Molecular Dynamic Simulation of 3-(5-Chloro-2, 4-dihydroxyphenyl)-pyrazole-4-carboxamide and HSP90 Molecular Chaperone Interaction

LEILA BARAMAKEH

Department of Chemistry, Marvdasht Branch,
Islamic Azad University, Marvdasht , Iran
baramakeh@yahoo.com

Received 13 November 2010; Accepted 21 January 2011

Abstract: The calculation of free energy differences of a system is of great importance as the rate and extent of many if not all chemical and biophysical processes are governed by the nature of underlying free energy landscape. In this study the preferential binding of 3-(5-chloro-2, 4-dihydroxyphenyl)-pyrazole-4-carboxamide (4BC) and Heat shock protein 90(Hsp90) molecular chaperone has been evaluated using molecular dynamics simulation. A soft core potential was used during the mutations to facilitate the creation and deletion of atoms. Trajectory analysis showed a stable equilibrium after energy minimization. Potential energy plot showed equilibrium around -69520 and -183859 kJ/mol for Hsp90 and Hsp90-4BC. Kinetic energy also was calculated for Hsp90 and Hsp90-4BC as 44500 and 65928.29 kJ/mol, respectively.

Keywords: Molecular chaperone, Heat shock protein 90, Dihydroxyphenyl pyrazole

Introduction

Molecular chaperones are proteins which have a key role in the maintenance of conformation, stability and function of client proteins within the cell. Heat shock protein 90 (HSP90) is a molecular chaperone that has several oncogenic client proteins involved in signal transduction, cell cycle regulation and apoptosis and has recently become a focus of interest as a potential anticancer drug target¹⁻⁶. It has been shown that several natural products and their derivatives have anti-tumor activity arising from inhibition of the intrinsic ATPase activity of Hsp90⁷⁻¹⁰. Inhibition of Hsp90 activity results in the proteasomal degradation of client proteins causing cell growth arrest and/or apoptosis in cancer cells¹¹.

The geldanamycin derived inhibitor 17-AAG (1b) has entered phase II clinical trials and initial results are encouraging, providing proof of principle for Hsp90 inhibitors as cancer therapeutics^{12–14}. However, 17-AAG has several potential limitations including poor solubility, limited bioavailability, toxicity and extensive metabolism¹⁵. These issues and the inherent chemical complexity of the compounds have led to significant efforts to identify small molecule inhibitors of Hsp90^{16–19}. Figure 1 shows some of the chemo types recently identified as potent inhibitors of Hsp90^{16,20,17}. Estimating differences in free energy is central to the process of rational molecular design. This is because all equilibrium properties of a system such as phase behavior, association-dissociation constants, solubility's, adsorption coefficients and conformational equilibrium depend on differences in free energy between alternative states. Free energy differences are essentially related to the relative probability of finding a system in a given microscopic state. Many empirical approaches have been developed to estimate interaction or binding free energies between proteins and ligands. However, only by using an approach that samples an appropriate thermodynamic ensemble of states, such as molecular dynamics (MD) and monte carlo (MC) simulation techniques, from which it is possible to get thermal averages over microscopic configurations at an atomic level, can differences in free energy between two states of a system, be estimated directly^{21–27}. In this manuscript, the relative binding affinity of 3-(5-chloro-2, 4-dihydroxyphenyl)-pyrazole-4-carboxamid to heat-shock protein 90, an anticancer target is evaluated using molecular dynamic simulation.

Figure 1. Structures of the identified dihydroxyphenylpyrazole compounds

Experimental

Topology of the blocker was generated by manually adapting the output of PRODRG³⁰ for the force field²⁹ GROMOS53a5. The starting structure for the heat shock protein 90 was a crystallographic coordinate obtained from protein data bank (PDB code 1YC1)³².

Molecular dynamic simulation

The protein molecule placed in a truncated octahedral box with an edge approx 0.5 nm from the molecule(s) and consequently solvated with 7295 water molecules per amino to give a system of around 7305 molecules per experiment. As each helix has a net charge of -8, positive sodium ions were also added to compensate for any extra charge within the simulation box. The solvated system was equilibrated for 200 ps with the protein restrained, followed by the production phase of fully unrestrained 200 ps MD simulation. For MD simulations GROMACS v 3.1¹ software was used. Simulations were run at 300° K in an isothermal isobaric ensemble (NPT)³². Periodic boundaries were present and a Berendsen temperature and pressure coupling was chosen to keep these parameters constant. The time step for the simulation was 0.002 ps. A Linear Constraint³³ algorithm was used to maintain the geometry of the molecules. Long-range electrostatics was calculated with the particle-mesh Ewald (PME)³⁴ method using standard GROMACS parameters: grid dimensions 0.12 nm,

interpolation order 4. Double counting correction for short-range forces was applied. Lennard Jones and short-range coulomb interactions were cut off at 1.1 and 0.9 nm, respectively. The simple point charge water model “SPC”³⁵ was used to describe the water in the simulation box. Simulations were performed on a Pentium M 725 processor with a dual Centrino 1.6 GHz processor.

Structure was visualized using VMD (Visual Molecular Dynamic 1.8.4, <http://www.ks.uiuc.edu/Research/vmd/>)³⁶. The trajectory was viewed using drawing methods of licorice and surface³⁷ for drug and amino acid, respectively. The coloring method of charge was used to draw the amino acid.

Results and Discussion

The simulation gave rise to stable trajectory, indicating that the system has equilibrated as seen in Figure 2. The root mean square deviation (RMSD) of the backbone atoms of amino acid (Figure 3), without blocker and in the presence of it remains below 0.12 nm with respect to their initial conformations.

Figure 2. Stereo view of the average MD structure of the simulated Hsp90 and its inhibitor (4BC); the stick models represent inhibitor. The protein active site region is shown as a surface model

Figure 3. Root-mean-square deviation (RMSD) of Hsp90s backbone without blocker (—●—), and in the presence of the inhibitor, 4BC (—▲—)

The presence of 4BC, the inhibitor, only has a little effect on RMSD. Over a time span of the first 20 ps the RMSD values for the protein in the presence of blocker are slightly higher than it without blocker. After that RMSD for the protein with the 4 BC remains approximately 0.15 nm higher than the value for the bundle without blocker. The drug RMSD (Figure 4) reach a plateau at ~ 0.1 nm after about 13 ps. The backbone RMSD indicates that the rigid protein structure equilibrates rather quickly but the drug does not equilibrate until after 15 ps. The RMSD for the drug is more variable indicative of its mobility within the binding pocket. Temperature fluctuation was calculated. The temperature plot (Figure 5) shows the normal oscillation behavior of the temperature about the desired average (300 K).

Figure.4. Root-mean-square atom positional deviation (RMSD) in nm from the x-ray (crystal) structure as function of time in ps for the simulation of 4BC using the GROMOS96 force field.

Figure 5. Time evolution of temperature for simulated Hsp90-4BC box

The pressure fluctuation also plotted (Figure 6). As it was expected it is variable around zero including no unexpected deviation, showing the stability of the simulation. 200 ps dynamics simulations were performed for amino acid alone and in the presence of the drug. Plots of potential and kinetic energy were shown in Figures 7 and 8 for amino acid and amino acid in presence of drug, respectively. The potential energy equilibrates about averages of ~ -69520 and -183859 kJ/mol for protein and protein with inhibitor, respectively. Kinetic energy was calculated around 44500 and 65928 KJ/mol for protein and protein with inhibitor, respectively. We have analyzed the MD trajectories for stable hydrogen bonds between the drug and the protein (Figure 9). Stability of hydrogen bonds leads us to conclude that it could be relatively important to the complex and it should be responsible in stability of drug –amino acid interaction.

Figure 6. Time evolution of pressure for simulated Hsp90-4BC box

Figure 7. Plot of gromacs energies of Hsp90-4BC using GROMOS96 force field

Figure 8. Plot of gromacs energies of Hsp90 using GROMOS96 force field

Figure 9. Number of hydrogen bonds between drug and amino acid

Conclusion

Over the past decade, heat-shock protein (Hsp90) has begun to draw increasing attention as a novel anticancer target with unique features. As a molecular chaperone, Hsp90 promotes the maturation and maintains the stability of a large number of conformational labile client proteins, most of which are involved in biologic processes that are often deranged within tumor cells, such as signal transduction, cell cycle progression and apoptosis. As a result and in

in contrast to other molecular-targeted therapeutics, inhibitors of Hsp90 achieve their promising anticancer activity through simultaneous disruption of many oncogenic substrates within cancer cells. A series of dihydroxyphenylpyrazole compounds were identified as a unique class of reversible Hsp90 inhibitors. In this study, we compared solvated Hsp90 and Hsp0 bonded 4BC (a dihydroxyphenylpyrazole) by MD simulations. The simulation was done using gromacs software and force field GROMOS 96. The simulations showed stable trajectory for both of them indicating a stable equilibrium after energy minimization. The atom-positional RMSD from the starting (x-ray) structure of the drug reach a plateau after a few nanoseconds indicating that it will reach a stable equilibrium after energy minimization although it is more variable indicative of its mobility within the binding pocket.

References

1. Jolly C and Morimoto R I, *J Natl Cancer Inst.*, 2000, **92(19)**, 1564-1572.
2. Banerji U, Judson I and Workman P, *Curr Cancer Drug Targets*, 2003, **3(5)**, 385-390.
3. Neckers L and Ivy S P, *Curr Opin Oncol.*, 2003, **15**, 419-424.
4. Solit D B, Scher H I and Rosen N, *Semin Oncol.*, 2003, **30**, 709-716.
5. Whitesell L, Bagatell R and Falsey R, *Curr Cancer Drug Targets*, 2003, **3(5)**, 349-358.
6. Kelland L R, Sharp S Y, Rogers P M, Myers T G and Workman P, *J Natl Cancer Inst.*, 1999, **91(22)**, 1940-1949.
7. Schulte T W and Neckers L M, *Cancer Chemother Pharmacol.*, 1998, **42(4)**, 273-279.
8. Schulte T W, Akinaga S, Soga S, Sullivan W, Stensgard B, Toft D and Neckers L M, *Cell Stress Chaperones*, 1998, **3(2)**, 100-108.
9. Neckers L, Schulte T W and Mimnaugh E, *Invest New Drugs*, 1999, **17(4)**, 361-373.
10. Kaur G, Belotti D, Burger A M, Fisher-Nielson K, Borsotti P, Riccardi E, Thillainathan J, Hollingshead M, Sausville E A and Giavazzi R, *Clin Cancer Res.*, 2004, **10(14)**, 4813-4821.
11. Hostein I, Robertson D, Distefano F, Workman P and Clarke P A, *Cancer Res.*, 2001, **61(10)**, 4003.
12. Banerji U, Donnell O, Scurr M, Pacey S, Stapleton S, Asad Y, Simmons L, Maloney A, Raynaud F, Campbell M, Walton M, Lakhani S, Kaye S, Workman P, Judson I, *J Clin Oncol.*, 2005, **23(18)**, 4152-4161.
13. Goetz M P, Toft D, Reid J, Ames M M, Stensgard B, Safgren S and Sloan J, Atherton P, Vasile V, Salazaar S, Adjei A, Croghan G and Erlichman C, *J Clin Oncol.*, 2005, **23**, 1078-1087.
14. Grem L, Morrison G, Guo X, Agnew E, Takimoto C H, Thomas R, Szabo E, Growchow L, Grollman F, Hamilton J M, Neckers L and Wilson R H, *J Clin Oncol.*, 2005, **23(9)**, 1885.
15. Workman P, *Cancer Lett.*, 2004, **206**, 149-157.
16. Vilenchik M, Solit D, Basso A, Huezo H, Lucas B, He H, Rosen N, Spampinato C, Modrich P and Chiosis G, *Chem Biol.*, 2004, **11(6)**, 787-797.
17. Cheung K M J, Matthews T P, James K, Rowlands M G, Boxall K J, Sharp S Y, Maloney A, Roe S M, Prodromou C, Pearl L H, Aherne G W, McDonald E and Workman P, *Bioorg Med Chem Lett.*, 2005, **15**, 3338.
18. Dymock B W, Barril X, Brough P A, Cansfield J E, Massey A, McDonald E, Hubbard R. E, Surgenor A, Roughley S D, Webb P, Workman P, Wright L and Drysdale M, *J Med Chem.*, 2005, **48(13)**, 4212-4215.
19. Llauger L, He H, Kim J, Aguirre J, Rosen N, Peters U, Davies P and Chiosis G, *J Med Chem.*, 2005, **48(8)**, 2892-2905.

20. Dymock B, Barril X, Beswick M, Collier A, Davies N, Drysdale M, Fink A and Fromont C, Hubbard R E, Massey A, Surgenor A and Wright L, *Bioorg Med Chem Lett.*, 2004, **14**(2), 325-328.
21. Van Gunsteren W F and Berendsen H J C, *Angew Chem Int Ed Engl.*, 1990, **29**, 992.
22. Kollman PA, *Chem Rev.*, 1993, **93**, 2395.
23. Mark A E, van Helden S P, Janssen L H M and Van Gunsteren W F, *J Am Chem Soc.*, 1994, **116**, 6293.
24. Mark A E, Free Energy Perturbation Calculations in Encyclopedia of Computational Chemistry, van Rague Schleyer; John Wiley & Sons, New York, 1998, **2**, 1070-1083.
25. Dixit S B and Chipot C, *J Phys Chem A.*, 2001, **105**, 9795-9799.
26. Van Gunsteren W F, Daura X and Mark A E, *Helv Chim Acta*, 2002, **85**, 3113-3129.
27. Chipot C and Pearlman D A, *Mol Sim.*, 2002, **28**, 1-12.
28. Van Aalten D M F, *J Comput Aided Mol Des.*, 1996, **10**, 255-262.
29. Oostenbrink C, Villa A, Mark A E and Van Gunsteren W F, *J Comput Chem.*, 2004, **25**, 1656-1676.
30. Kreusch A, Han S, Brinker A, Zhou V, Choi H S, He Y, Lesley S A, Caldwell J and Gu X, *Bioorg Med Chem Lett.*, 2005, **15**(5), 1475-1478.
31. Lindahl E, Hess B and van der Spoel D, *Mol Model.*, 2001, **7**, 306.
32. Berendsen H J C, Postma J P M, van Gunsteren W F, DiNola A and Haak J R, *J Chem Phys.*, 1984, **81**, 3584-3690.
33. Hess B, Bekker H, Berendsen H J C and Fraaije J G E M, *J Comp Chem.*, 1997, **18**, 1463-1472.
34. Darden T, York D and Pedersen L, *J Chem Phys.*, 1993, **98**, 10089-10092.
35. Berendsen H J C, Postma J P M, van Gunsteren W F and Hermans J, Interaction Models for Water in Relation to Protein Hydration in Intermolecular Forces, B. Pullman, Editor. D. Reidel Publishing Co., Dordrecht. 1981, 331-342.
36. Humphrey W, Dalke A and Schulten K, *J Mol Graphics*, 1996, **14.1**, 33-38
37. Varshney A, Jr Brooks F P and Wright W V, IEEE, Comp Graphics and Applications 1994, **14**(5), 19-25.