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Research Article

In Silico Based Approach to Identify Mura as A Potential Drug Target for Leprosy

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Abstract

The search for novel drugs to prevent or treat infections in *Mycobacterium* is gaining importance due to the emergence of drug resistance. In the present work, we have analyzed the complete genome of *Mycobacterium leprae* coding for 1605 proteins to identify some novel drug targets using computational methods. The enzymes were screened on the basis of their essentiality in the pathogen as well as on the basis of having least or negligible similarity for that enzyme in the host, *Homo sapiens*. These possible drug targets were identified for their role in the metabolic pathway of the pathogen. The results were then screened manually and further comparison of those targets against Tropical Disease Research (TDR) database selecting *M. tuberculosis* as a species of interest was carried out. This comparison against TDR database identified Mur A as a single outcome. Further, molecular modeling and docking was carried out for this enzyme with its natural substrate UDP-N-acetylglucosamine (UNAG) and a known inhibitor T6362 to understand the key residues that could be targeted for building broad spectrum antibiotics.

Keywords: Drug Targets; Homology Modeling; Leprosy; Mura; Mycobacterium Leprae

Introduction

Leprosy is a curable chronic infectious disease caused by the bacterium *Mycobacterium leprae.* Although an uncommon disease, it still remains an important disease with 250,000 cases diagnosed annually all around the globe [1]. Also called Hansen's disease, after G.H.A. Hansen, who in 1873 identified the causative agent, it causes skin sores, nerve damage, and muscle weakness that gets worse over time [2]. The disease has been a menace due to long incubation period which makes it very difficult to diagnose in the early stages, which is crucial to prevent the spread of the disease. Generally, the cases are diagnosed in late stages causing permanent nerve damage in the arms and legs. It can be commonly misdiagnosed as diabetes, Lupus vulgaris, vasculitis, sarcoid [1]. Over the past few years, the rate in the number of new cases has dropped by more than 18% while, some regions like the Mediterranean and South Asia have even seen a slight rise in the frequency of new cases diagnosed annually [3]. India alone represents around 76% of the global burden of the disease and 90% of newly detected cases in the South East Asian region. The standard treatment for Leprosy is a Multi Drug Treatment (MDT) with the antibiotics such as Dapsone, Rifampin, Clofazamine. All these factors along with the increasing drug resistance in the strains call for the search of new possible drug targets.

The whole genome sequence of *M. leprae* is known which can serve as a resource to identify the possible drug targets against Leprosy. The drug target identification requires that the target should be an essential enzyme and should be indispensable for the growth, replication and survival or viability of the pathogen. Another crucial criterion includes the effect of the drug on the host organism. The host should not have an enzyme which is homologous to the drug target [4]. Genes which are conserved thorough a wide species of pathogens are often essential targets [5].

Material and Methods

Drug target identification in *M. leprae*

The strain used in this study is *Mycobacterium leprae* TN from the nine-banded armadillo in Tamil Nadu, India. The whole genome was obtained from NCBI (National Centre for Biotechnology Information) and all the 1605 proteins of the organism were retrieved from "NCBI Bioproject" [6]. The selection of enzymes as drug targets was carried out in numerous steps. The essential genes in *Mycobacterium leprae* TN were identified using BLAST (Basic Local Alignment Search Tool) against the Database of Essential Genes (DEG) (http://tubic.tju.edu.cn/deg/). The database identifies all the essential genes which are indispensable for the survival of the microbe. This outcome was searched for similarity with the host (*Homo sapiens*) genome using BLAST tool. To increase the confidence level, sequences having identity greater than 30% and query coverage greater than 20% with the host were manually removed from selection to ensure that the target is non homologous to an enzyme in human.

KEGG pathway database [7] was used for metabolic pathway identification of the enzymes with "mle (*M. leprae* TN)" as the query organism. Blast search tool of the database was employed to find out the pathway (s) in which the proteins were playing functional roles. The identified targets were cross-checked against the draggability analysis by using Tropical Disease Research (TDR) targets database [8] selecting *M. tuberculosis* as a species of interest. The TDR target database exploits the availability of diverse datasets to facilitate the identification and prioritization of drug targets in pathogens.

Homology modeling and docking

Homology modeling was used to generate the model of *Ml* MurA. The 418 amino acids long sequence was retrieved from NCBI accession number CAC31531.1. The template was selected by performing protein BLAST of the query sequence against the PDB database. Modeller 9.10 was used to generate the 3D models. A total of 20 models were generated. Out of these, the best model was selected on the basis of their DOPE score and molpdf values. PROCHECK, ERRAT, VERIFY-3D was used to validate the models; PROCHECK to determine the stereochemical quality of a protein, ERRAT to analyze the statistics of non bonded interactions between different atom types and for finding the overall quality factor and VERIFY_3D to determine the compatibility of an atomic model with its own amino acid sequence (1D). This model was then subjected to energy minimization using Swiss PDB viewer [9] which was further used for carrying out docking analysis.

The substrate and ligand files were downloaded from NCBI Pub Chem Compound database. The optimized molecules were docked into the refined protein model using "Glide" (Grid-based LIg and Docking with Energetics) in Maestro. Glide uses hierarchical series of filters to assist in finding possible location of different ligand conformations molecule in the binding site based on grid based energy function for a given receptor molecule. The grid represents shape and properties of the receptor molecule by different sets of fields that give progressively more accurate scoring of the ligand poses. The comparison of ligand scores with those of a reference distinguishes between molecules that bind strongly in their optimal placement from those that bind weakly.

Results and Discussion

The analysis of the genome from *M. leprae* encoding 1605 proteins against DEG (database of essential genes) resulted in the identification of 652 genes essential for its survival. This was reduced to 282 after subjecting them for the absence of its close homologues in human. To increase the confidence level by keeping query coverage to less than 20% identified only 44 enzymes. The KEGG database search for those enzymes associated to metabolic pathway identified 24 enzymes and has been listed in table 1. The Tropical Disease Research (TDR) targets database search for draggability yielded UDP-N-acetylglucosamine-1-carboxyvinyltransferase (Mur A) as a single outcome among the above 24 enzymes.

UDP-N-acetylglucosamine-1-carboxyvinyltransferase (MurA) is an enzyme involved in the first step of peptidoglycan biosynthesis, an essential component of bacterial cell wall [10,11]. MurA catalyses the transfer of the enolpyruvyl of phosphoenolpyruvate (PEP) to the 3'-hydroxyl group of the glucosamine of UDP-N-acetylglucosamine (UNAG) [12,13] This reaction occurs through the formation of a tetrahedral intermediate of UNAG and PEP and the subsequent elimination of inorganic phosphate. The antibiotic fosfomycin has been reported as one of the most widely used inhibitors against MurA [14]. Its inhibitory action involves the formation of a covalent adduct with the Cys 115 residues of MurA [15,16]. However, development of resistance to fosfomycin has been observed in few gram positive bacteria by single amino acid mutation of Cys 115

Accession no.	Gene	Description
KEGG Pathway mlb01110: Biosynthesis of secondary metabolites		
Glutamyl-tRNA reductase gi|13093997|emb|CAC31938.1|		
L-serine dehydratase gi|13093493|emb|CAC30708.1|		
Ferredoxin-dependent glutamate synthase gi|13092454|emb|CAC29569.1|		
Isocitrate dehydrogenase gi|13093872|emb|CAC32204.1|		
5-methyltetrahydropteroyltriglutamate-homocysteinmethyltransferase i|13093010|emb|CAC31342.1|		
KEGG Pathway mlb00473: D-Alanine metabolism		
D-alanine-D-alanine ligase A gi|13093442|emb|CAC30631.1|		
KEGG Pathway mlb00550: Peptidoglycan biosynthesis		
UDP-N-acetylglucosamine-1-carboxyvinyltransferase, MurA gi|13093131|emb|CAC31531.1|		
UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapept pyrophosphoryl-undecaprenol N-acetylglucosamine transferase, MurG gi|13092984|emb|CAC31295.1 gi|13092978|emb|CAC31289.1| penicillin-binding protein 2		
mlb00061 Fatty acid biosynthesis		
Fatty acid synthase gi|13093156|emb|CAC31572.1|		
mlb00400 Phenylalanine, tyrosine and tryptophan biosynthesis		
3-dehydroquinate synthase gi|13092728|emb|CAC30026.1| Chorismate synthase gi|13092726|emb|CAC30024.1|		
mlb03030 DNA replication		
Gi|13093876|emb|CAC32212.1| replicative DNA helicase gi|13092915|emb|CAC30343.1| DNA primase		
cga00500 Starch and sucrose metabolism		
Gi|13093758|emb|CAC31210.1| probable trehalose-phosphate synthase		
mlb00730 Thiamine metabolism		
Gi|13093440|emb|CAC30629.1| probable thiamine-monophosphate kinase Gi|13092616|emb|CAC29802.1| putative thiamine biosynthesis protein		
mlb03420 Nucleotide excision repair		
Gi|13093278|emb|CAC31773.1| excinuclease ABC subunit A gi|13092761|emb|CAC30070.1| putative Excinuclease ABC subunit C		
mlb00230 Purine metabolism mlb00240 Pyrimidine metabolism		
Gi|13093163|emb|CAC31588.1| DNA polymerase III, [alpha] subunit		
mlb03060 Protein export		
Gi|13092889|emb|CAC30288.1| putative preprotein translocase subunit		
mlb02010 ABC transporters		
Gi|13092784|emb|CAC30097.1| putative membrane protein		
mlb02020 Two-component system		
Gi|13092535|emb|CAC29683.1| putative two-component system sensor kinase Gi|13092413|emb|CAC29509.1| putative chromosomal replication initiator protein		

Table 1:

to Asp [17,18,19]. This substitution of Cys by Asp also occurs in the MurA of *M. leprae*. Therefore, the need arises for new drugs identification/development against MurA in the strains having this mutation.

Thus, T6362 has been used as *Ml*-MuRA inhibitor in the current study as its mode of action is different from fosfomycin binding and works by blocking the transition of MurA from open to the closed state which is in general is most essential for the functionality of this enzyme. T6362 is a 5-sulfonyloxy-anthranilic acid derivative and targets the loop Pro 112 - Pro 121 of the enzyme [20]. The corresponding residue in *Ml*-MurA is Pro 114 - Pro 123. It is well known that the inhibition by T6362 is competitive with respect to UNAG with K_i of 16 µM [21].

Hence in the present work we took MurA as a target for homology modeling and docking with its substarte UNAG and T6362 inhibitor to identify the key residues that could be utilized further for drug development against leprosy.

Molecular Modeling of *M. leprae* MurA (*Ml*-MurA):

The primary amino acid sequence analysis of *M. leprae* MurA (*Ml*-MurA) against PDB database showed 48% identity with Mur A from *Listeria monocytogenes* (PDB ID:3R38). The structure consists of two globular domains with the active site located between them, and it was found to be similar to known architecture reported in *E.coli*. The structural superposition of the model with the template (3R38) showed that the structural motifs at the active site in *M. leprae* were highly conserved (Figure 1).

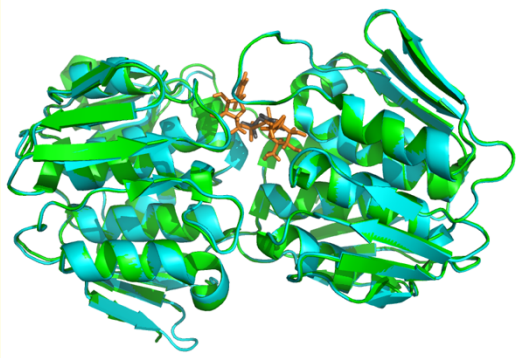

Figure 1: Superposition of M.leprae murA with the template L. monocytogenes (3R38) colored in green and cyan respectively. UNAG binding at the active site is shown, colored in orange stick model.

Binding site and Docking analysis

The model obtained after homology modeling and structure refinement was uploaded into the CASTp server calculation to identify surface accessible pockets as well as interior inaccessible cavities, for proteins and other molecules. The pocket corresponding to the binding site of substrates for MurA was compared with the pocket of the template used to build the model. Out of the residues common to both the pockets few were selected on the basis of the previously established results by other studies. These include N23 and D305, which are essential residues in the MurA binding site [22] and correspond to N24 and D305 in *Ml*-MurA. For the PEP binding site, Lysine 22 is crucial for enzymatic activity and formation of covalent adducts with PEP and fosfomycin [23-34]. R120 [20] corresponding to R122 in *M. leprae* is also involved in PEP binding. New inhibitors proposed for MurA interact with many residues [21], the most significant of which R91, P121, V163, G164 correspond to R93, P123, V164, G165 in our sequence. Thus, the residues K22, N24, R93, R122, P123, V164, G165, and D305 were chosen as the binding sites for docking.

The docking study of *Ml*-MurA was carried out with its natural substrate UNAG in order to compare the relative efficiency of binding of the inhibitor to the enzyme. The docked UNAG showed multiple interactions at the active site involving residues from both the domains (Figure 2, and Table 2).

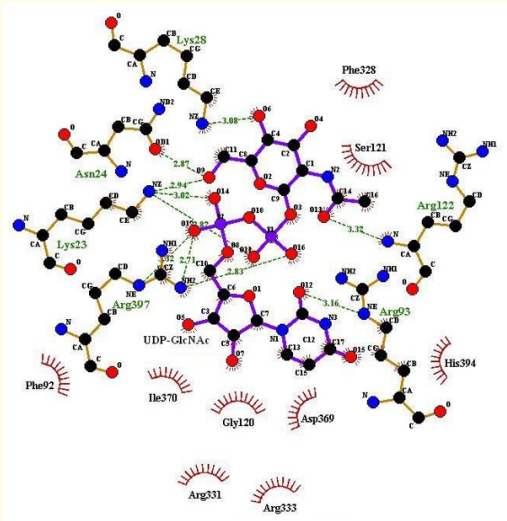

Figure 2: Ligplot diagram showing the docking of UNAG with Ml-MurA. The interacting residues can be seen in the figure.

Substrate/Ligand	Glide Score	Residues involved in hydrophobic/ non-bonding interaction	Residues involved in H-bond formation
51062364 (T6362)	-5.19	Asn24,Leu113,Pro114,Gly116,Ser121,Leu124, Val164,Glu 189,Thr304,Val327,Phe328	Lys28,Arg93, Gly115,Arg122
UNAG	-7.23	Phe92, Gly120, Ser121, Phe328, Arg331, Arg333, Asp369, Ile370, His394	Lys23, Asn24, Lys28, Arg93, Arg122, Arg397

Table 2:

The GLIDE scores generated were used for further analysis of these ligands. UNAG has the highest GLIDE score of -7.232 and GLIDE score of PEP was - 4.538. The inhibitor should have its score close to this value to be effective. Fosfomycin, the well accepted inhibitor against Mur A, has the second best score of - 5.25 but since it cannot be used as an inhibitor in the MurA of *M. leprae* due to resistance conferred by the mutated Cys residue, an inhibitor with a similar GLIDE score is required. The docking of selected inhibitor T6362 (has a different binding mechanism to MurA than that of Fosfomycin), had a comparable GLIDE score of -5.194 (Figure 3). T6362 can thus be used as a further target for lead identification against *M. leprae* MurA.

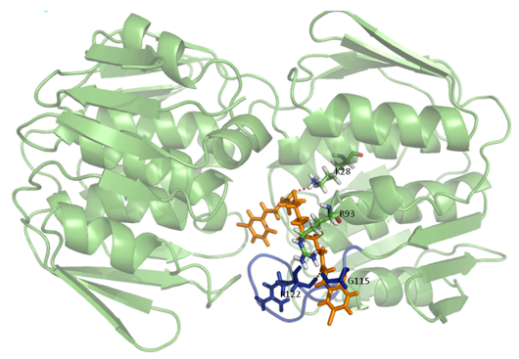

Figure 3: Docking of T6362 with Ml-MurA. T6362 has been shown in stick model, colored orange. The corresponding loop Pro 114 - Pro 123 of the enzyme targeted for its binding is shown in blue. Hydrogen bonds are colored with magenta dashes.

Conclusion

In this work, the complete genome of *M. leprae* has been studied and compared to its host *H. sapiens*. After eliminating the targets which are currently being used against leprosy, several new drug targets were identified which can be used in the treatment of leprosy. These included the enzymes for secondary metabolite synthesis, cell wall biosynthesis, fatty acid biosynthesis, alanine metabolism and shikimate pathway. Out of these, the cell wall biosynthesis enzyme (MurA) was found to be a common drug target against tuberculosis in the TDR database. The structure function analysis of *Ml*-MurA and docking study with inhibitor identified some highly conserved residues (Arg93, Ser121, Arg122, Gly165 and Phe328) which can be targeted to develop novel drugs against leprosy. T6362 was identified as a inhibitors against *Ml*-MurA based on their Glide score and designing of its analogs may lead to development of better drugs against leposy.

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