Journal of Theoretical Biology 351 (2014) 37–46

Contents lists available at ScienceDirect

Journal of Theoretical Biology

journal homepage: www.elsevier.com/locate/yjtbi

Molecular modeling and molecular dynamics simulations of GPI 14 in *Leishmania major*: Insight into the catalytic site for active site directed drug design

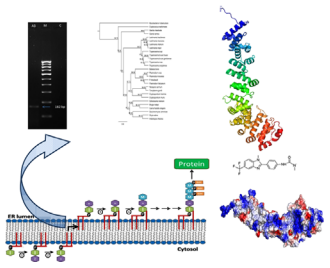
Sonali Shinde, Milsee Mol, Virashree Jamdar, Shailza Singh *

National Centre for Cell Science, NCCS Complex, Ganeshkhind, Pune 411007, India

HIGHLIGHTS

- *Leishmania major* expresses a putative GPI 14 gene which could be a possible therapeutic target.
- *In silico* modeling of *L. major* putative GPI14 protein and its docking with inhibitors.
- Putative GPI 14 in *L. major* shows substrate that is non-specific to human GPI 14.
- Phylogeny of GPI 14 in *L. major* shows close relation to *Trypanosoma* and *Schistosoma*.
- Multiple biosynthetic pathways can be targeted through GPI 14.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 November 2013
Received in revised form
10 February 2014
Accepted 17 February 2014
Available online 25 February 2014

Keywords:
Cutaneous leishmaniasis
GPI biosynthesis
Docking
Gene expression
Phylogeny

ABSTRACT

Leishmania major causes cutaneous form of Leishmaniasis affecting 21 million people in developing countries. Overuse of the chemotherapeutics against leishmaniasis has resulted in the development of drug resistance in the parasite. To surmount this emerging threat we have attempted to target the surface molecules. Glycosylphosphatidylinositol is one such molecule that is present abundantly and thus our work revolves around the enzyme mannosyltransferase (GPI 14), an enzyme essential to add mannose on the glycosylphosphatidyl. It has been targeted for drug discovery on account of growing resistance to miltefosine in *L. major*. This paper serves as the first attempt to detect GPI 14 gene in *L. major* supported with modeling and molecular dynamic analysis of complete three dimensional structure of GPI 14. The functional analysis revealed multiple transmembrane regions in GPI 14 and a close phylogenetic relation with *Trypanosoma* species and *Schistosoma mansoni* with highest bootstrap values. The protein model obtained was subjected to minimization for 14 ns simulation. Eight derivatives of *N*-4-(-5(trifluromethyl)-1-methyl-1*H* benzo[*d*]imidazole-2 yl) phenyl) were docked onto GPI 14. The

Abbreviations: CL, cutaneous leishmaniasis; MCL, mucocutaneous leishmaniasis; VL, visceral leishmaniasis; LPG, lipophosphoglycan; GIPLs, glycoinositolphospholipids; GPI, glycosylphosphatidylinositol; iNOS, inducible nitric oxide synthase; NO, nitric oxide; PPG, proteophosphoglycans; GlcN, glucosamine; PI, phosphatidyl inositol; Man, mannose; EtNP, ethanolamine phosphate; ER, endoplasmic reticulum; GlcNAc, *N*-acetyl glucosamine; PIG, phosphatidylinositol glycan; ORF, open reading frame; I-TASSER, iterative threading assembly refinement; PDB, protein databank; LOMETs, local meta threading server; MC, Monte Carlo; MD, molecular dynamic; PROSA, protein structure analysis; SAVS, structural analysis and verification server; SMART, simple modular architecture research tool; TM, transmembrane; NJ, neighbour joining; MCMC, Monte Carlo Markov chain; GROMACS, groningen machine for chemical simulations; SPC, simple point charge; NVT, number of moles, volume, temperature; NPT, number of moles, pressure, temperature; PME, particle Mesh Ewald; RMSD, root mean square deviation; RMSF, root mean square fluctuation; LGA, Lamarckian genetic algorithm; PE, potential energy; RT-PCR, reverse transcriptase polymerase chain reaction

* Corresponding author. Tel.: +9120 2570 8296/95; fax: +9120 2569 2259.
E-mail addresses: shailza_iitd@yahoo.com, singhs@nccs.res.in (S. Singh).

http://dx.doi.org/10.1016/j.jtbi.2014.02.017
0022-5193

contact frequency of GPI 14 with the docked compounds suggested the inhibition of mannosylation proposing the druggability for leishmaniasis therapy.

1. Introduction

Leishmaniasis is one of the most important neglected tropical disease of the world, ranking third on the scale of communicable diseases and second leading cause of parasite related death after malaria. According to the Global Burden of Disease Report it is endemic in 98 countries, putting 350 million people at risk of contracting the disease (Expert Committee, 2010). Around 300,000 deaths due to leishmaniasis are reported each year (www.dndi.org). Current chemotherapeutics available for the treatment of leishmaniasis are the standard pentavalent antimonial (sodium stibogluconate), fluconazole, pentamidine, paromomycin, and miltefosine. The overuse of these chemotherapeutics has resulted in the development of drug resistance in the parasite, with substantial side effects associated with them (Mcgwire and Satoskar, 2014; Croft et al., 2006). To overcome the resistance, there is a need to identify a novel strategy to distinguish a disease associated target in the causative agent. Omics data can be mined to identify 'druggable' targets in *Leishmania*. Computational analysis of these data can potentially change the way drugs are designed. For example gene function from gene expression data can be clustered together belonging to a particular metabolic pathway and perturbation experiments can be carried out, to define the importance of a molecular species (gene/protein/metabolites) in that pathway.

Lipid metabolism in *Leishmania major* covers about 19.80% of the entire metabolic pathway, of this 62% comprises of lipophosphoglycan (LPG) and glycoinositolphospholipids (GIPLs), which are the end products of the glycosylphosphatidylinositol (GPI) anchor biosynthetic pathway (Chavali et al., 2008; Hong and Kinoshita Trypanosome, 2009). LPG and GIPL are GPI anchored molecules that form a protective surface coat and mediate essential host-parasite interactions (Naderer et al., 2004). It has been shown that LPG and GIPLs modulate the synthesis of the inducible nitric-oxide synthase (iNOS) and hence nitric oxide (NO) in the macrophages is an important mediator of a variety of biological functions including microbicidal activity against *Leishmania* species. GIPLs inhibit the synthesis of NO in time and dose-dependent manner in contrast to LPG which is present in the promastigote stage that does not inhibit NO synthesis (Proudfoot et al., 1990, 1996). This data, suggests that GIPLs may contribute towards the survival of the parasite by evading the innate immune response. GPI-anchor is widely distributed and conserved in various eukaryotes and is essential for development in higher animals as well as for growth of yeast and protozoan parasites. According to (Ilgoutzl and McConville, 2001) GIPLs and LPGs of the trypanosomatids are unique molecular entities and have components that are not found either in other eukaryotes or in bacteria. These molecules collectively form a protective barrier, guarding promastigotes from microbicidal action of complement system, oxygen radicals and hydrolase of the mammalian immune system, thus gaining an easy intrusion into the macrophages to complete its life cycle. GPI anchors is densely present in all life stages of the parasite expressing up to 10–20 million copies making it a potential target for anti-leishmanial drugs. The prime function of GPI-anchor is to yield a stable association of proteins with the surface membrane lipid bilayer (Natalia and Sukhareva, 2003). It is a glycolipid consisting of phosphatidylinositol (PI), glucosamine (GlcN), mannose (Man) and ethanolamine phosphate (EtNP) and is synthesized in the endoplasmic reticulum (ER) by 10 sequential enzyme reactions explained (Ferguson, 1999) and diagrammatically represented in (Fig. 1). GPI-anchored proteins depart from the ER and are transported to the plasma membrane to be incorporated in the lipid rafts via Golgi apparatus (Takeda, 1995). The fourth reaction of the pathway that involves the addition of Man by mannosyltransferase I (PIG-M, PIG-V and PIG-B) is also called as GPI 14 (Maeda et al., 2001). Structurally, it has a DXD short conserved motif that is found in many glycosyltransferases that adds a range of different sugars to other sugars, phosphates and proteins and manganese dependent glycosyltransferases (Wiggins and Munro, 1998). The *L. major* open reading frame (ORF) encoding this enzyme has very limited sequence similarity to any structural proteins in any eukaryotes (Rogers, 2011). It is functionally different to mammalian pathway as the enzyme adds an extra ethanolamine phosphate group to the first mannose residue and side chain carbohydrate modification

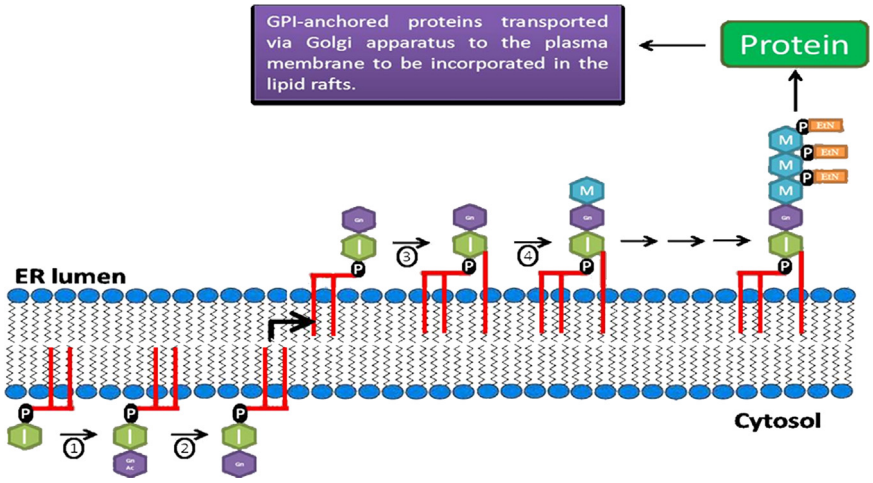


Fig. 1. GPI 14 in the biosynthesis of GPI anchor. Reaction 1 is catalyzed by enzyme complex consisting of seven components, PIG-A, PIG-C, PIG-H, PIG-P, GPI1, DPM2 and PIG-Y. Reaction 2 is catalyzed by PIG-L (de-*N*-acetylase). Reaction 3 is catalyzed by PIG-W and PIG-M (GPI 14/mannosyltransferase). Reaction 4 is catalyzed by PIG-V and PIG-B. Eventually the GPI anchor is attached to the protein to be transported via Golgi apparatus to the lipid rafts in the plasma membrane.