Insight into the Inhibitory Mechanism and Binding Mode Between D77 and HIV-1 Integrase by Molecular Modeling Methods

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Abstract

Integrase is an essential enzyme in the life cycle of Human immunodeficiency virus type 1 (HIV-1) and also an important target for designing integrase inhibitors. In this paper, the binding modes between the wild type integrase core domain (ICD) and the W131A mutant ICD with the benzoic acid derivative – D77 were investigated using the molecular docking combined with molecular dynamics (MD) simulations. The result of MD simulations showed that the W131A substitution affected the flexibility of the region 150-167 in both the monomer A and B of the mutant type ICD. In principle, D77 interacted with the residues around the Lens Epithelium-Derived Growth Factor (LEDGF/p75) binding site which is nearby the HIV-1 integrase dimer interface. However, the specific binding modes for D77-wild type integrase and D77-mutant integrase systems are various. According to the binding mode of D77 with the wild type ICD, D77 can effectively intervene with the binding of LEDGF/p75 to integrase due to a steric hindrance effect around the LEDGF/p75 binding site. In addition, we found that D77 might also affect its inhibitory action by reducing the flexibility of the region 150-167 of integrase. Through energy decomposition calculated with the Molecular Mechanics Generalized Born Surface Area approach to estimate the binding affinity, it seems likely that W131 and E170 are indispensable for the ligand binding, as characterized by the largest binding affinity. All the above results are consistent with the experimental data, providing us with some helpful information not only for the understanding of the mechanism of this kind of inhibitor but also for the rational drug design.

Key words: HIV-1 integrase; D77; Molecular docking; Molecular dynamics simulation; MM-GBSA.

Introduction

Inhibitors targeting protease and reverse transcriptase of human immunodeficiency virus type 1 (HIV-1) have become successful drugs to treatment of AIDS patients (1). However, efforts toward development of effective integrase (IN) inhibitors have been hampered by various reasons, of which the most important one is the absence of crystal structures of the full-length enzyme (2). So far, only one FDA-approved HIV IN inhibitor, i.e. Raltegravir, exists. HIV-1 IN is composed of 288 residues (32 kDa), and can be divided into three distinct functional domains: the N-terminal domain (NTD, residues 1-49), the catalytic core domain (CCD, residues 50-212), and the C-terminal domain (CTD, residues 213-288). The NTD contains a conserved “HHCC” motif that binds with a Zn$^{2+}$ ion. This domain has been shown to facilitate the multimerization of HIV-1 IN in vitro (3, 4). The CCD contains three highly conserved residues, namely D64, D116, and E152 (DDE), which coordinate divalent cations such as Mg$^{2+}$ or Mn$^{2+}$ (5-7). The DDE motif mainly serves
It is found that a variety of cellular proteins are important partners in the integration process. Lens Epithelium-Derived Growth Factor (LEDGF/p75) is one of these essential partners, and the first cofactor shown to function as a cellular co-factor for integration in vivo (10). As shown by previous experiments, it plays a crucial role in controlling the location of HIV integration in human cells (11). LEDGF/p75 belongs to the hepatoma-derived growth factor-related protein (HRP) family, and is originally isolated and characterized as a general transcriptional co-activator (12-14). It is characterized by a conserved N-terminal Pro-Trp-Trp-Pro motif (PWWP motif, residues 1-93) that is a putatively chromatin-binding fragment. The residues from 347 to 429 of LEDGF/p75 constitute the C-terminal domain, which is identified as integrase binding domain (IBD) (15, 16). The crystal structure of the HIV-1 IN CCD in complex with LEDGF/p75 IBD provides much more structural information about the interaction (17-19). For clear cognition, Figure 1 shows the IN CCD dimer interacting with LEDGF/p75 IBD (PDB code 2B4J) (19). Previous studies show that LEDGF/p75 acts through a tethering mechanism as a potent cofactor for HIV-1 integration with the N-terminal PWWP motif and A/T hook elements binding to chromatin, and the C-terminal IBD binding to IN (20-22). LEDGF/p75 plays the important role in HIV integration, and perturbing the interaction between LEDGF/p75 and IN may have therapeutic potential (22).

The compound D77 (Figure 2) is a benzoic acid derivative and it is found that this compound shows strong inhibitory activity against LEDGF/p75-IN interaction (23). According to this report, D77 can inhibit HIV-1 (IIIB) replication by EC_{50} value of 23.8 μg/mL in MT-4 cell, and exhibits a high specific binding affinity to HIV-1 IN CCD. Besides, it is also pointed out that D77 is located around the LEDGF/p75 binding site in HIV-1 IN CCD dimer interface, and the mutation of W131A nearly abolishes the D77 binding to IN. Although the rough binding region of D77 is identified, the inhibitory mechanism and the specific binding modes of the inhibitor remain elusive.

In this paper, we intend to investigate the binding modes and inhibitory mechanism of the small molecular compound D77 while interacting with HIV-1 IN. The molecular docking process involves the prediction of the ligand conformation and its orientation within the protein interaction site. As can be seen from recent publications in this area (24-54), diverse methodologies have been developed depending on the biological system under investigation. In our case, the binding modes of D77 with the wild type IN and the W131A mutant type IN, were predicted with the relaxed complex molecular docking method (55, 56), and then IN-D77 complex were
subject to molecular dynamics (MD) simulations. The key residues of binding were
analyzed through the Molecular Mechanics Generalized Born Surface Area (MM-
GBSA) approach (57, 58). According to the results of molecular docking and MD
simulations, we have explained the inhibitory mechanism of D77 against IN. Probing
the binding modes and inhibitory mechanism of D77 is helpful for our future
study on anti-HIV drug design, modification and HIV drug resistance.

Materials and Methods

System Preparation

The structure of D77 was generated and minimized with the Sybyl 7.0 program
package. The structure of the IN core domain was obtained from the Protein Data
Bank with the PDB entry of 2B4J (19), which includes a dimeric catalytic core
domain of HIV-1 IN, termed chain A and B. The missing functional 140s loop
on each monomer (residues 141-150 in chain A; 140-149 in chain B) was built
by using the Biopolymer module of Sybyl 7.0. The molecular models were con-
structed by using standard geometries (standard bond lengths and angles) from
the TRIPOS force field within the Sybyl 7.0 package. Charges were assigned
using the Gasteiger–Marsili method. Energy minimization was performed by 1000
steps steepest descent followed by 1000 steps of the conjugate gradient using Sybyl
7.0. Finally, the structure of the modeled IN dimer was checked by Ramachand-
ran map. Most of the residues were located at the most allowed regions. Then, all
hydrogen atoms were added with the xLeap program of AMBER 8 package accord-
ing to the Amber ff03 force field (59). The processed system was then merged into a
70 × 66 × 60 Å3 TIP3P (60) water box, and four Cl− ions were added to neutralize
the system. The wild type IN system is denoted as WT. After finishing the prepa-
ration of WT structure, the W131A mutated type IN system (denoted as W131A)
were prepared by introducing the W131A substitution with VMD 1.8.6 software
(61). The dimer of IN was used in subsequent MD simulations.

Molecular Dynamics Simulations

All MD simulations were performed with the NAMD 2.6 program (62), and used
the AMBER all-atom force field (59, 61). During the simulations, all bond lengths
containing hydrogen atoms were constrained utilizing the SHAKE algorithm (63),
and the integration time step was set to 2 fs. First, the two systems were respecti-
vely minimized by 20,000 steps with solutes constrained (restricting force =
10 kcal/mol·Å2), followed by 20,000 steps of minimization without any constraint.
Then, the minimized systems were slowly heated from 0 up to 300 K within 120 ps
while constraining all Cα atoms and the two Mg2+ ions. Finally, the nonconstrained
simulations were performed for 6 ns at a constant temperature of 300 K and a con-
stant pressure of 1 atm through the Langevin piston method (64). The subsequent
MD simulations of four independent MD simulations (WT, WT_D77 complex,
W131A and W131A_D77 complex) were performed with the same procedures
mentioned above as well. The number of atoms in WT, WT_D77, W131A and
W131A_D77 is 38112, 39390, 31728 and 39042, independently. The simulation
time for the four independent MD simulations was each 6 ns.

Molecular Docking with the Relaxed Complex Method

Presently, the flexibility of receptor is still not taken into consideration in most
molecular docking methods. In fact, the conformation of receptor, particularly the
active site, would change to some extent owing to the insertion of ligand. Conse-
sequently, the binding mode predicted by docking could be in better accordance with
the reality if the receptor flexibility can be partly taken into account. To accommo-
date the flexibility of receptor, the relaxed complex molecular docking approach
(55, 56) was employed in our study. First, a 6-ns MD simulation was performed for
WT and W131A systems, and then 12 snapshots were extracted for each system to dock with the D77. Among the 12 snapshots, the first and the last one are the first and the last recorded structures of the 6-ns simulation, respectively. The other 10 snapshots are extracted from the beginning to the end of the simulation according to their Cα RMSD values vs. the starting structure. Basically, we preferred to choose the snapshots with different conformations in the binding site. The multiple conformation molecular docking calculations were performed with the AutoDock 4.0 package (65, 66). The Gasteiger partial charges were assigned to the ligand and the receptor using Autodocktools 1.52 (66). All single bonds of the ligand were treated as rotatable during the docking calculation, and altogether 12 flexible torsions were defined. The maximum number of energy evaluations was increased to $2.5 \times 10^7$ to explore the conformational space sufficiently. The center of the grid box was set to the center of T174 of monomer A and Q125 and W131 of monomer B. The box size was set to 60 $\times$ 60 $\times$ 60 with grid spacing 0.375 Å in each dimension, which is large enough for the free rotation of the ligand. Each docking calculation generated 100 structures. All other docking parameters were set to default. The final solutions were selected according to the size of clusters according to RMSD and the estimated free energy of binding (FEB). Basically, the best solution of the largest cluster was selected as the best mode for each docking test. Therefore, the mode with the lowest FEB value was taken as the final bind mode and the starting structure for the following complex MD simulation. The two complex systems were then subjected to minimizations and MD simulations. The procedures have been described in detail above.

**MM-GBSA**

The MM-GBSA method (67, 68) has been successfully used for energy decomposition. To investigate the energetic contributions of each residue to ligand binding in different binding modes, energy decomposition was performed using MM-GBSA approach. The binding free energies ($G_{bind}$) were calculated by using MM-GBSA (69) method in AMBER 8.0 package. In this method, $G_{bind}$ is evaluated as a sum of the changes in the molecular mechanics gas-phase binding energy ($\Delta E_{MM}$), solvation free energy ($G_{solv}$), and entropy contribution ($T\Delta S$):

$$
G_{bind} = \Delta E_{MM} + G_{solv} + T\Delta S
$$

Where $\Delta E_{MM}$ and $\Delta G_{solv}$ denoted the electrostatic and van der Waals interactions, respectively. Note that Eq. [1] is an approximation under the assumption of $\Delta H_{bind}^{gas} = \Delta E_{MM}$ when the volume change during the binding process is negligible under constant temperature and pressure. $\Delta G_{solv}$ are computed in continuum solvent using GB methods (70). The non-polar part of the solvation energy ($G_{solv}^{non}$) is calculated using the function

$$
\Delta G_{solv}^{non} = \gamma \times \Delta SASA + b
$$

where $\Delta SASA$ is the solvent accessible surface area (71) of the complex, receptor or ligand, determined with LCPO (72) method; the parameters use typical values ($\gamma = 0.00542$ kcal/mol Å$^2$ and $b = 0.92$ kcal/mol).

It has been shown in earlier studies that neglecting the entropy effects of analogues binding to the same receptor results in good agreements between the calculated and experimental relative BFE (73, 74). Therefore, we omitted the entropy contributions.
in this study since we are only interested in the relative order of binding affinities. The decomposition is carried out on a pairwise per-residue basis, which lists the binding energy items for each residue-ligand pair.

**Results and Discussion**

**Comparative Analysis of the MD Trajectories of the IN Core Domains**

The root mean square deviations (RMSD) for Cα atoms of monomer A and B for the WT and W131A systems with regard to their respective starting structures are shown in Figure 3 (A) and (B). Illustrated by the Figure 3, the RMSD curves become flat within 1 ns, indicating that the conformations of the protein reached equilibrium. The average Cα RMSD values (from 1 ns to the end) for monomer A of the two systems are 0.7 ± 0.1 Å and 0.6 ± 0.1 Å, respectively. The average Cα RMSD values (from 1 ns to the end) of monomer B for both of the two systems are 0.7 ± 0.1 Å. According to the Figure 3 (A) and (B), it can be found that the overall RMSD of the two systems are similar. In order to compare the flexibility changes of the two systems, we also calculated their respective root mean square fluctuations (RMSF). The RMSFs for the WT and the W131A systems are depicted in Figure 3 (C) and (D), respectively. Based on Figure 3 (C) and (D), we note the differences between the flexibility of the segment containing residues 150-167 between these two systems. The residues 150-167, called as the amphipathic α4 helix, protrudes at

**Figure 3:** Comparative analyses of the MD trajectories of the WT and the W131A systems. (A) and (B) RMSD of the Cα atoms of monomer A and B for the two systems, respectively; (C) and (D) RMSF of the Cα atoms of monomer A and B for the two systems, respectively. The RMSF plot for 150-167 regions of the two systems is given as zoom-in inset for clarity. In this article, we apply a consistent coloring scheme to all Figures: blue for WT and red for W131A. All curves and graphs are represented by corresponding colors, if not otherwise addressed.
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As revealed by previous experiments, it bears the catalytic residue E152 and several other residues, such as K156 and K159, which have been shown to be important for the binding of IN to DNA and for virus survival (75-77). Besides, Fermandjian’s results strongly indicate that the $\alpha_4$ helix stability is essential to the specific recognition of viral DNA (78). According to our MD simulations, it is found that the 150-167 peptides of the W131A system are not as stable as that of the WT system for monomer A and B. It is suggested that the W131A mutant may interfere with the catalytic activity of IN by affecting the stability of the $\alpha_4$ helix. This speculation is basically in line with the fact that the W131 mutation affected both the process of 3'-processing and strand-transfer in catalytic reaction (79).

The Prediction of Binding Modes

The predicted binding modes of D77 with the WT and the W131A mutant are illustrated in Figure 4 (A) and Figure 6 (A), and the detailed information about the interactions between the ligand and the protein is exhibited by Figure 4 (B) and Figure 6 (B).

As illustrated in Figure 4 (A), D77 is located at the HIV-1 IN catalytic core domain dimer interface. This is in good agreement with result of the site-directed mutagenesis analysis (23). According to Figure 4 (B), it is found that the D77 makes hydrophobic interactions with the A98, L102, A128, A129, W131 and W132 of monomer B and A169 and M178 of monomer A. Simultaneously, it makes hydrophilic interactions with the Q95 and T125 of monomer B and E170, H171 and T174 of monomer A. Among these residues, the backbone nitrogen atoms of E170 and H171 form hydrogen bonds with the oxygen atoms of carboxyl group of D77. D77 binds to three regions of the wild type IN, including residues ranging from 165 to 179 in monomer A, from 94 to 103 and from 123 to 133 in monomer B. The benzene ring 1 forms two $\pi$-$\pi$ stacking interactions with the indole group of W131 and W132, respectively. Additionally, the benzene ring 2 and 3 insert into the deep trench formed at the interface of two monomers, thus possibly resulting in the destabilization of the IN dimer by causing a steric hindrance around the monomer interface. As revealed by previous experiments, D366 of p75, which is of great importance for the p75/IN binding, forms two hydrogen-bonds with E170 and H171 of IN, respectively (19, 80). Besides, I365, F406 and V408 of p75 make hydrophobic interactions with W131 of IN (19) (Figure 1 (B)). Figure 5 shows the binding site for D77 in WT is highly similar to that for the p75. Therefore, it may lead to a significant inhibition activity of D77 against the interaction of p75 with IN by causing a steric hindrance.

As shown in Figure 6 (A), the binding mode of D77 with the W131A is quite different from that with the wild type IN. Based on Figure 6 (B), there is a conversion in the orientations of the benzene rings 1 and 2 of D77. In the WT-D77 system, the benzene rings 1 and 2 point to the outside of the deep trench while pointing to the interior as observed in the W131A-D77 system. For the wild type IN, the
benzene ring 1 lies near by the aromatic side-chains of W131 and W132, whereas in the case of W131A mutant the Alanine substitution for Tryptophan breaks such equilibrium because Alanine lacks the indole side-chain. This may partly account for the conversion in the orientations of the benzene rings 1 and 2 in the W131A mutant. In addition, it can be found that the benzene rings 2 and 3 are far away from the dimer interface (see Figure 6 (B)). Although the benzene rings 2 and 3 also insert into the deep trench, this compound is probably not capable of interfering with the stability of the IN dimer as effectively as it does to the wild type IN, because it is far away from the dimer interface. Despite of the quite different binding mode, it should be noted that the D77 makes more residue contacts with WT than with W131A IN (see Figure 6 (B)). This finding confirms that D77 interacts with the three regions, 165 to 179 of monomer A, 94 to 103 and 123 to 133 of monomer B.

**Comparative Analysis of the MD Trajectories of the Complexes**

According to Figure 7(A) and (B), the $C_{\alpha}$ RMSD curves of the WT_D77 and W131A_D77 complexes relative to their respective starting structures reach equilibrium within the first 1 ns. The average $C_{\alpha}$ RMSD values (from 1 ns to the end) for monomer A of the two systems are $0.7 \pm 0.1 \text{ Å}$ and $0.6 \pm 0.1 \text{ Å}$, respectively. For monomer B, the average $C_{\alpha}$ RMSD values (from 1 ns to the end) are $0.9 \pm 0.1 \text{ Å}$ and $0.7 \pm 0.1 \text{ Å}$, respectively. The overall RMSD for monomer B of two systems is similar, but for monomer A, the W131A_D77 system is more stable than the WT_D77 system. This just validates the pre-speculation that D77 in the W131A_D77 system is probably not capable of interfering with the stability of the IN dimer as effectively as it does to the wild type IN. Figure 7 (C) shows RMSD of D77 in the two systems with regard to their respective starting structures, illustrating that D77 in the WT_D77 system is much more stable than in the W131A_D77 system with average RMSD value being only $2.1 \pm 0.3 \text{ Å}$. As for the W131_D77 system, the binding modes of D77 with W131A switch between two conformations and the average RMSD value for D77 is up to $3.6 \pm 1.4 \text{ Å}$. It is said that the interaction between D77 and the W131A mutant may be weaker and more instable. The structural alignment of the two conformations is showed by Figure 7 (D). It can be seen that the difference between two conformations is mainly due to the binding mode change of the benzene ring 1 in D77 to W131A, as the Alanine substitution for Tryptophan.

For the sake of comparing the differences in the flexibility of IN before and after inhibitor binding, the $C_{\alpha}$ RMSF of WT and W131A vs. their respective complex systems are given by Figure 8. Based on Figure 8 (A) and (B), it is found that the mobility of the region containing residues 150-167 is higher in the wild-type complex than in free WT system, especially for monomer A. It is pointed out that the stability of region 150-167 is essential to the specific recognition of viral DNA [46]. According to our result, it is suggested that the D77 binding may cut down the
stability of region 150-167, and interfere with the catalytic activity of IN by affecting the recognition of viral DNA. As illustrated by the Cα RMSF plots of the free W131A and its corresponding complex system (see Figure 8 (C) and (D)), differences in flexibility are still observed in the region 150-167. However, in contrast, the mobility of residues 150-167 in the complex run is much lower than that in the W131A. This may partially account for the drug resistance to D77 induced by the W131A substitution.

Table I lists all hydrogen bonds with the frequencies of occupancy between IN and D77 in the WT_D77 and W131A_D77 systems. As shown in Table I, there are five hydrogen bonds between D77 and residues E170, H171 and T174 of WT. The backbone nitrogen atom of residue E170 forms two N—H…O hydrogen bonds with the oxygen atoms of carboxyl group of D77. The frequency of occupancy of hydrogen bond between E170-main-N and D77-O36 is up to 92.67%, especially. This indicates that residue E170 may be indispensable for the binding of D77 to WT IN. The backbone nitrogen atom of residue H171 also forms two N—H…O hydrogen bonds with the oxygen atoms of carboxyl group of D77. The frequencies of occupancy of hydrogen bond are 43.83% and 30.30%, respectively. Therefore, H171 may also be important for the D77 binding to WT IN. These findings are in good agreement with the previous docking results. Although the hydrogen bond between residue T174 and D77 is a little bit weaker (the frequency of occupancy is only 22.67%) than others, the mutagenesis experiment shows that T174 is also important to the binding (23). For the W131A_D77 system, D77 forms four
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hydrogen bonds with IN. The corresponding residues are Q95, H171 and T174, respectively. Residue H171 still forms two hydrogen bonds with D77, confirming that H171 is important for the D77 binding. However, it is also worth noting that there is no hydrogen bond formed between E170 and D77. Besides, a new strong hydrogen bond, whose frequency of occupancy is up to 72.17%, formed between the oxygen atoms of carboxyl group of D77 and the side chain nitrogen atom of residue Q95. These were not found by previous docking calculations. As mentioned above, residue E170 is of great importance in the binding of LEDGF/p75 and IN (19, 80). Consequently, the absence of hydrogen bond between E170 and D77 in the W131A_D77 system, which induces the exposure of residue E170, may partly account for the resistance of the W131A mutant to D77. Additionally, the side chain of residue T174 also forms hydrogen bonds with D77, with the frequency of occupancy being merely 17.17%. This suggests that T174 may not be as important for the recognition between the W131A mutate and D77 as does in the wild type IN.

Energy Decomposition

Figure 9 (A) and (B) show the energy contribution of each residue in the WT and W131A systems to the D77 binding. Here, we nominate the residues that notably contribute (no matter positive or negative) to the D77 binding as binding residues, and define that the binding range is from the binding residue with the smallest residue index to the one with the largest.
It can be seen from Figure 9 that there are three regions which are mainly responsible for the ligand binding, noted I, II and III respectively. These include residues 166-178 in monomer A and residues 95-102 and 125-133 in monomer B, in good agreement with the previous docking results. In addition, D77 mainly interacts with the residues in Region I and III, and the binding affinity between D77 with Region II is much weaker. In the WT_D77 system, Region I of monomer A contains six key residues responsible for the D77 binding, namely Q168, A169, E170, H171, T174 and M178. Region II and III are located in monomer B. In these two regions, there are eight key residues for the binding, namely Q95, A98, L102, T125, A128, A129, W131 and W132. Among these binding residues, E170, A169 and H171 of monomer A and W132, W131, T125 and A128 of monomer B make the seven strongest interactions with the inhibitor. Characterized by the low binding energies of these residues, it is speculated that these residues are important for the binding of this inhibitor. All of these binding residues were obtained by the previous docking calculations (Figure 4 (B)).

As to the W131A_D77 system, it can be found that there are also three regions which are mainly responsible for the ligand binding. Comparison between Figure 9 (A) and (B), reveals that the contributions of Region I and III are weaker in the W131A_D77 system, otherwise, the contribution of Region II becomes much stronger. Besides, it is found from Figure 9 (A) that the binding range of the

<table>
<thead>
<tr>
<th>System</th>
<th>Donor(^a)</th>
<th>Acceptor(^b)</th>
<th>Dist.(^c)(Å)</th>
<th>Angle(^d)(°)</th>
<th>Freq.(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT_D77</td>
<td>E170-main-N</td>
<td>D77-O36</td>
<td>2.7 ± 0.1</td>
<td>160.59 ± 9.16</td>
<td>92.67%</td>
</tr>
<tr>
<td></td>
<td>H171-main-N</td>
<td>D77-O36</td>
<td>2.9 ± 0.1</td>
<td>170.61 ± 10.01</td>
<td>43.83%</td>
</tr>
<tr>
<td></td>
<td>H171-main-N</td>
<td>D77-O37</td>
<td>3.0 ± 0.1</td>
<td>155.98 ± 11.13</td>
<td>30.30%</td>
</tr>
<tr>
<td></td>
<td>E170-main-N</td>
<td>D77-O37</td>
<td>3.0 ± 0.1</td>
<td>162.13 ± 11.34</td>
<td>29.33%</td>
</tr>
<tr>
<td></td>
<td>T174-side-O</td>
<td>D77-O37</td>
<td>2.9 ± 0.1</td>
<td>148.72 ± 12.09</td>
<td>22.67%</td>
</tr>
<tr>
<td>W131A_D77</td>
<td>Q95-side-N</td>
<td>D77-O37</td>
<td>2.8 ± 0.1</td>
<td>169.34 ± 9.21</td>
<td>72.17%</td>
</tr>
<tr>
<td></td>
<td>H171-side-N</td>
<td>D77-O36</td>
<td>2.8 ± 0.1</td>
<td>178.12 ± 9.78</td>
<td>65.50%</td>
</tr>
<tr>
<td></td>
<td>H171-side-N</td>
<td>D77-O37</td>
<td>3.0 ± 0.1</td>
<td>156.23 ± 10.44</td>
<td>30.50%</td>
</tr>
<tr>
<td></td>
<td>T174-side-O</td>
<td>D77-O36</td>
<td>3.1 ± 0.1</td>
<td>145.12 ± 13.14</td>
<td>17.17%</td>
</tr>
</tbody>
</table>

\(^a\)Donor atoms.
\(^b\)Acceptor atoms.
\(^c\)The donor-receptor distance.
\(^d\)The angle of donor-hydrogen-receptor.
\(^e\)The probability of hydrogen bond occurring in the MD simulation.

Figure 9: Contribution of each residue of WT and W131A to free energy of D77 binding. The plots are focused on a small range of residues, residue index: 164 to 190 of monomer A (A), 94 to 137 of monomer B (B), respectively. The binding energy contribution vs. Monomer A and B for two systems are given as insets, respectively. The important residues for binding are marked by corresponding texts.
W131A mutant is narrower than that of wide type IN. This is due to the fact that the contributions of Q168, A169 and E170 to the D77 binding are much weaker than those in the WT_D77 system. Additionally, the contribution of K173 to the binding in W131 mutant (−1.29 kcal/mol) is nearly five times higher than in WT (merely −0.26 kcal/mol), possibly partially due to the closure of D77 towards K173. Of Region III, it is obvious that the contributions of A131 and W132 in W131A mutant are quite weaker than in WT. The contributions of A131 and W132 range from −2.53 to −0.54 kcal/mol and −3.27 to −1.31 kcal/mol, respectively. It means that the W131A substitution not only obviously affects the interaction of residue at position 131 with D77, but also reduces the contribution of W132 to binding affinity. Moreover, the contribution of A133 in W131A mutant (−2.11 kcal/mol) is much higher than in WT (−0.08 kcal/mol). This indicates that there is a strong hydrophobic interaction between A133 and D77 in the W131A_D77 system, as the benzene ring 1 is closer to A133 (see Figure 6(B)) than in the WT_D77 system. It is also worthwhile to note that the stronger contribution of the Region II is mainly due to the contribution of residue Q95, i.e., its binding energy in WT and W131A is −0.78 and −3.64 kcal/mol, respectively.

Conclusion

In this study, MD simulations and molecular docking were performed to evaluate the binding modes of the D77 with the wild type and the W131A mutant type of IN. According to the results of the MD simulations of the unbound IN, it is found that the W131A substitution affects the flexibility of the 150-167 segment in both of monomer A and B. For both WT and MT IN, although D77 binds at the interface of IN dimer, the variable binding modes can be observed for these two systems. Based on the binding mode of D77 with WT and the complex MD simulation, it is suggested that D77 can effectively interfere with the binding of LEDGF/p75 to IN by causing a steric hindrance at p75-binding site. This binding mode fits well with the previous experiment. Moreover, the inhibitor may also exert its function by cutting down the stability of the 150-167 residues. As for the W131A mutant, it seems that D77 cannot effectively interfere with the binding of LEDGF/p75 with IN dimer since D77 is far away from the interface and the hydrogen bonds between D77 and E170 disappear. Finally, three binding regions and seven key binding residues, that is, E170, A169 and H171 of monomer A and W132, W131, T125 and A128 of monomer B, are identified based on the calculation results of energy decomposition. Characterized by the largest binding affinity, W131 and E170 seem to be indispensable for the ligand binding. In conclusion, the binding modes of D77 to IN, and its inhibitory mechanism were explained in this work. Understanding of these will not only facilitate the subsequent IN inhibitor design and modification, but also aid in the elimination of possible drug resistance.

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