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2D-Molecular homology modeling of selected Enolase enzyme for Leishmaniasis

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ABSTRACT

In present study, BLAST search was performed and an identity with *Trypanosoma brueei* is 79% Based upon and Pfam results Enolase- 2PTZ (protein data bank) was considered as an ideal homologue and used as a template for homology modeling due to its higher X-ray resolution at 1.65. Sequence alignment between enolase and 2PTZ was done using align 123 followed by manual modification. The final alignment was carefully evaluated and evidenced to be matching the conserved residue data for chain A of enolase in *Trypanosoma brucei* well Superimposing of the model was done over the template 2PTZ. Further research on the comparison of the models for the inhibitors may eluciadate the mechanism of enolase ligands interactions. This study has shown that some formation of favorable hydrogen bonds, hence it is predicated from this study that these novel compounds may act as potent inhibitors for Leishmania Enolase. These compounds may be used further for synthesis, their wet lab activity against Enolase, animal model study and clinical trials and then can be implicated for the treatment of leishmaniasis. Those models are considered to be used in designing new leads for hopefully more active compounds.

Keywords: Enolase, Leishmaniasis, Enzyme, 2D-moleculare Modeling

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INTRODUCTION

Leishmaniasis is a disease caused by protozoan parasites that belong to the genus Leishmania and is transmitted by the bite of certain species of sand fly (subfamily phlebotomine) [1-2]. Two genera tranprit Leishmania to humans "Lutzomyia in the New World and Phlebotomus in the Old World" (1). The disease was named in 1901 after the Scottish pathologist William Boog Leishman. This disease is also known as Leichmaniosis, Leishmaniose, Leshmaniose, and formerly, Orient Boils, Aleppo Boil, Baghdad Boil, Kala azar, Black fever, sand fly disease, Dum-Dum fever, chiclero uleer or espundia[3-6].

Most forms of the disease are transmissible only from animals (zoonosis). But some can be spread between humans. Human infection is caused by about 21 of 30 species that infect mammals. These include the *L. donovani* complex with three species (*L. donovani*, *L. infantum* and *L. chagasi*); These include the *L. Mexicana* complex with 3 main species (*L. Mexicana*, *L. amazonensis* and *L. venezuelensis*); *L. tropica*: *L. major*: *L. aethiopica*; and the subgenus Viamia with four main species (L.(V.) braziliensis, L. (V) guyanensis, L. (V.) panamensis, and L. (V.) peruviana). The different species are morphologically indistinguishable, but they can be differentiated by isoenzyme analysis, DNA sequence analysis, or monoclonal antibodies[7-11].

Current drug treatment for Leishmania is either lacking or unsatisfactory and new drugs for clinical use are too expensive to develop through classical empirical screening methods. Antimonials (pentavalent) has 28-day intramuscular- or intravenous-injection regimen. Resistance reported in as many as 60 percent of cases in parts of India. Expensive (if name brand product) [12-15]. Otherwise the generic SSG is relatively cheap (US$ 30). Infusion side effects include nausea, pain, anorexia, myalgia arthralgia, headache, malaise, reversible cardiotoxicity and chemical and clinical pancreatitis. Amphotericin B has 4-8 week intravenous regimen but is expensive, side effects include fever, chills, hypokalaemia, renal toxicity, anaemia and cardiotoxicity, AmBisome has 5-day intravenous regimen, Liposomal Amphotericin B (encapsulated in liposomes) is well tolerated, but is expensive, Miltefosine has 28 day oral regimen but is expensive, risk of teratogenicity[16-17].

MATERIALS AND METHODS

Protein Structure Predication

Knowledge of the three-dimensional structure is a prerequisite for rational drug design. X-ray crystallography,

NMR spectroscopy and electron microscopy are most important experimental methods to obtain detailed structure information.

Protein and nucleic acid sequence methods are now well advanced and available in many laboratories. As a result sequence database such as Swiss-prot. TreMBL (http://www.expasy.ch/), and the protein information resource (PIR), (http://www- nbrf.georgetown.edu/), have been growing rapidly in recent years. In contrast the determination of protein structure by NMR or X-ray crystallography has tended to proceed much more slowly. Hence there are many important proteins where the sequence is available but the three-dimensional structure is not yet known. The gap between the number of known sequences and the number of known structure is widening rapidly and the most successful theoretical approach to bridging this gap is homology modeling.

The objective of homology modeling is to build a 3D model for a protein of which the structure is unknown (the target) on the basis of sequence similarity to proteins of known structure (the templates). The technique uses experimentally determined protein structure to predict the conformation of another protein that has a similar amino acid sequence. The method relies on the observation that in nature the structure conformation of a protein is more highly conserved than its amino acid sequence and that small or medium changes in sequence typically result in only small changes in the 3D structure. Homology modeling or comparative modeling methods are able to predict the 3D structure of a protein sequence by using information derived from a homologous protein of known structure.

RESULTS AND DISCUSSION

Sequence Retrieval Form SWISSPROT:

Protein sequence database which strives to provide a high level of annotation (such as the description of the function of a protein, its domains structure, post- translational modifications, variants, etc.), a minimal level of redundancy and high level of integration with other database.

Amino Acid Sequence of Target

>gi|190335775|gb|ACE74540.1|enolase[Leishmania donovani]

MPIRKVYAREVLDSRGNPTVEVELMTEAGVFRSAVPSGASTGV
HEACELRDGDKARYCGAGCTQAVKNVEILAPALVGKDESDQAG
LDKMMCELDGTKNKSKLGANAILGCSMAISKAAAAAKAGVPLYR
YIAGLAGTKDIRLPVPCFNVINGGKHAGNVLPFQEFMIAPTKAT
SFREALRMGSEVYHALKVHKSKYGQDAVNVGDEGGFAPPIKHI
DEPLPHMEAIEKAGHKGKFAICMDCAASEAYDAERKMYNLTFK
NPEPTYVSAAELQATYERWVAEYPLVSIEDPFAEDNFDEFSATT
MALAGKAQIVGDDLTVTNVERVKMAIEKSACNSLLLKINQIGTIS
ESIAAAKLCMENGWSVMVSHRSGETEDTYIADLSVGLGTGQIKT
GAPCRGERTAKLNQLLRIEEEIGSTATYGYPGWA

Amino Acid Sequence of Template

>gi|160877729|pdb|2PTZ|A Chain A, Crystal Structure of the T, Brucci Enolase Complexed with Phosphonoacetohydroxamate (path), His 156-Out Conformation

GSHMTIQKVHGREVLDSRGNPTVEVEVTTEKGVFRSAVPSGAS
TGVYEACELRDGDKKRYVGKGCLQAVKNVEVIGPALIGRDELKQ
EELDTLMLRLDGTPNKGKLGANAILGCSMAISKAAAAAKGVPLY
RYLASLAGTKELRLPVPCFNVINGGKHAGNALPFQEFMIAPVKA
TSFSEALRMGSEVYHSLRGIIKKKYGQDAVNVGDEGGFAPPIKDI
NEPLPILMEAIEEAGHRGKFAICMDCAASETYDEKKQQYNLTFK
SPEPTWVTAEQLRETYCKWAHDYPIVSIEDDPYDQDDFAGFAG
ITEALKGKTQIVGDDLTVTNTERIKMAIEKKACNSLLLKINQIGTI
SEAIASSKLCMENGWSVMVSHRSGETEDTYIADLVVALGSGQIK
TGAPCRGERTAKLNQLLRIEEELGAHAKFGFPGWS

BLAST (Basic Local Alignment Search Tool)

BLAST search was performed against protein data bank (PDB) with the default parameter to find suitable template for homology modelling. Sequence was aligned and the one that showed the maximum identity with high score and lower e- value and 79% sequence identity were used as a reference structure to build a 3D model for Enolase respectively. The Enolase was modelled by means of comparative modelling procedure using the PDB having ID "2ptz" as template for Enolase.

Query ID	gi|190335775|gb|ACE74540.1|
Description enolase	Leishmania donovani
Molecule type	amino acid
Query Length	429
Database Name	pdb
Description	PDB protein database
Program	BLASTP

Sequence alignment provides a powerful way to compare novel sequence with previously characterized genes. Both function and evolutionary information can be inferred form well designed queries and alignments. BLAST 2.0, (Basic Local Alignment Search Tool), provides a method for rapid searching of nucleotide and protein databases. Since the BLAST algorithm detects local as well as global alignments, regions of similarity embedded in otherwise unrelated proteins can detected. Both types of similarity may provide may provide important clues to the function of uncharacterized proteins.

Choice of Template

Comparative modeling usually starts with searching the PDB of known protein structure using target sequence as the Query. This search is generally done by comparing the target sequence with the sequence of each of the structure in the database. The most popular programs in the class include FASTA and BLAST.

Once a list of potential templates is obtained using searching methods, it is necessary to select one or more templates that appropriate for the particular modeling problem. The quality of a template increases with its overall sequence similarity to the target and decreases with the number and length of gaps in the alignment. The simplest template section rule is to select the structure with the highest sequence similarity to the modeled sequence.

BLAST Result of Enolase

PDB ID	Name of template	Identity
2ptz	Crystal structure of the *T. Brucei* Enolase complexed with phosphonoacetohydroxamate (pah)	79%

Sequence Allgnment by CLUSTAL W

Alignment of sequence with their templates structure was done using the Clustal W. The software also takes into account structural information from the template when constructing an alignment. The MODELLER script was used for alignment all target sequence s in the Ail file with their corresponding template structure in the PDB files. Finally, the alignment was written out in two formats, PIR and PAP. The PIR format is used by MODELLER I the subsequence model building stage, while the PAP alignment format is easier to inspect visually. In the PAP format, all identical positions are marked with a '*'. The details of modelling and sequence alignment scripts are submitted as supplementary material.

CLUSTAL W (1.83) multiple sequence alignment

CONCLUSION

Leishmania enolase is potential target for treating visceral Leishmaniasis. In order to understand structural features we carried out homology modelling of *Leishmania donovani* enolase. Developed model shows good overall structural quality as validated by using different validation tools like Procheck, verify 3d SCORE. In addition, model shows good native protein folding. The ligand active site was determined by sequence alignment and Q site finder analysis tool. The amino acid residues lys155, eys 147 and cys241 of enolase of *Leishmania donivani* are active site residues, which were exploited to design target specific inhibitors. The enolase model was taken for further screening of different chemical library to studying their enolase inhibitory activities; different public domain chemical libraries were accessed for this study.

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