Advances in Bioresearch
Adv. Biores., Vol 15 (3) May 2024: 283-289

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.3.283289

Advances in Bioresearch

ORIGINAL ARTICLE

A Study of Some Key Enzymes Within Leishmania Through Comparative Molecular Modelling Techniques

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ABSTRACT
Leishmaniasis, a protozoan disease caused by the Leishmania genus, poses a significant global health threat with diverse manifestations across various regions. Despite affecting millions worldwide, there's a scarcity of vaccines, and the available chemotherapeutic agents exhibit high toxicity. This study focuses on understanding pivotal enzymes within Leishmania crucial for its survival. Using computational approaches like comparative modeling and docking analyses, six key enzymes were explored: Mitochondrial DNA primase, Universal Minicircle Sequence Binding Protein (UMSBP), DNA primase large and small subunits, enolase, and trypanothione. Various tools like I-TASSER, Phyre2, and Swiss Model were utilized to generate structural models, emphasizing the challenges encountered due to low sequence identity in some enzymes. Particularly, the mitochondrial DNA primase, with less than 30% identity, was modeled via I-TASSER. The generated models serve as a foundation for further empirical studies and potential drug development against Leishmania, highlighting the urgency for therapeutic advancements against this neglected tropical disease.
Keywords: *Leishmania, enzymes, computational tools, I-TASSER, Phyre-2, Swiss model.*

Received 24.03.2024 Revised 06.04.2024 Accepted 14.05.2024

How to cite this article:
Pawitar Dulari, Ajay Bhushan, Brijender Bhushan, Vivek Chand Chandel. A Study of Some Key Enzymes Within Leishmania Through Comparative Molecular Modelling Techniques. Adv. Biores., Vol 15 (3) May 2024: 283-289.

INTRODUCTION

Leishmaniasis, a protozoan disease caused by the Leishmania genus, is a significant global concern. This genus, found within the Trypanosomatidae family, possesses unique mitochondrial DNA and a kinetoplast. Around 21 Leishmania species are known to harm humans, leading to diverse manifestations like skin, muco-cutaneous, or visceral presentations, varying by species and their pathogenicity. This disease affects approximately 12 million individuals across 98 countries, prevalent in tropical, subtropical, and even European regions. Annually, an estimated two million new Leishmania infections arise globally, including visceral and cutaneous types. The severity escalates notably among HIV-infected patients, increasing the risk of full-blown disease with high relapse and mortality rates [1-4]. Despite being the ninth most burdensome infectious disease, leishmaniasis remains a neglected tropical disease (NTD) concerning drug development. Vaccines against Leishmania are non-existent, and the primary treatment relies on chemotherapy, though the current chemotherapeutic agents are highly toxic to organisms [5-7]. These compelling facts underscore the urgent need for drug development against Leishmania. The current focus is on computationally understanding the crucial enzymes involved in this parasite's survival.

MATERIAL AND METHODS

Receptor enzymes

Six crucial receptor enzymes—Mitochondrial DNA primase, universal minicircle sequence binding protein (UMSBP), DNA primase large subunit putative, DNA primase small subunit putative, enolase, and

tryphanothione—have been retrieved in FASTA format from the ftp://ftp.ncbi.nlm.nih.gov/genomes/. These enzymes play pivotal roles and hold significance in various biological processes, forming essential targets for in-depth computational analysis and potential drug development against Leishmania.

Mitochondrial DNA Primase

MQRLTSARLF KGAFNAGQKR TTNASAGAAA AASRPQALPQ PHQASTMTPP AAKAAAAAPQ ASLTRSASLV APPSASVPPS GAAAGAAPPM RKKKIIRRII KRKKASAGAA TVETVHHAAA EAVSQPAAPA APSEPAFAAA TGAQPPRRGL AMLNEIRKHP EGSIEEKPHV ITEQHSEETA TPQKQYHEHT HDHHVESQET EKAHEHHHHH DAVSEKATSS RYKASSAEAE TDAAARSDDM YDAIYNNGSS SSRKTAMVKG YRIDEVGALC SSSDAMFARR LPSGGCQFFA WPGTPLPVAS

SAIVSMPDTI RTVHAVFGRA GTPIDIVMDI DAQVPQEYWT MSKIRVYQRK VLDDVLTPLK EEIEKIGEEI ETQVVLQSPN LKKASFHVHT RLKDAAFADF YSLHGFLFRF QDRLPNVDLQ IYRPNGMLRM FSCMKENRTS AIIVFDEPKW NIGFPGGKVS DEQAALHSIC VRDPSTFSRV LTFEAPRQHN MPAYGGSKGA CGDGNEGALR PPQVLLPRTE KEAIENASRW LRQATEVEVG EWRTWIGLGL CAYRVAYQFR NARNLPRPAM AEMLDAWTEA SRKCPLKFHS GECEARWAAF

DPEKLGSYSD WWSAYKRLGR LEVPMREAME REAAFAARCA SRYQAAPAAE VPLAPEPPQF AAAAGSVTSA SRKMKQQKKA FRRA*

Universal Minicircle Sequence Binding Protein(UMSBP)

MQRLTSARLF KGAFNAGQKR TTNASAGAAA AASRPQALPQ PHQASTMTPP AAKAAAAAPQ ASLTRSASLV APPSASVPPS GAAAGAAPPM RKKKIIRRII KRKKASAGAA TVETVHHAAA EAVSQPAAPA APSEPAFAAA TGAQPPRRGL AMLNEIRKHP EGSIEEKPHV ITEQHSEETA TPQKQYHEHT HDHHVESQET EKAHEHHHHH DAVSEKATSS RYKASSAEAE TDAAARSDDM YDAIYNNGSS SSRKTAMVKG YRIDEVGALC SSSDAMFARR LPSGGCQFFA WPGTPLPVAS

DNA Primase Large Subunit Putative

MQAITASTPS QQYSAGAGIE KPLGATTSAD WMTMYEQRPH GNSTLFELEA
MVAKRMEFLA WVDQQINSPQ AKSFDSVLDA IIARLPEERR SSVATDASRG
KMVLLGYDET AAGGCSDRRS SSRGSTGSAA GSSQAAGIVF APEEDVTSHL
LCRFAFCMSE HWRDWLVRTE RVLLTARIKM EMAKRPFTFL VDLMKRNGLP
CAPLTDKQLA DPMLQEYLEY RRVKAESARE SEGRAENYYA VPLSLATRLI
KKRYVLCRAG QTILFRDQVQ EVFLTVFCAR LNRGLHNAYL ARVKQQALEE
ETTKSTVMAM LDAFLQQFIS DPVDTLQEGV AGSVKAGDVQ RLAQAHFPPC
MRAIDWHLRR EGHLKHHGRF MYGLFLKSIG LSLEDSLELF ATLMKVKGGG
SVEAFAKTAY GYNVRHNYGM EGKKMSYSSA SCATILGLPP VVDQHDCHGC
PFRFRDEGAL RTMLGKETQN PKGCNYPSVR PTPGDIEDIV SDSKAQHYTR
ACYKYFMATH PGARRDTLFR SPYEYYSVSL EFETPSDSTE
SARPSAVPGK RTSVVLNEDV LRLRTSP*

DNA Primase Small Subunit Putative

MQAINENSLR EFYAHVYPVE LITQWLSYRL SQPRLSAGSS VARVKGEQTA VSGEESKDGV LHALGMKAEP CNAVGAASGA DDDKEWLDGK GAAAAEGYLA RREFCFTLIG DIFTRFRSYR SAEELRAELV RCFPEKIDVG AVYNIRPNQK QGLANVFPVE RELVFDIDMS DYDNVRSCCS GKSICSYCWA WMSCAAHVLH TLLRDDFGFK YILPVFSGRR GIHLWVCDRR ARRMTNDERT ALVGYLTVVA PKTLRSTIVA DLANHRLIHP TIRHVLRTQL DRAFTALFVA SSSDNPNNIQ HHPKAAYIVH DATSAVLKLG RRDALNRFQQ HVHFQQGNVL DWATYLRALG SEQEATDILH AVQLLLMYPR LDEHVSTRRD HLLKLPFCVH PGTASLCCPL EWEEVDGFNP TEDAPKLHEM LLSHSLDVRW TLPLERMLKA MRTDEDENSS *

Enolase

GDKARYCGAGCTQAVKNVNEILAPALVGKDESDQAGLDKMMCELDGTKNKSKLGANAILGCSMAISKAAAAKAG VPLYRYIAGLAGTKDIRLPVPCFNVINGGKHAGNVLPFQEFMIAPTKATSFREALRMGSEVYHALKVIIKSKYGQDA VNVGDEGGFAPPIKHIDEPLPILMEAIEKAGHKGKFAICMDCAASEAYDAERKMYNLTFKNPEPTYVSAAELQATY ERWVAEYPLVSIEDPFAEDNFDEFSAITMALAGKAQIVGDDLTVTNVERVKMAIEKSACNSLLLKINQIGTISESIAA AKLCMENGWSVMVSHRSGETEDTYIADLSVGLGTGQIKTGAPCRGERTAKLNQLLRIEEEIGSTATYGYPGWA

Tryphanothione

MSRAYDLVVLGAGSGGLEAGWNAAVTHKKKVAVVDVQATHGPPLFAALGGTCVNVGCVPKKLMVTGAQYMDLI RESGGFGWEMDRESLCPNWKTLIAAKNKVVNSINESYKSMFADTEGLSFHMGFGALQDAHTVVVRKSEDPHSDV LETLDTEYILIATGSWPTRLGVPGDEFCITSNEAFYLEDAPKRMLCVGGGYIAVEFAGIFNGYKPQGGYVDLCYRGD LILRGFDTEVRKSLTKQLGANGIRVRTNLNPTKITKNEDGSNHVHFNDGTEEDYDQVMLAIGRVPRSQALQLGKA GVRTGKNGAVQVDAYSKTSVDNIYAIGDVTNRVMLTPVAINEGAAFVETVFGGKPRATDHTKVACAVFSIPPIGTC GMTEEEAAKNYETVAVYASSFTPLMHNISGSKHKEFMIRIITNESNGEVLGVHMLGDSAPEIIQSVGICMKMGAKIS DFHSTIGVHPTSAEELCSMRTPAYFYESGKRVEKLSSNL

PDB BLAST-sequence searching

The protein structures of the selected six receptor enzyme sequences were individually analyzed using the PDB BLAST online software. Subsequently, the query coverage for each of these six protein sequences was assessed, and a list was compiled based on the percentage of query cover. Using this query coverage data, further processing of these sequences was performed, as outlined in Table-1.

I-Tasser

The I-Tasser server serves as an online resource catering to protein structure and function predictions. Its functionality enables academic users to generate precise 3D structure predictions and determine the biological function of protein molecules based solely on their amino acid sequences. Given the absence of structural evidence within the Protein Data Bank (PDB), an *ab initio* modelling approach was applied to the sequence. Due to the observed limited structural identity and query coverage templates from BlastP analysis, Molecular modelling via the I-TASSER (Iterative Threading Assembly Refinement) server was employed [8].

Phyre2

Phyre2 stands as a comprehensive suite of web-based tools designed for the prediction and in-depth analysis of protein structure, function, and mutations. This upgraded version supersedes its predecessor, Phyre, leveraging advanced homology detection techniques to construct 3D models, forecast ligand binding sites, and evaluate the impact of amino acid variants, including nonsynonymous SNPs, based on a user's protein sequence. The Phyre2 system amalgamates numerous diverse software components developed by its own group and others, employing multiple programming languages. Users can employ the Phyre2 server in various research contexts, with its most popular utility being the prediction of the 3D structure of individual submitted protein sequences. Recognized as one of the most widely used methods for protein structure prediction, Phyre2 has amassed over 1500 citations, attesting to its significance and reliability in scientific research.

Advanced facilities include:

The Phyre2 platform offers a spectrum of functionalities: (i) Backphyre, facilitating structure searches across a spectrum of genomes; (ii) Streamlined batch submission, enabling modeling for large sets of protein sequences; (iii) One-to-one threading, which aligns user sequences with user structures; (iv) Phyrealarm, automating weekly scans targeting challenging-to-model proteins; (v) Phyre investigator, providing comprehensive analysis of model quality, functional insights, and the impact of mutations [9].

Swiss model

Homology modeling constitutes a technique aimed at constructing three-dimensional protein structures by leveraging experimentally determined structures of related protein family members as templates. The Swiss Modeling Workspace stands as an integrated web-based expert system for modeling. It employs a library of experimentally derived protein structures to identify suitable templates for a given target protein. Through sequence alignment between the target protein and the template structure, a three-dimensional model for the target protein is generated. To gauge model reliability, tools for assessing model quality are employed. Homology modeling presently stands as one of the most precise computational methods for producing dependable structural models, extensively applied across various biological contexts. Typically, the computational endeavor for a modeling project spans less than 2 hours. However, this estimation excludes the time required for visualizing and interpreting the model, which can vary based on individual familiarity with working on protein structures [2].

Ligand search

We have studied from the literature that UTP is the best ligand. UTP stands for Uridine-5'-triphosphate. UTP also has the role of a source of energy or an activator of substrates in metabolic reactions, like that of ATP, but more specific. When UTP activates a substrate, UDP-substrate is usually formed and inorganic phosphate is released. UDP-glucose enters the synthesis of glycogen. UTP is used in the metabolism of galactose, where the activated form UDP-galactose is converted to UDP-glucose. UDP-glucuronate is used to conjugate bilirubin to a more water-soluble bilirubin diglucuronide.

AutoDock

Molecular docking strategy is widely used in computer aided drafting design (CADD) approach to analyze the orientation of drug compound against receptors to study the inhibitory and binding efficiency with the residue interacted [8].

Autodock vina has been used in the present study for docking the selected compound against the selected receptor enzyme molecules.

Table 1: Summary of receptor enzymes used, their template ids and software used

Sr. No.	Receptor enzyme	Template ID/Percentage	Software Used
1	Mitochondrial DNA primase		I-TASSER
2	UMSBP	c2k87_/22%	Phyre2 and Swiss model
3	DNA primase large subunit putative	c4rr2D_/26%	Phyre2 and Swiss model
4	DNA primase small subunit putative	c4bpxC_/33%	Phyre2 and Swiss model
5	Enolase	97%	No need
6	Trypanothionine	c2wDhA_/96%	No need

RESULTS AND DISCUSSION

Choice of software

We have used PDB BLAST for analysing six enzymes under study with template was selected on the basis of significant e-vale of 1e-04. Out of 6, 5 were showing more than 30% identity to respective templates (Table 1 and Table 2). However, we also observed that two proteins (enolase and tryanothionine) were showing more than 90% identity to the templates therefore was excluded from further analysis.

For more than 30%, we have used PHYRE-2 and Swiss model which is the widely used tool for building model. The following template for each protein is as follows (Table 2): for UMSBP, c2k87 (22%); DNA primase large subunit putative c4rr2D_(26%]; ,DNA primase small subunit putative , c4bpxC_[33%].

We also found that <30% and therefore deployed I-TASSER which is a popular tool for determination of model when homology modelling fails then subjected Mitochondrial DNA primase to I-TASSER software programe.

Table 2: Query coverage of receptor enzymes through PDB blast

S.No.	RECEPTORS ENZYMES	QUERY COVERAGE (%)
1.	Mitochondrial DNA primase	15%
2.	UMSBP	63%
3.	DNA Primase large subunit putative	81%
4.	DNA Primase small subunit putative	69%
5.	Enolase	97%
6.	Trypanothionine	96%

Phyre2 results for the receptor enzymes

The model selected from Phyre2 for each enzyme was downloaded and saved into the terminal and evaluated. We can procheck the model on the basis of confidence score (c-score). The c-score of the three models are as follows:

UMSBP

Normal mode, confidence in the model 27.3%, warning; very low confidence model. Treat this model as highly speculative.

b) DNA primase large subunit putative: Normal mode, confidence in the model: 100%.

c) DNA Primase small subunit putative: Normal mode, confidence in the model 100%.

I-TASSER Results for the receptor enzymes

The I-Tasser result will shows 5 models (Plate 1-5). The best Model is selected from 5 models on the basis of:

(i) Confidence score (C-score):

Estimating the quality of predicted modes by I-TASSER. It is calculated based on significance of threading alignment and convergence parameter of the structure assembly simulations**.** C-score typically ranges from {-5, 2}, where a C-score of higher value signifies a model with high confidence or vice versa positive confidence score is the best.

(ii) TM and RMSD score

TM score is a measure of similarity between two protein structures different tertiary structure. The TM score is intended as more accurate measure of the quality of full length of protein structures and often used RMSD and GDT (Geometric Dimensioning and Tolerancing) measures. The RMSD is an average distance of all residues pairs in two structures, a local error (e.g. misorientation of tail) will arise a big RMSD value although the global topology is correct. In TM score, however small distance is weighted stronger than the big distance which makes the score insensitive to local modelling error. A TM score >0.5 indicates a model of correct topology and TM score is <0.17 means random similarity. These cut off do not depend on the protein length.

(iii) Cluster density

The cluster density is defined as the number of structure decoys at a unit of space in the SPICKER cluster. A higher cluster density means the structure occurs more often in the simulation trajectory and therefore signifies a better quality model. The last column represents the density of clusters.

Plate-1

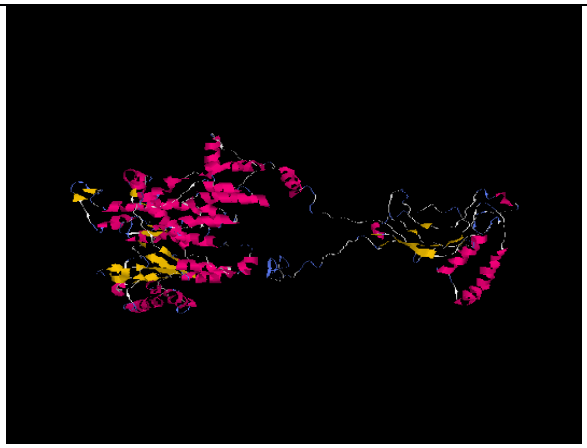

Plate-2

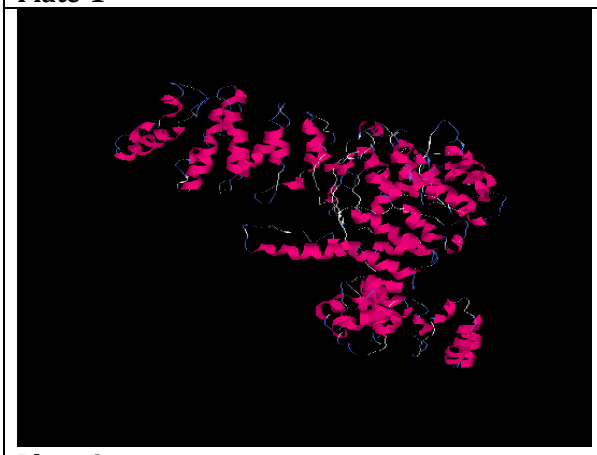

Plate-3

Plate-4

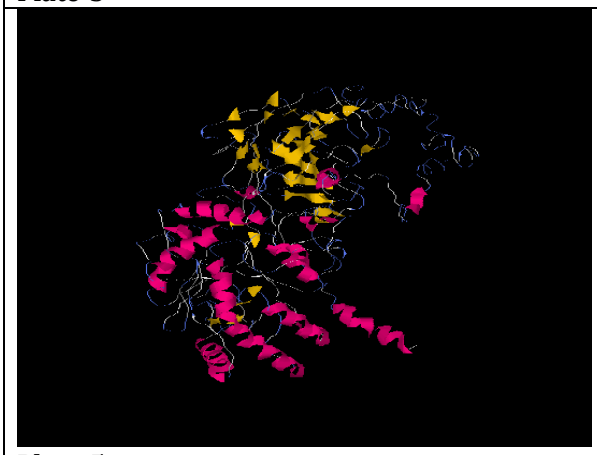

Plate-5

Plate 1-5: Showing five models obtained from I-Tesser

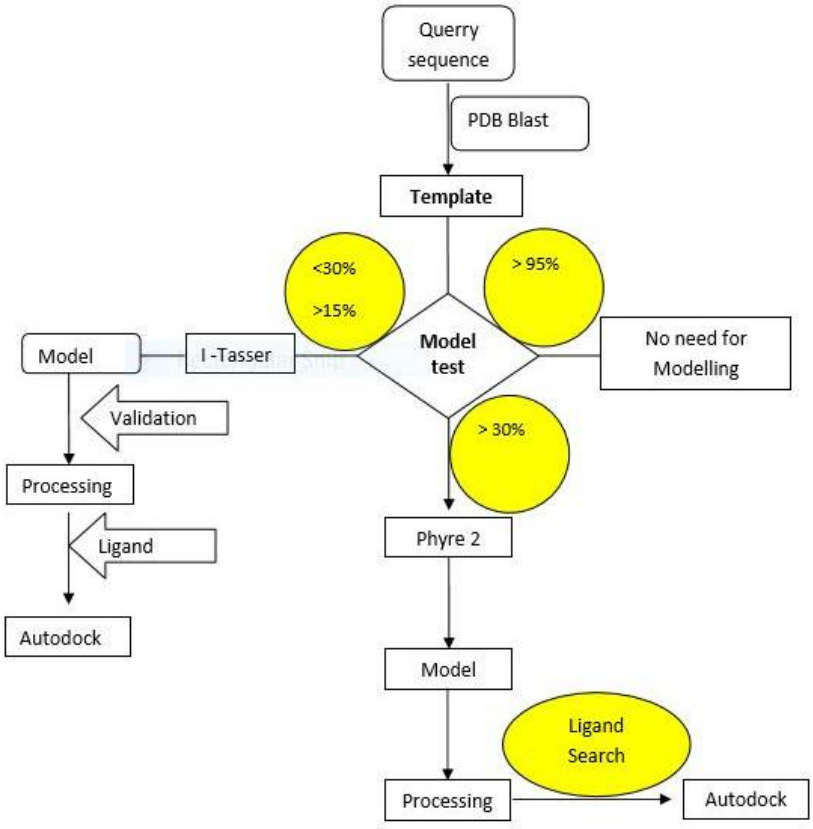

Plate 2: Flow chart for modelling six receptor enzymes

CONCLUSION

Till today, there are no vaccines available against any of the parasitic diseases, and chemotherapy is still the only option. Hence, there is an urgent need for development of vaccines against *Leishmania.* In the present work we have used the key enzymes which participates in the survival of this parasite and our study was to use these enzymes as a query for our comparative modelling and docking studies.

Likewise, we found one enzyme, mitochondrial DNA primase whose sequence identity was low as compared to the protein structure where there was a need for modelling. However, this sequence was showing less than 30% identity we therefore propose I-TASSER for our study. As a result, the model was selected and may be used for further empirical studies.

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