Original Article

Homology Modeling of *Leishmania donovani* Enolase and its Molecular Interaction with Novel Inhibitors

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**Introduction:** The treatment of Indian tropical disease such as kala-azar is likely to be troublesome to the clinicians as AmpB- and miltefosine-resistant *Leishmania donovani* has been reported. The rationale behind designed a novel inhibitors of model of *L. donovani* enolase and performing a binding study with its inhibitors to gain details of the interaction between protein residues and ligand molecules.

**Methods and Materials:** The *L. donovani* enolase model consists of two typical domains. The N-terminal one contains three-stranded antiparallel β-sheets, followed by six α-helices. The C-terminal domain composed of eleven-stranded mixed α/β-barrel with connectivity. The first α-helix within the C-terminal domain, H7, and the second β-strand, S7, of the barrel domain was arranged in an antiparallel fashion compared to all other α-helices and β-strands. The root-mean-square deviation between predicted model and template is 0.4 Å. The overall conformation of *L. donovani* enolase model is similar to those of *Trypanosoma cruzi* enolase and *Streptococcus pneumoniae* enolase crystal structures. **Result:** The key amino acid residues within the docking complex model involved in the interaction between model enolase structure and ligand molecule are Lys70, Asn165, Ala168, Asp17, and Asn213. **Conclusion:** Our theoretical prediction may lead to the establishment of prophylactic and therapeutic approaches for the treatment of kala-azar. This biomedical informatics analysis will help us to combat future kala-azar.

**Keywords:** Homology modeling, *Leishmania donovani* enolase, molecular docking, visceral leishmaniasis

**Introduction**

Visceral leishmaniasis (VL) is also known as kala-azar, a systemic protozoan disease which has been caused by *Leishmania donovani* under the effect of phlebotomine sand flies. The serious VL infections are being occurred in both young adults and children that frequently need proper treatment. This pathogen causes chronic fever that leads to severe weight loss and anemia. It infects not only our pulmonary system of infected person but other organ systems also affected simultaneously. At some conditions, extrapulmonary complications may occur in association with *L. donovani* infection as a result of direct invasion, especially central nervous system (CNS) during encephalitis. Encephalitis manifestations are greater severity and have more clinical importance than the primary respiratory infection. Over the past decade, the incidence of this organism that invades CNS has increased dramatically. Patients who suffer from persistent *L. donovani* infection are a continuous transmission source to others with in community. In fact, a number of recently developed therapies are no longer effective in treatments due to resistance from previously existed drugs. Recently, there have been a number of reports indicating that enolase enhances the virulence of some pathogens. The enolase is a cytoplasmic metalloenzyme which participates in glycolytic pathways. It is also termed as phosphopyruvate hydratase.

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**How to cite this article:** Mahato JP, Rana S, Kumar M, Sarsaiya S. Homology modeling of *Leishmania donovani* enolase and its molecular interaction with novel inhibitors. J Pharm Bioall Sci 2017;9:99-105.
It belongs to lyases family. The multifunctional protein basically serves as a plasminogen receptor over many epithelial, endothelial, and hematopoietic cells. It has a great role in invasive autoimmune disorder which was found recently in humans.\textsuperscript{[12]} There are several different enolase isozymes present within all organisms. Structurally, the enzyme consists of dimers of three different polypeptide chains, i.e., $\alpha$, $\beta$, and $\gamma$.\textsuperscript{[13-15]}

The dimer of $\alpha\alpha$ isozyme expresses in many tissues whereas $\beta\beta$ isozymes found exclusively in the muscular tissues. The $\gamma\gamma$ dimer is present in the neuroendocrine tissues and also in neurons and exclusively termed as neuron-specific enolase.\textsuperscript{[15,16]}

The enzyme enolase which participates in crucial biological metabolisms, especially glycolysis and gluconeogenesis, is highly conserved. It has overall similar fold and identical catalytic residues in archaea, bacteria, and eukaryotes. The ubiquitous presences of metabolic enzyme enolase under conserved manner in different phyla clearly indicate the existence of enolase gene as common in ancestor. The gene has been potentially diversified by speciation of organisms and smartly duplicated within organisms.\textsuperscript{[17]}

The enolase enzyme is actively engaged in such biological mechanisms in \textit{L. donovani} to cause their virulent effect in humans.\textsuperscript{[18]} It is generally found at cell membrane of \textit{Leishmania} that effectively plays infectious role host cells.\textsuperscript{[19]} \textit{Leishmania} oligopeptidase B initially infects the host macrophages and then regulates an enolase level that further facilitates the parasite to enter into the macrophages.\textsuperscript{[20]} Enolase is a key enzyme, a part of novel class of surface protein responsible for the reversible conversion of 2-phosphoglycerate and phosphoenolpyruvate in glycolysis and gluconeogenesis for vital cellular function.\textsuperscript{[12]} The microbial enolase is captured by inhibitors of known compounds, and its subsequent conversion to plasmin provides a mechanism to augment virulence, favoring host tissue invasion.\textsuperscript{[21-23]}

For such action, enolase protein must be located on the surface of microbial pathogens.\textsuperscript{[23,24]} In light of the above findings, this work is an attempt to predict molecular interaction of \textit{L. donovani} enolase with inhibitors of...
known compounds which would be useful for further investigation of the mechanism of *L. donovani* invasion to human brain.

**Materials and Methods**

The identification of the protein sequence

The primary protein sequence of *L. donovani* enolase sequence was obtained from GenBank (accession number: P75189). This protein sequence has been further entertained for computational analysis, molecular modeling, and predicted effective protein–ligand interaction with suitable ligand inhibitors. Modeling template was searched using BLAST. To analyze modeled complex of protein–protein interaction, sequence identity of 40%–50% between target and template may be required.

Multiple sequence alignment analysis

Multiple sequence alignment was performed using ClustalW. The ClustalW is a dynamic program widely used in identifying the sequence similarities between nucleotides and protein sequences. The atomic coordinates of *Escherichia coli* enolase were retrieved from Protein Data Bank (PDB: ID: 1E9I), which was most suitable for our work.

Three-dimensional structure prediction, model prediction, and protein stimulation

Molecular structures of *Mycoplasma pneumoniae* enolase were modeled using restraint-based modeling implemented in the program MODELLER. Several models were generated and then energy minimized using the molecular dynamics and simulation procedure CHARMM in program MODELLER. The structural quality and stereochemistry evaluation was carried out by Ramachandran Plot through using the program PROCHECK. The structure was also analyzed by RAMPAGE that further checks the allowed and disallowed regions of amino acid residues in Ramachandran plot. The final model was selected based on stereochemical quality. The main-chain conformations for 98.80% amino acid residues were within the favored or allowed regions of the Ramachandran plot, and the overall G-factor was 0.11, indicating that molecular geometry of the model is of good quality. The selected model was then added Mg$^{2+}$ and further refined by energy minimization by the NAMD program (http://www.ks.uiuc.edu/Research/namd/) by 2,000 steps of conjugate gradient minimization until the energy gradient root-mean-square (RMS) was <0.05 kcal (mol Å)$^{-1}$. Structural models were visualized by PyMol™ Molecular Graphics System version 0.97 (DeLano Scientific LLC, San Carlos, CA, USA, http://www.pymol.org). The VMD program was used to superimpose structure of *L. donovani* enolase model with crystal structures of enolases from *Trypanosoma cruzi* (PDB ID: 1E9I) and *Streptococcus pneumoniae* (PDB ID: 1W6T).

*L. donovani* enolase model was docked to inhibitors of known compounds using Hex 4.5 (http://www.csd.abdn.ac.uk/hex/). The atomic coordinates of inhibitors of known compounds were retrieved from PDB (PDB ID: 1B2I). Automate energy minimization was applied to each docking solution. The Chimera program (http://www.cgl.ucsf.edu/chimera/) was exploited to identify hydrogen bonds using default parameters and geometric criteria described previously. Mark Gerstein’s calc-surface program, which is implemented in the program Chimera, was used to calculate the solvent accessibility at the interface of *L. donovani* enolase and inhibitors of known compounds before and after docking.

**Results and Discussion**

Template identification and model quality crystal structures of enolases from many organisms, including those from bacteria, have already been determined and available in PDB. Based on sequence similarity analysis, *L. donovani* enolase shows 79% amino acid sequence identity with *T. brucei* enolase [Table 1]. The high degree of sequence identities between the three-dimensional (3D) coordinate structure of

| PDB_ID | Description | Max. score | Total score | Query cover (%) | E | Identities (%) |
|--------|-------------|------------|-------------|----------------|---|----------------|
| 4G7F   | Chain A, crystal structure of enolase from *T. cruzi* | 718 | 718 | 100 | 0 | 79 |
| 1OEP   | Chain A, structure of *T. brucei* enolase reveals the inhibitory divalent metal site | 713 | 713 | 100 | 0 | 79 |
| 2PTW   | Chain A, crystal structure of the *T. brucei* enolase complexed with sulfate, identification of a metal binding site | 712 | 712 | 100 | 0 | 79 |
| 3QTP   | Chain A, crystal structure analysis of *E. histolytica* enolase | 530 | 530 | 99 | 0 | 61 |
| 3UCC   | Chain A, asymmetric complex of human neuron specific enolase-1-PgaPEP | 516 | 516 | 98 | 1.00E-180 | 61 |

PDB: Protein Data Bank, *T. cruzi*: *Trypanosoma cruzi*, *T. brucei*: *Trypanosoma brucei*, *E. histolytica*: *Entamoeba histolytica*
enolase and crystal structure of Leishmania donovani. Practically, at this level of sequence identity, it is good enough to use crystallographic structures of T. cruzi enolase as a template, to obtain high-quality alignment for structure prediction by homology modeling. A T. cruzi enolase crystal structure 4G7F_A[30] was specifically selected on the basis of BLAST result and was utilized as a template for L. donovani enolase structure modeling.

Structural models for L. donovani enolase were built by dynamic MODELLER program[30] based on their atomic coordinates of 1E9I and were then energy minimized with the help of other computational programs.[4]

The model with the lowest discrete optimized protein energy[34] score (−348.7318), which was considered as the best one, was selected and subjected to quality evaluation. The PROCHECK Ramachandran plot analysis shows that the main-chain conformations for 98.80% of amino acid residues are within the most favored, 1.4% allowed regions and 0.2% outlier [Figure 1]. The G-factors, indicating the quality of covalent and bond-angle distance, were −0.07 for dihedrals, −0.22 for covalent, and overall −0.11. The overall main- and side-chain parameters, as evaluated by PROCHECK, are all very favorable. The comparable Ramachandran plot characteristics and G-factors confirm the quality of predicted model. VERIFY 3D of model protein is 90.44% of the residues which had an average 3D–1D score >=0.2 which pass at least 80% of the amino acids that have scored >=0.2 in the 3D/1D profile. Overall quality factor of 94.537 of ERRAT plot [Figure 2] and ProSA result have shown that the model has a good quality (Z-Score: −9.78) [Figure 3]. The L. donovani enolase model consists of typical two domains. The N-terminal one contains three-stranded antiparallel β-sheets, followed by six α-helices. The C-terminal domain comprises of eleven-stranded mixed α/β-barrel with connectivity. The first α-helix within the C-terminal domain, H7, and the second β-strand, S7, of the barrel domain was arranged in an antiparallel fashion compared to all other α-helices and β-strands [Figure 4]. The RMS deviation between predicted model and template is 0.4 Å (Angstrom). The overall conformation of L. donovani enolase model is similar to those of T. cruzi enolase crystal structure[21,30] as observed by the superposition analysis (0.04Å). The structural model of L. donovani enolase is shown in Figure 4. The secondary structure was predicted and shown in Figure 5. The Mg$^{2+}$, a metal ion cofactor, was encircled by Asp256, Glu310, and Asp337 which located in active site of enolase [Figure 6]. Although Mg$^{2+}$ is required for catalytic activity of the enzyme, it may also play a role in stabilizing enolase conformation.[30]

**Interaction of Leishmania donovani enolase and inhibitors of known compounds**

Based on rigid-body docking using HEX 4.5, both proteins were analyzed for shape complementary, hydrophobic effects resulting from a decrease in the solvent accessible surface, and electrostatic interactions. The homology model of the hypothetical protein (enolase) shows alpha (α), beta (β) and flexible loops. [Figure 4].

These residues were determined based on intermolecular hydrogen bond lengths of amino acid residues interacted between inhibitors of known compounds and L. donovani enolase. All hydrogen bond lengths appear to be shorter than 3.4 Å. This suggests that hydrogen bonds can be plausibly formed. The docking result indicated that

![Figure 4: Model structure of Enolase protein of Leishmania donovani](image1)

![Figure 5: Secondary structure of model protein](image2)
the complex could be stabilized by hydrogen bonding. Electrostatic potential surface area showed that ten amino acid residues of *L. donovani* enolase appeared to be available for making contact with pindolol [Figures 6 and 7].

These also include Lys99 and Glu94 and around the binding site are Glu 352, Val 325, Cys 93, Thr 98, and Lys 90 [Figure 8]. The positive charge residues located at the opposite end of the binding pocket. Considering *L. donovani* enolase, a significant change of accessible surface area of Lys 90 occurred. Noticeably, hydrogen bonding was observed on this residue. For inhibitors of known compounds in the complex, the model showed large decrease in the accessible surface area involving residues Lys 99 and Glu 94. Some of these appeared to form hydrogen bonds with corresponding residues of *L. donovani* enolase. The results suggest that model of the interaction complex between *L. donovani* enolase and inhibitors of known compounds can be practicable. The 2D view of enolase with pindolol gives the suitable information about the hydrogen bond interaction [Figure 8]. In addition, the proposed interaction between inhibitors of known compounds and *L. donovani* enolase agreed with previous experimental investigations.[21,24,35]

The interaction between library of inhibitors of known compounds and *L. donovani* enolase proposed in this study is useful for understanding the possible mechanism used by *L. donovani* to invade human brain tissue. For instance, the interaction between inhibitors of known compounds and *L. donovani* enolase might provide a vehicle for targeting cells. This line of work may lead to insight into host-pathogen interaction and provide valuable information for prophylactic strategies in combating infections at a very early stage.

**Conclusion**

The rationale in building a *L. donovani* enolase model and performing a binding study with inhibitors of known compounds is to gain details of interaction between the ligand and protein. *L. donovani* enolase modeling was conducted using homology modeling. Comparison of the obtained model with experimentally derived crystal structures of *T. cruzi* enolase and *S. pneumoniae* enolase revealed that they were all basically similar. The docking studies revealed the important residues involving in the interaction of *L. donovani* enolase with inhibitors of known compounds. Analyses of the interaction model

![Figure 6: The best-scoring compounds which show the pindolol structure of highest scoring compounds](image)

![Figure 7: Ligplus two-dimensional structure visualization and final docked structure (pindolol with enolase structure)](image)
between pindolol and *L. donovani* enolase, based on distances of hydrogen bonds, changes of solvent accessible surface, and electrostatic potentials, showed that this binding complex was reliable. Our theoretical prediction may lead to the establishment of prophylactic and therapeutic approaches.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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