed by the PTRAJ module. The pictures were executed by the Chimera program [36].

2.3 MM-GB/SA calculations

The Molecular Mechanics Generalized Born Surface Area (MM-GB/SA) method [37, 38] was used to calculate the binding free energies between the protein and SWCNT. For each system, a total number of 1000 snapshots without water and Cl⁻ were extracted from the last 20 ns of the MD simulation trajectory of the protein–ligand complex with an interval of 20 ps. The binding free energy (ΔG_{bind}) of the ligand to the protein was calculated by the MM-GB/SA method as follows:

$$\Delta G_{\text{bind}} = G_{\text{complex}} - (G_{\text{receptor}} + G_{\text{ligand}}) \quad (1)$$

G_{complex} , G_{receptor} , G_{ligand} are the binding free energy of the respective complex, receptor, and ligand in the solution. ΔG_{TOT} consists of the molecular mechanics free energy (ΔE_{MM}), the solvation free energy (ΔG_{sol}), and the conformational entropy effect on binding ($-T\Delta S$) in the gas phase. It can be expressed as:

$$\Delta \Delta G_{\text{TOT}} = \Delta E_{\text{MM}} + \Delta G_{\text{sol}} - T\Delta S \quad (2)$$

The free energy ΔE_{MM} consists of electrostatic interactions ΔE_{ele} and van der Waals ΔE_{vdw} in the gas phase:

$$\Delta E_{\text{MM}} = \Delta E_{\text{ele}} + \Delta E_{\text{vdw}} \quad (3)$$

The solvation free energy (ΔG_{sol}) can be divided into two components; the polar (ΔG_{GB}) and non-polar contributions (ΔG_{SA}):

$$\Delta G_{\text{sol}} = \Delta G_{\text{GB}} + \Delta G_{\text{SA}} \quad (4)$$

ΔG_{sol} was calculated with the GB module (IGB = 2) of the AMBER12 suite of programs. In this work, the dielectric constant was set to 1.0 for the interior solute and 80.0 for the exterior solvent. The same atomic radii and charges as in the MD simulations were used to calculate the binding energy. The non-polar contribution of the solvation free energy (ΔG_{SA}) is determined according to:

$$G_{\text{SA}} = \gamma \times \text{SASA} + \beta \quad (5)$$

where the solvent-accessible surface area (SASA) was estimated by the MSMS algorithm, with a solvent probe radius of 1.4 Å. The values of empirical constants γ and β were set to 0.005 kcal/(mol · Å [2]) and 0.0, respectively. The entropy term ($-T\Delta S$) was calculated through a NMA with the NMODE module in the AMBER12 program. The calculation of entropy was based on the 1000 snapshots extracted from the MD simulation trajectories.

3 Results and discussion

3.1 VP35 and SWCNT binding modes

To obtain insight into the conformational stability and the convergence of the dynamical properties of the VP35-SWCNT complex, the RMSD values for the C_α atom of VP35 during all MD simulations relative to the initial structure were calculated and plotted in Fig. 1a. The RMSD plots indicate that the conformations of the VP35-free and VP35-SWCNT complexes

Fig. 1 **a** The root-mean-square deviation (RMSD) of VP35-free and VP35-SWCNT plot for backbone C_{α} atoms relative to the starting structure as a function of time. **b** The RMSFs of the C_{α} atoms around their average positions versus the residue number of VP35-free and VP35-SWCNT. The K248-I297 C_{α} (**c**) and K248-C307 C_{α} (**d**) distance of VP35-free and VP35-SWCNT as a function of time

are in good equilibrium. According to the RMSD data obtained from the last 20 ns trajectories, VP35-SWCNT has a higher mean (2.04 Å) than VP35-free (1.83 Å), with respective deviations of 0.16 and 0.25 Å. The result suggests that the RMSD of the two complexes were similar from the starting structures but the trajectory of the VP35-SWCNT complex fluctuates more than the VP35-free complex.

A detailed analysis of root-mean-square fluctuation (RMSF) versus the residue number for VP35-free and VP35-SWCNT is shown in Fig. 1b. A different RMSF may provide direct insights into the structural fluctuation of different regions of VP35-free and VP35-SWCNT. Figure 1b indicates that the most rigid regions in both VP35-free and VP35-SWCNT complexes are located in the α -helix and β -folded regions. Moreover, the RMSF plots of the two complexes are different from each other. Furthermore, VP35-SWCNT shows higher fluctuations than VP35-free, indicating that binding with SWCNT could change the flexibility of some residues inside the binding pocket; moreover, a changed mobility of the residues, especially for the residues 248–262 and 298–310 in the binding pocket is observed. The mean of the RMSF values of residues 248–262 and 298–310 of VP35-free (VP35-SWCNT) are 0.91 (1.14) and 1.37 Å (1.92 Å), respectively. This indicates that these residues play an important role in the binding of SWCNT.

In order to study the transformation of the binding pocket, the distances (K248 C_{α} , I297 C_{α}) and (K248 C_{α} , C307 C_{α}), which are located within the binding pocket, were calculated. Figure 1c and d shows the time series plot for the distances. As shown in Fig. 1c, the distance

(K248C_α, I297C_α) of VP35-free and VP35-SWCNT almost overlap with a few exceptions for VP35-SWCNT, where the distance goes down to a range of 3–6 Å around 40 ns to 50 ns. The mean and SD of the last 20 ns trajectories of VP35-free (VP35-SWCNT) are 17.96 and 1.04 Å (17.70 and 0.56 Å), indicating that VP35 binding with SWCNT leads to a smaller pocket space than VP35-free with a reduced value of 0.26 Å. We can see from Fig. 1d that the distance (K248C_α, C307C_α) of VP35-SWCNT assembles a different type of structure as compared with the VP35-free complex. The distance (K248C_α, C307C_α) of WT-SWCNT sharply goes down to about 4 Å at 71 ns. The mean of the last 20 ns trajectories of VP35-free and VP35-SWCNT are 17.38 and 15.46 Å, with a SD of 0.81 and 0.63 Å. Compared with the VP35-free complex, the distance (K248C_α, C307C_α) of the VP35-SWCNT complex decreases to about 1.92 Å. In conclusion, the binding of SWCNT results in a smaller binding pocket space.

3.2 Comparing the complex proteins from RMSD and RMSF

Previous research found that the C-terminal domain of VP35 is significant for the replication of EBOV via binding with NP and suggested that double mutation residues K248 and I295 have a significant effect on the binding of inhibitor with VP35 but residues outside the FBP had no impact on inhibitor binding [11]. In order to compare the dynamical behavior of key residues mutated in VP35-SWCNT binding systems with the WT VP35-SWCNT binding complex, we performed two MD simulations for single mutated systems (160 ns of K248A system, 180 ns of I295A system) and one MD simulation for a double mutated system (170 ns of K248A/I295A system).

In order to explore the effect of mutations on the conformational stability of VP35-SWCNT complexes, RMSD values for the VP35 C_α atoms during all MD simulation trajectories relative to the initial structure were calculated and are shown in Fig. 2. This figure indicates that the conformations of four complexes have achieved equilibrium without distinct fluctuations and are similar to the starting structures during the MD simulations. According to the data obtained from the RMSD values of the last 20-ns trajectories of the WT-SWCNT and mutant-SWCNT complexes, the mean values of the last 20 ns trajectories of the four complexes (WT-SWCNT, K248A-SWCNT, I295A-SWCNT, and K248A/I295A-SWCNT) are 2.04, 2.04, 2.66, and 1.58 Å, with a deviation of respectively 0.16, 0.14, 0.16, and 0.15 Å. Compared with the WT-SWCNT complex, I295A-SWCNT has a higher mean value but the double mutant complex (K248A/I295A-SWCNT) has a lower mean value. The RMSD plots indicate that the four systems share a similar variable trend in the first 90 ns but the trajectory of the I295A-SWCNT complex fluctuates more than the other three trajectories after the first 90 ns and K248A/I295A-SWCNT shows a lower fluctuation than WT-SWCNT. In general, the I295A single mutant has a greater effect on VP35 conformation than K248A. Interestingly, the double mutant complex has a lower RMSD mean value than WT-SWCNT.

To investigate the detailed residual atomic fluctuations, the RMSF calculations of C_α atoms were performed for all complexes as illustrated in Fig. 2e. Overall, the RMSF values of WT-SWCNT are higher than the other three mutant-SWCNT complexes, except for the residues 219–236 and 330–335 of the I295A-SWCNT complex. The residues 219–236 and 330–335 are far away from the FBP, so they have very little effect on the interaction of VP35 and SWCNT. As shown in Fig. 2e, the mutant-SWCNT complexes show similar fluctuations, which are lower than WT-SWCNT, indicating that mutations change the flexibility of some residues inside the binding pocket; moreover, residue mobility is reduced, especially for the residues in the binding

Fig. 2 RMSD of WT (a), K248A (b), I295A (c), and K248A/I295A (d) plot for backbone C_{α} atoms relative to the starting structure as a function of time. e The RMSFs of C_{α} atoms around their average positions versus the residue number of WT, K248A, I295A, and K248A/I295A

pocket (residues 238–252 and 295–309). The mean values of the RMSF of residues 238–252 (295–309) of WT-SWCNT, K248A-SWCNT, I295A-SWCNT and K248A/I295A-SWCNT are 1.02 (1.80), 0.6 (0.93), 0.69 (1.23), and 0.60 Å (1.09 Å), respectively, which suggest that the flexibility of the residues in the FPB of VP35 are reduced after mutation. The result indicates that residues K248, I295, or the K248/I295 mutant have a significant effect on the flexibilities compared to the WT-SWCNT complex.

3.3 Local fluctuations for complex proteins

In order to study the effect of mutants on the binding pocket, we calculated the distances ($K248C_{\alpha}$, $I297C_{\alpha}$) and ($K248C_{\alpha}$, $C307C_{\alpha}$) that are located within the binding pocket. The time series plots and frequency distribution plots for ($K248C_{\alpha}$, $I297C_{\alpha}$), ($K248C_{\alpha}$, $C307C_{\alpha}$) are shown in Fig. 3.

The distance between K248 and I297 (Fig. 3a) shows that the four complexes almost overlap, with a few exceptions around 40 ns to 50 ns for WT-SWCNT, where the distance goes down to a range of 3–6 Å. The mean and SD of the last 20 ns trajectories of WT-SWCNT distribution are 17.71 and 0.56, of K248A-SWCNT are 19.28 and 0.55, of I295A-SWCNT are 19.97 and 0.70 and of K248A/I295A-SWCNT are 20.13 Å and 0.6 Å, respectively. This reflects that mutants have a great effect on the distance of the binding pocket and lead to a bigger pocket space than WT-SWCNT with the values being respectively 1.57 (K248A-SWCNT), 2.26 (I295A-SWCNT) and 2.42 Å (K248A/I295A-SWCNT). By analyzing the frequency distribution plot (Fig. 3c), the distribution peaks of K248A-SWCNT, I295A-

Fig. 3 The K248-I297 C α distance (a) and the K248-C307 C α distance (b) of WT, K248A, I295A, and K248A/I295A. Histogram distributions of K248-I297 C α distance (c) and K248-C307 C α distance (d) for WT, K248A, I295A and K248A/I295A

SWCNT and I295A-SWCNT are located at about 20, 21, and 20 Å, respectively, whereas for WT-SWCNT the peak is located at about 19 Å, suggesting that mutant residues are very important for the binding.

The results and the frequency distribution plot for the (K248C α , C307C α) distance are shown in Fig. 3b and d. It is clearly seen that with respect to the (K248C α , C307C α) distance, WT-SWCNT assembles a different type of structure as compared with the mutant complexes. For K248A-SWCNT, I295A-SWCNT, and K248A/I295A-SWCNT there is one peak around 17, 20 and 20 Å, respectively, whereas for WT-SWCNT, one peak is located around 16 Å and another is located around 20 Å. It can be seen from Fig. 3b that the distance K248-C307 of WT-SWCNT sharply goes down to about 4 Å at 71 ns. The mean of the last 20 ns trajectories of the four complexes (WT-SWCNT, K248A-SWCNT, I295A-SWCNT, and K248A/I295A-SWCNT) are 15.46, 16.76, 19.7, and 19.13 Å, with a SD of respectively 0.63, 0.62, 0.54 and 0.39 Å. The larger residues (K248C α , C307C α) distance for the I295 and K248/I295 mutants leads to a more spacious FBP as compared to the WT.

In addition, the superimposition of the lowest-energy structure of the last 20-ns trajectories for the WT-SWCNT and mutant-SWCNT complexes is shown in Fig. 4, from which we find some different conformations between WT-SWCNT and mutant-SWCNT, especially for I295A-SWCNT and K248A/I295A-SWCNT. Compared with WT-SWCNT, the binding pockets of I295A-SWCNT and K248A/I295A-SWCNT change significantly and the binding pockets of I295A-SWCNT and K248A/I295A-SWCNT are more spacious than for WT-SWCNT. We also find, by analysis of SWCNT RMSD and RMSF, that the SWCNT conformation has a limited variation. The conformation change of the protein may be due to the change in the van der Waals (VDW) interactions between the inhibitor and residues of VP35. For clarification, we also calculated the binding energy and decomposed the binding energy per-residue, which will be discussed with the related issues of the present data.

Fig. 4 Superimposition of the lowest-energy structure of the last 20 ns trajectories for WT-SWCNT and mutant-SWCNT complexes

3.4 SWCNT-VP35 interactions

Displacement of the SWCNT to the binding pocket (FBP) of VP35 is coupled with the complex motion of the entire protein. In order to obtain the VP35-SWCNT interactions, we used a simple way to calculate the average distance between the residue K248 and the center of mass of SWCNT as shown in Fig. 5. The time series plot for the interaction shows that the trajectories of WT-SWCNT and K248A-SWCNT are different from those of I295A-SWCNT

Fig. 5 The K248-SWCNT distance of WT (a), K248A (b), I295A (c), and K248A/I295A (d). e Histogram distributions of the K248-SWCNT distance for WT, K248A, I295A, and K248A/I295A

and K248A/I295A-SWCNT. The distance (K248, SWCNT) of single mutant K248A sharply drops down to 7 Å from 11 Å at the beginning of MD simulation and the distance of WT sharply drops down to 6.5 Å from 10.5 Å at 71 ns. Then the four trajectories run parallel to each other. The mean distance of the last 20 ns for (K248, SWCNT) in the WT-SWCNT complex is 6.24 Å, for K248A-SWCNT is 7.25 Å, for I295A-SWCNT is 10.51 Å and for K248A/I295A-SWCNT is 11.07 Å with their respective SD of 0.33, 0.39, 0.27 and 0.27 Å. The obtained result signifies that the SWCNT tries to fit properly inside the FBP of VP35 at the beginning of simulations for mutant-SWCNT and in the first 71 ns for WT-SWCNT and then accommodates well inside the binding pocket. Moreover, the result also indicates that the positions and the orientations of SWCNT of the four complexes are different from each other, which suggest that the mutated residues have significant influence on the SWCNT binding.

3.5 Binding free energy

In order to understand the contribution spectrum of binding energy for SWCNT with WT, K248A mutant, I295A mutant and K248A/I295A double mutant, absolute binding free energies were calculated for all the complexes by using the MM-GB/SA method. All the used trajectories were extracted from the last 20 ns because the structures of all the complexes have achieved equilibrium in the last 20 ns. To describe the interaction micromechanism of the SWCNT binding, it is necessary to calculate the binding free energy and energy decomposition based upon the trajectories that have achieved equilibrium. The absolute binding free energies were calculated as the sum of gas phase energy ($\Delta E_{\text{vdw}} + \Delta E_{\text{ele}}$), solvation free energy ($\Delta G_{\text{SA}} + \Delta G_{\text{GB}}$) and entropy contribution ($-T\Delta S$). Contributions of the binding free energies are summarized in Table 1. The binding free energies of the WT-SWCNT, K248A-SWCNT, I295A-SWCNT, and K248A/I295A-SWCNT complexes are -30.89 , -27.70 , -25.29 , and -28.28 kcal/mol, respectively, which suggest that the binding free energies for the four complexes are considerably higher than the binding affinities of the GA017 to WT (-6.36), K248A (-7.70), I295A (-5.36) and K248A/I295A (-1.30 kcal/mol) [18]. For all of the three mutants their bindings to the SWCNT decrease as compared to the WT complex; the binding free energies decrease by 3.19, 5.60 and 2.61 kcal/mol for the K248A, I295A and K248A/I295A mutants, respectively.

Table 1 Binding free energy (kcal/mol) components for the protein-inhibitor complex by using the MM-GB/SA method

Component (kcal/mol)	WT		K248A		I295A		K248A/I295A	
	mean	SD	mean	SD	mean	SD	mean	SD
ΔE_{ele}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ΔE_{vdw}	-55.47	3.01	-50.31	3.25	-50.42	3.19	-52.46	2.52
$\Delta G_{\text{nonpolar}}$	-2.71	0.14	-2.56	0.15	-2.82	0.15	-2.79	0.11
ΔG_{polar}	10.89	1.08	9.08	0.95	10.24	0.98	9.77	0.81
ΔH	-47.29	2.72	-43.80	2.90	-43.01	3.10	-45.45	2.39
$-T\Delta S$	16.40	5.66	16.10	5.78	17.72	6.10	17.20	5.71
$\Delta\Delta G_{\text{TOT}}$	-30.89		-27.70		-25.29		-28.28	

ΔE_{ele} , electrostatic energy in the gas phase; ΔE_{vdw} , van der Waals energy; $\Delta G_{\text{nonpolar}}$, non-polar solvation energy; ΔG_{polar} , polar solvation energy; $\Delta H = \Delta E_{\text{ele}} + \Delta E_{\text{vdw}} + \Delta G_{\text{polar}} + \Delta G_{\text{nonpolar}}$; $T\Delta S$, entropy contribution; $\Delta\Delta G_{\text{TOT}} = \Delta H - T\Delta S$

Comparisons of the binding free energy components (ΔE_{vdw} , ΔE_{ele} , $\Delta G_{\text{nonpolar}}$ and ΔG_{polar}) and entropy ($-T\Delta S$) between the WT-SWCNT and the mutant-SWCNT complexes were carried out to elucidate the interaction mechanism. Detailed comparisons of the different free energy components between the WT complex and mutant complexes are shown in Fig. 6. In accordance with the energy components of the binding free energy in Table 1 and Fig. 6, for the WT complex and mutant complexes, the VDW energies (ΔE_{vdw}) in the gas phase provide the major forces for affinity binding. The contributions of VDW energies in the gas phase of all the systems are -55.47 , -50.31 , -50.42 , and -52.46 kcal/mol for the WT, K248A, I295A and K248A/I295A complexes, respectively. The results indicate that the K248A and I295A single mutants and the K248A/I295A double mutants have unfavorable influences on the VDW as the energies decrease by 5.16, 5.05 and 3.11 kcal/mol as compared with the WT complex, which suggests that the K248A/I295A double mutant has a lesser effect on the VDW interaction. The relatively small non-polar solvation energies ($\Delta G_{\text{nonpolar}}$), resulting from the burial of the SWCNT solvent accessible, which are similar to each other in all the complexes, have favorable contributions to binding affinity. The relatively small $\Delta G_{\text{nonpolar}}$ indicates that the packing of the cavity region is quite closed in all the systems. The electrostatic component of enthalpy of solvation (ΔG_{polar}) and the entropy ($-T\Delta S$) contribute an unfavorable force to the binding of the SWCNT. The results show that the entropy contribution for all the systems is rather approximated (16.10–17.72 kcal/mol) but the I295A single mutant and the K248A/I295A double mutant are slightly higher than the WT systems. We conclude that the mutant has a negative effect on the binding of the SWCNT, especially for the I295A single mutant and the K248A/I295A double mutants.

3.6 Decomposition of the binding free energy

To analyze the effect of the mutants on the binding affinity, the binding free energy decomposition was used to decompose the binding free energy into energy per residue, which could be summed over an atom group to obtain different energy contributions coming from the residues side-chain and backbone by the MM-GB/SA method [39–41] in the AMBER12 module. Quantitative information about binding free energy decomposition is significantly useful for the located key residues that contribute to the ligand and protein binding. The

Fig. 6 Free energy components for the binding of SWCNT to WT, K248A, I295A and K248A/I295A. ΔE_{ele} , electrostatic energy; ΔE_{vdw} , van der Waals energy; $\Delta G_{\text{nonpolar}}$, non-polar solvation energy; ΔG_{polar} , polar solvation energy; $\Delta H = \Delta E_{\text{ele}} + \Delta E_{\text{vdw}} + \Delta G_{\text{polar}} + \Delta G_{\text{nonpolar}}$; $-T\Delta S$, entropy contribution; $\Delta G_{\text{binding}} = \Delta H - T\Delta S$, absolute binding free energy

binding free energy decomposition into energy per residue consists in molecular mechanics and solvation energy but does not include entropy changes.

To understand the effect on the binding affinity due to mutants, a comparison of binding free energy per residue between the WT-SWCNT and mutant-SWCNT systems is shown in Fig. 7a. As shown in Fig. 7a, the WT-SWCNT and mutant-SWCNT systems have similar interaction spectra, though there exists a difference in the individual residue interaction spectra. In general, the residues that have a strong interaction with the SWCNT are mainly located in the FBP, such as subdomains of α -helices (α 2, residues 238–252; α 5, residues 305–309) and β -strands (β 1, residues 294–297; β 3, residues 325–330). We can conclude that the residues Q241, Q244, V245, K248, L249, P293, I295, I297, I303, P304, C307, C326, F328, and L338 play an important role in the binding of the SWCNT from the distributions of free energy decomposition. All of these residues have strong favorable contributions to the binding affinity.

The decomposition of binding free energy on a per-residue basis upon contribution from the VDW energy (ΔE_{vdw}), the polar solvation energy (ΔG_{polar}) and non-polar solvation energy ($\Delta G_{\text{nonpolar}}$) for the key residues of the four systems mentioned above is shown in Fig. 7. Table 2 further represents the per-residue energy contributions from the backbone and side-chain atoms. We can note from Fig. 7 and Table 2 that the electrostatic interaction in the gas phase (ΔE_{ele}) has not contributed to the binding free energy of the four systems and that the major driving force for the binding of the SWCNT to VP35 is VDW as well as a comparatively small amount of non-polar solvation energies, which agrees well with Table 1 and Fig. 6.

Fig. 7 a Comparison of binding free energy per residue between the WT and the mutants systems. Decomposition of binding free energy on a per-residue basis upon contribution from VDW energy (ΔE_{vdw}), polar solvation energy (ΔG_{polar}) and non-polar solvation energy ($\Delta G_{\text{nonpolar}}$) for the key residues: WT (b), K248A (c), I295A (d), and K248A/I295A (e)

Table 2 Decomposition of the binding free energy on a per-residue basis

Residue	S _{vdw}	B _{vdw}	T _{vdw}	S _{ele}	B _{ele}	T _{ele}	S _{polar}	B _{polar}	T _{polar}	T _{nonpolar}	T _{GBTOT}
WT											
235	-0.01	0	-0.01	0	0	0	0.01	0.03	0.03	0.00	0.02
241	-0.68	-0.79	-1.47	0	0	0	0.33	0.22	0.55	-0.06	-0.99
244	-1.56	-1.13	-2.69	0	0	0	0.96	0.57	1.52	-0.17	-1.33
245	-1.31	-1.30	-2.61	0	0	0	0.18	-0.06	0.11	-0.10	-2.60
248	-2.21	-1.00	-3.21	0	0	0	1.60	0.50	2.10	-0.24	-1.35
249	-1.26	-0.91	-2.17	0	0	0	0.14	-0.04	0.10	-0.11	-2.18
293	-1.44	-0.55	-1.99	0	0	0	0.24	0.11	0.34	-0.12	-1.77
295	-2.35	-0.43	-2.78	0	0	0	0.05	0.10	0.14	-0.25	-2.89
297	-0.13	-0.03	-0.16	0	0	0	0.00	0.02	0.02	0.00	-0.14
303	-0.62	-0.16	-0.78	0	0	0	0.05	0.12	0.17	-0.05	-0.66
304	-0.50	-0.14	-0.64	0	0	0	0.11	-0.07	0.04	-0.10	-0.69
307	-0.69	-0.12	-0.81	0	0	0	0.18	0.05	0.23	-0.07	-0.65
326	-1.30	-0.30	-1.59	0	0	0	0.22	0.06	0.29	-0.08	-1.38
328	-0.94	-0.06	-1.00	0	0	0	0.13	0.01	0.15	-0.06	-0.92
338	-0.48	-0.05	-0.53	0	0	0	0.05	-0.02	0.03	-0.02	-0.51
K248A											
235	-0.02	0.00	-0.02	0	0	0	0.01	0.04	0.04	0.00	0.02
241	-0.68	-0.83	-1.51	0	0	0	0.40	0.20	0.60	-0.07	-0.98
244	-1.74	-1.10	-2.84	0	0	0	1.22	0.57	1.79	-0.20	-1.25
245	-1.32	-1.28	-2.60	0	0	0	0.14	0.00	0.14	-0.10	-2.56
248	-0.71	-1.02	-1.73	0	0	0	-0.02	0.51	0.49	-0.17	-1.41
249	-1.19	-0.81	-2.00	0	0	0	0.13	0.00	0.13	-0.11	-1.99
293	-1.57	-0.53	-2.10	0	0	0	0.23	0.10	0.33	-0.14	-1.91
295	-2.20	-0.25	-2.45	0	0	0	0.05	0.06	0.10	-0.24	-2.58
297	-0.09	-0.02	-0.11	0	0	0	0.00	0.01	0.02	0.00	-0.09
303	-0.57	-0.15	-0.72	0	0	0	0.05	0.12	0.17	-0.03	-0.58
304	-0.59	-0.16	-0.75	0	0	0	0.13	-0.07	0.07	-0.12	-0.81
307	-0.69	-0.12	-0.81	0	0	0	0.18	0.05	0.23	-0.06	-0.64
326	-1.30	-0.29	-1.60	0	0	0	0.21	0.04	0.25	-0.07	-1.43
328	-0.91	-0.05	-0.96	0	0	0	0.13	0.01	0.14	-0.06	-0.88
338	-0.36	-0.05	-0.41	0	0	0	0.03	-0.01	0.02	-0.01	-0.40
I295A											
235	-0.75	-0.12	-0.87	0	0	0	0.17	0.12	0.29	-0.08	-0.66
241	-2.56	-0.77	-3.33	0	0	0	1.16	0.09	1.24	-0.18	-2.27
244	-2.01	-0.87	-2.88	0	0	0	1.02	0.52	1.53	-0.19	-1.54
245	-1.01	-0.87	-1.88	0	0	0	0.14	0.01	0.15	-0.08	-1.81
248	-1.24	-0.19	-1.43	0	0	0	1.24	0.06	1.30	-0.25	-0.38
249	-0.13	-0.06	-0.20	0	0	0	0.03	0.00	0.03	-0.01	-0.17
293	-1.18	-0.34	-1.52	0	0	0	0.18	0.05	0.22	-0.13	-1.43
295	-0.78	-0.52	-1.30	0	0	0	-0.01	0.22	0.21	-0.13	-1.22
297	-0.59	-0.17	-0.76	0	0	0	0.01	0.06	0.07	-0.05	-0.74
303	-0.74	-0.27	-1.01	0	0	0	0.06	0.19	0.26	-0.08	-0.83
304	-0.65	-0.18	-0.83	0	0	0	0.19	-0.13	0.06	-0.11	-0.88
307	-0.50	-0.11	-0.60	0	0	0	0.07	0.08	0.16	-0.03	-0.48
326	-1.29	-0.28	-1.57	0	0	0	0.20	0.08	0.28	-0.08	-1.37
328	-1.10	-0.05	-1.15	0	0	0	0.11	0.04	0.15	-0.06	-1.06
338	-0.98	-0.09	-1.07	0	0	0	0.05	-0.02	0.04	-0.04	-1.08
K248A/I295A											
235	-0.03	-0.01	-0.04	0	0	0	0.01	0.06	0.07	0.00	0.03
241	-2.51	-0.67	-3.18	0	0	0	1.37	0.11	1.48	-0.19	-1.89
244	-2.10	-0.85	-2.95	0	0	0	1.33	0.54	1.87	-0.21	-1.30
245	-1.07	-0.84	-1.91	0	0	0	0.13	0.01	0.13	-0.08	-1.86
248	-0.25	-0.19	-0.45	0	0	0	0.00	0.09	0.09	-0.11	-0.47
249	-0.13	-0.06	-0.18	0	0	0	0.03	0.02	0.05	-0.01	-0.14
293	-1.26	-0.40	-1.66	0	0	0	0.18	0.11	0.29	-0.13	-1.50

Table 2 (continued)

Residue	S _{vdw}	B _{vdw}	T _{vdw}	S _{ele}	B _{ele}	T _{ele}	S _{polar}	B _{polar}	T _{polar}	T _{nonpolar}	T _{GBTOT}
295	-0.84	-0.45	-1.29	0	0	0	-0.01	0.15	0.15	-0.14	-1.28
297	-0.85	-0.14	-1.00	0	0	0	0.03	0.08	0.12	-0.11	-0.99
303	-1.25	-0.35	-1.60	0	0	0	0.05	0.20	0.25	-0.12	-1.47
304	-0.43	-0.18	-0.61	0	0	0	0.18	-0.15	0.03	-0.08	-0.66
307	-0.57	-0.14	-0.71	0	0	0	0.03	0.12	0.15	-0.02	-0.58
326	-1.47	-0.44	-1.91	0	0	0	0.26	0.04	0.31	-0.10	-1.70
328	-1.88	-0.12	-1.99	0	0	0	0.15	0.05	0.21	-0.07	-1.85
338	-1.25	-0.17	-1.42	0	0	0	0.10	-0.02	0.07	-0.06	-1.41

Energies shown as contributions from van der Waals energy (vdw), electrostatic energy (ele), polar solvation energy (polar), non-polar solvation energy of side-chain atoms (S), back-bone atoms (B) and their total value (T) of protein-inhibitor complexes. All values are given in kcal/mol

Some residues have rather high unfavorable polar solvation energies, such as Q241, Q244 and K248, which cancel out the net binding free energies due to their solvation in aqueous solutions.

Figure 8 shows the geometries of SWCNT in a binding complex with important residues, which interact strongly with SWCNT by using the lowest-energy structure from the last 20 ns of the MD simulations. Figure 8 shows that the I295A single mutant and the K248A/I295A double mutants lead to the sites and orientations of the SWCNT, having significant variation to accommodate the change of the conformation of VP35. Thus, the VDW interactions of the above mutants are different from that of the WT complex. As can be seen from Figs. 4 and 7, the I295A mutant and the K248A/I295A double mutants have similar interaction spectra and the sites and orientations of the SWCNTs are very close to each other. For the I295A mutant, the contributions of the residues V245, K248, L249, P293, and I295 of the I295A mutant decrease compared to the WT complex; the energy contributions decrease by 0.79, 0.97, 2.01, 0.34, and 1.67 kcal/mol, respectively. The VDW energy contribution of the side-chain of the

Fig. 8 Geometries of SWCNT in the binding complex with important residues by using the lowest-energy structure from the last 20 ns of the MD simulations

I295A mutant decreases by approximately 1.57 kcal/mol, which suggests that the mutated residue I295 has a direct effect on the loss of binding affinity. The I295A mutant also has a significant effect on the loss of contribution of residues V245, K248 and L249. But the energy contributions of the residues Q241, I297, I303, and L338 of the mutant I295A complex show increases of 1.28, 0.60, 0.17 and 0.57 kcal/mol, respectively, as compared to the WT complex. Table 2 and Fig. 8 show that the binding variation is mainly due to change of van der Waals interactions, which indicates that the binding site of the SWCNT of the I295A mutant is different from that of the WT system. The changed VDW for the I295A complex is most likely due to the cooperative C/N-H $\cdots\pi$ interactions between the SWCNT and VP35 residues. In addition, the residue F235 of the I295A mutant contributes favorable affinity of 0.66 kcal/mol, while the residues F235 of WT, K248A and K248A/I295A contribute unfavorable forces to the binding of 0.02, 0.02, and 0.03 kcal/mol, respectively.

4 Conclusions

In this work, the molecular dynamics (MD) simulation approach was used to study the interaction mechanisms of SWCNT and WT VP35 as well as a few primary mutants (K248A, I295A and K248A/I295A). The structure analyses indicate that the binding pockets of I295A-SWCNT and K248A/I295A-SWCNT are more spacious than that of WT-SWCNT. Moreover, the sites and orientations of the SWCNT binding to the I295A mutant and the K248A/I295A double mutants have changed significantly to accommodate the variation of the VP35 conformation. According to the calculated results, the binding free energies of the mutant systems decrease by 3.19, 5.60 and 2.61 kcal/mol for the K248A, I295A and K248A/I295A complexes, respectively, as compared to the WT complex. By analyzing the energy terms constituting the absolute binding free energy, it was found that the electrostatic energy of polar solvation and entropy terms contribute an unfavorable force to the binding of SWCNT and that the VDW energies in the gas phase provide the major forces for affinity binding. The results also indicate that the K248A single mutant, I295A single mutant and K248A/I295A double mutants have unfavorable influences on VDW with the energies decreasing by 5.16, 5.05 and 3.11 kcal/mol as compared with the WT complex. In addition, the MM-GBSA method was employed to calculate decompositions of the binding free energies of all the systems at an atomic level. The results indicate that residues Q241, Q244, V245, K248, L249, P293, I295, I297, I303, P304, C307, C326, F328, and L338 play an important role in the binding of the SWCNT. The VDW energy contributions of these key residues of the I295A single mutant and the K248A/I295A double mutants systems are different from that of the WT system. In accordance with the energy components of binding free energy, for all the systems the VDW energies provide the main contributions to SWCNT binding.

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Author Contributions Yan-Jun Zhang, designed research, performed research, contributed analytic tools, analyzed data, and wrote the paper.

Jing-Na Ding partly analyzed data.

Hui Zhong partly analyzed data.

Chang-Ping Sun partly analyzed data.

Ju-Guang Han modified the paper.

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