Parasitology International xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Parasitology International

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

Molecular cloning, characterization and expression profile of a glutathione peroxidase-like thioredoxin peroxidase (TPx_{Gl}) of the rodent malaria parasite *Plasmodium berghei*

Q1 Kyle J. Haselton [a,1], Robin David [a,1], Katherine Fell [a], Emily Schulte [a], Matthew Dybas [a,b], Kenneth W. Olsen [b], Stefan M. Kanzok [a,*]

[a] *Loyola University Chicago, Department of Biology, 1032 W Sheridan Rd, Chicago, IL 60660, USA*
[b] *Loyola University Chicago, Department of Chemistry & Biochemistry, 1032 W Sheridan Rd, Chicago, IL 60660, USA*

ARTICLE INFO

Available online xxxx

Keywords:
Malaria
Plasmodium berghei
Glutathione peroxidase-like
Enzymatic activity
Ookinete
Cellular expression
Protein modeling

ABSTRACT

Glutathione peroxidases (GPx) comprise an important group of redox active proteins with diverse functions, including antioxidant defense and signaling. Although the genome of the malaria parasite *Plasmodium* does not contain a genuine GPx gene a *glutathione peroxidase-like thioredoxin peroxidase* (TPx_{Gl}) has recently been identified and biochemically characterized in the human malaria parasite *P. falciparum*. To gain more insight into the potential biological function of this enzyme we have cloned and expressed TPx_{Gl} of the rodent model system *P. berghei* ($PbTPx_{Gl}$). Biochemical characterization confirmed that the protein is redox active with the *P. berghei* thioredoxin system. We compared PbTPxGl to recently characterized thioredoxin-dependent GPx-type proteins of other organisms, and generated the first hypothetical 3D model of a *Plasmodium* TPx_{Gl}, which indicates the conservation of the thioredoxin-fold and the spatial orientation of a classic GPx catalytic tetrad. Utilizing the *P. berghei* system we demonstrate that $PbTPx_{Gl}$ is continuously expressed in all asexual blood stages, gametocytes and in early mosquito-stage parasites. Confocal microscopy indicates a cytoplasmic localization of $PbTPx_{Gl}$ in all investigated life stages, specifically in mature ookinetes. Our data provides new insights into the structure and ubiquitous expression of *Plasmodium* TPx_{Gl} and warrants further investigation into this potentially important redox enzyme.

© 2014 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Glutathione peroxidases (GPx) and GPx-like proteins comprise a family of redox active enzymes that belong to the thioredoxin superfamily of proteins. They are highly conserved in eukaryotes from fungi to mammals and plants. GPx were originally identified in mammalian cells as low molecular weight, non-heme peroxidases that catalyze the glutathione (GSH)-dependent detoxification of cytotoxic reactive oxygen (ROS) and reactive nitrogen species (RNS) with high efficiency [1]. It was subsequently determined that these enzymes contain a highly reactive selenocysteine (SeCys) in their active sites (SeCys-GPx) while homologous GPx-type proteins, mostly in lower organisms, regularly possess the less active cysteine (Cys) in their active sites (Cys-GPx) [2,3]. Due to the high reactivity of the selenoproteins it has been argued that their cellular function lies in cellular antioxidant defense [4].

A GPx gene has recently been described in the human malaria parasite *Plasmodium falciparum* [5]. The primary sequence exhibits significant similarities with mammalian selenoproteins, including human GPx-1. Most notably, the amino acids that comprise the characteristic active site triad are conserved with the difference that the parasite protein contains a Cys instead of a SeCys. It was hypothesized that the parasite utilizes antioxidant enzymes for protection against cytotoxic ROS. Consequently, an effective enzyme like GPx would represent an attractive target for potential drug development. However, biochemical characterization of the putative *Plasmodium* GPx revealed that the enzyme is up to 1000 times less efficient in detoxifying ROS than the homologous selenoproteins. Furthermore, the enzyme was more efficiently reduced by the small redox protein thioredoxin (Trx) than by the eponymous tripeptide glutathione (GSH) [2]. The protein was thus described as a *glutathione peroxidase-like thioredoxin peroxidase* (TPx_{Gl}). Analysis of the completed *P. falciparum* genome revealed that the parasite does not possess a genuine classical GPx gene [6]. In PlasmoDB the gene (PlasmoDB-ID: PF3D7_1212000) is currently annotated as *Trx-G1* in the *P. falciparum* genome and as *putative glutathione peroxidase* in all other *Plasmodium* species [7]. To remain consistent

* Corresponding author. Tel.: +1 773 508 3790; fax: +1 773 508 3646.
E-mail address: skanzok@luc.edu (S.M. Kanzok).
[1] KJH and RD contributed equally to this work.

http://dx.doi.org/10.1016/j.parint.2014.02.004
1383-5769/© 2014 Elsevier Ireland Ltd. All rights reserved.

with the current literature, however, we will refer to this gene and its homologs as TPx_{Gl} [2,8,9].

Antioxidant efficiency of the cysteine-containing $PfTPx_{Gl}$ is more than 100 times lower compared to that of homologous selenoproteins, such as human GPx-1 [2]. This led to the hypothesis that TPx_{Gl} may have only a peripheral role as an antioxidant enzyme in the malaria parasite [10]. The prediction of a putative targeting sequence at the N-terminus of $PfTPx_{Gl}$ prompted two independent studies on its subcellular localization in the parasite [8,9]. Overlapping observations in *P. falciparum* trophozoites showed the protein in several cellular compartments, including the cytoplasm, the apicoplast, and the mitochondrion. The biological role of this enzyme remains unclear.

In recent years additional Trx-dependent GPx-type proteins have been identified and characterized in diverse organisms [4]. The structures for some of these proteins have been resolved, including yeast [11], the trematode *Schisotosoma* [12] and the poplar tree [13], and have provided a better understanding of the reaction mechanism and substrate specificity of these enzymes. Yet, the cellular functions for most of these proteins have also not yet been determined. The only exception represents glutathione peroxidase 3 (GPx3/Hyr1/Orp1) of *Saccharomyces cerevisiae*, which is hypothesized to function in the antioxidant stress response of the yeast cell as a cellular hydrogen peroxide sensor [14].

To gain more insight into the potential function of *Plasmodium* TPx_{Gl} we have cloned and expressed a putative GPx of the rodent malaria model organism *P. berghei*. Biochemical characterization confirmed that the gene codes for a TPx_{Gl} ($PbTPx_{Gl}$), the functional ortholog of $PfTPx_{Gl}$. Using structural information of homologous proteins we generated the first 3D model of a *Plasmodium* TPx_{Gl}. Working with *P. berghei* allowed us to conduct gene and protein expression studies of $PbTPx_{Gl}$ in several developmental stages of the parasite, including asexual and sexual blood stages, as well as in developing ookinetes from culture and mosquito midguts. Our data represents a first step in determining the cellular function of this potentially important protein.

2. Material and methods

2.1. Parasite maintenance and mosquito infections

Plasmodium berghei parasites (ANKA 2.34) were maintained in Harlan ND4 mice for a maximum of four serial passages and regularly passed through *Anopheles stephensi* mosquitoes. *A. stephensi* mosquitoes were reared under standard conditions (26 °C, 80% RH, 12 h light–dark cycle, 5% sucrose solution). Female mosquitoes (5–10 days post emergence) were used in all experiments. Mosquitoes were fed on *P. berghei* infected mice (10%). Exflagellation (2–4/20×) of parasites was tested prior to feeding to ascertain maturity. Fed mosquitoes were maintained at 21 °C and 80% RH. Midguts of 50–60 mosquitoes were dissected and transferred to Tri-Reagent ® (MRC gene). Following each feed we maintained 20 mosquitoes to test for infection analysis by oocyst count (mercurochrome stain) nine days post infectious blood meal.

2.2. RNA extraction and quantitative real time RT-PCR (RT-qPCR)

Total RNA from blood fed mosquito midguts was extracted using Tri-Reagent (MRC) according to manufacturer's instructions. Isolated RNA was treated with DNAse I (Ambion) and subsequently quantified using the Qubit RNA assay kit with the Qubit fluorometer (Invitrogen). RNA-samples were either immediately used for cDNA synthesis or flash frozen and stored at −80 °C. cDNA was synthesized from total RNA with the High Capacity RNA-to-cDNA kit (Applied Biosystems) using random hexamer primers. Sequences of target genes for primer design were acquired from PlasmoDB (plasmodb.org). RT-qPCR was performed on a StepOnePlus machine (Applied Biosystems) using the Fast SYBR Green Master Mix. Each sample was run in triplicate and yielded highly comparable C_t values (cycle threshold). No primer dimers were detected and amplicons exhibited optimal efficiencies. To test specificity all primer pairs were tested on uninfected mouse blood, non-fed and uninfected blood fed mosquitoes. No amplification products could be detected. Expression data was subsequently analyzed with the StepOne Software v2.2 (Applied Biosystems) and normalized against the expression of 18s rRNA A-Type, which has been established as internal standard for expression analysis in *Plasmodium* mosquito stages [15,16]. For analysis of time course expression data the $\Delta\Delta C_t$ method was applied using the earliest experimental time point as reference sample (RQ = 1).

2.3. Cloning, expression and purification of recombinant PbTrxR and $Pb\Delta TPx_{Gl}$

The following gene specific primers for *pbtpx*$_{Gl}$ (PlasmoDB ID: PBANKA_061050, NCBI accession #: XP_673343.1) were generated according to sequence information on PlasmoDB: Fwd 5′-CCGGATCCATCACAAAAAAATAACTG-3′ which contained a BamHI restriction site and Rev 5′-CCAAGCTTTTATATCCATGTTTGATG-3′, which contained a HindIII restriction site. The forward primer binds 60 bp downstream of the start codon, eliminating the predicted targeting sequence (AA1-17). This resulted in a shortened sequence of 559 bp, designated *pbΔtpx*$_{gl}$. PCR was performed using the following conditions: 35 cycles of 95 °C for 30 s, 54 °C for 1 min, and 63 °C for 45 s. Following sequence verification we cloned *pbΔtpx*$_{Gl}$ into the bacterial pQE30 expression vector (Qiagen) which introduced a 6xHIS tag at the N-terminus of the recombinant protein. The resulting plasmid was transformed into *Escherichia coli* M15 cells. For cloning and expression of *pbtrxr* (PlasmoDB ID: PBANKA_082470, NCBI accession#: 3428560) the following primers were used: Fwd 5′-GGGGATCCTGTAACGATAATAAAA GAATCCTAT CAATG-3′ which contained a BamHI restrictions site and Rev 5′-GGAAGCTTTTATCCA CATTTCCCTCCAC CAC-3′, which contained a HindII restriction site. The resulting fragment was cloned into the bacterial pRSET-A expression vector (Life Tech), which also adds an N-terminal 6xHIS tag to the recombinant protein. The *pbtrxr* plasmid was transformed into BL21 *codon plus* expression cells (Fisher). Protein expression was induced (1 mM IPTG) and bacteria were harvested after a 24 hour incubation period at 37 °C. Recombinant proteins were purified using Ni-NTA resin (Thermo Scientific). Purity of the proteins was assessed via SDS PAGE. Protein concentrations were measured using the Nano-drop 2000 (Fisher). Average yields for $rPb\Delta TPx_{Gl}$ and rPbTrxR were 3 mg and 8 mg per liter of *E. coli* culture, respectively.

Q2 Q3

2.4. Enzyme assays

All enzymatic assays were carried out in 1 ml volume at 25 °C using a Genesys6 UV–Vis spectrophotometer (Fisher). The *insulin reduction* assay was conducted as described [17]. Enzymatic activity of the recombinant protein was determined by adding varying amounts of purified $Pb\Delta TPx_{Gl}$ or PbTrx-1 to a reaction mixture containing bovine insulin (44 μg/ml) and DTT (1 mM) in a potassium phosphate buffer (100 mM potassium phosphate, 2 mM EDTA, pH 7.4). The reduction of the insulin disulfide bonds was monitored as an absorbance increase at 650 nm. Activity of the enzyme was detected after 13 min at a rate of 0.02 Abs/min. Insulin reduction could be detected already after 11 min when enzyme concentration was increased. Recombinant *P. berghei* Thioredoxin-1 (rPbTrx-1) was used as positive control [18] and was detectable after only 3 min post-addition at a rate of 0.05 Abs/min. Coupled enzymatic assays were conducted as described [19,20]. Oxidation of NADPH was followed at 340 nm. All assays were performed in assay buffer containing 100 mM KH_2PO_4, 2 mM EDTA, pH 7.4, 200 μM NADPH ($\varepsilon_{340\ nm} = 6.22\ mM^{-1}\ cm^{-1}$), and 20 μM PbTrx-1. The reaction was started with PbTrxR and the decrease of absorption at 340 nm was monitored during the linear phase.

UNCORRECTED PROOF

Please cite this article as: Haselton KJ, et al, Molecular cloning, characterization and expression profile of a glutathione peroxidase-like thioredoxin peroxidase (TPx_{Gl}) of the rodent malaria ..., Parasitology International (2014), http://dx.doi.org/10.1016/j.parint.2014.02.004

Download English Version:

https://daneshyari.com/en/article/6136517

Download Persian Version:

https://daneshyari.com/article/6136517

Daneshyari.com