HEAT SHOCK PROTEIN (HSP 83) OF LEISHMANIA DONOVANI A POTENTIAL DRUG TARGET

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Abstract - Leishmania donovani (a protozoan parasite) poses a serious health threat on human populationsin large parts of the world. Few antileishmanial drugs have been developed, while the drug-resistance problem is also increasing. There is thus a pressingneed to develop new effective antileishmanial. Emerging evidence indicates that the HSP83 could be proved as a potent lead molecule for drug targeting. In the search for effective antileishmanial drug target here, a three-dimensional (3-D) model for Leishmania donovani HSP83 was built using homologymodeling. The model was validated by PROCHECK and Ramachandran plot. Further the 3-D model of HSP83 was analyzed using in silico approach. Through Ramachandran plot analysis it was found that the portion residues falling into the most favored was 68.4%. This is thefirst study dealing with the modeling of L. donovani HSP83. Earlier, few researchers have identified and reported HSP83 as a potent drug target through mass spectrometry based proteomics study. The 3-D model could prove usefulin further functional characterization of this protein. The predicted model allows initial inferences about the unexplored 3-D structure of the HSP83 and may be further used in rational design of molecules for structure-function studies because it was reported as a potent drug target for antileishmanial drug discovery. The predicted 3-D model of the L. donovani HSP83 will be very useful in wet laboratory for drug target study.

Keywords - Leishmania Donovani, Heat Shock Protein 83, Homology Modeling, Ramachandran Plot, 3-D Model.

I. INTRODUCTION

Visceral Leishmaniasis or Kala-azar (caused by Leishmania donovani) remains a major public health problem throughout much of the tropical and sub-tropical world and fatal [1]. In India, as many as 100, 000–200, 000 new cases of visceral leishmaniasis are diagnosed each year. To date, there is no vaccine against Leishmania and chemotherapy is the main weapon in our arsenal with alarming rise of drug-resistance problem [2]. There is an urgent need to identify novel chemotherapeutic targets and better drugs against Leishmania. The major challenge is to take the process from discovery of drug candidates all the way along the arduous path to the marketplace. The complete sequencing of the Leishmania genome hasprovided a better understanding of the biology of parasite and also unraveled potential targets thatmay be of utility in prophylactic and therapeutic interventions.One such target is HSP83 which belongs to a family of heat shock proteins. Heat shock proteins (HSPs), are called asstress proteins, are a group of proteins that are present in all cells in all life forms. HSPs induced when a cell undergoes various types of environmental stresses like heat, cold and oxygen deprivation[3].They act like 'chaperones,' making sure that the cell's proteins are in the right shape and in the right place at the right time. There are four major subclasses: HSP90, HSP70, HSP60 and small HSPs. The heat shock response first observed in Drosophila has been studied extensively [3].

Earlier it was reported that Heat Shock Protein 90 (also known as HSP83)controls stage differentiation in L. donovani by Wiesgigl and Clos in 2001 [4]. They also showed the involvement of Hsp83 in cell cyclecontrolhttps://doi.org/10.1111/j.1574-6968.2006.00105.x and cellular stress response. Some evidence also demonstrates that HSPs are also participating in other functions of the immune system. Thus, HSPs are involved in antigen processing and presentation [5], in inflammation processes [6], in autoimmunity [7], in immune responseto cancer [8], and as antigensof bacterial and parasitic pathogens [9].Kumar et al., 2010 reported HSP83 as potential drug targets [10].Rico et al., 1999 also advocated the immunostimulatory properties of the Leishmania infantum HSP8311. Identifying suitable potential drug targets is essential for effective drug development [11]. Emerging evidence indicates that HSP83 is an important target molecule that is why an effort for modeling work on HSP83 was undertaken here. Comparative modeling or homology modeling (HM) is becoming a useful technique in the field of bioinformatics because the knowledge of the 3-D structure of a protein would be an invaluable aid to understand the details of a particular protein [12]. By using the bioinformatics tools, a 3-D structure model of L. donovani HSP83 protein was constructed through HM. Insights into the 3-D structure of a protein are of great assistance when planning experiments aimed at the understanding of protein function and during the drug design process. The experimental elucidation of the 3-Dstructure of proteins is however often hampered by difficulties in obtaining sufficient protein, diffracting crystals and many other technical aspects. In this context it is not surprising that predictive methods have gained much interest. Comparative model building consists of the extrapolation of the structure for a new (target) sequence from the known 3-Dstructure of related

family members (templates). While the high precision structures required for detailed studies of protein-ligand interaction can only be obtained experimentally, theoretical protein modeling provides the molecular biologists with "low-resolution" models which hold enough essential information about the spatial arrangement of important residues to guide the design of experiments. In silicoapproach has been employed in this study for modeling of L. donovaniHSP83 protein, which further gives a lead towards structure based antileishmanial drug design.

II. DETAILS EXPERIMENTAL

2.1. Search and Selection of Protein Sequence

The protein sequence of L. donovani HSP83 was searched by usingSwiss prot (http://expasy.org/sprot). It was ascertained that the 3-D structure of the protein was not available in Protein Data Bank (http://www.rcsb.org/pdb/home/home.do); hence the present exercise of developing the 3-D model of L. donovani HSP83was undertaken.

2.2. L. donovaniHSP83 sequence alignment

The FASTA sequence of L. donovani HSP83was retrieved from the Swiss prot that has 452 amino acids (P27890).Comparative modeling usually starts by searching the Protein Database (PDB)of known proteinstructures using the target sequence as the query [13]. This search is generallydone by comparing the target sequence with the sequence of each of thestructures in the database. The target sequence was searched for similarsequence using the BLAST (Basic Local Alignment Search Tool) [http://blast.ncbi.nlm.nih.gov].

2.3. Characters and secondary structure prediction of L. donovaniHSP83

The biochemical and physiological characters were predicted based on http://web.expasy.org/protparam. The secondary structure of L. donovani HSP83 was predicted according tohttp://npsa-pbil.ibcp.fr/cgi-bin/secpred_gor4.pl. Hydrophilicity plot was predicted by http://web.expasy.org/cgi-bin/protscale/protscale.pl?1.

2.4. Molecular homology modeling

The obtained amino acid sequence was used to find template proteins through Geno3dD (http://www.geno3d-pbil.ibcp.fr), which is an onlinetool forautomatic modeling of protein 3-D structure, based on sequence andstructure-wise similarity. CHARMM [14]energy minimizationwas applied using Swiss-Pdb-Viewer(SPDBV) program (http://spdbv.vitalit.ch).

2.5. Analysis and validation of HSP83 model

The stereo-chemical properties of models were validated using PROCHECK (http://www.biochem.ucl.ac.uk/~roman/procheck/procheck.html). PROCHECK checks the stereochemical quality of a protein structure [15]. The respective energies and other statistics of the model wasalso studied and reviewed from Geno3D and the PROCHECK.Analysis of protein structure and models was done on the basis of verifying parameters like Models energy, Ramachandran plot quality, structural agreement between models etc. Backbone conformation was evaluated by the inspection of the Psi/Phi Ramachandran plot. The stereochemical and quality of the final models were assessed bythe PROCHECK.Structural modelswere visualized bySPDBV [14].

III. RESULTS AND DISCUSSION

3.1. Analysis of L. donovani HSP83 amino acid sequences

The amino acid sequences of L. donovani HSP83 were taken from swiss prot [HSP83_LEIDO (P27890)]. The protein is 452 amino acids in length with a molecular weight of 52,691 Da.Template taken for modeling wasHSP83_LEIDO (P27890) and their sequence was as follows

TKKVKEVTKEYEVQNKHKPLWTRDPKDVTKE
EYAAFYKAISNDWEDPMATKHFSVEGQLE
FRSIMFVPKRAPFDMFEPNKKRNNIKLYVRRVF
IMDNCEDLCPDWLGFVKGVVDSEDLPL
NISRENLQQNKILKVIRKNIVKKCLEMFDEVAE
NKEDYKQFYEQFGKNIKLGIHEDTANP
KKLMEFLRFYSTESGLEMTTLKDYVTRMKEGQ
KSIYYITGDSKKKLESSPFIEQARRRGL
EVLFMTEPIDEYVMQQVKDFEDKKFACLTKEG
VHFEESEEEKQQREEEKAACEKRCKTMK
EVLGDKVEKVTVSDRLSTSPCILVTSEFGWSAH
MEQIMRNQAVRDSSMSAYMMSKKTMEL
NPRHPIIKVLRRRVEADENDKAVKDLVFLLFDT
SLLTSGFQLEDPTGYAKRINRMIKLGL
SLDEEEEAAEATVVETAPAEVTVGTSSMEQVD

The primary requirement for reliable homologymodeling is a detectable similarity between the sequence of interest (targetsequence) and a known structure (template) by BLASTp query. The BLAST result yielded that HSP83 of L. donovanishowed 62% similarity with HumanHeat shock protein (HSP 90-alpha). In general, 30% sequence homology is required for generating useful models [12].

3.2. Secondary structure ofL. donovani HSP83

The secondary structure of HSP83 includedα-helix (49.78%), β- strands (11.73%),andrandomcoil(38.50%) (Figure 1).

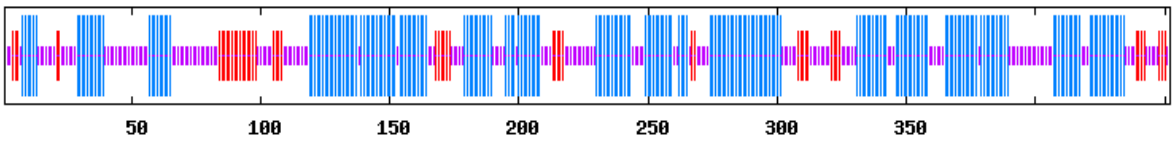


Figure 1: The secondary structure of Leishmania donovani HSP83.The vertical lines from long to short as follows: α-helix, β-strands and random coil.

3.2. Biochemical and Physiological characters of HSP83

The predicted isoelectric point of HSP83 was 5.64 and the molecular weight was 52651.3 Da. The total average hydrophobicity was -0.669.The hydrophilicity plot (**figure 2**) was predicted by ProtScale platform (Kyte-Doolittle).X-axis represents protein length from N- to C-terminal. Y-axis represents the score computed by certain algorithm.

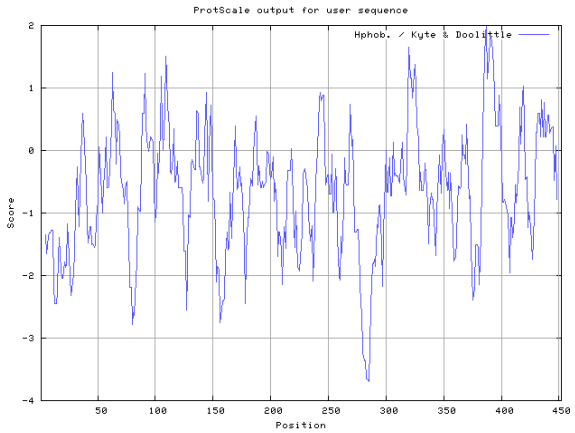


Figure 2: Hydrophilicity plot of HSP83 (Kyte-Doolittle).

Total numbers of negatively charged residues (Asp + Glu) were 82 and positively charged residues (Arg + Lys) were 74. The estimated halflifeof protein predicted in mammals, yeast and Escherichiacoli were > 7.2 h, > 20 h, > 10 h, respectively, and its instability coefficient was 56.09.

3.3. Comparative HSP83 structure modeling using Geno3D server

The theoretical structure of HSP83 from L. donovani was generated using Geno3D a comparative modeling program [16]. Geno3D implements comparative protein structure modeling by satisfaction of spatial restraints. The program was designed to use as many different types of information about the target sequence as possible. Geno3D provided 3 models as a result against submitted query. The respective energies, and other statistics of the model was studied and reviewed from Geno3D and all the parameters provided by Geno3D is given below:

Models energy (kcal/mol) :

Model 1 : -19919.60
Model 2 : -19301.90
Model 3 : -19561.00

Structural agreement between models (RMSD-Root Mean Squared Deviation in angstrom):Mean deviation: 1.018131

	Model-1	Model-2	Model-3
Model-1	0.00	1.14	1.04
Model-2	1.14	0.00	0.88
Model-3	1.04	0.88	0.00

Number of intrachain restraints deduced from templates:

7141 on MChain atoms (CA,C,N)

8137 on other atoms (CB,CE,OG,...)

Number of violation of these intrachain restraints:

M Chain	Other
Model 1	8(0.10%) 6(0.10%)
Model 2	22(0.30%) 8(0.10%)
Model 3	3(0.10%) 3(0.00%)

After analyzing above data it was found that Model 2 has maximum number of residues in the core region of ramachandran plot [17], so model 2 was more acceptable and preferred model for further analysis by PROCHECK [18].

3.4. PROCHECK analysis

Ramachandran plot was generated through PROCHECK **(figure 3).**

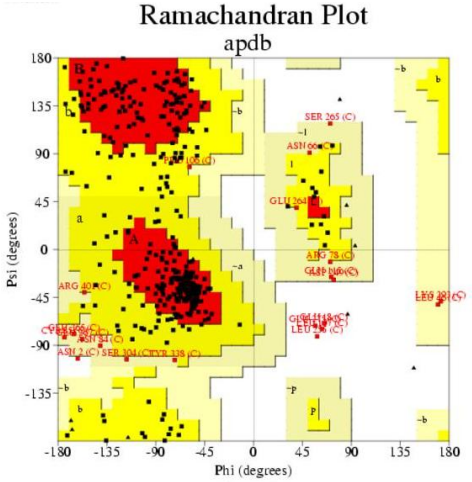


Figure 3:Ramachandran's plot of Heat Shock Protein 83 model from *Leishmania donovani.*

Ramachandran plot analysis was done to validate the predicted Model of HSP83. The Ramachandranplot statistics were discussed in **table 1.** The Φ and Ψ dihedral angles in the homo-dimer lie withinthe allowed regions of the Ramachandran plot. The plot statistics were; 68.4% residues in most favored regions; 26.3% residuesin additional allowed regions; 4% residues in generously allowed regions and 1.3% residue in disallowed regions. Number of non-glycine and non-proline residues was 376, no. of glycine residue was 15 and no. of proline residue was also 15. The analysis indicated that the model has a good quality. Stereo chemical quality of models with PROCHECK :

	Core	Allowed	Generously	Disallowed
Model 1	66.8%	27.1%	2.9%	3.2%
Model 2	68.4%	26.3%	4.0%	1.3%
Model 3	61.5%	33.7%	2.4%	2.4%

Residues in quadrangles	Total number of residues	% of residues
Residues in most favored regions [A, B, L]	257	68.4
Residues in additional allowed regions [a, b, 1, p]	99	26.3
Residues in generously allowed regions [-a, ~b, —1, ~p]	15	4
Residues in disallowed regions	5	1.3

Table 1: Ramachandran plot statistics for 3-D model of Heat Shock Protein 83 of *Leishmania donovani* computed with the PROCHECK program.

3.5. Three dimensional modeling

The 3-D model was built by adopting an approach of comparative modeling using SPDBV (Figure 4).The secondary structure (α-helices and β–sheets represented by ribbons) of the model thus obtained is shown in the figure 4.

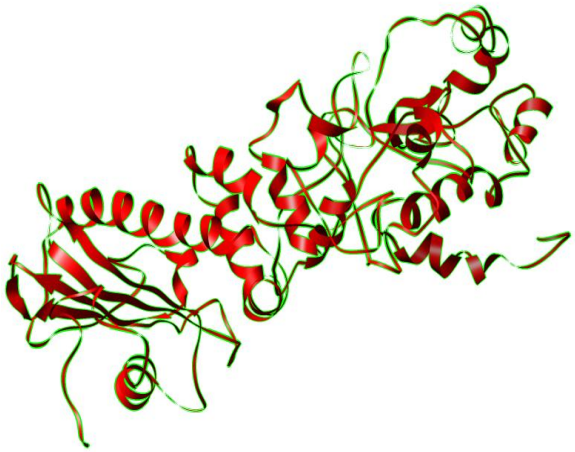


Figure 4:3-D model of Leishmania donovaniHeat Shock Protein 83.

IV. CONCLUSION

Thermal fatigue behavior of plasma sprayed YSZ Homology modeling helps in predicting the 3-D structure of a macromolecule with unknown structure (target) by comparing it with a known template from another, structurally highly similar, macromolecule [19]. Homology modeling is a viable technique because it produces models that can be used for further research [20]. The structure of the target protein is structurally similar with the template if both the target and template sequences are similar. A satisfactory model for HSP83 from L. donovani was obtained by adopting of an approach to comparative modeling. The Model presented here allows initial inferences about the structure of the HSP83 and will allow the rational design of peptide derivatives for structure-function studies. 3-D structure details of proteins are of major importance in providing insights into their molecular functions. This modeled structure can be used to predict the molecular function and the active sites. The predicted 3-D model of L. donovani HSP83 will be very useful in wet laboratory while studying the real structure of the protein. Thus authors carried out an interesting bioinformatics study to determine the 3-D model of L. donovani HSP83. The predicted 3-D model may be further used in characterizing the HSP83 protein of L. donovani in wet laboratory. Additional studies of HSP83 with known inhibitors may be docked to evaluate the efficacy of model and to compare/contrast with human protein.

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