CONFORMATIONS AND VALIDATIONS OF P38 MAP KINASE BOUND TO INHIBITOR

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Abstract - P38 mitogen-activated protein (MAP) kinases signaling pathway plays an important role in inflammation. The aims of this work are to study the interaction and conformational flexibility between the p38 MAP kinase and the SB-203580 inhibitor, which is located in the ATPbinding pocket by the molecular dynamics (MD) simulations. The crystal structures of p38 MAP kinase bound to SB-203580 (pdb code 1A9U, 3MPA, 3OBG, 3GCP, 3ZS5 and 2EWA) are investigated. The six kinases exist in the DFG-in and DFG-out conformations and SB-203580 conformations share the similar binding mode. From the results, in the ATP active site, the SB-203580 forms two hydrogen bonds with the backbone NH group of Met106 and O atom of His104 in the hinge region and the NH group of Asp165 of the DFG-in loop movement. Phe166 flips out the DFG loop, which it is formed with SB-203580 by the pi-pi stacking. The SB-203580 has a stable binding with DFG-in and DFG-in mutate of P38 MAP kinase. However, it is unstable in DFG-out MAP kinase. It is also found that SB-203580 in the protein 2EWA changed conformation from DFG-in to DFG-out. The information from this study can be used to develop the new inhibitor.

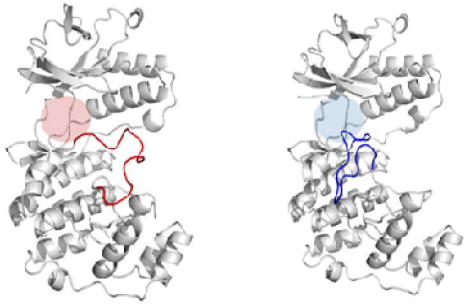
Keywords - P38 mitogen-activated protein (MAP) kinases; Molecular dynamics simulations (MD), DFG, SB-203580

I. INTRODUCTION

Kinases are involved in a wide range of disease conditions from inflammation to oncogenesis and as such are increasingly viewed as major targets for therapeutic intervention. Different protein kinases recognize a narrow range of substrates a factor that can be exploited pharmaceutically to create specificity. p38 mitogen-activated protein (MAP) kinases signaling pathway plays an important role in inflammation and other physiological processes that play a central role in the regulation of pro-inflammatory stress response pathways[1] such as cytokines, ultraviolet irradiation, heat shock, and osmotic stress, arsenite[2-4], and are involved in cell differentiation, apoptosis and autophagy. Furthermore, this pathway controls the production and secretion of pro-inflammatory cytokines such as tumor necrosis factor-α (TNFα), interleukin-6 (IL-6) and interleukin-1β (IL-1β)[5]. The p38 MAP kinase has been a target for anti-inflammatory therapy[6] and important targets for drug discovery.The structure of protein kinase consists of two major subdomains which are the N-terminal and C- terminal lobes[7]. The active loop belongs to the C-terminal lobe and closes to the hinge region controls the conformational transition between the active conformation and the inactive conformation of the kinase[8]. Most kinase inhibitors are ATP-competitive molecule, which form hydrogen bond with the hinge region (ATP binding site) of the active Asp-Phe-Gly (DFG)-in kinase conformation and the ATP binding site is well-known for type I inhibitors affinity and selectivity profiles of kinases[9]. The crystal structures of kinase have shown that the kind of kinase inhibitors occupy both the ATP-binding pocket and the allosteric pocket (hydrophobic pocket) and are named as type II inhibitors [10]. The kinase activation can be blocked by the type II inhibitors, which bind to the inactive (DFG-out) conformations of kinase. The type-I and type-II inhibitors bind to binding sites with different conformations of the DFG in kinases [11]. P38 MAP kinases are characterized the DFG-loop conformation and typically found in the active, or DFG-in conformation. The DFG-loop transition is triggered by the shift of the Phe169 side chain by 10 Å and 180º flip from DFG-in conformation to DFG-out conformation[12]. The DFG-in conformation accesses to the deep pocket in hindered by Phe169[13], whereas in the DFG-out conformation, Phe169 points away from the nearby αC helix and projects into the ATP binding pocket[14]. Therefore, analysis of kinase structures is the outstanding interest to the structure between the active and inactive kinase conformations. The inhibitors are a key of therapeutic strategy for the treatment of a diverse range of pathological[15]. The conformations of DFG-in and DFG-out are shown in Figure 1.

Considering the nature of the amino acids in the ATP pocket, one would expect methods for discriminating between DFG-in and DFG-out binders to guide innovative compound design. Because the

stabilization of type I inhibitor kinase conformations is demonstrated by protein X-ray crystallography. Therefore, the model system for conducting comparison of simulations and experiment, we have chosen to study the interaction between the p38 MAP kinase and the inhibitor SB-203580, which binds in the ATP–binging pocket of kinase that the X-ray structures point to marked conformational flexibility of the p38 MAP kinases [16].



(A) (B)

Fig.1: Structures of (A) DFG-in and (B) DFG-out conformations of p38 MAP kinase.

II. METHODS

All simulations are performed using the GROMACS 3.3.3[17] simulation package in conjunction with the GROMOS 54A7 [18] force field. The initial structures of p38 MAP kinase (379 residues) complex the inhibitors [SB203580 Pyridinylmidazole (4-[5-(4-Fluoro-Phenyl)-2-(4-Methanesulfinyl-Phenyl)-3H-Imidazole-4-YL]-Pyridine)] are taken from the PDB entries 1A9U, 2EWA, 3GCP, 3MPA, 3OBG and 3ZS5 respectively [16, 19-21]. The structure of SB203580 can be usefully decomposed into five principal moieties: (I) imidazole, (II) fluorobenzene, (III) pyridine, (IV) benzene, and (V) sulfoxide. The initial structure of p38 MAP kinase (360 residues) is taken from the PDB entry 1KV1 complexed with BMU (1-(5-Tert-Butyl-2-Methyl-2h-Pyrazol-3-Yl)-3-(4-Chloro-Phenyl)-Urea). The topologies of ligand are generated using the 'automated topology builder' (ATB) and optimized manually[22]. P38 MAP kinase inhibitors are structurally and biophysical well-characterized (Table 1). Each complex is placed in a dodecahedral periodic box and solvated with simple point charge (SPC) water molecules[23].The protonation state of titratable groups is chosen appropriate to pH 7.0 giving a total charge on the system of -8 e. Each complex is energy minimized and the system equilibrated for 200 ps with the heavy atoms of the protein positionally restrained before commencing a series of unrestrained molecular dynamics (MD) simulations. All simulations are performed at constant temperature (298 K) and pressure (1 atm) using a Berendsen thermostat (coupling time of 0.1 ps) and barostat (coupling time of 1.0 ps and isothermal compressibility of 4.575 × 10-4 (kJ/mol/nm3)-1) [24]. A triple-range cutoff is used. Interactions within a shorter-range cutoff of 0.8 nm are updated every step (0.002 ps). Interactions within the longer-range cutoff of 1.4 nm are updated vary 0.010 ps together with the pair list. To correct for the truncation of the electrostatic interactions beyond the 1.4 nm long-range cutoff a reaction-field correction is applied using a dielectric permittivity of 78. The equations of motion are integrated using the leapfrog scheme. Initial velocities at a given temperature are taken from a Maxwell-Boltzmann distribution. All bonds are constrained using the SHAKE algorithm with a geometric tolerance of 0.0001[25].

III. RESULTS AND DISCUSSION

3.1. Validation of p38 MAP kinase bound to SB-203580

The crystal structures of the type I inhibitor (SB-203580) in complex with p38 MAP kinases are 1A9U, 3MPA, 3OBG, 3GCP, 3ZS5 and 2EWA [16] that the six kinases exist in the DFG-in and DFG-out conformations and SB-203580 share the similar binding mode. MD simulations for six complexes in aqueous solutions are run for duration of 10 ns, they are stabilized in the protein conformation (Figure 1A). The type I inhibitor conformations change at 5 ns, however after 5 ns they were stable and similar the conformations with the starting structures (Figure 1B). In the ATP active site, the type I inhibitor forms two hydrogen bonds with the backbone that are NH of Met106 and O of His104 in the hinge region and the NH of Asp165 of the DFG-in loop movement [30]. The Phe166 structure flipping out in the DFG loop, [31] which is formed with SB-203580 by the pi-pi stacking. However, SB-203580 binds to p38 MAP kinase in the DFG-in and DFG-out form reported by the different group [32], [33]. To explore the dynamic stability of complexes and confirm the rotation of the SB-203580 by the sampling methods, root-mean-square deviations (RMSD) from the starting structure are analyzed, show in Figure 2. The RMSD of each system except SB-203580-p38 MAP kinase complex tend to converge, indicating that the system is stable and equilibrated. The SB-203580-p38 MAP kinase complex show the highest fluctuations among the six, the average RMSD were 2.9, 3.5, 2.5, 3.3, 3.6, and 2.7 Å for protein respectively, indicated that the type I inhibitor (SB-203580) has a stable binding with DFG-in and DFG-in mutate P38 MAP kinase and is an unstable in DFG-out MAP kinase. However, SB-203580 in the protein 2EWA changes conformation after 7 ns that show in Figure 2f because of the conformation structure of 2EWA changes from DFG-out to DFG-in.

Analyses of root-mean-square fluctuation (RMSF) versus the residue number for complexes are shown in Figure 3. The RMSF indicate that the residues with in higher fluctuation values are those in the flexible loops. The observations are in agree with the

experimental results from x-ray crystallographic data. The fluctuation of the active site for type I inhibitor is more significant, it shows relatively the ATP active site region (residues 32, 50, 104, 106, 151, and 165-167). All proteins have similar interaction mechanism with SB-203580.

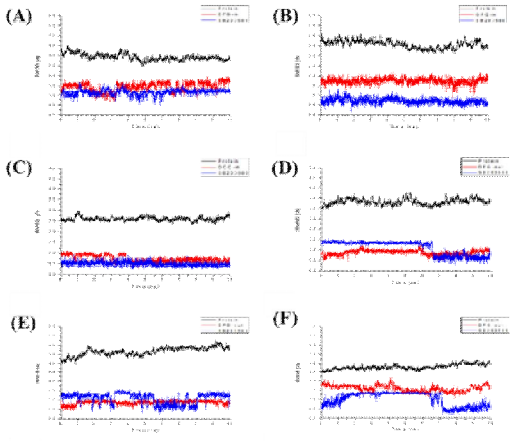


Fig.2: RMSDs of the protein, DFG, and SB203580 for (A) 1A9U, (B) 3MPA, (C) 3OBG, (D) 3GCP, (E) 3ZS5 and (F) 2EWA bound to SB203580 as a function of simulation time

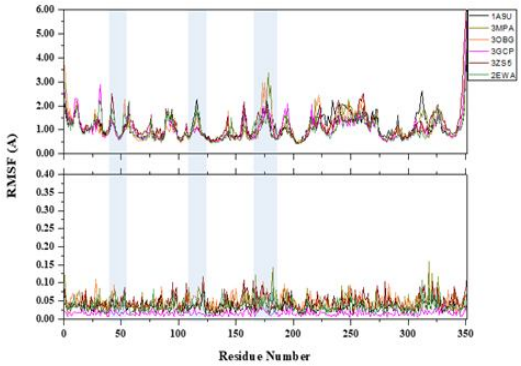


Fig.3: RMSF of each residue of the protein for all six complexes obtained from crystal structure (bottom) and 10 ns MD simulation (top).

3.2. Conformational of the ATP active site region

To future investigate inhibitors-p38 MAP kinase interactions in the binding site process, the snapshots are taken from last 10 ns MD trajectory. Figure 4 shows the interactions at the active site for six p38-bound complexes. It can be seen that the NH of Met106 formed hydrogen bond with N atom of imidazole of inhibitors, has a similar in all systems. The DFG-in (1A9U) and mutant (3MPA and 3OBG) proteins form hydrogen bond with SB-203580 at Gly33, Lys50 and Ser151. In the part of DFG-out, 3GCP and 3ZS5 are stably the hydrogen bond and pi-stacking networks among Lys50, Tyr32 and Phe166 (Figure 5D and 5E). The DFG-in and -out conformation of 2EWA form hydrogen bond and pi-stacking at Lys50, Tyr32 and Phe166 (Figure 5F).

The hydrogen bond plays important role in type I inhibitor binding to ATP site of kinase. For the six protein-inhibitor systems, the hydrogen bond interaction with key residues in the binding pocket are shown in Figure 5. The SB-203580 inhibitor binds with the p38 MAP kinase proteins, they form a stable hydrogen bond with Gly33, Lys50 and Ser151. The inhibitor interacted with Met106 in the hinge region that are a stable hydrogen bond. And the SB-203580 formed pi stacking interactions with Tyr32, Lys50 and Phe166 that are found in the DFG-out conformation. The position of fluorobenzene, imidazole, pyridine and sulfoxide are importance in SB-203580 structure for interaction with DFG-in and DFG-out of kinase that are shown in Figure 6.

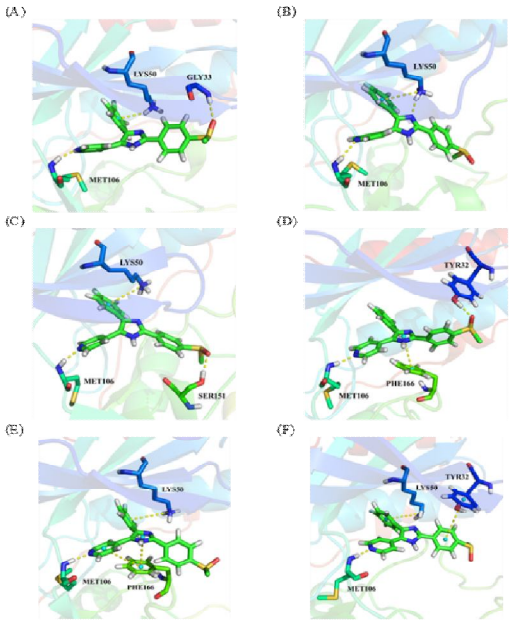


Fig.5:Sructural representative snapshot from MD of: (A) 1A9U, (B) 3MPA, (C) 3OBG, (D) 3GCP, (E) 3ZS5 and (F) 2EWA with SB203580. Yellow dots represent hydrogen bond and stacking interactions

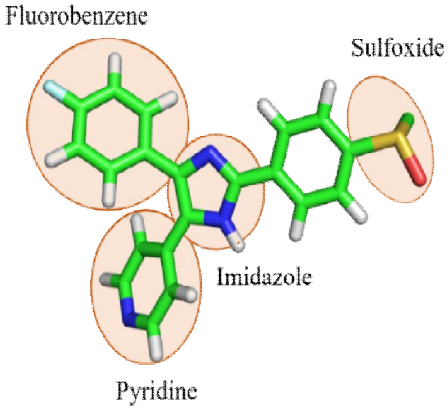


Fig.6: Structure of type I inhibitor of kinase (SB-203580).

CONCLUSIONS

Significant differences between six structures of P38 MAP kinase and type I inhibitor are restricted to the ATP -bind pocket, where ligand-induced conformational differences are observed in the hinge

region (His104 and Met106) and the DFG motif. SB-203580, type I inhibitor is bound mostly in region that are conserved in protein kinase for the bonding of ATP. The DFG-out strongly inhibited by SB-203580 that the interaction is the aromatic stacking interaction between the phenyl group of the inhibitor and Tyr32, which point into the ATP pocket. Experiments or calculations and use the reported results. Although not everything need be disclosed, a paper must contain new, useable, and fully described information. For example, a specimen's chemical composition need not be reported if the main purpose of a paper is to introduce a new measurement technique. Authors should expect to be challenged by reviewers if the results are not supported by adequate data and critical details.

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REFERENCES

[1] Bukhtiyarova, M., Karpusas, M., Northrop, K., Namboodiri, H.V.M., Springman, E.B., Biochem., 2007; 46: 5687-96.
[2] Freshney, N.W., Rawlinson, L., Guesdon, F., Jones, E., Cowley, S., Hsuan, J., and Saklatvala, J., Cell., 1994; 78: 1039-49.Han, J., Lee, J., Bibbs, L., Ulevitch, R., Sci., 1994; 265: 808-11.Han, J., Jiang, Y., Li, Z., Kravchenko, V.V., Ulevitch, R.J. Nat., 1997; 386: 296-9.
[3] Lee, J.C., Laydon, J.T., McDonnell, P.C., Gallagher, T.F., Kumar, S., Green, D., McNulty, D., Blumenthal, M. J., Keys, J. R., Land vatter, S. W., Strickler, J. E., McLaughlin, M. M., Siemens, I. R., Fisher, S. M., Livi, G. P., White, J. R., Adams, J. L. and Young, P. R., Nat., 1994; 372: 739-46.Kumar, S., Boehm, J., Lee, J.C., Nat. Rev. Drug Discov., 2003; 2: 717-26.Huse, M., Kuriyan, J., Cell., 2002; 109: 275-82.Sun, H., Tian, S., Zhou, S., Li, Y., Li, D., Xu, L. Shen, M., Pan P. and Hou, T., Sci. Rep., 2015; 5: 8457.Apsel, B., Blair, J.A., Gonzalez, B., Nazif, T.M., Feldman, M.E., Aizenstein, B., Hoffman, R., Williams, R. L., Shokat, K. M. and Knight, Z. A., Nat. Chem. Biol., 2008; 4: 691-9.
[4] Schindler, T., Bornmann, W., Pellicena, P., Miller, W.T., Clarkson, B., Kuriyan, J., Sci., 2000; 289: 1938-42.
[5] You, W., Chang, C.-e.A., J. Chem. Inf. Model., 2018.
[6] Badrinarayan, P., Sastry, G.N., J. Chem. Inf. Model., 2011; 51: 115-29.
[7] Atzori, A., Bruce, N.J., Burusco, K.K., Wroblowski, B., Bonnet, P., Bryce, R.A., J. Chem. Inf. Model., 2014; 54: 2764-75.
[8] Kornev, A.P., Haste, N.M., Taylor, S.S., Ten Eyck, L.F., Proc. Natl. Acad. Sci., 2006; 103: 17783-8.
[9] Janne, P.A., Gray, N., Settleman, J., Nat. Rev. Drug Discov., 2009; 8: 709-23.
[10] Azevedo, R., van Zeeland, M., Raaijmakers, H., Kazemier, B., de Vlieg, J., Oubrie, A., Acta Crystallogr., Sect. D:, 2012; 68: 1041-50.
[11] Vogtherr, M., Saxena, K., Hoelder, S., Grimme, S., Betz, M., Schieborr, U., Pescatore, B., Robin, M., Delarbre, L., Langer, T., Wendt, K. U. and Schwalbe, H., Angew. Chem. Int. Ed., 2006; 45: 993-7.
[12] Regan, J., Breitfelder, S., Cirillo, P., Gilmore, T., Graham, A.G., Hickey, E., Klaus, B., Madwed, J., Moriak, M., Moss, N., Pargellis, C., Pav, S. Proto, A., Swinamer, A., Tong L. and Torcellini, C., J. Med. Chem., 2002; 45: 2994-3008.
[13] Van Der Spoel, D., Lindahl, E., Hess, B., Groenhof, G., Mark, A.E., Berendsen, H.J.C., J. Comput. Chem., 2005; 26: 1701-18.
[14] Schmid, N., Eichenberger, A., Choutko, A., Riniker, S., Winger, M., Mark, A., and van Gunsteren, W., Eur. Biophys. J., 2011; 40: 843-56.
[15] Wilson, K.P., McCaffrey, P.G., Hsiao, K., Pazhanisamy, S., Galullo, V., Bemis, G.W., Fitzgibbon, M. J., Caron, P. R., Murcko, M. A. and Su, M. S. S., Chem. Biol., 1997; 4: 423-31.
[16] Simard, J.R., Getlik, M., Grütter, C., Pawar, V., Wulfert, S., Rabiller, M. and Rauh, D., J. Am. Chem. Soc., 2009; 131: 13286-96.
[17] Malde, A.K., Zuo, L., Breeze, M., Stroet, M., Poger, D., Nair, P.C., Oostenbrink, C. and Mark, A. E., J. Chem. Theory Comput., 2011; 7: 4026-37.
[18] Berendsen, H.J.C., Postma, J.P.M., van Gunsteren, W.F., and, Hermans, J., Intermol. Forces. 1981; 331-42.
[19] Berendsen, H.J.C., Postma, J.P.M., van Gunsteren, W.F., DiNola, A., Haak, J.R., J. Chem. Phys., 1984; 81: 3684-90.
[20] Ryckaert, J.-P., Ciccotti, G., Berendsen, H.J.C., J. Comput. Phys., 1977; 23: 327-41.
[21] van Gunsteren, W.F., Beutler, T.C., Fraternali, F., King, P.M., Mark, A.E., Smith, P.E., ESCOM Sci. Publ., Leiden. 1989.
[22] Beutler, T.C., Mark, A.E., van Schaik, R.C., Gerber, P.R., van Gunsteren, W.F., Chem. Phys. Lett., 1994; 222: 529-39.
[23] Zacharias, M., Straatsma, T.P., McCammon, J.A., J. Chem. Phys. 1994; 100: 9025-31.
[24] Allen, M.P., Tidesley, D.J., Oxford Univ. Press Inc., New York. 1989.
[25] Horjales, S., Schmidt-Arras, D., Limardo, Ramiro R., Leclercq, O., Obal, G., Prina, E., et al., Struct., 2012; 20: 1649-60.
[26] Hari, Sanjay B., Merritt, Ethan A., Maly, Dustin J., Chem. Biol., 2014; 21: 628-35.
[27] Huang, Y.-ming M., Chen, W., Potter, Michael J., Chang, C.-en A., Biophys. J., 2012; 103: 342-51.
[28] Simard, J.R., Grütter, C., Pawar, V., Aust, B., Wolf, A., Rabiller, M., and Rauh, D., J. Am. Chem. Soc., 2009; 131: 18478-88.

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