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Molecular insight into the interaction mechanisms of inhibitors BEC and BEG with HIV-1 protease by using MM-PBSA method and molecular dynamics simulation

Shu-Hua Shi, Jian-Zhong Chen, Guo-Dong Hu, Chang-Hong Yi, Shao-Long Zhang, Qing-Gang Zhang *

College of Physics and Electronics, Shandong Normal University, Jinan 250014, China

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ABSTRACT

HIV-1 protease has been an attractive drug target for the antiretroviral treatment of HIV infection over the years. Molecular dynamics (MD) simulations coupled with Molecular Mechanics Poisson–Boltzmann Surface Area (MM-PB/SA) method have been carried out to investigate the bindings of inhibitors BEC and BEG to HIV-1 protease. The results suggest that van der Waals energies mostly drive the binding of this class of inhibitors to HIV-1 protease. The analyses of structure–affinity relationship by using the free energy decomposition provide a more-detailed insight into the mechanisms driving the bindings of BEC and BEG to HIV-1 protease. It is found that a number of C–H…π and C–H…H–C interactions exist between the hydrophobic groups of BEC and BEG and the hydrophobic residues of the binding pocket in HIV-1 protease, and these interactions and the hydrogen bond interactions of BEC and BEG with HIV-1 protease play important roles in the bindings of BEC and BEG to HIV-1 protease. The improvement and optimization of these interactions are helpful to the rational design of potent inhibitors combating AIDS.

1. Introduction

Human immunodeficiency virus protease (PR) has been an important target for the development of antiviral therapeutics [1]. The PR, which has been shown to be essential in the production of mature and infectious virions, processes the *gag*- and *gagpol*-encoded polyproteins into structural and functional enzymes [2,3], hence inhibition of this enzyme has become an attractive target for effective antiviral agents. Currently, nine HIV-1 protease inhibitors (PIs) have been approved by the FDA [4]. Despite the initial success of these PIs, there is an urgent need for improved drugs that can combat HIV protease because of increasing viral resistance and unfavorable side effect. HIV patients in the world are looking forward to the new potent PIs, which must be provided with lower susceptibility to viral resistance and fewer side effects than the existing drugs [5].

Understanding the interaction mechanisms of PIs with the PR at an atomic level may provide useful information that can lead to the development of PIs with better potency. Just for this reason, two PIs BEC and BEG were selected to investigate the PR–PI interactions, and the structures of these two PIs are plotted in Fig 1. BEC was synthesized with two central hydroxy groups, while BEG with only one [6]. In fact, although H.O. Andersson et al. have applied X-ray crystallography method to investigate the binding of this class of inhibitors to the PR, our analyses of binding free energies also provide details on the structure–affinity relationship of these two PI–PR complexes.

In this work, molecular dynamics (MD) simulations coupled with molecular mechanics Poisson–Boltzmann surface area (MM-PBSA) [7] method, which has widely been applied to the computation of protein–ligand binding free energies currently [8–15], are carried out to explore the interactions of two PIs BEC and BEG with the PR. Detailed binding free energies between two inhibitors and individual PR residues are calculated by using a per-residue basis decomposition method [16]. The information of detailed interaction energies combined with the analyses of hydrogen bonds through the MD simulations provides insights into the PI-PR binding mechanisms and also helps elucidate the structure–affinity relationship with the interpretation of the structural and energetic results from the simulation.

2. Theory and method

2.1. Constructions of the initial models of PR–PI complexes

The crystal structures of the PR complexed with two inhibitors were obtained from the protein Data Bank (PDB). The PDB entries

* Corresponding author. Tel.: +86 531 86180892; fax: +86 531 86182521.
E-mail address: zhangqg@sdnu.edu.cn (Q.-G. Zhang).

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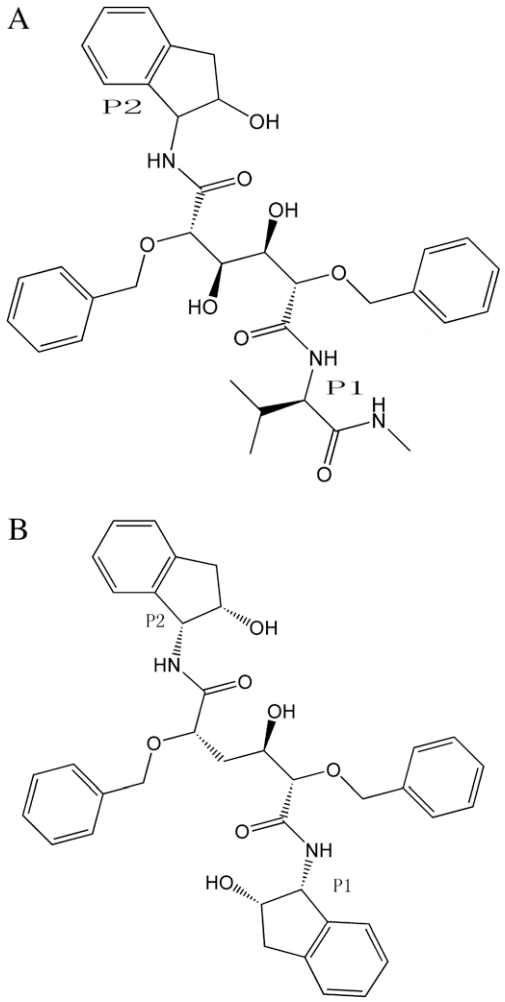

Fig. 1. Molecular diagram of two inhibitors. (A) BEC, (B) BEG.

are: 1EBZ for BEC and 1D4I for BEG [6]. These crystal structures were used as starting models for two MD simulations. All missing hydrogen atoms were added by using the leap module in the AMBER 9 software package [17]. Due to the importance of the protonation of Asp25/Asp25′ in the PR, the OD2 of Asp25 was protonated [18]. All crystal water molecules were kept in the starting module.

The starting structures and force field parameters for these two inhibitors were obtained as follows. Geometric optimization was performed with Gaussian 98 [19] at the Hartree–Fock level with 6-31∗ basis functions to adjust the bond length involving hydrogen atoms, which were added to the starting module by taking into account the hybridization of the covalent bonds. Single-point calculations with Gaussian 98 were subsequently carried out to obtain the electrostatic potentials around each complex by using the same basis set and level of theory as those used in the geometric optimization step. Partial atomic charges of inhibitors were derived by using RESP fitting procedure in the AMBER. The force field ff03 was used to produce the force field parameters of the protein and the crystal water molecules. The general AMBER force field (GAFF) [20] was adopted to obtain the force field parameters of BEC and BEG, including the Lennard–Jones, torsion, bond angle terms.

The next step was to soak the system in a truncated octahedral periodic box of TIP3P water molecules. The distance between the edges of the water box and the closest atom of the solutes was at least 10 Å. To neutralize the charge of systems, an appropriate number of chloride counterions were placed to grids with the largest positive Coulombic potentials around the complexes.

2.2. Molecular dynamics simulations

Energy minimization and MD simulation were carried out by using the sander module of the AMBER 9 with the Cornel force field. The entire system was subject to energy minimization in two stages to remove bad contacts between the complex and the solvent molecules. Firstly, the water molecules were minimized by holding the solute fixed with a harmonic constraint of a strength of 100 kcal/(mol Å^2). Secondly, the entire system was minimized without restriction, and each stage was performed by using the steepest descent minimization of 500 steps followed by a conjugate gradient minimization of 2500 steps. The system was then heated from 0 to 300 K in 200 ps and equilibrated at 300 K for another 200 ps. After the minimization and heating, 3-ns dynamics simulations without restriction were performed at a constant temperature of 300 K and a constant pressure of 1 atm. Finally, root-mean-square deviation (RMSD) of the protease C_α atom was computed from the MD trajectory relative to the initial structures to judge the stabilization of the system. During the minimization and MD simulations, particle mesh Ewald (PMD) method [21] was employed to treat the long-range electrostatic interactions in a periodic boundary condition. The Langevin dynamics with a collision frequency of 1.0 ps^{-1} was applied to control the temperature of the system. The SHAKE method was used to constrain hydrogen atoms. The time step for all MD simulations is 2 fs, with a direct-space, non-bonded cutoff of 10 Å. Initial velocities were assigned from a Maxwellian distribution at the initial temperature.

2.3. The MM-PBSA method

Although free energy perturbation (FEP) and thermodynamic integration (TI) calculations may give accurate binding free energies, they are extremely time-consuming and require sufficient statistical sampling, which prevents FEP and TI from being applied to free energy calculations in structure-based drug design [22–23]. In this study, the binding free energies were calculated by using MM-PBSA [24] and normal mode methods. 100 snapshots were extracted from the last 800-ps MD simulation, and the binding free energies were calculated between two inhibitors and the PR. In general, the binding free energies in condensed phase can be calculated according to the following equations.

$$\Delta G_{\text{bind}} = G_{\text{complex}} - [G_{\text{protein}} + G_{\text{inhibitor}}] \quad (1)$$

$$G = E_{\text{gas}} + G_{\text{solve}} - \text{TS} \quad (2)$$

$$E_{\text{gas}} = E_{\text{bond}} + E_{\text{angle}} + E_{\text{torsion}} + E_{\text{vdW}} + E_{\text{ele}} \quad (3)$$

$$G_{\text{solve}} = G_{\text{PB}} + G_{\text{SASA}} \quad (4)$$

where G_{complex}, G_{protein} and $G_{\text{inhibitor}}$ are the free energies of the complex, the protein and the inhibitor, respectively. E_{gas} is standard force field energy that consists of strain energies (E_{bond}, E_{angle} and E_{torsion}). The solvation free energy (G_{solve}) is further divided into a polar component (G_{PB}) and a nonpolar one (G_{SASA}). The polar component was calculated by using the PBSA program in AMBER 9.0, and the dielectric constant was set to 1 inside solute and 80 in solvent in this work. The nonpolar component was determined by $\Delta G_{\text{nonpol}} = \gamma\text{SASA} + \beta$, in which SASA is the solvent-accessible surface area and was calculated with the MSMS program [25]. In our calculations, the values for γ and β were set to 0.00542 kcal/(mol Å^2) and 0.92 kcal/mol, respectively. The contribution of entropy (TS) to binding free energies, which arises from changes of the translational, rotational and vibrational degrees of freedom, are generally calculated by using classical statistical thermodynamics [26] and normal-mode analysis. Since the normal mode calculation of entropy is extremely time-consuming for large systems, only 25 snapshots for each complex were used to estimate the contribution