 DOI: 10.18231/2394-2797.2017.0016

Molecular dynamics simulations: A mechanistic probe for understanding antibacterial activity

Arati Prabhu

Associate Professor, Dept. of Pharmaceutical Chemistry, Viva Institute of Pharmacy, Thane, Maharashtra

Email: aratisp@gmail.com

Abstract

Introduction: Molecular dynamics, in which molecules are allowed to interact over time at a given temperature following the laws of classical mechanics has been shown to be highly successful in simulating various biological phenomena down to atomic detail. When applied to complexes of protein targets and potential binders, this technique, provides a detailed description of the stability of the protein-ligand complexes, thereby throwing light on the binding potential of the ligands to probable target proteins.

Materials and Method: In the present study we have explored the mechanism of protein synthesis inhibition by the antibiotic thiostrepton with the combination of manual docking followed by molecular dynamics simulations to two molecular targets – the L11/23S-rRNA interface and the elongation factor Ef-Tu. Cross-docking runs on the two native co-crystallised ligands of the target proteins were done as a further probe.

Results: Docking of thiostrepton at the L11/23S-rRNA interface as well as Ef-Tu indicated stable binding during 10-ns molecular dynamics simulations, whereas LFF571 binds stably only to its native protein EF-Tu which is in accordance to literature reports. Thus, molecular dynamics simulation studies indicate that thiostrepton has binding potential to two targets of protein synthesis translation, the elongation factor (Ef-Tu) and L11 protein and 23S-rRNA interface.

Conclusion: This study corroborates that thiostrepton inhibits Ef-Tu in addition to the L11-ribosomal RNA complex. Binding to multiple targets probably enhances its inhibitory effect on protein translation.

Keywords: Thiazolyl peptide, Macrocyclic, Ef-Tu, Molecular dynamics simulations, Thiostrepton, Ribosome

Introduction

Thiostrepton, a thiazolyl peptide antibiotic that is produced by several strains of streptomyces such as Streptomyces azureus and Streptomyces laurentii exhibits potent antibacterial and antimalarial activities.[1,2,3]

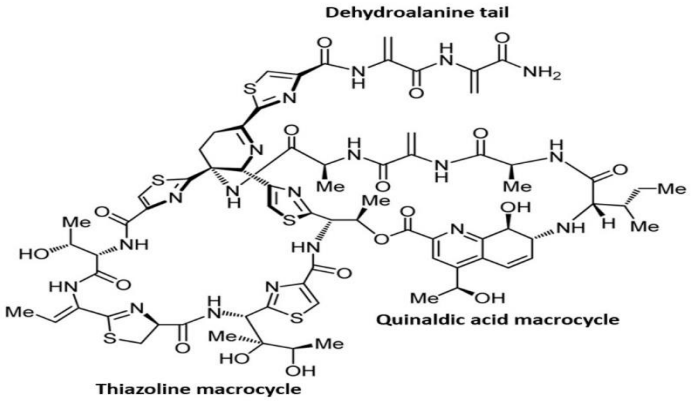

Fig. 1: Structure of thiostrepton

It belongs to a class of macrocyclic thiopeptide antibiotics.[4] Structurally, it consists of one macrocycle containing three thiazoles and a dihydrothiazole (thiazoline macrocycle), fused via a reduced pyridine ring to a second macrocycle containing a quinaldic acid ring structure and a dehydroalanine tail. (Fig. 1)

The thiazolyl peptide class of antibiotics inhibit bacterial growth by impeding its protein biosynthesis. Functionally, they are known to target the GTPase-associated center (GAC) of the ribosome to inhibit translation factor function.[5] Thiostrepton is best known as an inhibitor of mRNA–tRNA translocation at the ribosome junction.[6,7] The antibiotic binds to the 23S rRNA of the 50S ribosomal subunit at the same site as the L11 ribosomal protein.[8] This prevents the normal conformational transition that occurs from 23S-L11 interaction and results in a stalling of translation.[9,10] An older study also reports Thiostrepton as a target for elongation factor EF-Tu (GTP)–catalyzed aa-tRNA delivery.[11]

Thus, while maintaining a common mechanism of action of inhibiting protein biosynthesis, two different targets have been reported for the thiostrepton in separate studies. The present work was undertaken to

understand the binding and interaction dynamics of thiostrepton to the two reported targets, elongation factor, EF-Tu and the L11-23S rRNA interface region in the 50S subunit of the ribosome. Molecular dynamics simulations (MDS) were carried out on complexes of thiostrepton with EF-Tu as well as within the L11-23S rRNA interface region in the 50S ribosomal subunit and the stability of the complexes was observed. Comparison was done with parallel MDS studies done on the semisynthetic thiazolyl peptide, LFF571[12,13] which is known to bind exclusively to EF-Tu.

Materials and Method

The NMR structure of the thiostrepton/ L11/RNA complex (pdbid- 2jq7) and the crystal structure of LFF571-bound Ef-Tu (pdbid- 3u2q) were available and downloaded from the Protein Data Bank and used for this study. The cross-complexes of thiostrepton in EF-Tu and LFF571 at L11/RNA complex were built within Maestro-v10.5 (Schrodinger Suite 2016-1 – Academic License) by manual docking of the ligands within the respective binding sites and performing energy minimization using steepest descents until a gradient threshold of 1.0 kcal/mol/Å was reached. Desmond-v4.5 as implemented in the Schrodinger package was used for the minimization exercise. The OPLS 2005 force field was used to model protein-ligand interactions, and the SPC model was used for simulating water. The particle-mesh Ewald method (PME) was used to calculate long-range electrostatic interactions with a Ewald tolerance of $1e^{-09}$. Van der Waals and short range electrostatic interactions were smoothly truncated at 9.0.

Molecular dynamics (MD) simulations on the minimized complexes was performed using Desmond-v4.5 as implemented in Schrödinger package with 10ns (nanoseconds) simulation time. Tesla K40 GPU (NVIDIA Corporation) was used for performing the 10ns simulations. Nose–Hoover thermostats were utilized to maintain the constant simulation temperature and the Martyna–Tobias–Klein method was used to control the pressure. The equations of motion were integrated using the multistep RESPA integrator with an inner time step of 2.0 fs for bonded interactions and non-bonded interactions within the short range cutoff. An outer time step of 6.0 fs was used for non-bonded interactions beyond the cutoff. Periodic boundary conditions were applied throughout.

Each system was equilibrated with the default protocol provided in Desmond, which consists of a series of restrained minimizations and molecular dynamics simulations designed to slowly relax the system, while not deviating substantially from the initial protein co-ordinates. 10-ns Molecular dynamics simulation was then run on both the relaxed systems with a NPT ensemble using a Berendsen thermostat at 300 K and the trajectory was recorded after every 4.8 ps.

Root Mean square deviations (RMSD) of the ligands thiostrepton and LFF571 within the L11/RNA interface and the EF-Tu binding pocket with respect to the simulation time were assessed for evaluating the stability of the docked complexes.

Crossdocking of thiostrepton within Ef-Tu protein and LFF571 at the L11/RNA interface were also performed using the same procedure.

Results and Discussion

The stability of LFF571 and Thiostrepton in their native proteins and potential for binding to each other's parent was checked by using molecular dynamics. The mean ligand atom RMSD as compared to the starting structure over the 10ns simulation period are shown in Fig. 2.

LFF571 showed a stable pose only in its native protein EF-Tu (3U2Q) during the 10ns simulation with a mean ligand heavy atom rmsd of 1.636 + 0.2 with the starting pose. Whereas at the L11/RNA interface in 2JQ7, the ligand showed a consistently increasing deviation from its original position with a mean ligand heavy atom rmsd of 2.839 + 0.47 which demonstrates its poor binding potential for this target.

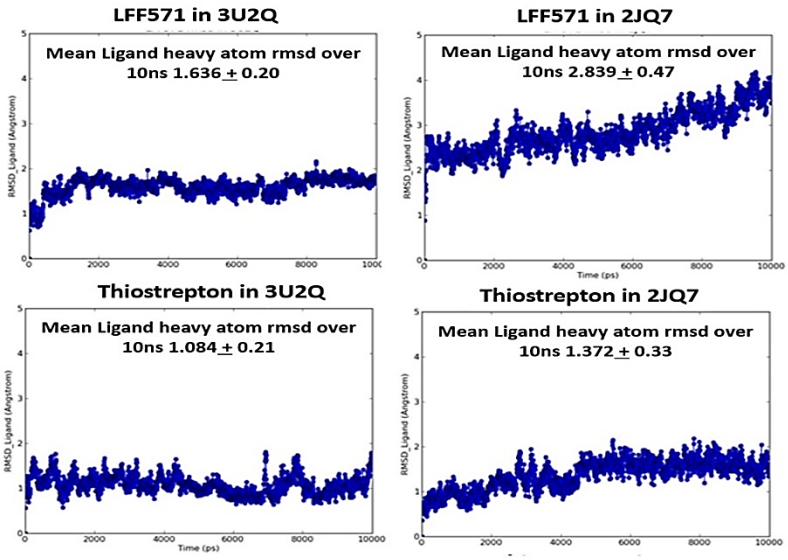

Fig. 2: Root Mean Square Deviation of LFF571 (top panel) and Thiostrepton (bottom panel) in EF-Tu (pdb - 3U2Q) and L11/RNA interface (pdb - 2JQ7)

Thiostrepton on the other hand showed stable poses in both self-docking to L11/RNA interface (mean ligand heavy atom rmsd of 1.372 + 0.33 in 2JQ7) and cross-docking to EF-Tu (mean ligand heavy atom rmsd of 1.084 + 0.21 in 3U2Q) environments after 10ns, thereby consolidating the previous reports of thiostrepton binding at both the interface of L11 /RNA as well as the elongation factor EF-Tu.

Conclusion

The thiazolyl peptide class of antibiotics are known to inhibit the translation stage of protein synthesis. Within translation, two different targets have been reported for these peptides. Peptides like LFF571 bind to the prokaryotic elongation factor, EF-Tu while thiostrepton falls under the group that directly target the L11-23S rRNA interface. In addition, older literature reports also indicate a probable inhibition of elongation factor EF-Tu by thiostrepton.

Our studies show that thiostrepton can bind stably to both the proteins implicated in translation pathways which points towards the interesting possibility of multiple targeting of thiostrepton in inhibiting protein synthesis.

Acknowledgements

The support provided by NVIDIA Corporation in the form of donation of Tesla K40 GPU used for this research is gratefully acknowledged.

References

1. Donovick R, Pagano JF, Stout HA, Weinstein MJ. Thiostrepton, a new antibiotic. I. In vitro studies. Antibiot Annu. 1955; 3: 554–9.
2. Aminake MN, Schoof S, Sologub L, Leubner M, Kirschner M, Arndt HD, Pradel G. Thiostrepton and derivatives exhibit antimalarial and gametocytocidal activity by dually targeting parasite proteasome and apicoplast. Antimicrob. Agents Chemother. 2011; 55:1338–1348.
3. Clough B, Strath M, Preiser P, Denny P, Wilson IR. Thiostrepton binds to malarial plastid rRNA. FEBS Letters. 1997; 406:123–125.
4. Mocek U, Zeng Z, O'Hagan D, Zhou P, Fan L-DD, Beale JM, Floss HG. Biosynthesis of the modified peptide antibiotic thiostrepton in Streptomyces azureus and Streptomyces laurentii. J. Am Chem. Soc. 1993; 115: 7992 – 8001.
5. Harms JM, Wilson DN, Schluenzen F, Connell SR, Stachelhaus T, Zaborowska Z, Spahn CMT, Fucini P. Translational regulation via L11: molecular switches on the ribosome turned on and off by thiostrepton and micrococcin. Mol. Cell 2008; 30: 26–38.
6. Pestka S. Thiostrepton: A ribosomal inhibitor of translocation. Biochem. Biophys. Res. Commun. 1970; 40:667–674.
7. Rodnina MV, Savelsbergh A, Matassova NB, Katunin VI, Semenkov YP, Wintermeyer W. Thiostrepton inhibits the turnover but not the GTPase of elongation factor G on the ribosome. Proc. Natl. Acad. Sci. 1999; 96: 9586–9590.
8. Thompson J, Cundliffe E. The binding of thiostrepton to 23S ribosomal NA. Biochimie 1991; 73:31-35.
9. Xing Y, Draper DE. Cooperative interactions of RNA and thiostrepton antibiotic with two domains of ribosomal protein L11. Biochemistry 1995; 35: 1581 – 1588.
10. Porse BT, Leviev I, Mankin AS, Garrett RA. The antibiotic thiostrepton inhibits a functional transition within protein L11 at the ribosomal GTPase centre. J. Mol. Biol. 1998; 276:391-404.
11. Modolell J, Cabrer B, Parmeggiani A, Vazquez D. Inhibition by siomycin and thiostrepton of both aminoacyl-tRNA and factor-G binding to ribosomes. Proc. Natl. Acad. Sci. 1971; 68: 1796–1800.
12. LaMarche MJ, Leeds JA, Amaral A, Brewer JT, Bushell SM, Deng G, Dewhurst JM, Ding J, Dzink-Fox J, Gamber G, Jain A, Lee K, Lee L, Lister T, McKenney D, Mullin S, Osborne C, Palestrant D, Patane MA, Rann EM, Sachdeva M, Shao J, Tiamfook S, Trzasko A, Whitehead L, Yifru A, Yu D, Yan W, Zhu. Discovery of

LFF571: an investigational agent for Clostridium difficile infection. J Med Chem. 2012; 55: 2376-2387.

13. Leeds JA, Sachdeva M, Mullin S, Dzink-Fox J, LaMarche MJ: Mechanism of action of and mechanism of reduced susceptibility to the novel anti-Clostridium difficile compound LFF571. Antimicrob. Agents Chemother. 2012; 56: 4463-4465.