Journal of Molecular Graphics and Modelling 79 (2018) 59–64

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

journal homepage: www.elsevier.com/locate/JMGM

Molecular insight into mutation-induced conformational change in metastasic bowel cancer BRAF kinase domain and its implications for selective inhibitor design

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ARTICLE INFO

Article history:
Received 10 September 2017
Received in revised form 31 October 2017
Accepted 6 November 2017
Available online 9 November 2017

Keywords:
BRAF kinase
V600E mutation
Conformational change
Metastasic bowel cancer
Molecular modeling

ABSTRACT

Oncogenic BRAF V600E mutation confers constitutive activation for the kinase and is closely related to the pathogenesis of metastasic bowel cancer (MBC). Here, the V600E-induced conformational change in MBC BRAF kinase domain is characterized systematically at structural, energetic and dynamic levels. The mutation is observed to cause a conformational conversion of the kinase's activation loop from DFG-out to DFG-in, thus activating the kinase. Electrostatic force is primarily responsible for the conformational conversion and stabilization of DFG-in associated with the mutation. Molecular docking calculations are employed to analyze the binding mode difference of mutant-selective inhibitors between the DFG-out and DFG-in conformations of BRAF kinase. It is revealed that the mutation can reshape inhibitor selectivity profile by altering kinase loop conformation. Several compounds are determined to have a high or moderate selectivity for mutant over wild-type kinase. The selectivity is primarily originated from hydrogen bond interactions of inhibitor ligands with mutant rather than wild type due to the conformational difference in kinase domain.

1. Introduction

The serine/threonine-specific kinase BRAF is an oncogenic protein belonging to the Raf kinase family of growth signal transduction protein kinases. This protein plays a key role in regulating the MAP kinase/ERKs signaling pathway, which affects cell division, differentiation and secretion [1]. BRAF is 766 amino acids long and composed of three conserved domains characteristic of the Raf kinase family: a Ras-GTP-binding self-regulatory domain, a serine-rich hinge region, and a catalytic protein kinase domain that phosphorylates a consensus sequence on protein substrates [2]. In active conformation, BRAF forms dimers *via* hydrogen-bonding and electrostatic interactions of its kinase domains [3].

BRAF somatic missense mutations have been observed in a wide range of human cancers. All mutations are within the kinase domain, with a single substitution (V600E) accounting for 80%. Mutated BRAF proteins have elevated kinase activity and are transforming in NIH3T3 cells (Davies et al., 2002). The V600E mutation is caused by a single nucleotide substitution of glutamic acid for valine (nucleotide 1799 T > A; codon GTG > GAG) (Ascierto et al., 2012). The mutation results in substitution of hydrophobic Val600 residue with negatively charged amino acid Glu at activation loop, which confers constitutive activity to the kinase by >5-fold more activity than its wild-type counterpart and is highly associated with familial metastasic bowel cancer (MBC) [4]. Therefore, the $BRAF^{V600E}$ kinase mutant has been recognized as an attractive druggable target for the molecular therapy of MBC [5]. Previously, a variety of mutant-selective inhibitors have been discovered to treat MBC [6]. However, since the position of residue 600 locates at BRAF activation loop where is out of the kinase's activity site. Therefore, the mutation may not determine inhibitor affinity directly; instead, it can only influence inhibitor binding indirectly *via* regulation of activation loop conformation [7].

It is supposed that the mutation causes a conformational change in the kinase domain, which results in two effects that promote the kinase activation: (i) the mutation induces conformational flipping of the activation loop from inactive DFG-out state to active DFG-in form, directly conferring constitutive activation for the kinase, and (ii) the mutation reshapes the location and configura-

Abbreviations: DFG-in, Asp-Phe-Gly-in; DFG-out, Asp-Phe-Gly-out; GAFF, general AMBER force field; HF, Hartree-Fock; MD, molecular dynamics; MM/GBSA, molecular mechanics/generalized Born surface area; PDB, protein data bank; PME, particle mesh Ewald; MBC, metastasic bowel cancer.

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https://doi.org/10.1016/j.jmgm.2017.11.005
1093-3263/

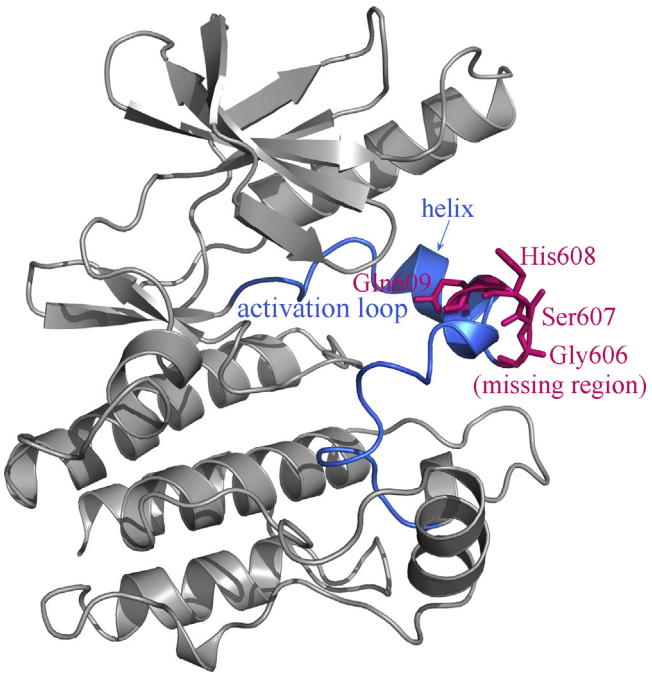

Fig. 1. The chain A of PDB crystal structure 4WO5, where only 4 residues 606–609 (Gly606-Ser607-His608-Gln609, red region) are missing in the crystal structure, which were modeled using the GalaxyLoop server (Ko et al., 2012).

tion of residues in dimerization interface, which could stabilize the kinase dimer and is indirectly responsible for the pathogenic function of MBC-associated BRAF mutants with high intrinsic kinase activity [8]. Previously, the allosteric regulation of residue mutation and phosphorylation on protein kinases has been investigated in detail [9–12]. However, the dynamics behavior and biological implication underlying kinase activation still remain largely unexplored. Recently, Yao et al. characterized the molecular mechanism of Vemurafenib, a $BRAF^{V600E}$ mutant-selective inhibitor approved for treating melanoma and candidating for treating MBC, and identified several structurally similar compounds as potential mutant-selective inhibitors by chemical similarity search against a large library of drug/lead-like compounds [13]. Here, long-term molecular dynamics (MD) simulations were performed to characterize V600E-induced conformational change in the activation loop of BRAF kinase domain. We also carried out molecular docking calculations and binding free energy analyses to investigate the structural basis and energetic property of selective inhibitors to both the wild-type and mutant kinases.

2. Materials and methods

2.1. Crystal structure of BRAF kinase domain

The activation loop covers amino acid residues 593–622 of BRAF kinase domain, which is highly flexible and intrinsically disordering. We have surveyed the PDB database [14] and found that all solved crystal structures of BRAF kinase domain are incomplete in this loop; most crystal structures miss 10 or more loop residues. The crystal structure of wide-type BRAF kinase solved by Thevakumaran et al. [15] is an asymmetric unit containing two chains A and B; each represents a kinase domain. The chain B misses 15 residues (560–614) in the structure, while the chain A only misses four residues (606–609, Gly606-Ser607-His608-Gln609) in this region, which can be readily molded using bioinformatics approach [16] (Fig. 1). The residue Val600 was computationally mutated to Glu600 with structure-based side-chain substitution [17].

2.2. Mutant-selective inhibitors in complex with BRAF kinase domain

The crystal structure is a cocrystallized complex of BRAF kinase domain with small-molecule inhibitor PLX4720, a 7-azaindole derivative that inhibits the kinase mutant with IC_{50} value of 13 nM [18]. In addition, there are a series of PLX4720 analogs that have been found to selectively inhibit the kinase mutant, including PLX4032 [19], PLX7904 [20] and PLX8394 [21]; these compound are structurally similar to each other (Fig. 2), and thus we can readily mutate the PLX4720 in cocrystallized structure to obtain the complex structure models of BRAF kinase domain with PLX4032, PLX7904 and PLX8394.

2.3. Molecular dynamics simulation

Molecular dynamics (MD) simulations were carried out at neutral pH using AMBER 14 package [22]. The Lys and Arg residues are positively charged, and the Asp and Glu residues are negatively charged. The ionizable/protonatable residues were assigned according to the calculated pK_a values. Each of the simulated systems was placed in the centre of a cubic simulation box filled with water molecules. The TIP3P solvent model [23] was used for the explicit water molecules and the buffering distance was set to 10 Å. Counter ions were added to maintain electroneutrality of the simulated system. The partial charges and the force-field parameters for the ligands were automatically generated using Antechamber program. The AMBER ff03 force field [24] and general AMBER force field (GAFF) [25] were applied for kinase protein and inhibitor ligands, respectively. Firstly only the water molecules and ions were minimized for 500 steps while keeping the protein structure fixed. Secondly, a 4500 step minimization with the conjugate gradient method to convergence criterion of 0.5 kcal mol^{-1} $Å^{-1}$ was performed on the whole system without any constraint [26]. The simulated systems were then gradually heated from 0 to 300 K, equilibrated for 500 ps, and finally production runs were performed [27,28]. A 2 fs integration time step was used. The SHAKE algorithm was used for all bonds involving hydrogen atoms [29], and the particle-mesh Ewald (PME) method [30] was employed to treat the long-range electrostatic interactions. The binding free energy between kinase and inhibitor was calculated using the semiquantitative MM/GBSA method [31]. The total free energy change ΔG_{ttl} upon the binding consists of intermolecular interaction energy ΔE_{MM} and desolvation penalty ΔG_{GBSA} [32]; the former was calculated through fore field approach, while the latter was described using implicit generalized Born solvent model [33,34].

2.4. Molecular docking calculation

The chemical structures of inhibitor compounds PLX4720, PLX4032, PLX7904 and PLX8394 were minimized with MMFF94 force field [35]. Polar hydrogen atoms and Kollman charges were added to the system by using the AutoDock Tools [36]; the tool was also utilized to prepare the input files for investigated compounds and to set the size and center of a grid box covering the kinase's active site, which can be defined by the cocrystallized ligand PLX4720. Next, molecular docking calculations were implemented in AutoDock Vina software [37]; the docking employed Lamarckian genetic algorithm to generate thousands of potential binding modes for an inhibitor ligand, which were then clustered into few representatives.