BMB
reports

Modeled structure of trypanothione reductase of *Leishmania infantum*

Bishal K. Singh[1], Nandini Sarkar[1], M.V. Jagannadham[2] & Vikash K. Dubey[1,]*

[1]Department of Biotechnology, Indian Institute of Technology Guwahati, Assam-781039, India, [2]Molecular Biology Unit, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, India

Trypanothione reductase is an important target enzyme for structure-based drug design against *Leishmania*. We used homology modeling to construct a three-dimensional structure of the trypanothione reductase (TR) of *Leishmania infantum*. The structure shows acceptable Ramachandran statistics and a remarkably different active site from glutathione reductase(GR). Thus, a specific inhibitor against TR can be designed without interfering with host (human) GR activity. [BMB reports 2008; 41(6): 444-447]

INTRODUCTION

Trypanothione reductase (E.C. 1.6.4.8) is a member of the disulfide oxidoreductase family of enzymes (1) that presents an attractive target for the development of new drugs by rational inhibitor design. TR is an NADPH-dependent flavoprotein unique to protozoan parasites from the genera *Trypanosoma* and *Leishmania*.

Mammals use their most abundant thiol, the tripeptide glutathione (L-γ-glutamyl-L-cysteinyl glycine; GSH), in a range of metabolic functions (2). In particular, GSH regulates the intracellular reducing environment and protects biological molecules from the oxidizing by-products of aerobic metabolism, and GSH is therefore often oxidized to glutathione disulfide (GSSG). The flavoenzyme GR (E.C. 1.6.4.2) ensures high levels of GSH by reducing GSSG (3). Glutathione is not the principal thiol in trypanosomes, and these protozoa do not possess GR. Instead, the metabolites trypanothione (T(SH)2) and N^1-glutathionyl-spermidine (GspdSH) and the auxiliary enzyme TR maintain the reducing environment in protozoan cells. TR and GR are both homodimeric, with a subunit molecular weight of approximately 52 kDa, and catalyze the transfer of electrons from NADPH to their specific substrates via an FAD prosthetic group and a redox-active cysteine disulfide (3, 4). TR and mammalian GR share approximately 40% sequence identity and are mutually exclusive with respect to disulfide substrate specificity (1), indicating a difference in substrate binding pocket geometry.

The trypanothione system is necessary for protozoan survival because the dithiol trypanothione is required for the synthesis of DNA precursors, the homeostasis of ascorbate, the detoxification of hydroperoxides, and the sequestration/export of thiol conjugates. Moreover, the majority of peroxidases that eliminate the reactive oxygen species (ROS) generated in the aerobic metabolism are trypanothione dependent (5). In addition, the absence of this pathway from the mammalian host and the sensitivity of trypanosomatids to oxidative stress make it an attractive target for structure-based drug design.

The leishmaniasis are caused by 20 species pathogenic for humans belonging to the genus *Leishmania*, a protozoa transmitted by the bite of a tiny 2 to 3 millimeter-long insect vector, the *phlebotomine* sandfly. Leishmaniasis results in millions of human deaths each year worldwide. The chemotherapeutic treatments currently available have a number of limitations due to poor efficacy, unacceptable host toxicity and drug resistance, and new targets drug targets are required. Trypanothione reductase may be an ideal target for structure based drug design. In the current report, we describe the structure of trypanothione reductase *Leishmania infantum*. The PDB file of the protein is submitted to the journal as supplementary material.

RESULTS AND DISCUSSION

Choice of template

The closest trypanothione reductase (TR) sequence to that of *Leishmania infantum* comes from *Crithidia fasciculate* (78% sequence identity), with the next closest from *Trypanosoma cruzi* (66% sequence identity). We therefore selected two templates from the structures of *Crithidia fasciculate* (PDB accession code 1FEA) and *Trypanosoma cruzi* (PDB accession code 1AOG) and performed an alignment (Fig. 1). Out of 491 residues, 300 amino acids are conserved across these sequences.

Quality of homology model

The Ramachandran plot (submitted as supplementary material) for trypanothione reductase shows that most non-glycine resi-

*Corresponding author. Tel: 91-361-2582203; Fax: 91-361-2582249; E-mail: vdubey@iitg.ernet.in

Received 10 December 2007, Accepted 11 January 2008

Keywords: Drug design, Structure-Function, Reductase activity, Homology Modeling

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                        10        20        30        40        50        60
TryR/C.fasciculata      -SRAYDLVVIGAGSGGLEAGWNAASLHKKRVAVIDLQKHHGPPHYAALGGTCVNVGCVPKKLMVTGAN
TryR/T.cruzi            -SKIFDLVVIGAGSGGLEAAWNAATLYKKRVAVIDVQMVHGPPFFSALGGTCVNVGCVPKKLMVTGAQ
TryR/L.Infantum         MSRAYDLVVLGAGSGGLEAGWNAAVTHKKKVAVVDVQATHGPPLFAALGGTCVNVGCVPKKLMVTGAQ
                         *   **** ********* ****   ** *** * *  ****   ********************

                        70        80        90        100       110       120       130
TryR/C.fasciculata      YMDTIRESAGFGWELDRESVRPNWKALIAAKNKAVSGINDSYEGMFADTEGLTFHQGFGALQDNHTVL
TryR/T.cruzi            YMEHLRESAGFGWEFDRTTLRAEWKNLIAVKDEAVLNINKSYDEMFRDTEGLEFFLGWGSLESKNVVN
TryR/L.Infantum         YMDLIRESGGFGWEMDRESLCPNWKTLIAAKNKVVNSINESYKSMFADTEGLSFHMGFGALQDAHTVV
                        **   *** ***** **      ** *** *   *  ** **  ** ***** *  * * *     *

                         140       150       160       170       180       190       200
TryR/C.fasciculata      VRESADPNSAVLETLDTEYILLATGSWPQHLGIEGDDLCITSNEAFYLDEAPKRALCVGGGYISIEFA
TryR/T.cruzi            VRESADPASAVKERLETEHILLASGSWPHMPNIPGIEHCISSNEAFYLPEPPRRVLTVGGGFISVEFA
TryR/L.Infantum         VRKSEDPHSDVLETLDTEYILIATGSWPTRLGVPGDEFCITSNEAFYLEDAPKRMLCVGGGYIAVEFA
                        ** * ** * * * * ** ** * ****      *   ** *******   * * * **** *  ***

                           210       220       230       240       250       260       270
TryR/C.fasciculata      GIFNAYKARGGQVDLAYRGDMILRGFDSELRKQLTEQLRANGINVRTHENPAKVTKNADGTRHVVFES
TryR/T.cruzi            GIFNAYKPKDGQVTLCYRGEMILRGFDHTLREELTKQLTANGIQILTKENPAKVELNADGSKSVTFES
TryR/L.Infantum         GIFNGYKPCGGYVDLCYRGDLILRGFDTEVRKSLTKQLGANGIRVRTNLNPTKITKNEDGSNHVHFND
                        **** **   * * * ***  ******   *  ** ** ****   *  ** *   * **   * *

                             280       290       300       310       320       330       340
TryR/C.fasciculata      GAEADYDVVMLAIGRVPRSQTLQLEKAGVEVAKNGAIKVDAYSKTNVDNIYAIGDVTDRVMLTPVAIN
TryR/T.cruzi            GKKMDFDLVMMAIGRSPRTKDLQLQNAGVMI-KNGGVQVDEYSRTNVSNIYAIGDVTNRVMLTPVAIN
TryR/L.Infantum         GTEEDYDQVMLAIGRVPRSQALQLDKAGVRTGKNGAVQVDAYSKTSVDNIYAIGDVTNRVMLTPVAIN
                        *   * * ** **** **   ***  ***   ***   ** ** * * ********* **********

                                350       360       370       380       390       400
TryR/C.fasciculata      EGAAFVDTVFANKPRATDHTKVACAVFSIPPMGVCGYVEEDAAKKYDQVAVYESSFTPLMHNISGSTY
TryR/T.cruzi            EAAALVDTVFGTTPRKTDHTRVASAVFSIPPIGTCGLIEEVASKRYEVVAVYLSSFTPLMHKVSGSKY
TryR/L.Infantum         EGAAFVETVFGGKPRATDHTKVACAVFSIPPIGTCGMTEEEAAKNYETVAVYASSFTPLMHNISGSKH
                        * ** * ***   ** **** ** ******* * **  ** * * *  **** ********  ***

                        410       420       430       440       450       460       470
TryR/C.fasciculata      KKFMVRIVTNHADGEVLGVHMLGDSSPEIIQSVAICLKMGAKISDFYNTIGVHPTSAEELCSMRTPAY
TryR/T.cruzi            KTFVAKIITNHSDGTVLGVHLLGDNAPEIIQGIGICLKLNAKISDFYNTIGVHPTSAEELCSMRTPSY
TryR/L.Infantum         KEFMIRIITNESNGEVLGVHMLGDSAPEIIQSVGICMKMGAKISDFHSTIGVHPTSAEELCSMRTPAY
                        * *   * **   * ***** ***  *****   ** *  ******  ***************** *

                          480       490
TryR/C.fasciculata      FYEKGKRVEKI---D
TryR/T.cruzi            YYVKGEKMEK----P
TryR/L.Infantum         FYESGKRVEKLSSNL
                         *  *   **
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Fig. 1. Alignment of amino acid sequences of trypanothione reductase (TR) of *C. fasciculate, T. cruzi, and L. infantum*. '*' indicates a site that is conserved in alignment; '-' represents a gap introduced to optimize alignment. Key residues needed for function are shown in bold.

dues are within the allowed regions, with 92.9% of these residues in the energetically most favored area. No residues are in disallowed regions of the plot (Fig. 2A). The modeled TR from *L. infantum* has 0.47 Å and 0.44 Å RMS deviation to all C^{α} atoms in TR from *C. fascuculate* and *T. cruzi*, respectively.

Overall structure

The comparison of our modeled TR with *T. cruzi* and *C. fasciculata* shows that the secondary structure and domain organization is fairly well conserved. Earlier, a four-domain model was constructed along the amino acid sequence (6), followed by a three-domain model based on hydrophobic cores (7-9). Domain I is composed of two segments and binds FAD, domain II binds with NADPH, and domain III forms an interface with partner subunit. A ribbon diagram of these domains is presented in Fig. 2B to illustrate the domain organization of the modeled protein.

Eleven amino-acid residues of domain I of TR from *Trypanosoma* directly interact with the FAD molecule via 17 interactions (10), with Met333, Gly196, Arg222, Arg228, Tyr198, and Tyr 221 involved in NADPH binding (10). These residues are conserved in TR of *L. infantum*. However, the TR from *L. infantum* and host (mammalian) GR show different co-factor binding site geometry (Fig. 3). Tyr114 and Tyr197 are highly conserved residues in the active site of human GR; Tyr114 in the substrate (glutathione disulfide) binding site and Tyr197 in the NADPH site. Although these two residues are conserved in TR at position 110 and 198, a hydrophobic residue, Leu337, is replaced by an ionizable side chain (Met333), Met202 is replaced by Phe203, and position 287 has an additional basic side chain in TR (Fig. 3). Thus, a rationally designed inhibitor may block TR co-factor binding/substrate binding site without interfering with GR activity. *E.coli* GR can be converted to an enzyme with TR activity by mutating the substrate recognition site (11). The crystal structure of *T. cruzi* TR shows direct enzyme-substrate hydrogen bonds with side chains of Glu19 and Tyr111 and the peptide link between the GlyI and spermidine components (12). All these key residues are conserved in *L. infantum* TR and indicate similar interactions.

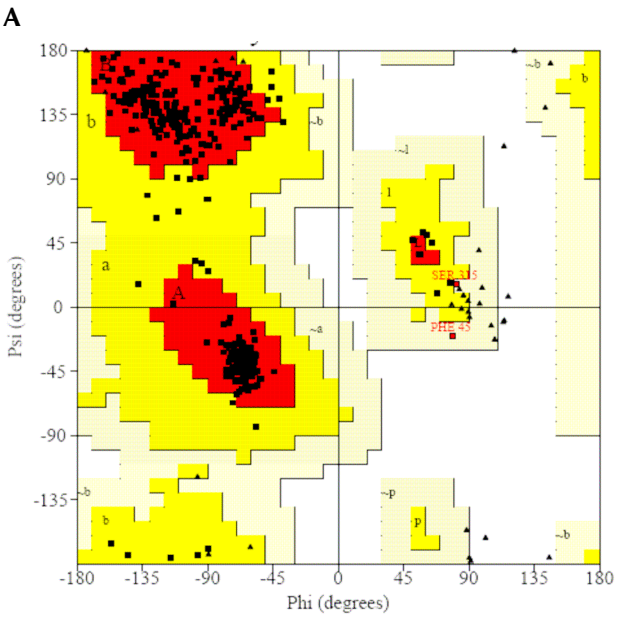

Fig. 2. (A) Ramachandran plot of the modeled structure (B) Ribbon drawing of modeled trypanothione reductase monomer from the *L. Infantum*. FAD binding domain I (red), NADPH binding domain II (yellow), and Interface domain III (blue).

METHODS

Choice of template

Homologs to the target enzymes sequences were identified with PSI-BLAST (13) for the PDB databank on NCBI. The chosen templates were the sequence from the latest version of the PDB databank with the lowest expected value and highest score after four iterations.

Sequence alignment

Alignment of sequences with their templates structure was done using the alignment.salign() command in MODELLER9V1 (14). The software also takes into account structural information from the template when constructing an alignment. The MODELLER script was used for aligning all target sequences in the .ali file with their corresponding template structures in the PDB files.

Finally, the alignment was written out in two formats, PIR and PAP. The PIR format is used by MODELLER in the subsequent 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 modeling and sequence alignment scripts are submitted as supplementary material.

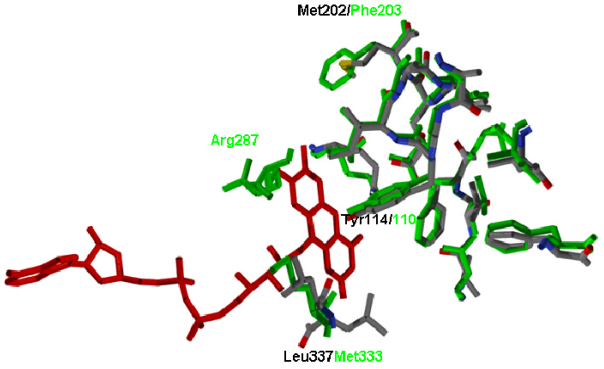

Fig. 3. Differences in active site geometry of human glutathione reductase (PDB ID: 3GRS) and modeled TR of *L. infantum*. FAD is shown in red. Green and black show active site geometry of TR and GR respectively.

Homology modeling

A 3D model of the target sequence was constructed with the automodel class of MODELLER9V1 to generate five similar iterative models of the target sequence based on its template structure and the alignment input file 'filename.ali' (PIR format). The 'best' model was selected by picking the model with the lowest MODELLER objective function value, which is reported in the second line of the model PDB file.

Quality of homology model

The quality of the structures were analyzed with the PROCHECK (15) program to calculate the main-chain torsional angle, i.e., a Ramachandran plot (16).

Supporting information

The PDB file of the Homology-Modeled Structure of Trypanothione Reductase (TR) of *Leishmania infantum* was submitted to the Protein Model Data Base (http://mi.caspur.it/PMDB/). The PMBD id of the submission is PM0075098. Scripts for modeling and sequence alignments are submitted as supporting information.

Acknowledgements
The infrastructural facilities provided by the Indian Institute of Technology, Guwahati are acknowledged.

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