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Mechanism of inhibitor ADS-J1 and ADS-J2 binding to HIV-1 gp41

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Abstract: In order to analyze and explain the mechanism of the two small inhibitors (ADS-J1 and ADS-J2) binding to HIV-1 gp41, a computational study is carried out to help identifying possible binding modes by docking these compounds onto the hydrophobic pocket on gp41 and characterize structures of binding complexes. The binding interactions of gp41-molecule and free energies of binding are obtained through molecular dynamics simulation and molecular mechanic/Poisson-Boitzmann surface area (MM/PBSA) calculation. Specific molecular interactions in the gp41-inhibitor complexes are identified. The present computational study complements the corresponding experimental investigation and helps establish a good starting point for further refinement of small molecular gp41 inhibitors.

Key words: HIV-1 entry inhibitor; binding modes; gp41; binding free energy

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Human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein gp120 and gp41 play a critical role in the early stages of HIV entry. The transmembrane subunit gp41 of HIV-1 mediates fusion of the virus with the target cell[1-2]. The gp41 molecule consists of three domains: the cytoplasmic domain, the transmembrane domain, and the extracellular domain (ectodomain). The ectodomain contains three major functional regions: the fusion peptide (FP), the N-terminal heptad repeat (NHR or HR1), and the C-terminal heptad repeat (CHR or HR2). The crystal structure of the six-helix bundle of gp41 shows that three helices from the N-peptides (the N-helices) associate to form the central trimeric coiled-coil and the other three helices from the C-peptide region (the C-helices) pack obliquely in an anti-parallel configuration into the highly conserved hydrophobic grooves on the surface of the central coiled-coil. There exists a highly conserved hydrophobic cavity in each of the grooves, formed by the cavity-forming sequence in the NHR region, which is critical for viral fusion and stability of the 6-HB (helix bundle)[3]. Since the inhibition of gp41 can effectively block HIV-1 entry into human cells and, thus, prevent new infection of AIDS virus, gp41 is an important target for developing anti-HIV drugs besides the available classes of drugs targeted at reverse transcriptase, protease and integrase[4]. One of the C-peptide analog, T-20 (Fuzeon, Trimeris Inc.), is licensed by the US Food and Drug Administration as the first member of a new class of anti-HIV fusion drugs. The discovery of fusion inhibitor T-20 is a great breakthrough in the development of anti-HIV drugs since it can be used for the treatment of HIV-infected individuals who fail to respond to the currently available antiretroviral drugs, such as HIV reverse transcriptase and protease inhibitors. However, the application of T-20 is limited because of the lack of oral availability, high costs of production, and the rapid emergence of resistant HIV-1 strains in patients receiving T-20. Therefore, it is essential to develop small-molecule anti-HIV-1 fusion inhibitors with a mechanism of action similar to that of C peptides but without the disadvantages of the peptidic drugs.

Much effort has been devoted toward developing effective small molecular inhibitors of gp41 with little success. The unique 6-HB structure of gp41 makes it difficult to identify a suitable small molecule inhibitor that can bind effectively to gp41. By a combined experimental and theoretical study, oleuropein and hydroxytyrol are shown to be effective gp41 inhibitors and they can bind to the N-terminal hydrophobic cavity. Computational study shows that the hydrogen bonds formed by oleuropein/hydroxytyrol with Gln577 of gp41 play important roles in determining the binding affinities[5]. Recently, Jiang et al.[6-7] reported several natural and synthetic small inhibitors targeting gp41, including two small inhibitors (ADS-J1 ADS-J2) (see Fig. 1). These molecules are found to be active inhibiting HIV-1 entry but detailed binding structures and interaction with gp41 are not clear. Theoretical characterization of possible binding modes and

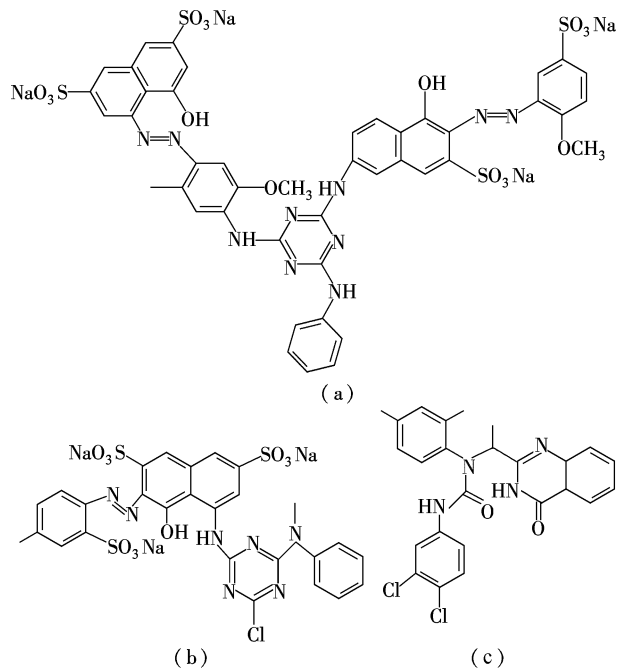

Fig. 1 Chemical structure of inhibitors of gp41. (a) ADS-J1; (b) ADS-J2; (c) ADS-J13

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binding affinity of these compounds with gp41 should be very helpful in the guidance of experimental research. To complement the experimental investigation, we perform systematic computational studies to investigate possible binding complexes of compounds/gp41 through molecular docking, molecular dynamics(MD) simulation and free energy calculations. Specific binding modes from docking studies are analyzed and MD simulation is performed to study molecular interaction, binding mechanism, stability of the binding complexes and binding affinities for the compounds with gp41.

1 Computational Methods

Each of the ligands is first optimized at the PM3 level and the minimized structure is used for electrostatic potential (ESP) calculation by B3LYP/6-31G* using the Gaussian03 package. The atomic charges of the ligands are derived from the ESP by using the RESP[8] program implemented in the AMBER 9.0 package. The atomic coordinates of HIV-1 gp41 NHR trimer core structure are taken from Protein Data Bank (PDB) with PDB entry ID 1AIK. For the ligands we study here, the binding orientations are first estimated using AutoDock 4.0. There is a highly conserved large hydrophobic cavity located at the N-terminal of the N-36 coiled-coil core structure, including conserved residues such as Leu565, Leu566, Gln567, Leu568, Thr569, Val570, Trp571, Gly572, Ile573, Lys574, Gln575, Leu576, Gln577, Ala578, Arg579, Ile580 and Leu581. Occupying the cavity by small molecules will interrupt the formation of six-helix fusion structure, and thus inhibit the HIV membrane fusion process. The preliminary docking studies show that the hydrophobic cavity located at the N-terminal of the N36 trimer is a relatively favorable site for the docking of small molecules. We thus use this hydrophobic cavity to generate the receptor site and the energetic grids for the docking calculations. To obtain all the possible binding orientations, flexible docking is performed, in which single bonds outside the rings are set free to rotate. During the docking process, conformational search is performed using the Solis and Wets local search method, and the Lamarckian genetic algorithm is applied to find the minimum energy structure of the ligand-receptor complexes. The docking structures are minimized in the fixed protein using 200 steps of Steepest Descent followed by 2 000 steps of conjugate gradient.

Molecular dynamics simulations are carried out using the SANDER module of the AMBER9.0 program with the ff03 version of the Amber force field. For each gp41-ligand binding mode obtained from AutoDock, about 8 000 TIP3P water molecules with 0.8 nm buffer are added around the complex. Eleven Na^+ counterions are added to maintain the neutrality of the system. The simulations are carried out at 300 K with a time step of 2.0 fs. The non-bonded cutoff is set to 1.0 nm and the SHAKE algorithm is applied for all the bonds involving hydrogen atoms. After minimization of 1 500 steps and equilibration for 150 ps, complex conformations are collected every 1 ps for the following 3 ns simulation. Finally, 100 snapshots are collected from the region with stable fluctuation for post-processing analysis and free energy calculation.

For each snapshot collected during the MD simulation, both protein-ligand interaction energies (ΔE^{vdw}, ΔE^{elec}) and the electrostatic contribution (ΔG^{PB}) to the solvation energy are calculated with the PBSA program of AMBER 9.0. Here, the single trajectory approach is applied to estimate the energies. This approach means that the thermodynamic data are extracted from a single trajectory of the protein-ligand complex based on the assumption that the bound protein and bound ligand conformations are similar to their free conformations. Estimation of energies in this manner has proved successful in many studies. Part of the reason for the success of this approach is the cancellation of errors that hides the effect of incomplete sampling. A logically better approach, limited in practice due to larger fluctuations and errors, is the use of separate trajectories where the energies are estimated from three separate MD trajectories of the protein-ligand complex, free protein, and free ligand. Due to sampling limitations, the separate trajectory approach appears to be significantly less stable in numerical study. The non-polar part of the solvation energy ΔG^{SA} is estimated using the simple empirical relation: $\Delta G^{SA} = \gamma A + b$, where A is the solvent-accessible surface area that is estimated using the Sanner algorithm implemented in the MSMS program with a solvent probe radius of 0.14 nm and the PARSE atomic radius parameters. γ and b are empirical constants and are set to be 0.005 42 and 3.85 kJ/mol, respectively. The energy terms obtained with the MM-PBSA approach are then averaged over 100 time frames. The normal mode calculation to estimate the entropy contribution is somewhat problematic and time-consuming. One normal mode calculation is performed by the Nmode module in the AMBER 9.0 for each ligand and only residues at the nearest two N36 helices are included in the entropy calculations.

2 Results and Discussion

Studies are carried out to dock these small inhibitors to the hydrophobic pocket of gp41. In general, molecular dockings give quite different lowest energy orientations, especially for a molecule with many degrees-of-freedom (DOF). Cluster analysis is performed on the docking result and different binding modes with the best scores and the largest populations for each molecule are selected for further MD study and analysis. Three nanosecond molecular dynamics simulations are performed for all the binding modes of small ligands. For most of the binding modes, the complexes are well equilibrated after 400 ps MD simulations (see Fig. 2). Thus, 100 snapshots (every fourth snapshot of the 400 collected snapshots) after equilibration are taken for further analysis.

All the binding energies and entropies from the normal mode analysis are calculated and listed in Tab. 1. The binding mode that has the lowest binding free energy is supposed to be the most favorable binding mode (see Fig. 3). In Tab. 1, only those binding modes that have the lowest binding free energies from our calculation are listed. Our results show that most binding modes with the lowest free energies have large negative electrostatic interaction energies, implying that the electrostatic interaction plays a dominant role in stabilizing the ligand—gp41 complex. We notice that, however, ADS-J13 is possesses a relatively less attractive electrostatic interaction as shown in Tab. 1. Because of sha-

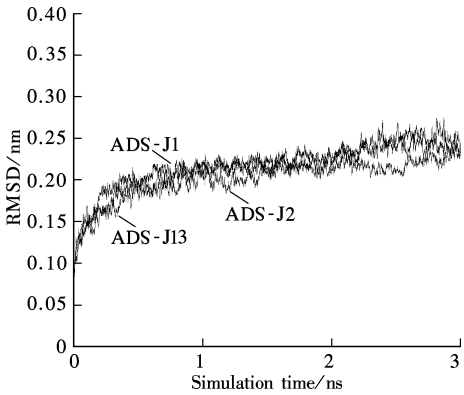

Fig. 2 Root-mean-square deviation of all compounds for 3 ns MD simulations

ring similar structures, all the compounds display close manners in solvent surface areas and van der Waals interactions. Clearly, the absences of negatively polarized groups in ADS-J13 result in dramatically less negative binding free energy, as claimed by the experimentalists; the compound is not capable of inhibiting the 6-HB formation. It is obvious that the electrostatic interaction energy term plays a crucial role in determining the binding affinities of the ligands. ADS-J1 and ADS-J2 are similar in interacting with positively charged groups on the HIV-1 gp41 NHR trimer core. As the purpose of designing these two compounds, the electrostatic interaction energy terms of all the models of these inhibitors are more negative than ADS-J13. Fig. 3 displays how ADS-J1 and ADS-J2 interact with the HIV-1 gp41 NHR trimer core, and the sulfonic acid group is pointing to the amino group of Lys574. They have many similar features in binding with the HIV-1 gp41 trimer core. They both occupy largely in the cavity on the NHR trimer surface, such as Leu568, Trp571, Ile573. The cavity closely contacts with both inhibitors, and the van der Waals interaction also plays an important role in the gp41-ADS-J1 complex, ΔE^{vdw} for gp41-ADS-J1 is −161.4 kJ/mol, which is much lower than that for gp41-ADS-J2(−139.3 kJ/mol). The inactive compound ADS-J13 has hydrophobic groups to interact with the hydrophobic residues (such as Leu567, Trp571, Ile573) in N-peptides, but gp41-ADS-J13's ΔE^{vdw} is the highest, which can ensure the binding affinities of ADS-J1 and ADS-J2.

Tab. 1 Binding free energies of different modes of inhibitor series and those metabolites of HIV-1 gp41 kJ/mol

Ligand	ΔE^{vdw}	ΔE^{ele}	ΔE^{gas}	ΔG^{PB}	ΔG^{SA}	ΔG^{MMPBSA}	$T\Delta S$	ΔG
ADS-JI	−161.4	−408.4	−569.8	441.5	−19.1	−147.4	−95.6	−52.7
ADS-J2	−139.3	−381.9	−521.2	424.5	−18.1	−114.8	−84.3	−30.5
ADS-J13	−126.1	−34.1	−160.1	91.8	−17.2	−85.6	−75.1	−10.5

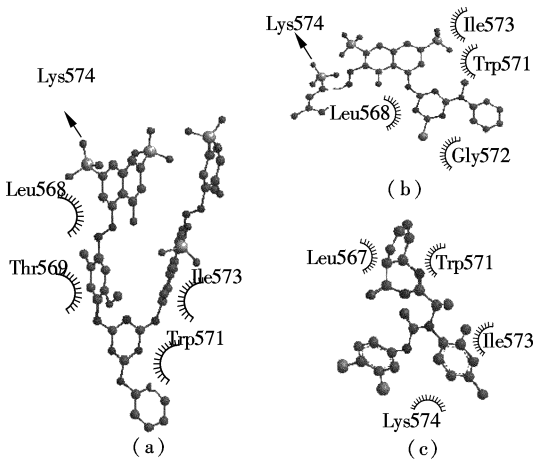

Fig. 3 The binding modes analysis of small molecules with gp41. (a) ADS-J1; (b) ADS-J2; (c) ADS-J13

ADS-J1 and ADS-J2 form a salt bridge with charged residue Lys574 at the N36 core structure. The gp41 core structure is a stable six-helix bundle formed by its N- and C-terminal heptad repeat sequences. Notably, the negatively charged residue Asp632 located at the pocket-binding motif in the C-terminal heptad repeat interacts with the positively charged residue Lys574 in the pocket formation region of the N-terminal heptad repeat to form a salt bridge.

3 Conclusion

The binding complexes of the compounds with the HIV-1 gp41 five-helical core structure are simulated from docking and MD calculations. Free energy analysis of the protein-ligand ensemble using the MM/PBSA model is used to identify the most possible binding modes. Our simulation shows that the conserved hydrophobic cavity located at the N-terminal of the gp41 core N36 trimer structure is the most possible binding site, which is considered to play a crucial role in the 6-HB formation and used as an important target for designing HIV-1 fusion inhibitors. The electrostatic interaction plays the key role in determining the affinities of the ligand binding. Besides the high hydrophobic properties of this large cavity, residue Lys574 carries positively charged sidechains, which are targeted by effective fusion inhibitors and directly determine the binding affinities of designed molecules. Moreover, most residues forming the cavity are highly conserved residues. Targeting this cavity and two charged residues may greatly reduce the drug-resistance caused by mutations. Our MD simulation and MM/PBSA calculation prove that ADS-J1 and ADS-J2 fit well into this hydrophobic cavity and are favorable to form strong electrostatic interactions with gp41 NHR trimer, and thus effectively interrupt the 6-HB formation and inhibit virus infections.

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抑制剂 ADS-J1 和 ADS-J2 与艾滋病病毒蛋白 gp41 的结合机理

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摘要:为分析和解释 2 个小分子抑制剂 ADS-J1 和 ADS-J2 与艾滋病病毒蛋白 gp41 的抑制机理,通过采用理论计算的方式把抑制剂和 gp41 蛋白疏水性空穴对接,得到了可能的结合方式,并通过采用分子动力学和 MM/PBSA 相结合的方法,确定蛋白质和抑制剂的具体结合方式和结合自由能,并且获得了抑制剂和蛋白的精确结合构型和相互作用力,明确了抑制剂和蛋白质之间的抑制机理. 理论研究证实了相关实验的推测,并且为以后设计新型的 gp41 小分子抑制剂提供了良好的基础.

关键词:艾滋病病毒进入细胞抑制剂;结合模式;gp41 蛋白;结合自由能
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