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Molecular dynamics simulation and free energy calculation studies of kinase inhibitors binding to active and inactive conformations of VEGFR-2

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ABSTRACT

Vascular endothelial growth factors receptor-2 (VEGFR-2) inhibitors have been proved as very effective anticancer agents. Structurally similar ligands **1** and **2** show almost the same inhibitory activities against VEGFR-2, but they bind to the enzyme in distinct binding mode. Ligand **1** targets DFG-in active conformation of VEGFR-2, known as Type I inhibitor. On the other hand, ligand **2** targets DFG-out inactive conformation of VEGFR-2, known as Type II inhibitor or allosteric kinase inhibitor. Ligand **2** shows high inhibitory activity, while the compound **3**, a close analog of **2** with the cyclopropylamide replaced by *tert*-butylamide, exhibits drastically diminished potency. In this work, molecular dynamics simulations and free energy calculations were performed on inhibitors **1**–**3** binding to active and inactive conformation of VEGFR-2. Molecular dynamics simulations find that the active conformation binding to Type I inhibitor **1** appears more flexible when compared to the unbound form. In contrast, binding of Type II inhibitor **2** to the inactive conformation helps to stabilize the inactive conformation of the protein. Binding free energy calculations verify that inhibitors **1** and **2** have almost the same activities against VEGFR-2, and that ligand **1** binds to and stabilizes the DFG-in conformation of VEGFR-2, which is in agree with the experimental observation. Molecular dynamics simulations and binding free energy calculations of **3** binding to VEGFR-2 can give a good explanation of the drastically diminished potency. Free energy analysis revealed that van der Waals interactions provided the substantial driving force for the binding process. The important hydrophobic property of the terminal 4-Cl phenyl was required to be Type II inhibitors. Furthermore, per-residue free energy decomposition analysis revealed that the most favorable contribution came from Leu840, Val848, Ala866, Lys868, Leu889, Val899, Thr916, Phe918, Cys919, Leu1035, Cys1045, Asp1046, and Phe1047. These results are expected to be useful for future rational design of novel potent VEGFR-2 inhibitors.

1. Introduction

Vascular endothelial growth factors (VEGFs) play a very important role in the regulation of angiogenesis, the process that lead to the formation of new blood vessels [1,2]. Abnormal regulation of angiogenesis has been shown to get involved in many diseases, such as diabetic retinopathy, psoriasis, rheumatoid arthritis, and cancer. Particularly, it is widely recognized that the growth and metastasis of solid tumors is dependent on angiogenesis [3,4]. The VEGFs exert its effects through receptor tyrosine kinase (VEGFR-1, -2, and -3). Among them, VEGF receptor-2 (VEGFR-2) plays crucial roles in vessel sprouting and new vessel initiation in early stage of angiogenesis.

It is well established that inhibiting of VEGFR-2 leads to suppression of angiogenesis and tumor growth. A number of preclinical and clinical studies have shown that many small-molecule VEGFR-2 inhibitors are capable of inhibiting angiogenesis, tumor progression, and dissemination [5–9]. Most kinase inhibitors discovered to date are ATP-competitive and classified as Type I inhibitors. Such inhibitors target the ATP binding pocket in its active conformation of the activation loop. This conformation is normally referred to as DFG-in based on the position of the conserved triad aspartate-phenylalanine-glycine (DFG) at the entrance of the activation loop.

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Type I inhibitors typically function in the DFG-in conformation of VEGFR-2 through hydrogen bonding with the hinge region as well as hydrophobic interactions in and around the adenine region. On the other hand, Type II inhibitors bind to and stabilize an inactive kinase form that features the DFG motif in a DFG-out conformation. The different position of the DFG residues in the DFG-out form creates a new hydrophobic binding pocket that is adjacent to the ATP-binding site. This pocket, also known as the allosteric site, is characteristic of kinase in an inactive conformation. Type II inhibitors predominantly occupy the ATP binding site, but they also exploit unique hydrogen bonding and hydrophobic interactions with the allosteric site. Compared with Type I kinase inhibitors, Type II inhibitors have several advantages, including great cellular potency and improved kinase selectivity [10–15]. Therefore, DFG-out conformation has gained great interest in discovery and development of Type II kinase inhibitors for the past few years. However, problems pursuing this type of inhibitor also exist. Mutations at or around allosteric pocket are more likely to occur that at evolutionary conserved regions, potentially leading problems associated with drug-resistance.

Recently, Amgen Inc. reported a dimethoxyyquinoline template targeting the hinge region of VEGFR-2 [16,17]. The X-ray co-crystal structure of VEGFR-2 in complex with ligands **1** and **2** were reported. Ligands **1** and **2** (Fig. 1) with the same scaffold and similar substituents bind to the enzyme in distinct DFG-in and DFG-out mode, respectively. Accordingly, ligands **1** and **2** were classified as Type I and Type II inhibitors, respectively. Despite the difference in the binding mode, ligands **1** and **2** show almost the same inhibitory activities against VEGFR-2. Ligand **2** is a potent inhibitor, while the compound **3**, a close analog of **2** with the cyclopropylamide replaced by *tert*-butylamide, exhibits drastically diminished potency. To improve our understanding of the structural determinants for Types I and II kinase inhibitors binding to and stabilizing the DFG-in and DFG-out conformation, a combined computational approach by molecular dynamics (MD) simulations, molecular mechanics Poisson–Boltzmann surface area (MM-PBSA) and molecular mechanics Generalized-Born surface area (MM-GBSA) free energy calculations and MM-GBSA free energy decomposition analysis was performed in the present study. We investigated Type I inhibitor **1** binding to active conformations and Type II inhibitor **2** binding to inactive conformations. For comparison, we also investigated **1** binding to the inactive conformation, compound **3** binding to active and inactive conformations, and the unbound forms of both protein conformations. We expect that this study will be helpful for the rational design of VEGFR-2 kinase inhibitors.

2. Materials and methods

2.1. Initial structure preparation

The atomic coordinates of the crystal structure for ligands **1** and **2** in complex with VEGFR-2 in active and inactive conformations were retrieved from the protein data bank (PDB entries: 3B8R, and 3B8Q, respectively) [16,17]. The structures are in a dimer former, only one chain is kept. The complex protein structure models were created by removing all ions, and all water molecules. The regions encompassing residues 842–846, 1050–1066 in 3B8R, and 1048–1066 in 3B8Q, not solved in the crystal structures, were modeled using the loop building routine in Modeler [18]. The reliability of the generated model was evaluated by QMEAN4 scoring function (Fig. S1 in supporting information) [19]. The 3B8R and 3B8Q based models are henceforth referred to as **1**-VEGFR-2(active) and **2**-VEGFR-2(inactive), respectively. A model of **1** binding to the inactive conformation of VEGFR-2 was generated for the purpose of comparison. This model was created by replacing ligand **2** with **1** in the inactive 3B8Q based VEGFR-2 model. Superposition of the ligands was performed based on the 3B8R-3B8Q Cα-atom alignment. This structure is termed as **1**-VEGFR-2(inactive). No model of **2** bound to the active VEGFR-2 conformation was generated as the absence of the back-pocket prevents the inhibitor from binding. The models of **3**-VEGFR-2(active) and **3**-VEGFR-2(inactive) were prepared by modifying the structure of compound **1** using the sketch module of Sybyl 7.3 [20]. Unbound structures of the active and inactive VEGFR-2 conformations were created by removing the ligands from the **1**-VEGFR-2(active) and **2**-VEGFR-2(inactive) models, respectively. These models are termed as VEGFR-2(active, unbound) and VEGFR-2(inactive, unbound) models, respectively. The treated structures were used as the initial structures of MD simulations.

Geometry optimization and the electrostatic potential calculations for compounds **1**–**3** were performed at the HF/6-31G* level of the Gaussian 03 suite [21]. The atomic partial charge of the three ligands was obtained by using RESP fitting method [22] implemented in AmberTools [23]. The force field parameters for the ligands were generated by the general amber force field (GAFF) [24] using the Antechamber program. The AMBER 99SB force field [25] was used to simulate all protein structures and the ionization state of amino acid residues was set according to the standard protocol. All models were neutralized by adding suitable counterions and were solvated in a truncated octahedron box of TIP3P [26] water molecules with a margin distance of 12 Å.

2.2. MD simulation

The MD simulations were performed using the PMEMD module of AMBER12 software package [27,28]. Initially, the solvated systems used as the starting structure for subsequent MD simulations were minimized by three steps. First, a harmonic constraint potential of 2.0 kcal mol^{-1} $Å^{-2}$ was applied to all the protein atoms. Second, the protein backbone atoms were restrained with a force of 2.0 kcal $mol^{-1}Å^{-2}$. Third, all atoms were allowed to move freely. In each step, energy minimization was executed by the steepest descent method for the first 4000 steps and the conjugated gradient method for the subsequent 1000 steps. After minimization, the MD simulations performed in this study followed a standard protocol consisting of gradual heating, density equilibration, equilibration, and production procedures. Firstly, the systems were gradually heated in the NVT ensemble from 0 to 300 K in 50 ps using a Langevin thermostat with a coupling coefficient of 1.0 ps with a force constant 2.0 kcal mol^{-1} $Å^{-2}$ on the complex. And then 50 ps of density equilibration with a force constant 2.0 kcal mol^{-1} $Å^{-2}$ on the complex were performed. Subsequently the systems were again equilibrated for 1 ns by releasing all the restrains. Finally, the actual MD production run of 80 ns in the NPT ensemble for each system was performed at 300 K with 1.0 atm pressure. During the MD simulations, the long-range Coulombic interactions were handled using the particle mesh Ewald (PME) method [29]. The cutoff distance for the long-range van der Waals (vdW) energy term was set at 10.0 Å. Periodic boundary conditions were applied to avoid edge effects in all calculations. The SHAKE algorithm [30] was employed on all atoms covalently bond to hydrogen atoms, allowing for an integration time step of 2 fs. Coordinate trajectories were recorded every 10 ps throughout all equilibration and production runs, and 1000 snapshots of the simulated structures within the last 10 ns stable MD trajectory at 10 ps intervals were extracted to perform the following binding free energy calculations.

2.3. Binding free energy calculations

The binding free energies (ΔG_{bind}) of the inhibitors with VEGFR-2 were calculated by using MM-PB/GBSA [31] procedure