Journal of Molecular Graphics and Modelling 27 (2009) 665–675

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

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

Comparative modeling of thioredoxin glutathione reductase from *Schistosoma mansoni*: A multifunctional target for antischistosomal therapy

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ARTICLE INFO

Article history:
Received 18 June 2008
Received in revised form 7 October 2008
Accepted 27 October 2008
Available online 6 November 2008

Keywords:
Schistosoma mansoni
Thioredoxin glutathione reductase
Glutathione disulfide
Homology modeling
Docking

ABSTRACT

Schistosoma mansoni, a trematode parasite, which causes schistosomiasis and affects more than 200 million people worldwide, lives in an aerobic environment and therefore needs an effective redox mechanism for surviving reactive oxygen species from its host. Although, the host has two different redox systems: glutaredoxin and thioredoxin, the parasite has only one unique multifunctional enzyme, thioredoxin glutathione reductase (TGR) involving a fusion of two proteins, glutaredoxin (Grx) and thioredoxin reductase (TR), for performing all the redox activities. This dependence of *S. mansoni* on a single protein, TGR, for its protection from oxidative stress, makes it a promising drug target. Here, we describe a suitably validated, homology model for *S. mansoni* TGR (SmTGR), developed using both TR and Grx templates, functionally complete in the dimeric form with cofactors NADP(H) and FAD. Comparative analysis of substrate and inhibitor binding pockets of our model with crystal structures of parent TR as well as with that of glutathione reductase (GR), which is an essential component of the Grx system, appears to provide greater insight into the functioning of TGR. This also augments recent observations reported on the basis of X-ray structure data on SmTGR monomer lacking the C-terminal selenocysteine tail.

1. Introduction

Schistosomiasis, or bilharzia, is a tropical disease that is endemic in 74 developing countries and infects more than 200 million people (mostly children under 14) in rural and sub-urban areas according to the World Health Organization (WHO) [1]. The causative organisms of schistosomiasis are trematode parasites that belong to the genus *Schistosoma*. Species like *Schistosoma mansoni*, *S. japonicum*, *S. mekongi* cause intestinal schistosomiasis, whereas *S. haematobium* causes urinary schistosomiasis. Schistosomiasis is second only to malaria in public health importance and hence, demands urgent attention [1]. Currently available drugs include praziquantel (effective in the treatment of all forms of disease), oxamniquine (for intestinal type caused by *S. mansoni*) and metrifonate (for urinary schistosomiasis) [1]. Since the chemotherapy is limited, temporary and prone to resistance, development of vaccines is a challenging alternative [2]. Sequencing of the parasite genomes, manipulating gene expression and understanding gene function, promise faster identification of targets for diagnostics, drugs and vaccines [3].

S. mansoni lives in an aerobic environment and therefore needs an effective redox mechanism for its survival in the host. In eukaryotes, there are two major thiol-dependent redox pathways: glutaredoxin (Grx) and thioredoxin (Trx) [4,5]. The Grx system consists of (i) glutathione reductase (GR), a member of the pyridine nucleotide disulfide oxidoreductase family, which is a homodimer of 55 kDa subunits having a disulfide CXXXXC motif in the active site [6,7], (ii) glutathione or GSH, a γ-Glu-Cys-Gly tripeptide, (iii) Grx, an 11 kDa thiol/disulfide oxidoreductase with CXXC redox motif and (iv) GSH peroxidase (GPx). GR catalyzes the reduction of diglutathione or glutathione disulfide (GSSG), bound at the GR dimer interface, to two reduced GSH molecules *via* a series of electron transport processes shown in Fig. 1. The Trx system is

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Abbreviations: WHO, World Health Organization; Grx, Glutaredoxin; Trx, Thioredoxin; GR, Glutathione Reductase; GSH, Glutathione; GPx, Glutathione Peroxidase; GSSG, Glutathione disulfide; TR, Thioredoxin Reductase; TPx, Thioredoxin Peroxidase; SeC, Selenocysteine motif; TGR, Thioredoxin Glutathione Reductase; SmTGR, *Schistosoma mansoni* TGR; FAD, Flavin Adenine Dinucleotide; NADP(H), Nicotinamide Adenine Dinucleotide Phosphate; RMS, Root Mean Square deviation; SSM, Secondary Structure Method of superposition; SMD, Steered Molecular Dynamics.

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doi:10.1016/j.jmgm.2008.10.009

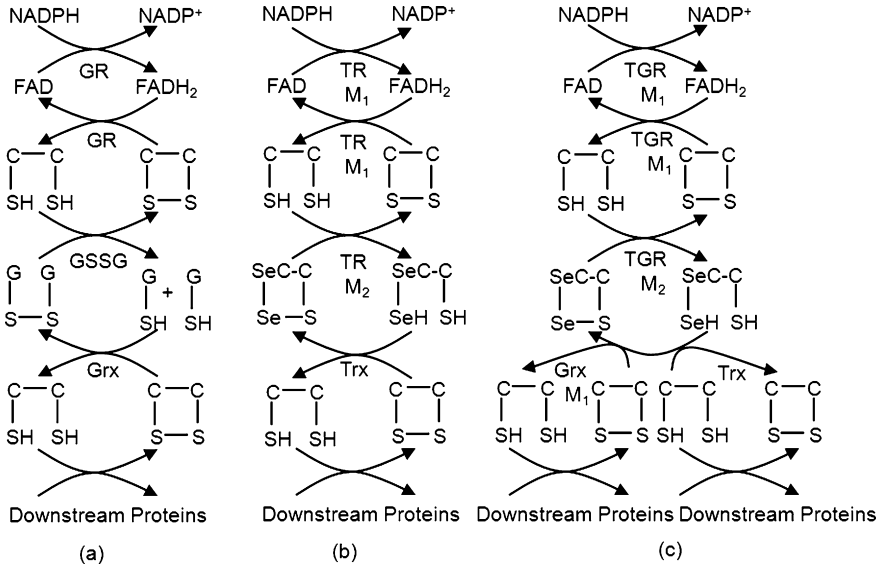

Fig. 1. Electron transport process in (a) GR: Grx pathway—the reaction proceeds with the concomitant oxidation of NADP(H) *via* FAD and CXXXXC disulfide motif. Reduced GR goes on to reduce GSSG resulting in two GSH molecules and in the process it gets oxidized, regenerating the disulfide bridge. GSH further reduces other proteins like GPx and Grx. Reduced Grx performs various Grx-dependent reactions like reduction of various substrates and substrate-related reductases like ribonucleotide reductase (b) TR: Trx pathway—electron transfer mechanism is the same in TRs as GRs except for additional involvement of the SeC motif. The amino terminal redox active disulfide (CXXXXC) of one subunit is reduced by NADP(H) *via* the FAD of the same monomer (M1), thereafter interacting with the C-terminal SeC of the other monomer (M2) which lies close to it. TR reduced at the SeC motif, reduces Trx and itself gets back to the oxidized sate. Reduced Trx performs various Trx-dependent reactions such as providing reducing equivalents to other redox enzymes like TPx and ribonucleotide reductase. (c) TGR: the redox disulfide in TR domain of one monomer (M1) of TGR is reduced by the NADP(H) *via* FAD which reduces the SeC motif of the second monomer (M2). The reduced SeC motif at the C-terminal in TGR then serves the role of a protein-linked GSSG and shuttles electrons to either the Grx domain of M1 or Trx. Thus TGR can coordinate both Grx and Trx-dependent reactions.

composed of (i) thioredoxin reductase (TR), homodimer of 56 kDa subunits, analogous to GR, (ii) Trx, a 12 kDa thiol/disulfide oxidoreductase with redox active CXXC motif and (iii) Trx peroxidase (TPx). Three mammalian TR isozymes TR1 (cytosolic), TR2/TGR and TR3 (mitochondrial) are known. Unlike GR, TRs contain a C-terminal extension that terminates with a conserved tetrapeptide sequence Gly-Cys-SeC-Gly, known as SeC motif (Fig. 2), which is an important component of the electron transfer process [8,9] (Fig. 1).

Unlike TR1 and TR3, TGR or thioredoxin glutathione reductase [10,11], is a fusion of TR with Grx at the N-terminus (Fig. 2). Since Grx is a part of a related albeit different redox system, TGR exhibits specificity for both redox systems (Fig. 1) and this provides TGR with wider substrate specificity [12–14]. SmTGR thus appears to be a single major redox enzyme in *S. mansoni*, completely replacing TR and GR which are functional in its host [15], and is therefore an important drug target for *S. mansoni* [16]. There are a few biochemical studies throwing light on the functional aspects of this protein, reported so far [15,16]. However, a thorough understanding of the functional mechanism, elucidated through extensive biochemical studies and validated on the basis of three-dimensional (3D) structure of the fully functional target, is essential for exploiting the potential of TGR as a therapeutic target. The recently solved crystal structure of SmTGR is that of a monomer with only one cofactor, FAD and without the other cofactor, NADP(H) as well as the SeC tail [17]. Though the structure has shed greater light on the mechanistic details, several issues are yet unclear:

(a) In the species wherein separate Grx and Trx systems operate, there are three essential oxidoreductase activities: (1) TR—Trx (Trx-dependent reactions), (2) GR—GSSG/GSH (GSH-dependent

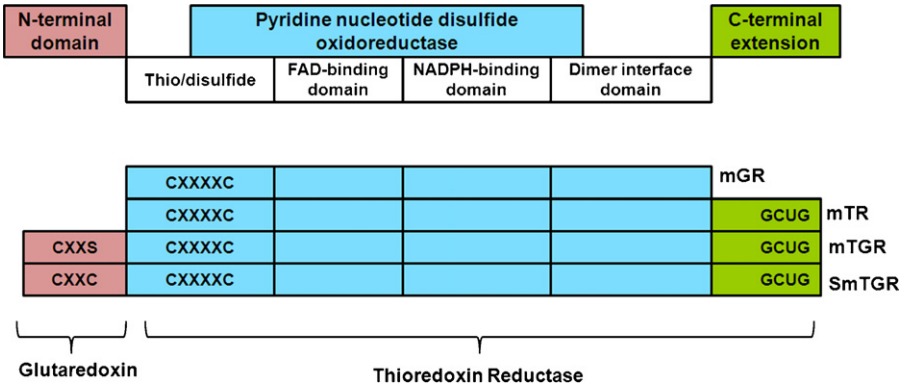

Fig. 2. Comparative domain organization of mammalian GR, TR, TGR and SmTGR. GR contains FAD and NADP(H) binding sites, active site redox center and dimer interface domains. TR has an additional C-terminal extension of GCUG or the SeC motif (U is selenocysteine residue). TGR is a fusion of TR with glutaredoxin at the N-terminus. mTGR and SmTGR differ in the CXXC disulfide of Grx domain wherein one of the cysteines is replaced by a Serine in mTGR.