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Investigation the Mechanism of Interaction between Inhibitor ALISERTIB with Protein Kinase A and B Using Modeling, Docking and Molecular Dynamics Simulation

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ABSTRACT: The high level of conservation in ATP-binding sites of protein kinases increasingly demands the quest to find selective inhibitors with little cross reactivity. Kinase kinases are a recently discovered group of Kinases found to be involved in several mitotic events. These proteins represent attractive targets for cancer therapy with several small molecule inhibitors undergoing different phase of clinical trials. Alisertib, a synthetic inhibitor of Kinase kinases, acts as an ATP-competitive compound which has been proved to be selective for Kinase A and is currently being evaluated in the phase I trial for patients with advanced solid tumors. However, the structural details on the selectivity of Alisertib towards Kinase-A over Kinase B are still not resolved. To investigate the structural details of this selectivity, the complexes of Kinase A and B with Alisertib were modeled and evaluated using molecular dynamics simulation and docking techniques. The predicted free energy for the binding of Alisertib to Kinase A and B suggests stronger interactions between Alisertib and Kinase A. Results also indicate that there are a strong attraction and anion- pi stacking interaction between the Phe144 in Kinase A and CLBB atom and benzazepine scaffold of Alisertib. As well as it seems a desired anion-pi stacking interaction was created between the carboxyl group of the side chain of Asp274 and fluoro methoxyphenyl ring of Alisertib. Furthermore, Kinase kinases contain a conserved hydrophobic ligand-binding pocket that is highly involved in ligand binding specificity. Taken together it seems that the mentioned difference in the binding pockets of Kinase A and B are the key factors responsible for selectivity.

Keywords: *Alisertib, ATP-binding site, Glycine-rich loop, Kinase.*

INTRODUCTION

The kinase kinases, a family of serine/threonine kinases, play a critical role in multiple features of mitosis in eukaryotic cells [1]. Humans express three members of Kinase kinases: Kinase A, B, and C which exhibit 67-76% amino acid sequence identity in their catalytic domain, but differ in amino acid length and sequence at the N-terminal domain [2,29]. Kinase A, the polar

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kinase, localizes on centrosomes, and plays a crucial role in each step during mitosis. Kinase B, the equatorial kinase, is a chromosomal passenger protein that moves from centromeres to the spindle midzone during mitosis. Although little is known about the importance of Kinase C, it is specifically expressed in the testis and plays a role in spermatogenesis [3]. Deregulation of Kinase kinases due to gene amplification and overexpression results aneuploidy and may contribute to tumor genesis. A large amount of evidence indicates that Kinase kinases are over expressed in a variety of solid tumors, including colon, breast, pancreas, prostate, and thyroid cancers [4]. As a result, Kinase kinases have attracted considerable attention as potential targets for cancer chemotherapy. In recent years, a number of small molecule inhibitors targeting Kinase kinases are developed which can be subdivided in to three general classes [5]: selective Kinase A inhibitors such as MLN8054 [6] and Alisertib [7,8,10,11,12], selective Kinase B inhibitors such as hesperidin [9] and AZD1152 [13], and Kinase A/B inhibitors such as VX-680 [14] and ZM447439 [15]. The great majorities of these synthetic Kinase kinase inhibitors are ATP-competitive and has a planar heterocyclic ring system that can occupy the adenine-binding region and mimic adenine-kinase interactions. Alisertib, a synthetic inhibitor of Kinase kinases, acts as an ATP-competitive compound which has been proved to be selective for Kinase A and is currently being evaluated in phase I and II trials for patients with advanced solid tumors. However, the structural details on the selectivity of Alisertib towards Kinase-A over Kinase B are still not resolved. On the other hand, a thorough understanding of ligand binding sites may aid the rational design of novel antitumor drugs and accelerate important therapeutic breakthroughs. To obtain new insights into this specific Inhibitor-Kinase, kinase interactions, we have carried out a theoretical study employing modeling, molecular docking and molecular dynamics simulation.

MATERIAL AND METHODS

Modeling

All starting atomic coordinates of proteins were modeled with Modeler 9.9 software [16].

The active model of Kinase A

For the preparation of this model, we used the human structure 1LO5 recorded in the PDB database. Afterward, we refined 1-6 and 22-29 (related toTPX2) and 140-149 (related to glycine-rich loop) regions. For this purpose, were used for 3HA6, 3E5A and 4C2V, respectively. Moreover, to generate active form of Kinase kinase A, the ultimate structure was phosphorylated in the Thr 287 and Thr 288 locations [17, 18].

The active model of Kinase B

For the preparation of this model, we used Human Kinase B (PDB: 4AF3) as a template structure, and missing areas (Activation loop and Glycine-rich loop) were refined by Xenopus Kinase kinase (PDB: 2BFX and 4C2V, respectively).

Model of Alisertib

Since no recorded three- dimensional structure for Alisertib in PDB, its analog structure, MLN8054 (PDB: 2X81) was used as a template. Then this structure was modeled and energy minimized with Hyperchem software [19].

Molecular dynamics (MD) simulation

All Molecular dynamics simulations were performed using the GROMACS simulation package version 4.5.3 and modified GROMOS 43A1 force field. Each protein was centered in a cubic box and immersed in SPCE water molecules so that the shortest distance between the protein and the box boundaries was 1.1 nm and periodic conditions were applied. To achieve a neutral simulation box, the net charge of the protein was neutralized by replacing water molecules with necessary Cl^- or Na^+ ions. Each solvated and neutralized was energy minimized using the steepest descent algorithm until the maximum force was smaller than 500 kJ/mol.nm. After energy minimization, two-position- restrained MD simulations were sequentially carried out to adjust temperature and velocities and to equilibrate the solvent and ions around the protein. First, to adjust the system temperature, an NVT MD simulation was performed for 400 ps at 300 K by imposing thermal energy in a constant volume con-

dition using the velocity rescale algorithm (modified Berendsen thermostat) with τ_T = 0.1 ps (ref-15). After arrival at the correct temperature, the resulting atom velocities and coordinates were used to start an NPT MD simulation at 300 K and 1 bar for 400 ps by parrinello-Rahman algorithm with τ_p = 2.0 ps during which density of the system was stabilized at around 1000 kg/m³ [20,21]. Finally, the production MD period of 600000 ps at constant pressure (1 bar) and temperature (300 K) without position restraints was performed on four systems. Bond length was constrained using LINCS algorithm [22]. In Gromos 43A1 the Lennard-Jones and short-range electrostatic interactions are optimized with a cut-off radius of 1.0 nm. The particle mesh Ewald algorithm was used for the long range electrostatic interactions [23]. The neighbor list was updated every 5 steps. Each component of the system was coupled separately to a thermal bath, and isotropic pressure coupling was used to keep the pressure at the desire value. A time step of 2 fs was used for the integration of equation of motion.

Molecular Docking

To estimate the binding Energy, flexible ligand docking was initially performed in the Auto Dock 4.0 software package. Polar hydrogen atoms were added, nonpolar hydrogen atoms were merged and Gasteiger charges were assigned by default. All docking calculations were performed with the Lamarckian genetic algorithm (LGA) [24]. A population size of 300 and 25,000,000 energy evaluations were used for 10 runs. The grid dimensions were 40×40×40 points along the x-, y- and z- axes. And grid spacing of 0.375 Å was used. The conformation with the lowest docked energies and reasonable orientations in light of the nature of the ATP-binding pocket were chosen for subsequent analysis [24,26,28].

RESULTS AND DISCUSSION

Molecular dynamics simulation for both Kinase kinase A/B as well as their complexes with Alisertib was performed during 60 ns. After the simulation, using data from the simulation tools in Gromacs 4.6.7 package to verify the accuracy of the simulation, some parameters were examined throughout all four structures. After that to determine the equilibrium, Structural changes, and changes in the interaction region of all four structures were assessed in the presence and absence of inhibitor. Finally, the range of 10 -60 ns was chosen for all analysis.

Structural analysis

To investigate the structural changes of proteins, initially, Radius of gyration was computed for all four systems. The radius of gyration is a scale for measuring the concentration of protein. The calculation of this parameter was performed during the final 50 ns of simulation. The results showed that in the presence of inhibitors Radius of gyration values of all four proteins were increased substantially. On the other hand, to assess the stability of protein, root- mean- square deviation (RMSD) of backbone atoms of protein, ligand-binding site, and ligand was estimated. The structure of proteins and ligands was stable except for the glycine-rich loop. In other words, in the presence of ligand, glycine-rich loop being opened so that ligand can place in the ligand-binding site. This is while the RMSD of Kinase-A ligand binding site were decreased substantially after ligand binding.

Binding modes of Kinase A and –B with Alisertib

Because of two Kinase proteins are highly homologous and conservative in the binding site, the interaction modes of Alisertib with Kinase A and –B should be very similar. To throw light on the differences between two Kinase kinases, we performed a comparative study of the structures of the Kinase A-Alisertib and Kinase B-Alisertib complexes. In the other words, there are some specific interactions between Kinase-A/ B and inhibitor. Hence amino acids of the ligand-binding site were divided into three categories.

Specific amino acids in Kinase A ligand binding site

In Kinase A, binding site, Phe144, Lys162, Thr217, and Asp274 constitute specific interactions with Alisertib. So that HD2, CD2, CE2, O and CA atoms of Phe144 undergo favorable hydrophobic interactions with HAI atom of inhibitor. Also, the phenyl ring of Phe144 establishes favorable anion –pi stacking interaction with CLBB atoms of Alisertib. It seems that

there is a strong attraction between N atom of Phe144 and CLBB atom of Alisertib. On the other hand, the oxygen atom of Phe144 created an anion- pi stacking interaction with the benzazepine scaffold of inhibitor. That the gatekeeper residue leucine is identical in the three identical sequences (Leu210 in kinase A and Leu156 in kinase B). Moreover, there are only four residues located in the vicinity of the ATP binding site that is not identical between kinase A and kinase B. Particularly, Leu215, Thr217, Val218, and Arg220 of kinase-A are replaced by Arg159, Glu161, Leu162, and Lys164 in kinase B. According to 3D structures and properties of the amino acids, these replacements are compared to gain insight into their influence on the binding pocket. The replacement of Val 218 by Leu162 seems to be the least important since both valine and leucine are nonpolar and lipophilic with side chains pointing away from the pocket. In contrast, the replacement of Thr217 by Glu161 should be considered great importance. In this case, a polar threonine residue is replaced by an acidic glutamate with a longer and more flexible side chain. And this residue is located near the edge of the catalytic pocket in the ribose binding region. The importance of this replacement was validated by the experimental results reported by Dodson et al [25]. In their work, the individual T217E point mutant of kinase-A is almost 20-fold less potently inhibited by MLN8054 than the wild-type kinase A. Our result showed that there is favorable hydrophobic interaction between CG2 of Thr217 and HAY atom of Alisertib RTIB but it seems that there is an electrostatic repulsion between side chains of Glu161and CLBB and NAQ atoms of Alisertib. The remaining two replacements seem to be of medium significance. Although the replacement of Leu215 by Arg159 has a nonpolar residue replaced by a basic one, the side chains of both leucine and arginine are directed toward the solvent and away from the catalytic pocket. In the replacement of Arg220 by Lys164, both residues share the same basic character and their side chains point to the solvent. As well as it seems a desired anion-pi stacking interaction was created between the carboxyl group of the side chain of Asp274 and fluoro methoxyphenyl ring of Alisertib, while this amino acid is far from Alisertib in kinase B.

Specific amino acids in Kinase B ligand binding site

Since Alisertib is also kinase B inhibitor, we analyzed Kinase B binding site and found that the interactions between residue Gly84 and Alisertib are stronger than corresponding residues in kinase B. In the other word, N, H, and CA atoms of Gly84 were created desired hydrophobic interactions with HAI atom of Alisertib with average distances of 3.5 Å. While the average distances between atoms of Gly140 in kinase A and atoms of Alisertib are 6.02 Å.

Table 1. Hydrophobic interactions between Benzazepin Scaffold of inhibitor and specific amino acids of Protein

Kinase A:	Atoms of Benzazepin Scaffold	Distance (Å)	Contact (for each frame)
Leu139 side chain	CAI,CBC,HAI,HBC	4.5	15
Leu263 side chain	NAV,CAB	4.7	6
Gly140 side chain	CBC,CAI,CAH	3.5	28
Kinase B:			
Val91 side chain	CAI,HAI,CBC,HBC	4.3	11
Leu207 side chain	NAV,CAB	5.1	5

Table 2. Comparison of the number of hydrophobic contacts between the amino acids present in the A and B interaction zone with ALISERTIB during the analyzed time frame.

Model	Total Contact (for each frame)
Kinase A-Alisertib	779
Kinase B- Alisertib	749

Table 3. Distance (Å) and number of hydrophobic contacts between specific amino acids Kinase kinase A and B with Alisertib during the analyzed time frame

Amino acids in Kinase A:	Atoms of Alisertib	Total contact (for each frame)	Distance (Å)
Thr217 Side chain	(FAX)	3	3.4
Leu139 Side chain	(HAI,HBC,CAI,CBC)	15	4.5
Gly140	(CL,HAI,CAI,CBC,CAH,HBC)	28	3.8
Lys141 Side chain	(CBK,OAZ)	14	4.1
Phe275 Side chain	(Phenyl Ring)	58	4.8
Leu263 Side chain	(FAX,CAB,NAV)	10	4.3
Amino acids in Kinase B:			
Val91(Side chain)	(CAI,HAI,CAH,CL)	17	3.8
Phe88(Side chain)	Phenyl Ring	72	4.1
Leu207(Side chain)	(CAB,NAV,FAX)	9	4.4
Ala217(Side chain)	(FAX,HAY)	4	5.5

Non-specific amino acids in Kinase A and B ligand binding site

It can be seen that the interactions between Alisertib and kinase A are mainly determined by residues Ala213, Leu263, Arg137, Tyr212, Leu139, Val147, Arg220, Leu194, Gly216, Leu210, Leu215, Ala160, and Asp261. Residues such as Leu83, Val91, Leu138, Glu155, Tyr156, Ala157, Arg159, Asn205, and Leu207 of kinase B also undergo strong interactions with Alisertib. Correspondingly, the residue Glu-161causes an unfavorable contribution to kinase B, compared with Thr217 which is favorable to kinase A. It indicates that the interaction spectra of Kinase A and Kinase B are quite similar. However, interactions with kinase-A are stronger than those with Kinase B, which is consistent with the experimental result that Alisertib shows stronger activities towards kinase-A over kinase B.

Hydrogen bond between Kinase A / B and Alisertib

The conservative residues from H-bond interactions with Alisertib, playing a key role in stabilizing the binding of the conformation. The two H-bond between Alisertib and Ala in the hinge region were strongly formed with occupancies of over 90% for both kinase A / B complexes during the 50 ns MD simulation And the distances of the two H-bonds were almost the same for both proteins, with average distances of 2.9 ±0.12 Å for Ala213/157 C=O…H-N, and 3.02 ±0.13 Å for Ala213/157 N-H…N. Therefor the H-bonds with the hinge region are essential for Alisertib binding to Kinase kinases.

Mechanism of selectivity for ALISERTIB over kinases

Here we mainly discuss its selectivity mechanism by considering two individual factors. It is worth mentioning that there are primarily three different residues in active sites between Kinase A and kinase B, namely the corresponding residues: L215/R159, T217/E161, and R220/K164. Meanwhile, the inhibitor Alisertib undergoes similar interactions with the two proteins in the binding pockets. Based on the structural and the dynamic information, the conformational changes at

Table 4. Comparison of the percentage of hydrogen bonds formed between the protein and the inhibitor.

Protein	Donor	Acceptor (inhibitor)	Percentage of hydrogen bonds = Occupancy (%)	Distance (Å)
Kinase A:	Ala213(O)	(NAQ)	96.8	2.9
	Ala213(N)	(N1)	80.17	3
Kinase B:	Ala213(O)	(NAQ)	96.05	2.9
	Ala213(N)	(N1)	75	3

the active sites were compared, especially for the different residues and free energy calculations ranked the activities reasonably and analyzed the differences and energy components.

CONCLUSIONS

In the current work, we mainly aimed to clarify the molecular mechanism of selective inhibitor and provide useful guidance for the rational design of new potential selective inhibitors of Kinase kinase A. Our simulation results show that the different properties of the binding pocket from polar to hydrophobic arising from the induced favorable interactions with two important variable residues within the active site play a key role in the binding selectivity. Alisertib, a modified compound of MLN8054, is reported as a second-generation kinase-A selective inhibitor with increased anti-tumor activity [1, 25]. Compared with its predecessor MLN8054, the structure of Alisetib is improved by introducing methoxyl to the benzoic acid and difluorophenyl groups. These modifications could strengthen the interactions in the binding site.
Molecular dynamics simulation, binding free energy calculation, and per-residue decomposition analysis were performed to study the inhibition and subtype selectivity mechanism of Alisertib to kinase A and B protein kinases. To improve the selectivity for Kinase A over kinase B, enhancing the interactions with Phe 144 and Asp274 should be an effective way. The residue Glu161 in kinase B contributes unfavorably to Alisertib with electrostatic repulsion, while the corresponding Thr217 in kinase-A has favorable interactions with Alisertib. The information gained from this study should help lead to the design and discovery of new inhibitors with improved binding properties.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: NM. Performed the experiments: ME. Analyzed the data: ME FY NM. Contributed reagents/ materials/analysis tools: NM. Wrote the paper: ME.

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