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REGULAR ARTICLE

Free Energy Profiles of Binding Processes of HIV-1 Protease-2AH/4AH by Potential of Mean Force Simulations

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Abstract: Inhibitors targeting HIV-1 protease are quite efficient in HIV/AIDS therapy. In this paper, we employed potential of mean force (PMF) simulations to obtain the free energy profiles of HIV-1 Protease-2AH and HIV-1 Protease-4AH binding processes, and some dynamic details of the binding processes are presented. The binding free energies of HIV-1 Protease-2AH and HIV-1 Protease-4AH are -31.2kcal/mol and -30.9kcal/mol, respectively. These two values are very close, qualitively consistent with experimental results.

AMS subject classifications: 92C40, 82C99, 97R30

Keywords: HIV/AIDS, HIV-1 protease, PMF simulations, binding free energy

Introduction

HIV/AIDS, has deprived the life or life quality of millions of people worldwide, since the beginning of the global pandemic of it in the early 1980s [1-4]. Thanks to the development of multiple therapeutic agents targeting HIV-1 reverse transcriptase (RT), protease (PR), integrase(IN), and so on, which is critical to some step of the HIV-1 life cycle, HIV-1 infection has been transformed from an inevitably fatal disease into a manageable chronic ailment [1].

HIV-1 protease is essential for HIV-1 replication because in the maturation step of HIV-1

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life cycle, HIV-1 protease must be employed to process Gag and Gag-Pol gene product into some series of structural proteins(p17, p24, p7, p6, p2, p1) and functional proteins(PR, RT, IN). Therefore, inhibitors targeting HIV-1 protease are quite efficient for therapy of HIV/AIDS.

HIV-1 protease is homodimer of two 99 amino acid subunits (**Figure1**). Asp25-Thr26-Gly27, catalytic triad from one monomer, together with Asp25'-Thr26'-Gly27', catalytic triad from another monomer, form the catalytic active site of the enzyme. The active site was covered by two flexible glycine-rich β flaps. When a substrate is binding the HIV-1 protease, the β flaps tranform from an open-state to a closed-state. If an inhibitor entered the active site, the HIV-1 protease will lose its activity now that the enzyme couldn't accept any substrate any more.

Up to now, 9 inhibitors for HIV-1 protease have been approved by the FDA, including saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, atazanavir, tipranavir and darunavir [1-5] . In 2006, Anders Hallberg reported a class of HIV-1 protease inhibitors which were structurally related to both atazanavir and indinavir. 2AH and 4AH (**Figure 2**) are two of them [5]. As shown in **Figure 2**, 2AH and 4AH are almost the same except that a nitrogen atom is at the ortho and meta position of pyridine, respectively [5-6].

Always there are many studies, experimental or computational, concerning the binding process of HIV-1 protease-inhibitors, however, the major works only focus on calculating the binding free energy, through experimental K_i or MMPB (GB)SA method, etc. By contrast, studies regard the dynamic details of the binding process is relatively rare. In this study, we employed PMF (potential of mean force) simulations to obtain the free energy profiles of HIV-1 Protease-2AH and HIV-1 Protease-4AH binding processes, hoping to discover some dynamic details of the binding processes.

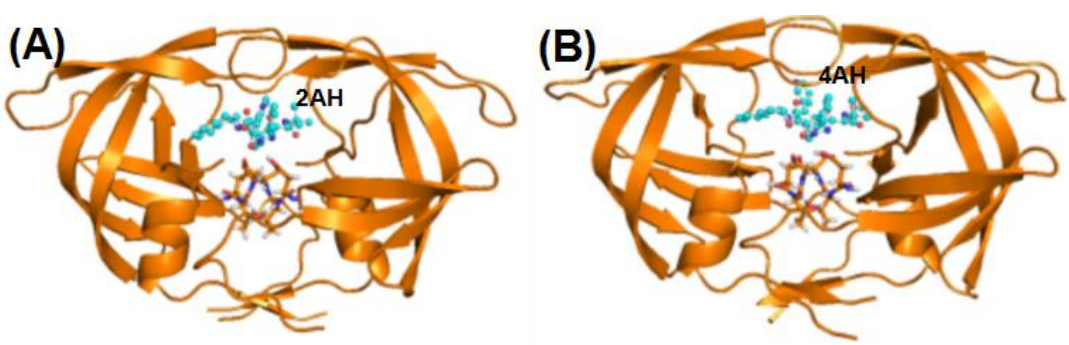


Figure 1: MD-simulated HIV-1 Protease-2AH/4AH binding structures. The enzyme is shown as golden ribbons, and 2AH/4AH is in ball-and-stick style. The two symmetric Asp25-Thr26-Gly27 catalytic triads of HIV-1 Protease are shown in stick style and colored by atom types. (**A**) HIV-1 Protease-2AH; (**B**) HIV-1 Protease-4AH.

Methods

MD simulation. The initial structures of HIV-1 Protease-2AH and HIV-1 Protease-4AH were obtained from RCSB PDB(http://www.rcsb.org/pdb， PDB ID:2cem，2cen) [5]. The two systems were dealt with the same MD simulations and PMF simulations procedure, respectively.

First, the partial charges of 2AH/4AH atoms were calculated by using the restrained electrostatic potential-fitting (RESP) protocol implemented in the Antechamber module of the Amber 12 program, following the electrostatic potential (ESP) calculation at *ab initio* HF/6-31G** level using Gaussian 03 program [7]. Considering the general accepted action mechanism of HIV-1 Protease, we chosen Asp25 of HIV-1 protease to be protonated [6]. And the bridging water W301 is reserved [6]. The complexes were solvated in an orthorhombic box of TIP3P water molecules [8] with a minimum solvent-wall distance of 10 Å. Counter ions were added to neutralize the solvated system.

Then, the two systems, with proteins, inhibitors and W301 restrained, were energy-minimized by using the Sander module of Amber 12 program [9] *via* a combined use of the steepest descent/conjugate gradient algorithms, with a convergence criterion of 0.0001 kcal mol^{-1} $Å^{-1}$, and the non-bonded cutoff distance was set to 8.0 Å. MD simulation was performed by using the Sander module of the Amber 12 program package. The solvated systems were gradually heated (with proteins, inhibitors and W301 restrained) to 300 K by the Langevin method in 500ps and equilibrated for 2 ns, from which step proteins, inhibitors and W301 were free. During the MD simulations, a 8.0 Å non-bonded interaction cutoff was used and the non-bonded list was updated every 25 steps. The motion for the mass center of the system was removed every 1,000 steps. The particle-mesh Ewald (PME) method [10, 11] was used to treat long-range electrostatic interactions. The lengths of covalent bonds involving hydrogen atoms were fixed with the SHAKE algorithm [12], enabling the use of a 2-fs time step to numerically integrate the equations of motion. Finally, the production MD was kept running for ~2.0 ns with a periodic boundary condition in the NTP ensemble at T = 300 K with Langevin method, and at P = 1 atm with isotropic molecule-based scaling [10].

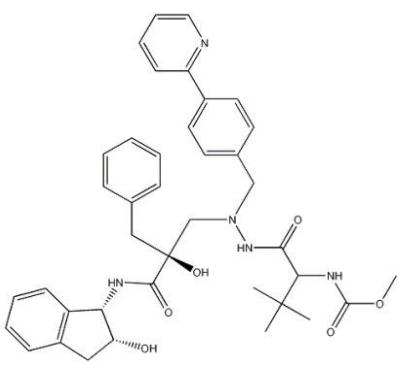
Potential of mean force (PMF) simulation. In order to explore the free energy profiles for the process of HIV-1 Protease binding with 2AH/4AH, the PMF simulation was carried out by using umbrella-sampling [13] MD simulation. The classic PMF definition [45] can be represented by a function of reaction coordinate as

$$\omega(\chi) = -RT \ln\langle \rho(\chi) \rangle - U(\chi) + F \quad (1)$$

in which $\rho(\chi)$ is the probability density along the reaction coordinate χ, R is the gas constant,

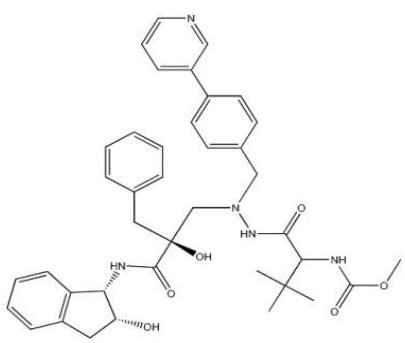
T is the simulation temperature, $U(\chi)$ is the biasing potential applied in the umbrella-sampling MD simulation, and F is the normalization constant. According to this approach, the reaction coordinate is usually divided into different regions, *i.e.*, windows, and each window is sampled separately. A biasing (umbrella) potential, *i.e.* $U(\chi)$, is applied for each window in order to obtain nearly uniform sampling of the potential energy surface. In the present study, the reaction coordinate was defined as the distance from the mass center of the non-hydrogen atoms of 2AH/4AH to the mass center of the non-hydrogen atoms on the side chains of the two symmetric Asp25-Thr26-Gly27 catalytic triads of HIV-1 Protease. The total number of windows was 28, with a window size of 0.5 Å, covering the reaction coordinate values from ~7.400 Å to ~21.400 Å. The biasing force constant applied in different windows of umbrella-sampling was 5.0 kcal/(mol•Å^2)[14]. For each umbrella-sampling window, the initial complex structure was selected from the last snapshot of the PMF simulations of the previous window. The selected structure for each window was first equilibrated for 200 ps and then kept running for 800 ps for production sampling. The frequency for data collection was set to 1 fs, which was the same as that of the time step of the umbrella-sampling MD. After the umbrella-sampling MD simulations for all windows were completed, the data collected from separate simulation windows were combined along the reaction coordinate. These data were then used to calculate the PMF for the whole binding process with the weighed histogram analysis method (WHAM) [15, 16] using the code developed by Alan Grossfield (http://membrane.urmc.rochester.edu/Software/WHAM/WHAM.html).

All of the MD and umbrella-sampling MD simulations were performed on a supercomputer (a DELL Cluster with 388 nodes or 4,816 processors) at the University of Kentucky's Computer Center.



methyl N-[(2S)-1-[2-[(2S)-2-hydroxy-3-[[(1S,2R)-2-hydroxy-2,3-dihydro-1H-inden-1-yl]amino]-3-oxo-2-(phenylmethyl)propyl]-2-[(4-pyridin-2-ylphenyl)methyl]hydrazinyl]-3,3-dimethyl-1-oxo-butan-2-yl]carbamate

Inhibitor 2AH



methyl N-[(2S)-1-[2-[(2S)-2-hydroxy-3-[[(1S,2R)-2-hydroxy-2,3-dihydro-1H-inden-1-yl]amino]-3-oxo-2-(phenylmethyl)propyl]-2-[(4-pyridin-3-ylphenyl)methyl]hydrazinyl]-3,3-dimethyl-1-oxo-butan-2-yl]carbamate

Inhibitor 4AH

Figure 2: Molecular structures of inhibitors 2AH/4AH.

Results and Discussion

Free energy profiles of the HIV-1 Protease-2AH/4AH binding processes. As shown in **Figure 3**, based on our PMF simulations, the binding free energies of the two processes are -31.2kcal/mol and -30.9kcal/mol, respectively. These two values are very close (the former is only 0.3kcal/mol higher than the latter), although the details of the free energy profiles of these two systems are distinguishing apparently. We can imagine the binding progress which is inverse to our PMF simulations. From the starting point of the binding progress, corresponding to the reaction coordinate of about 12.5Å, the free energy profile of HIV-1 Protease-2AH is steeper than that of HIV-1 Protease-4AH at first, but, after the point corresponding to the reaction coordinate of about 12.5Å, the free energy profile of HIV-1 Protease-2AH is smoother than that of HIV-1 Protease-4AH, until the end of the binding process.

As reported in previous literature, the experimental K_i for HIV-1 Protease-2AH and HIV-1 Protease-4AH is 12nM and 5nM [5], respectively. Considering the following formula:

$$\Delta G_{bind}(Expt) = RT \ln K_i \tag{2}$$

We can calculate the free energy for each system. That is, -10.9kcal/mol for HIV-1 Protease-2AH, and -11.4kcal/mol for HIV-1 Protease-4AH, respectively. Obviously, the experimental binding free energies for these two systems are very close, which is qualitively consistent with our PMF results. However, our computational values (-31.2kcal/mol and -30.9kcal/mol) are about three times of the experimental values (-10.9kcal/mol and 11.4kcal/mol). The difference of the computations and experiments could be induced by the limitations inherent in the MD simulations and PMF simulations.

In our earlier work, we have employed MMGBSA method, ignoring entropy, to determine the binding free energies for HIV-1 Protease-2AH and HIV-1 Protease-4AH. The binding free energy for HIV-1 Protease-2AH is -52.1kcal/mol, and that for HIV-1 Protease-4AH is -62.0kcal/mol [6]. These results are in reasonable agreement with Peter V. Coveney's results for the binding free energies of HIV-1 Protease-Atazanavir, -66.22kcal/mol, and HIV-1 Protease-Indinavir, -61.19kcal/mol [3]. Indeed, when synthesized, the inhibitors 2AH and 4AH were structurallly related to both ataanavir and indinavir, which may lead to the proximity of the corresponding binding free energies. We can see that the MMGBSA calculated binding free energies are 5-6 times of the experimental results, less accurate than PMF results.

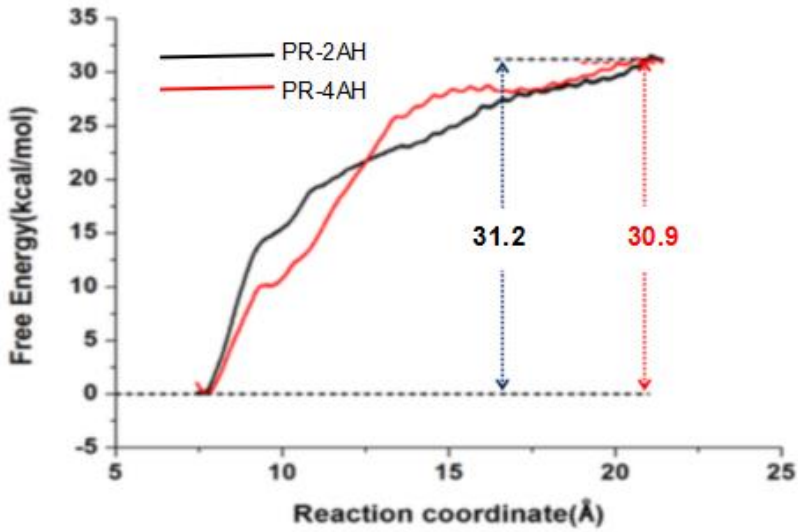


Figure 3: Simulated free energy profile of the HIV-1 Protease-2AH binding process in comparison with that of the HIV-1 Protease-4AH binding process. "PR" refers to HIV-1 Protease. The reaction coordinate was defined as the distance between the mass center of the non-hydrogen atoms of 2AH/4AH and the mass center of the non-hydrogen atoms of the two symmetric Asp25-Thr26-Gly27 catalytic triads of HIV-1 Protease.

Conclusion

Our results for binding free energies of HIV-1 Protease-2AH and HIV-1 Protease-4AH, computed by the PMF MD simulations, are 31.2kcal/mol and -30.9kcal/mol, respectively. The difference (~0.3kcal/mol) between 2AH and 4AH is negligible, which is qualitatively consistent with the very close experimental K_M values of the HIV-1 protease for the two substrates (K_M=12nM for 2AH and K_M=5nM for 4AH). Moreover, we obtained the free energy profiles of the binding processes of HIV-1 Protease-2AH and HIV-1 Protease-4AH. Which is a characteristic of PMF MD simulation method outshining experimental methods and some other computational methods such as MMPB(GB)SA method. Our insights could benefit the development of novel HIV-1 Protease inhibitors.

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processors.

References

[1] A. K. Ghosh, H. L. Osswald, G. Prato, Recent progreee in the development of HIV-1 protease inhibitors for the treatment of HIV/AIDS, J. Med. Chem., 59 (2016), 5172-5208.

[2] M. Kozisek, J. Bray, P. Rezacova, K. Saskova, J. Brynda, J. Pokorna, F. Mammano, L. Rulisek, J. Konvalinka, Molecular analysis of the HIV-1 resistance development: enzymatic activities, crystal structures, and thermodynamics of nelfinavir-resistant HIV protease mutants, J. Mol. Biol., 374(2007), 1005-1016.

[3] D. W. Wright, B. A. Hall, O. A. Kenway, S. Jha, P. V. Coveney, Computing clinically relevant binding free energies of HIV-1 protease inhibitors, J. Chem. Theory Comput., 10(2014), 1228-1241.

[4] A. Hosseini, A. Alibes, M. N. Julian, V. Gil, R. Paredes, R. Soliva, M. Orozco, V. Guallar, Computational prediction of HIV-1 resistance to protease inhibitors, J. Chem. Inf. Model., 56(2016), 915-923.

[5] J. K. Ekegren, N. Ginman, A. Johansson, H. Wallberg, M. Larhed, B. Samuelsson, T. Unge, A. Hallberg, Microwave-accelerated synthesis of P1'-extended HIV-1 protease inhibitors encompassing a tertiary alcohol in the transition-state mimicking scaffold, J. Med. Chem., 49(2006), 1828-1832.

[6] Y. X. Zhang, S. L. Zhang, Q. G. Zhang, Hybrid QM/MM study on interfacial water effect to the binding of HIV-1 protease and inhibitors, Shandong Sciences, 23(1)(2010), 1-5.

[7] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery Jr, T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople, Gaussian 03, rev A.1, Gaussian Inc., Pittsburgh, PA, (2003).

[8] W.L. Jorgensen, J. Chandrasekhar, J.D. Madura, R.W. Impey, M.L. Klein, COMPARISON OF SIMPLE POTENTIAL FUNCTIONS FOR SIMULATING LIQUID WATER, J Chem Phys, 79 (1983), 926-935.

[9] D.A. Case, T.A. Darden, T.E. Cheatham Iii, C.L. Simmerling, J. Wang, R.E. Duke, R. Luo, R.C. Walker, W. Zhang, K.M. Merz, B. Roberts, S. Hayik, A. Roitberg, G. Seabra, J. Swails, A.W. Goetz, I. Kolossváry, K.F. Wong, F. Paesani, J. Vanicek, R.M. Wolf, J. Liu, X. Wu, S.R. Brozell, T. Steinbrecher, H. Gohlke, Q. Cai, X. Ye, J. Wang, M.J. Hsieh, G. Cui, D.R. Roe, D.H. Mathews, M.G. Seetin, R.

Salomon-Ferrer, C. Sagui, V. Babin, T. Luchko, S. Gusarov, A. Kovalenko, P.A. Kollman, AMBER 12, University of California, San Francisco, (2012).

[10] T. Darden, D. York, L. Pedersen, Particle mesh Ewald—an N_log(N) method for Ewald sums in large systems, J. Chem. Phys., 98 (1993), 10089-10092.

[11] A. Toukmaji, C. Sagui, J. Board, T. Darden, Efficient particle-mesh Ewald based approach to fixed and induced dipolar interactions, J. Chem. Phys., 113 (2000), 10913-10927.

[12] J.P. Ryckaert, G. Ciccotti, H.J.C. Berendsen, NUMERICAL-INTEGRATION OF CARTESIAN EQUATIONS OF MOTION OF A SYSTEM WITH CONSTRAINTS - MOLECULAR-DYNAMICS OF N-ALKANES, J. Comput. Phys., 23 (1977), 327-341.

[13] G.M. Torrie, J.P. Valleau, Nonphysical sampling distribution in Monte Carlo free-energy estimation: umbrella sampling, J. Comput. Phys., 23 (1977), 187-199.

[14] Y. X. Zhang, X. Q. Huang, K. L. Han, F. Zheng, C.-G. Zhan, Free energy profiles of cocaine esterase-cocaine binding process by molecular dynamics and potential of mean force simulations, Chemico-Biological Interactions, 259(2016), 142-147

[15] S. Kumar, D. Bouzida, R.H. Swendsen, P.A. Kollman, J.M. Rosenberg, THE WEIGHTED HISTOGRAM ANALYSIS METHOD FOR FREE-ENERGY CALCULATIONS ON BIOMOLECULES .1. THE METHOD, Journal of Computational Chemistry, 13 (1992), 1011-1021.

[16] B. Roux, The calculation of the potential of mean force using computer simulations, Comput. Phys. Commu., 91 (1995), 275-282.