

Full Length Research Paper

***Plasmodium* and host glutathione reductase: molecular function and biological process**

Viroj Wiwanitkit

Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok Thailand 10330.

Accepted 15 September, 2006

Glutathione (GSH) is a cysteine-containing tripeptide with reducing and nucleophilic properties which play an important role in cellular protection from oxidative damage of lipids, proteins and nucleic acids. Glutathione reductase (GR) is an NADPH-dependent enzyme that reduces oxidized glutathione (GSSG) to GSH. Naturally, GR is present in human and in *Plasmodium* spp. However, the function of the GR in malarial infection is not well characterized. Here, the author used a new gene ontology technology to predict the molecular function and biological process. Using GoFigure server, the molecular function and biological process in human and *P. falciparum* GR is predicted. Comparing to the human GR, the *P. falciparum* GR has similar molecular functions as glutathione disulfide reductase activity, oxidoreductase activity, disulfide oxidoreductase activity and metal ion binding.

Key words: Human, *Plasmodium falciparum*, glutathione reductase, function.

INTRODUCTION

Glutathione (gamma-glutamyl-cysteinyl-glycine or GSH) is a cysteine-containing tripeptide with reducing and nucleophilic properties which play an important role in cellular protection from oxidative damage of lipids, proteins and nucleic acids (Gerard-Monnier and Chaudiere, 1996). GSH regulates the metabolism of proteins and their activities by means of thiol-disulfide exchange (Gerard-Monnier and Chaudiere, 1996). When present as a trophozoite in human erythrocytes, the malarial parasite *Plasmodium falciparum* exhibits an intense GSH metabolism (Becker et al., 2003). GSH plays a role not only in antioxidative defense and in maintaining the reducing environment of the cytosol (Becker et al., 2003). Many of the known glutathione-dependent processes are directly related to the specific lifestyle of the parasite (Becker et al., 2003). Proteins involved in GSH-dependent processes include glutathione reductase (GR), glutaredoxins, glyoxalase I and II, glutathione S-transferases, and thioredoxins (Becker et al., 2003).

GR is an NADPH-dependent enzyme that reduces oxi-

dized glutathione (GSSG) to GSH. Naturally, GR is present in human and in *Plasmodium* spp. During developmental stages of plasmodia, profound alterations of the structure and function of host erythrocytes take place, in order to support the development and/or survival of the parasite (Mishra et al., 1994). In addition an oxidant stress is also induced by the parasite (Mishra et al., 1994). There is also an increased production of reactive oxygen species (ROS) by the parasite. This may deplete the erythrocyte of its defense mechanisms namely, superoxide dismutase (SOD), catalase, glutathione peroxidase, NADPH, NADH, glutathione (GSH) and GR (Mishra et al., 1994). Malarial glutathione reductase, a homodimer of 110 kDa with a pH optimum of 6.8 and a high preference for NADPH over NADH, was shown to contain FAD as its prosthetic group (Krauth-Siegel et al., 1996). The N-terminal sequence, VYDLVIGGGSGGMA, which can be aligned with residues 20-34 of human glutathione reductase, represents the first beta strand and the diphosphate-fixing helix of the FAD domain (Krauth-Siegel et al., 1996). Methylene blue, an inhibitor of the structurally known *P. falciparum* GR, appears to be a promising antimalarial medication when given in combination with chloroquine (Mishra et al., 1994). However,

*Corresponding authors E-mail: vwiroj@pioneer.netserv.chula.ac.

A) Human GR

EFMHDHADYG FPSCEGKFNR VIK EKRDAYV SRLNAIYQNL TKSHIEIIRG
 HAAFTSDKPT IEVSGKKYTA PHILIAGGMP STPHESQIPG ASLGISDGFF
 QLEELPGRSV IVGAYIAVEM AGILSALGSK TSLIRHDKVL RSFDSMISTN
 CTELENAGVE VLKFSQVKEV KTLSGLEVSMVTAVPGRLPVTMIPDVD
 CLLWAIGRVPNTD LSLNKLGIQT DDKGHIIEVF

B) *P. falciparum* GR

VYDLIVIGGG SGGMAAARRA ARHNAKVALV EKSRLGGTCV
 NVGCVPKIMFNAASVHDIL ENSRHYGFD T KFSFNLPLLV ERRDKYIQR L
 NNIYRQNLSKDKVDLYEGTA SFLSENRLI KGTKDNNNKD NGPLNEEILE
 GRNILI AVGNKPVFPVKGI ENTISSDEFF NIKESKKIGI VSGGYIAVEL
 INVIKRLGIDSYIFARGNRI LRKFDES VIN VLENDMCKNN INIVTFADV V
 EIKKVSDKNLSIHLS DGR IY EHF DHV IY CV GRSPDTENLN LGKLN VETNN
 NYIVVDENQRTSVNNIYAVG DCCMVKKSKE IEDLNLLKLY NEETYLNKKE
 NVTEDIFYNVQLTPVAINAG RLLADRLFLK KTRKTNYKLI PTVIFSHPI
 GTIGLSEAAIQIYGKENVK IYESKFTNLF FSVDIEPEL KEKTYLKLVC
 VGKDELIKGLHIIGLNADEI VQGFAVALKM NATKKDFDET IPIHPTAAEE
 FLTLQPWMK

Figure 1. Sequence of human and *P. falciparum* GR.

Table 1. The summary on the molecular function and biological process comparing between human and *P. falciparum* GR.

Summary	Molecular function	Biological process
Human GR	Gluthathione disulfide reductase activity Electron transporter activity Oxidoreductase activity Disulfide oxidoreductase activity Metal ion binding	Electron transport Glutathione metabolism Response to stress Response to pest/pathogen/parasite
<i>P. falciparum</i> GR	Gluthathione disulfide reductase activity Oxidoreductase activity Disulfide oxidoreductase activity Metal ion binding	Electron transport Glutathione metabolism

the function of the GR in malarial infection is not well characterized. A full understanding of host and parasite GR promises advances in malarial treatment. Here, the author used a new gene ontology technology to predict the molecular function and biological process of this enzyme.

MATERIALS AND METHODS

Getting the sequence

The database Unitprot (Bairoch et al., 2005) was used for data mining of the amino acid sequence for human host and *P. falciparum* GR (Figure 1).

Prediction of molecular function and biological process

The author performs prediction of molecular function and biological process of human and *P. falciparum* GR using a novel gene ontology prediction tool, GoFigure (Khan et al., 2003). GoFigure is a computational algorithm tool which is recently developed in gene ontology (Khan et al., 2003). The tool accepts an input DNA or protein sequence, and uses BLAST to identify homologous sequences in gene ontology annotated databases (Khan et al., 2003). The approach is to use a BLAST search to identify homologs in public databases that have been annotated with gene ontology terms (Khan et al., 2003). These include: SwissProt, Flybase (Drosophila), the Saccharomyces Genome Database (SGD), Mouse Genome Informatics (MGI) and Wormbase (nematode) (Khan et al., 2003). The contents of the results will show results for molecular function as well as biological process of the studied protein (Khan et al., 2003). The prediction of molecular function and biological process were presented and compared.

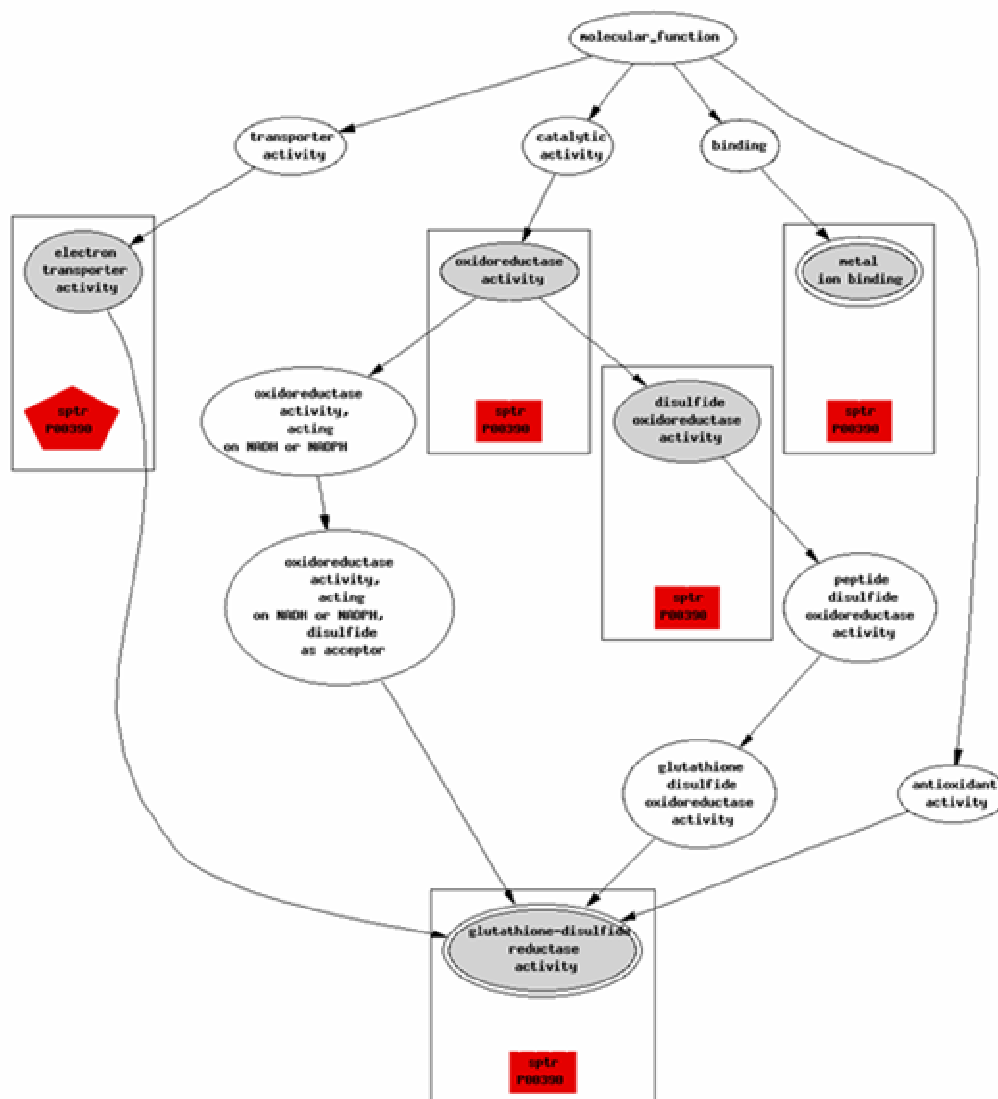


Figure 2. Expected molecular function of human GR.

RESULTS

Sequence of bacterial hemoglobin

From searching of the database Uniprot, sequence of human and *P. falciparum* GR was derived as shown in Table 1.

Prediction of molecular function and biological process

Using GoFigure server, the molecular function and biological process in human and *P. falciparum* GR is predicted. The molecular function and biological process

of human and *P. falciparum* GR are presented in Figures 2 and 3, respectively. The summary on the molecular function and biological process comparing between human and *P. falciparum* GR is presented in Table 1.

DISCUSSION

GR is an enzyme that is believed to have a significant role in malarial infection. Roles of both host and parasite GR in cellular level metabolism during a malarial infection have been proposed. Physiologically, GSH, which is known to guard *P. falciparum* from oxidative damage, may have an additional protective role by promoting heme catabolism (Davioud-Charvet et al., 2001). An ele-

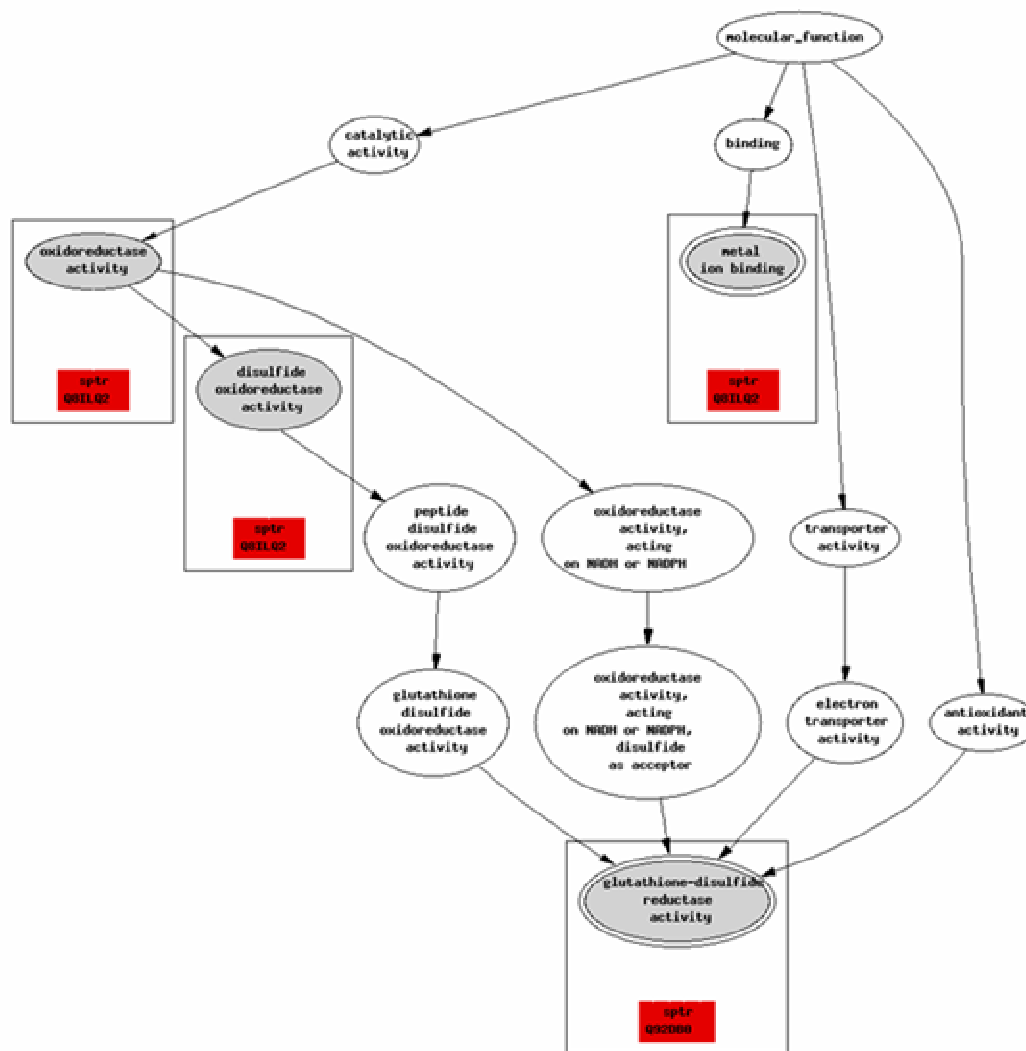


Figure 3. Expected biological process of *Plasmodium falciparum* GR.

vation of GSH content in parasites leads to increase resistance to chloroquine (CQ), while GSH depletion in resistant *P. falciparum* strains is expected to restore the sensitivity to CQ (Davioud-Charvet et al., 2001). High intracellular GSH levels depend on the efficient reduction of GSSG by glutathione GR (Davioud-Charvet et al., 2001). Since the malarial parasite *P. falciparum* is known to be sensitive to oxidative stress, and thus the antioxidant enzyme GR has become an attractive drug target for antimalarial drug development (Sarma et al., 2003). Until present, the functional of *P. falciparum* GR, correlating to human GR, is not well explored and there is a need for better understanding on these proteins' function.

Based on the recent advance in the genomics technology, current microarray technologies permit the examination of gene expression patterns of tens of

thousands of genes (Bairoch et al., 2005). One challenge facing the biologist interpreting such data is recognizing the function of many of the hits identified in a single experiment (Khan et al., 2003). While one can check the literature, a rapid means to get some idea of potential function of a gene product is to obtain the ontology terms that describe the gene (Khan et al., 2003). The gene ontology is developed for this specific purpose. Many genes ontology tools have been constructed and launched. Here, the author used a gene ontology tool to perform a comparative study on the predicted function of human and *P. falciparum* GR.

Comparing to human GR, the *P. falciparum* GR has similar molecular functions as glutathione disulfide reductase activity, oxidoreductase activity, disulfide oxidoreductase activity and metal ion binding. Although the

basic sequences for human and *P. falciparum* GR are totally different, the molecular functions are similar. This implies that any treatment aiming at blocking the functions of *P. falciparum* GR can affect human GR. Thus any drug targeting at *P. falciparum* GR might not be a magic bullet. The more specific structural antagonist that can directly block at amino acid of *P. falciparum* GR is more favorable. However, further experimental studies are needed before making a conclusion on this topic. The finding in this study is not only supports the previous knowledge on malarial GR but also gives the new view on the function of malarial GR.

REFERENCES

- Gerard-Monnier D, Chaudiere J (1996). Metabolism and antioxidant function of glutathione. *Pathol. Biol. (Paris)* 44:77-85.
- Becker K, Rahlfs S, Nickel C, Schirmer RH (2003). Glutathione--functions and metabolism in the malarial parasite *Plasmodium falciparum*. *Biol. Chem.* 384:551-66.
- Mishra NC, Kabilan L, Sharma A (1994). Oxidative stress and malaria-infected erythrocytes. *Indian J Malariol.* 31:77-87.
- Krauth-Siegel RL, Muller JG, Lottspeich F, Schirmer RH (1996). Glutathione reductase and glutamate dehydrogenase of *Plasmodium falciparum*, the causative agent of tropical malaria. *Eur J. Biochem.* 15; 235(1-2):345-50.
- Bairoch A, Apweiler R, Wu CH, Barker WC, Boeckmann B, Ferro S, Gasteiger E, Huang H, Lopez R, Magrane M, Martin MJ, Natale D, O'Donovan C, Redacschi N, Leh LL (2005). The Universal Protein Resource(UniProt). *Nucleic Acids Res. Jan* (1)33:154-9.
- Khan S, Situ G, Decker K, Schmidt CJ (2003). GoFigure: automated Gene Ontology annotation. *Bioinformatics* 19:2484-5
- Davioud-Charvet E, Delarue S, Biot C, Schwobel B, Boehme CC, Mussigbrodt A, Maes L, Sergheraert C, Grellier P, Schirmer RH, Becker K (2001). A prodrug form of a *Plasmodium falciparum* glutathione reductase inhibitor conjugated with a 4-anilinoquinoline. *J. Med. Chem.* 44:4268-76.
- Sarma GN, Savvides SN, Becker K, Schirmer M, Schirmer RH, Karplus PA (2003). Glutathione reductase of the malarial parasite *Plasmodium falciparum*: crystal structure and inhibitor development. : *J. Mol. Biol.* 328:893-907.