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Detailed conformation dynamics and activation process of wild type c-Abl and T315I mutant

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HIGHLIGHTS

- The effect of T315I mutation on properties of wild-type c-Abl was determined.
- The TMD simulations of activation process of Abl were carried out.
- The non ATP pockets for ligand binding were predicted.

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ABSTRACT

Bcr-Abl is an important target for therapy against chronic myelogenous leukemia (CML) and acute lymphocytic leukemia (ALL). The synergistic effect between myristyl pocket and the ATP pocket has been found. But its detailed information based on molecular level still has not been achieved. In this study, conventional molecular dynamics (CMD) and target molecular dynamics (TMD) simulations were performed to explore the effect of T315I mutation on dynamics and activation process of Abl containing the N-terminal cap (Ncap). The CMD simulation results reveal the increasing flexibility of ATP pocket in kinase domain (KD) after T315I mutation which confirms the disability of ATP-pocket inhibitors to the Abl-T315I mutant. On the contrary, the T315I mutation decreased the flexibility of remote helix αI which suggests the synergistic effect between them. The mobility of farther regions containing Ncap, SH3, SH2 and SH2-KD linker were not affected by T315I mutation. The TMD simulation results show that the activation process of wild type Abl and Abl-T315I mutant experienced global conformation change. Their differences were elucidated by the activation motion of subsegments including A-loop, P-loop and Ncap. Besides, the T315I mutation caused decreasing energy barrier and increasing intermediate number in activation process, which results easier activation process. The TMD and CMD results indicate that a drug targeting only the ATP pocket is not enough to inhibit the Abl-T315I mutant. An effective way to inhibit the abnormal activity of Abl-T315I mutant is to combine the ATP-pocket inhibitors with inhibitors binding at non-ATP pockets mainly related to Ncap, SH2-KD linker and myristyl pocket.

Introduction

Abelson tyrosine kinase (c-Abl) is a non-receptor tyrosine kinase, which is involved in various cell activities such as cell proliferation and differentiation, apoptosis, DNA damage repair and infection immunity [1]. Its fusion protein, Bcr (breakpoint cluster region)-Abl, results in a deregulated and constitutively active tyrosine kinase, which contributes to several diseases including chronic myelogenous leukemia (CML) and acute lymphocytic leukemia (ALL) [2,3]. Hence, Bcr-Abl is one of the important targets for CML therapy. Its tyrosine kinase core is composed of regions including N-terminal cap (Ncap), an SH3 domain, an SH2 domain, and a kinase domain (KD) [1] (Fig. 1). When Abl is in the autoinhibited conformation, the SH3 and SH2 domains are bound to the distal side of the KD, and the helix αI bends to form helix αI′. Thus, the SH2 domain forms a tight protein–protein interface with the carboxyl-terminal lobe of the KD through a set of interlocking hydrogen bonds (dotted box in Fig. 1). Similar interactions exist between the SH3 domain and the SH2-KD linker. Disruption of

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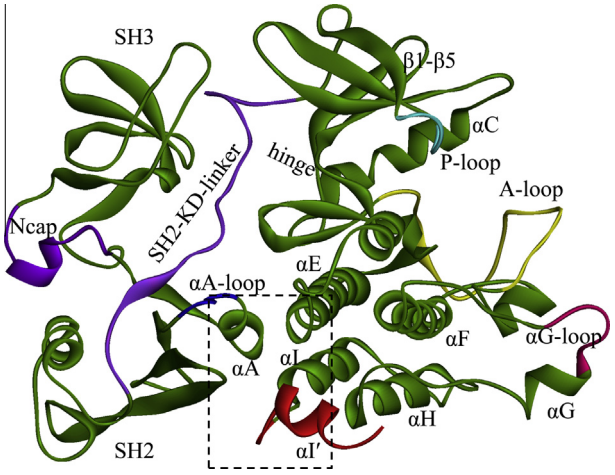

Fig. 1. Structure of inactive Abl^{W} with Ncap, an SH3 domain, an SH2 domain, and a kinase domain.

these intramolecular interactions or ligand binding leads to the activated c-Abl where helix αI is in an unbent conformation and the SH2 domain docks on top of the KD [4,5].

Imatinib [6] is the first generation inhibitor of Bcr-Abl approved by FDA to therapy the CML in 2001. However, emerging TKI resistances caused by mutations [7] especially T315I [8] are hindrances to prevent complete cure. The residue Thr315 is at the gatekeeper position of Abl. In 2006, the second generation inhibitors, dasatinib [9] and nilotinib [10], were granted FDA approval for treatment of CML in patients resistant to or intolerant of imatinib. But they are ineffective to the T315I mutation. The inhibitors developed recently, such as bosutinib [11], INNO-406 and AZD530 [12], fall into the same trouble. The inhibitors mentioned above all locate in the ATP pocket of Abl. Therefore, alternative strategies targeting non-ATP regulatory modules of Abl are good choices to deal with T315I mutation [13]. At present, several ligands binding to the myristyl pocket (MP) of Abl have been reported, e.g. GNF-2, GNF-5 [14], MS7 [15], MS9 and DPH [16]. Besides the myristyl pocket, Grebien et al. have shown that the SH2-kinase interface is an allosteric target for therapeutic intervention, where an engineered Abl SH2-binding fibronectin type III monobody inhibited Bcr-Abl kinase activity [17]. These results verify that potential non-ATP pockets can be targeted to inhibit the kinase activity. But what causes the ineffectiveness of ATP-pocket inhibitors to T315I-mutation Abl (Abl^{T315I})? How the T315I mutation influences the properties of Abl? Where are the other promising allosteric sites for ligand binding? Despite recent progress in computational [18] and experimental studies on Abl kinase structure and function, the mechanism and dynamics of the activation pathway remain unclear for the wild type Abl (Abl^{W}) and Abl^{T315I}. To address these questions, the dynamic conformation changes and activation process of Abl were explored in this study. To our knowledge, the current study performed for the first time CMD and TMD analyses of a near full-length Abl containing the Ncap and SH3 domains, which are important to the activation motion of Abl.

There are few techniques that allow protein dynamics to be conveniently investigated. Conventional molecular dynamics (CMD) techniques [19] can offer an alternative to experimental approaches for exploring properties, because they can treat macromolecules at an atomic level. However, CMD methods cannot simulate a large-scale conformational change like the global activation of Abl. The target molecular dynamics (TMD) method [20] can accelerate the process of large-scale conformational motion between two existing states by continuously decreasing the target values. In the current study, we used this method to address the probable activation pathway of Abl, as it has provided in detail the calculated activation path of several proteins. For example, to predict the complete conformational transition pathway of the juxtamembrane region and the activation loop in the c-Kit kinase activation process [21], to investigate the conformational mechanism of the archaeal RadA proteins [22], and to investigate mechanistic aspects of long-range communications in Abl and EGFR kinases [23].

Methodology

Computational modeling of Abl

The initial structures of both inactive and active Abl containing Ncap were prepared based on X-ray crystal structures deposited in the Protein Data Bank with PDB code 2FO0 [5] and 1OPL [1]. The sequence of Abl in 2FO0 was regarded as a referential sequence. 1OPL is a dimmer form constituted of chain A and chain B. Chain A possesses the same conformation as 2FO0 except for the missing Ncap. Chain B is in a particularly active conformation, hence only Chain B was chosen to serve as the computationally active model. The missing segments in chain B (Ala65-Asn139, Lys238-Tyr251, and Ser519-Gly530) were built on the template structure of 2ABL [18,24], 2FO0, and 3OXZ [25] respectively by homology modeling with the Discovery Studio (DS) 2.5 program (Accelrys, San Diego, CA, USA). To avoid the influence of uncertain factors, Abl^{T315I} in the inactive and active form were constructed by directly mutating Thr315 to Ile315 with the DS program.

Parameters of simulated systems

The parameters for phosphoserine in the -2 charge state of residue pSer69 within the Ncap were assigned according to Homeyer et al. [26]. The proteins with hydrogen atoms were solvated in a rectangular box of TIP3P water [27] with the buffering distance set to 14 Å, which is large enough for the conformation changes. The solvent molecules were allowed to move freely and to follow the dynamics of the protein. The water shells remained in contact with the protein throughout the entire simulations. To ensure the charge neutrality of ionizable groups, chloride (Cl^-) and sodium (Na^+) counterions at a concentration of 0.15 mol L^{-1} were added to the box in random positions. All Na^+ and Cl^- ions were placed more than 8 Å away from any protein atoms and from each other. Four systems were modeled, and the parameters of these simulated systems are listed in Table S1.

Molecular dynamics simulations

All the MD simulations were performed using NAMD 2.7 [28] and the CHARMM27 force field [29,30]. The ionized systems were first minimized for 50,000 integration steps with a constrained solute, and then minimized for 50,000 integration steps with an unconstrained solute. Then the systems were heated from 0 K to 310 K for 100,000 integration steps. The obtained systems were equilibrated for 28–30 ns with 2 fs time step for subsequent trajectory analysis, with frames stored each 20 ps. The SHAKE algorithm [31] was used to constrain the lengths of all chemical bonds involving hydrogen atoms at their equilibrium values, and the water geometry was rigidly restrained by the SETTLE algorithm [32]. Constant temperature (T = 310 K) and constant pressure (p = 1 atm) were maintained by the Langevin piston method [33]. Van der Waals interaction cut-off distances were set at 12 Å (smooth switching function beginning at 10 Å) and long-range electrostatic forces were computed using the Particle-Mesh Ewald [34] with a grid size of less than 1 Å.

TMD simulation was utilized to achieve a large-scale structural transition from a starting structure to a final target structure by