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Insight into the structural mechanism for PKBα allosteric inhibition by molecular dynamics simulations and free energy calculations

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ABSTRACT

Protein kinase B (PKB/Akt) is an attractive target for the treatment of tumor. Unlike PKB's ATP-competitive inhibitors, its allosteric inhibitors can maintain PKB's inactive state via its binding in a pocket between PH domain and kinase domain, which specifically inhibit PKB by preventing the phosphorylations of Thr308 and Ser473. In the present studies, MD simulations were performed on three allosteric inhibitors with different inhibitory potencies (IC_{50}) to investigate the interaction modes between the inhibitors and PKBα. MM/GB(PB)SA were further applied to calculate the binding free energies of these inhibitors binding to PKBα. The computed binding free energies were consistent with the ranking of their experimental bioactivities. The key residues of PKBα interacting with the allosteric inhibitor were further discussed by analyzing the different interaction modes of these three inhibitors binding to PKBα and by calculating binding free energy contributions of corresponding residues around the binding pocket. The structural requirements were then summarized for the allosteric inhibitor binding to PKBα. A possible structural mechanism of PKBα inhibition induced by the binding of allosteric inhibitor was formulated. The current studies indicate that there should be an optimum balance between the van der Waals and total electrostatic interactions for further designing of PKBα allosteric inhibitors.

1. Introduction

Protein kinase B (PKB, also known as Akt) is a key effector in the PI3K–Akt–mTOR pathway and plays a pivotal role in cell survival and proliferation [1,2]. As a member of AGC protein kinase superfamily, PKB shares 65% homology in the whole sequence and 80% residue identity in the ATP-binding pocket with protein kinase A (PKA). There are three subtypes of human PKBs, including PKBα/Akt-1, PKBβ/Akt-2, and PKBγ/Akt-3, which are encoded by different genes and share more than 80% sequence homology with each other [3,4]. PKB contains three domains, an N-terminal pleckstrin homology (PH) domain, a central catalytic domain, and a hydrophobic motif at the C-terminals. The PI3K–Akt–mTOR pathway starts from the phosphatidylinostiol-3-Kinase (PI3K) activation induced by G-protein coupled receptor or protein tyrosine kinase [5]. Activated PI3K catalyzes the phosphorylation of PtdIns(3,4)P2 (PIP2) to form PtdIns(3,4,5)P3 (PIP3) which can interact with the PH domain of PKB. PIP3-bound PKB is recruited to the plasma membrane for 3′-phosphoinositide-dependent kinase-1 (PDK1) catalyzed phosphorylation of Thr308 (in PKBα) located in the PKB activation loop. Then, mTORC2 promotes the phosphorylation of Ser473 located at the C-terminals next to the catalytic domain [4,6]. The phosphorylations at the residues of Thr and Ser of PKB are responsible for the full activation of PKB to regulate the survival, growth, proliferation, and metabolism of cell.

The aberrant activation of PKB has been widely implicated in many human cancers through several different mechanisms, including alterations of PI3K [7–11], alterations of the tumor suppressor phosphatase and tensin homolog (PTEN) [12–14], and alterations of PKB [10,15–19]. Therefore, it is expected that inhibition of PKB might prevent cellular growth and proliferation, and reverse the repression of apoptosis and resistance to cytotoxic agents in cancer cells [20]. Up to now, there are several kinds of compounds discovered to have good inhibitory activity to target PKBs. Some PKBα inhibitors have been in phase I or II clinical studies. According to the difference of their binding pockets in PKB, PKB's inhibitors can be classified into ATP-competitive inhibitor and allosteric inhibitor. The research group of Merck developed the allosteric inhibitors of PKBα and the corresponding inhibition model in 2005 [21]. Unlike ATP-competitive inhibitor docking to

Abbreviations: PKB/Akt, protein kinase B; MD, molecular dynamics; PI3K, phosphatidylinostiol-3-kinase; PDK1, 3′-phosphoinositide-dependent kinase-1; PTEN, phosphatase and tensin homolog; MM/PBSA, molecular mechanics/Poisson Boltzmann surface area; MM/GBSA, molecular mechanics/generalized Born surface area; PH, pleckstrin homology; RMSD, root-mean-square deviations; SMD, steered molecular dynamics.

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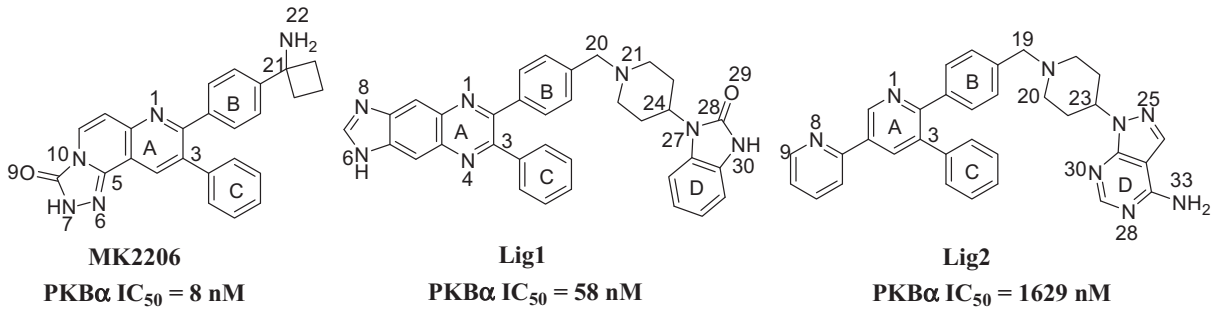

Fig. 1. The structures and IC_{50} values of PKBα's allosteric inhibitors MK2206 [22], Lig1 [23,24], and Lig2 [25].

the ATP-binding pocket of PKB, the allosteric inhibitor binds to a site that exists only in the presence of the PH domain for the stabilization of PKB in a "closed" conformation to prevent Thr308's phosphorylation promoted by PDK1 [1]. The allosteric inhibitor is selective against closely related kinases, like PKA or PKC, since it is non-competitive with respect to ATP and peptide substrate. Therefore, the PH domain-dependent allosteric inhibitor has gained great interest in the discovery and development of PKBα inhibitors in the past few years. Anticancer drug MK2206 [22] is an allosteric inhibitor currently studied in phase II trials for a combination treatment of metastatic solid tumors. As MK2206's analogs, inhibitors Lig1 (1-(1-(4-(7-phenyl-1H-imidazo[4,5-g]quinoxalin-6-yl)-benzyl)-piperidin-4-yl)-1H-benzo[*d*]-imidazol-2(3H-one) [23,24] and Lig2 (1-(1-(4-(5′-phenyl-2,3′-bi-pyridin-6′-yl)-benzyl)-piperidin-4-yl)-1H-pyrazolo[3,4-*d*]-pyrimidin-4-amine) [25] were also developed to be PKBα's allosteric inhibitors with modest and low bioactivity, respectively. The reported X-ray co-crystal structure [24] of PKBα with Lig1 showed the binding feature of Lig1 in the allosteric pocket of PKBα. Fig. 1 displays the chemical structures of these three inhibitors and their experimental bioactivities against PKBα.

The research group of Merck has successfully designed a series of allosteric inhibitors of PKBα [2,22,25–38]. Only one X-ray co-crystal structure [24] was reported for the allosteric inhibitor binding to PKBα. Calleja et al. elucidated the mechanism of PKBα allosteric inhibition through theoretical studies [39]. They used a multidisciplinary approach including molecular modeling and classical biochemical assays to depict the interactions of Lig1 with PKBα. It was found that Trp80 and Gln218 were important residues for maintaining the quaternary structure of PKBα in its inactive conformation. The PH domain induced cavity for the inhibitor's binding in PKBα was not formed in PKBγ. Gln218 was identified as an important residue for the stability of the Ser473 phosphorylation by molecular modeling and biochemical assays [24,39]. Due to the missing residues of Gly299–Thr312 in the activation loop of PKBα in its X-ray crystal structure, the Thr308 phosphorylation feature was analyzed and tested by biochemical assays to supply experimental data for the inhibitory effects of its allosteric inhibitors [39].

As reported, most allosteric PKBα inhibitors consist of an aromatic fused ring A, a six membered ring B, and a benzene ring C (Fig. 1). Recent studies [2,22,25–38] have shown that the biological activities vary with the substitution positions of ring and nitrogen atom on the aromatic fused ring A and the primary amino group of MK2206, indicating that the structural difference of inhibitors affects significantly their binding processes to PKBα. This may be caused by a specific conformation in the kinase domain which is induced by the inhibitor's binding to a pocket near PH domain of PKB. Therefore, the allosteric inhibitor has an absolutely pharmacophoric requirement for its binding to PKBα. It needs a detailed perspective into the interactions happened between PKBα and allosteric inhibitors. It would be significant to understand the influence of a specific conformation of PKB's allosteric inhibitor on the dynamic characteristics of its bound state.

As an important technology and tool to study interaction mechanism of ligand-receptor complex, molecular dynamics (MD) simulations and free energy calculation studies on the binding mechanism of different kinase inhibitors have received much attention in recent years [40,41]. By integrating molecular mechanics energy and continuum solvation models, molecular mechanics/Poisson Boltzmann surface area (MM/PBSA) [42] and molecular mechanics/generalized Born surface area (MM/GBSA) [43] were developed for the free-energy calculations and molecular docking studies of ligand binding to its target protein. MM/PBSA and MM/GBSA are indeed more specific than most empirical or knowledge-based scoring functions. In the current study, MD simulations and MM/PB(GB)SA free energy calculations were performed to three allosteric inhibitors MK2206, Lig1, and Lig2, respectively, binding to PKBα. We investigated the interaction features between PKBα and these inhibitors to understand the specificity of the allosteric inhibitor binding to PKBα. The key residues interacting with the allosteric inhibitor were discussed by analyzing the different interaction modes of these three inhibitors, with different inhibitory potencies, binding to PKBα and by comparing the different binding free energy contributions of corresponding residues around the binding pocket. The pharmacophoric requirements were then summarized for the allosteric inhibitor binding to PKBα. It is expected that this work will provide helpful information for the design of novel and potent allosteric inhibitors of PKBα.

2. Methods and computational details

2.1. System preparation

The X-ray co-crystal structure of PKBα-Lig1 (PDB entry: 3O96 [24]), downloaded from the RCSB Protein Data Bank (PDB), was applied to be the initial structure for the current molecular dynamic simulations. The missing residues (Asp46–Arg48, His89–Glu91, Glu114–Arg144, Lys189–Glu198, Gly299–Thr312) of PKBα in the crystal structure were inserted by using the loop search algorithm in BIOPOLYMER module of SYBYL x1.3 [46]. The structure was then finalized by adding hydrogen atoms, assigning partial charges and protonation states using the BIOPOLYMER module of SYBYL x1.3 [46] and the energy minimization was performed on the generated structure with 1000 steps of steepest descent to relax amino residue side chains. The obtained structure was regarded as the starting point for the following calculations. The initial structural models of complexes PKBα-MK2206 and PKBα-Lig2 were derived from the above treated structure of PKBα-Lig1. Either MK2206 or Lig2 was respectively prepared by modifying the structure of Lig1 extracted from above PDB file using the Sketch module of SYBYL x1.3 [46]. The in silico generated conformation of Lig2 or MK2206 was then merged into the binding pocket of the complex PKBα-Lig1