Biochimica et Biophysica Acta 1824 (2012) 1476–1483

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

# A leishmaniasis study: Structure-based screening and molecular dynamics mechanistic analysis for discovering potent inhibitors of spermidine synthase ☆

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ARTICLE INFO

*Article history:*
Received 9 February 2012
Received in revised form 18 May 2012
Accepted 31 May 2012
Available online 8 June 2012

*Keywords:*
Leishmania
Spermidine synthase
Inhibitors
Docking
Molecular dynamics simulations

ABSTRACT

Protozoa *Leishmania donovani (Ld)* is the main cause of the endemic disease leishmaniasis. Spermidine synthase (SS), an important enzyme in the synthetic pathway of polyamines in *Ld*, is an essential element for the survival of this protozoan. Targeting SS may provide an important aid for the development of drugs against *Ld*. However, absence of tertiary structure of spermidine synthase of *Leishmania donovani* (LSS) limits the possibilities of structure based drug designing. Presence of the same enzyme in the host itself further challenges the drug development process. We modeled the tertiary structure of LSS using homology modeling approach making use of homologous X-ray crystallographic structure of spermidine synthase of *Trypanosoma cruzi* (TSS) (2.5 Å resolution). The modeled structure was stabilized using Molecular Dynamics simulations. Based on active site structural differences between LSS and human spermidine synthase (HSS), we screened a large dataset of compounds against modeled protein using Glide virtual screen docking and selected two best inhibitors based on their docking scores (−10.04 and −13.11 respectively) with LSS and having least/no binding with the human enzyme. Finally Molecular Dynamics simulations were used to assess the dynamic stability of the ligand bound structures and to elaborate on the binding modes. This article is part of a Special Issue entitled: Computational Methods for Protein Interaction and Structural Prediction.



## 1. Introduction

Leishmaniasis is endemic in areas of the tropics, subtropics, and southern Europe, in settings ranging from rain forests in the Americas to deserts in western Asia, and from rural to periurban area [1]. Leishmaniasis is mainly caused by *Ld*, an obligate intramacrophage protozoa and spread by the bite of the sandfly [1]. Polyamine biosynthetic pathways have been target of interest for the development of antiprotozoal pharmaceutical molecules. Polyamines are the low molecular weight aliphatic polycations and are essential for growth and development of prokaryotes and eukaryotes [2,3]. Eflornithine, also known as D, L-α-difluoromethylornithine (DFMO) has been successfully tested in the treatment of few of the protozoan infection such as *Trypanosoma brucei gambiense* infection (West African sleeping sickness) [4–7]. DFMO binds irreversibly with ornithine decarboxylase (ODC), the initial and the rate limiting enzyme in the polyamine biosynthetic pathway and is involved in the conversion of ornithine to putrescine [8]. However, DFMO failed to show promising results against *Ld* infection [9]. Another enzyme spermidine synthase (SS), the enzyme immediately downstream of ODC, is also used as a target to check the polyamine biosynthesis in parasitic protozoa including *Ld* [10–15]. SS has been reported as an essential enzyme for the virulence of *Ld* [10] and can be used to develop an effective drug against *Ld* infections. SS is also known as S-adenosylmethioninamine: putrescine, 3-aminopropyltransferase. SS catalyzes the transfer of an aminopropyl moiety of decarboxylated S-adenosylmethionine on putrescine leading to the production of spermidine and methylthioadenosine [16].

Structure based drug development is an effective and popular approach to search inhibitors against a protein target [17,18]. But such an approach is inapplicable in case of LSS as the crystal structure of LSS has not yet been solved experimentally. However, structure of orthologous SS from other species such as Trypanosome, Plasmodium, Human, Escherichia, and Arabidopsis has been studied by using X-ray crystallographic techniques [19–22]. SS from these species is a homomeric protein which may contain two to four identical chains in quaternary form. One monomer unit of SS from *Thermotoga*

*Abbreviations: Ld*, *Leishmania donovani*; SS, Spermidine synthase; LSS, Spermidine synthase of *Leishmania donovani*; TSS, Spermidine synthase of *Trypanosoma cruzi*; HSS, Spermidine synthase of human; DFMO, D, L-α-difluoromethylornithine; ODC, Ornithine decarboxylase; MD, Molecular dynamics; HTVS, High throughput virtual screening; RMSD, Root mean square deviation; ROG, Radius of gyration

☆ This article is part of a Special Issue entitled: Computational Methods for Protein Interaction and Structural Prediction.

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doi:10.1016/j.bbapap.2012.05.016

*maritima* consists of two domains, a six β-stranded N-terminal domain and a Rossmann-like C-terminal domain [20]. LSS shares significant sequence similarity with its orthologs, such as ~99% sequence similarity with TSS and ~96% sequence similarity with HSS. High sequence similarity of LSS with its orthologs can be used to predict its tertiary structure by using the computational homology modeling technique [23]. Since spermidine is one of the most ubiquitous polyamines and is catalyzed by SS [22], its ubiquitous nature limits in the process of antiprotozoal drug development in humans. High sequence similarity between LSS and HSS (~96%) indicates the high structural similarity between both the orthologs. Targeting LSS under such circumstances may also damage host SS. One approach to eradicate *Ld* by targeting LSS may be based on the stability of LSS and lability of HSS as present in the case of other polyamine biosynthetic pathways enzymes [13]. Since SS is a stable enzyme in both *Ld* and human cells [24], inhibitors of both enzymes are likely to affect parasite and host cells similarly, unless structural differences can be exploited to selectively target the parasite enzyme [13]. Hence, considering all the limitations in the drug development approach against *Ld*, it becomes a necessity to know the structure of LSS.

In our work, we search for effective inhibitors against LSS using structure based drug designing approach. The first step is to predict the tertiary structure of LSS using homology modeling and molecular dynamic techniques. A comparative study between HSS and LSS was then performed to investigate the structural difference between LSS and HSS proteins. Based on these structural differences, we report novel small molecule inhibitors of LSS protein but showing poor or no inhibition of HSS protein, which are thus specific for the inhibition of only LSS.

## 2. Materials and methods

### 2.1. Homology modeling

A 300 amino acid long protein sequence of LSS was retrieved from the NCBI protein database (Accession no. AAG24612). Position-Specific Iterated BLAST (PSI-BLAST) was performed against the PDB database to know the homologous sequences [25–28]. Crystal structure from *Trypanosoma Cruzi* at 2.5 Å resolutions (3BWB) was selected as template based on the 99% query coverage, best e-score ($2e^{-114}$), and highest 65% identity with the protein sequence of LSS. Amino acid sequence of selected template (TSS) was retrieved from PDB and aligned with the amino acid sequence of LSS by using CLUSTAL X stand alone software [29–31]. Aligned sequences were carefully checked manually to avoid deletions or insertions in conserved regions and corrected wherever necessary. MODELLER version 9.7 was used to build homology model of LSS from crystal structure of TSS. Loops of generated models were refined by using loop refinement protocol with DOPE potential [32] of MODELLER. The root mean square deviation (RMSD) of the models relative to the template (3BWB) was calculated using PYMOL [33]. Out of several models, the one with best values of Verify3D [34,35], Errat [36], and RAMPAGE [37] was selected for further stabilization of modeled protein by using molecular dynamics simulations [38].

### 2.2. Molecular Dynamics simulations

All Molecular Dynamics (MD) simulations were performed on Desmond Molecular Dynamics system [39,40] using OPLS all-atom force field 2005 [41,42]. All protein complex systems for MD simulations were prepared by Desmond set up wizard. Prepared system was neutralized using an appropriate number of ions and was solvated by octahedral periodic box of SPC water molecules. The distance between box wall and protein complex was set to more than 10 Å to avoid direct interaction with its own periodic image. Energy of prepared systems for MD simulation was minimized up to maximum 3000 steps using steepest descent method until a gradient threshold (25 kcal/mol/Å) is reached. The systems were equilibrated with the default protocol provided in Desmond. Further MD simulations were carried on the equilibrated systems for desired period of time at constant temperature of 300 K and constant pressure of 1 atm with a time step of 2 fs. During the MD simulations smooth particle Mesh–Ewald method was used to calculate Long range electrostatic interactions. A 9 Å radius cut-off was used for coulombic short range interaction cutoff method. Frames of trajectory were captured at each 4.8 ps time step.

Model protein and crystallized HSS were prepared for MD simulation using parameters described above. Prepared proteins were then continuously simulated for a time period until the proteins become stabilized. Stability of docked ligands with modeled protein was also investigated through MD simulation. Docked protein–ligand complexes were simulated for 10 ns time period using similar parameters as described for other simulations in this study.

RMSD of all atoms as well as selected molecules has been calculated through the simulation with reference to first frame. The hydrophobic interactions and H-bonds were calculated using Ligplot program [43] where the H-bonds were defined as acceptor–donor atom distances of less than 3.3 Å, hydrogen-acceptor atom distance of maximum 2.7 Å, and acceptor-H–donor angle greater than 90°. Average structures were generated over last few frames from the simulation for both the modeled LSS protein as well as HSS protein.

### 2.3. Structure validation

The overall stereochemical quality of the final developed model was assessed by the program RAMPAGE. Environment profile of final developed model was checked using Verify-3D (Structure Evaluation Server). Reliability of the modeled protein was computed using ERRAT protein structure verification algorithm.

### 2.4. Active site comparison of LSS and HSS

CASTp calculation server was used to calculate the volume and shape of active site cavity in LSS and HSS [44,45]. Stabilized modeled protein structures of LSS and HSS (generated average structures) were compared by superimposition technique. Superimpositions were performed on *Schrödinger's maestro* interface [46] provided along with Desmond Molecular Dynamics system.

### 2.5. Binding site identification

LSS share a highly conserved binding site with SS of other organisms. High sequential and structural similarity of LSS with other SS was used to identify its binding site accurately as binding sites have already been identified in SS of other organisms. Conserved binding site residues were identified by multiple sequence alignment of LSS protein sequence with the SS protein sequences of the other organisms by CLUSTAL X software. Location of ligand binding site in the 3D structure of protein was determined by the superimposition of AdeDATO bound tertiary structure of SS from *Thermotoga maritima* (1JQ3). AdeDATO is a reported transition state analogue and irreversible inhibitor of SS which interacts with both substrate and well as product binding residues.

### 2.6. High throughput virtual screening (HTVS) and docking studies

A dataset of approximately one million compounds taken from a 7.9 million compounds database [47] was prepared using LigPrep's ligand preparation protocol [4]. Ligand preparation involved generation of tautomeric, stereochemical, and ionization variations, as well as energy minimization and flexible filters. HSS and Modeled LSS protein structures were docked virtually against prepared dataset of compounds. As a preparation step of protein complexes, hydrogens