APPENDIX

Research Publications
Glutathione mediated regulation of oligomeric structure and functional activity of \textit{Plasmodium falciparum} glutathione S-transferase

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Abstract

\textbf{Background:} In contrast to many other organisms, the malarial parasite \textit{Plasmodium falciparum} possesses only one typical glutathione S-transferase. This enzyme, PfGST, cannot be assigned to any of the known GST classes and represents a most interesting target for antimalarial drug development. The PfGST under native conditions forms non-covalently linked higher aggregates with major population (~98\%) being tetramer. However, in the presence of 2 mM GSH, a dimer of PfGST is observed. Recently reported study on binding and catalytic properties of PfGST indicated a GSH dependent low-high affinity transition with simultaneous binding of two GSH molecules to PfGST dimer suggesting that GSH binds to low affinity inactive enzyme dimer converting it to high affinity functionally active dimer. In order to understand the role of GSH in tetramer-dimer transition of PfGST as well as in modulation of functional activity of the enzyme, detailed structural, functional and stability studies on recombinant PfGST in the presence and absence of GSH were carried out.

\textbf{Results:} Our data indicate that the dimer – and not the tetramer – is the active form of PfGST, and that substrate saturation is directly paralleled by dissociation of the tetramer. Furthermore, this dissociation is a reversible process indicating that the tetramer-dimer equilibrium of PfGST is defined by the surrounding GSH concentration. Equilibrium denaturation studies show that the PfGST tetramer has significantly higher stability compared to the dimer. The enhanced stability of the tetramer is likely to be due to stronger ionic interactions existing in it.

\textbf{Conclusion:} This is the first report for any GST where an alteration in oligomeric structure and not just small conformational change is observed upon GSH binding to the enzyme. Furthermore we also demonstrate a reversible mechanism of regulation of functional activity of \textit{Plasmodium falciparum} glutathione S-transferase via GSH induced dissociation of functionally inactive tetramer into active dimers.
Structural and stability characteristics of a monothiol glutaredoxin: Glutaredoxin-like protein 1 from Plasmodium falciparum

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1. Introduction

Glutaredoxins (Grxs) are small ubiquitous proteins, which are highly conserved in different organisms throughout evolution. As thiol dependent oxidoreductases they contribute in maintaining the intracellular redox thiol-disulfide equilibrium. Mechanically, Grxs transfer electrons from glutathione to an inter- or intra-molecular disulfide and from there to their oxidizing substrate liberating the native functional electrons from glutathione to an inter- or intra-molecular disulfide and cellular redox thiol-disulfide equilibrium. Mechanistically, Grxs transfer the mixed protein–GSH-disulfides. In yeast, three monothiolic Grxs are found namely Grx3, Grx4 and Grx5, displaying specific regulatory functions. The absence of any of these proteins leads to the decrease in cellular Grx activity even though they contain a CGFS-motif at the amino-terminal region instead of the conserved active site residues of dithiol Grxs [5]. In fact, most knowledge on GLPs has so far been obtained using yeast as a model organism. Grx1/Grx2 act cooperatively with the cysteolic GSTs of yeast and are involved in the detoxification of herbicides [11]. Grx3 and Grx4 function as regulators of the nuclear localization of the Aft1 transcription factor [12]. Absence of yeast Grx5 causes severe growth defects indicating that the protein is essential for proper viability and growth [5]. In vivo gene disruption studies demonstrated that Grx5 of Saccharomyces cerevisiae is involved in the synthesis, assembly or repair of Fe-S clusters [13] and also in protein de-glutathionylation [14]. A null Grx5 mutant in yeast shows hypersensitivity to external oxidants, increased protein oxidative damage, inability for respiratory growth and high sensitivity to menadione [5,12,13]. Protein homologues to yeast monothiolic Grxs have been shown to exist in all type of organisms ranging from bacteria to human [5,6,12,15,16]. Recent studies suggest that various prokaryotic and eukaryotic monothiol Grxs can substitute the biological function of Grx5 in yeast mitochondria indicating a functional conservation between Grx5 and its evolutionary homologues [12,16–19].

Three genes encoding monothiol Grxs (PGLP1, PGLP2, and PGLP3) were detected in the malarial parasite P. falciparum. The respective proteins contain the conserved active site cysteine in a CGFS- motif, respectively [68]. Interestingly, PGLP1 shows 41.5%
Structural, functional and unfolding characteristics of glutathione S-transferase of Plasmodium vivax

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ABSTRACT
Glutathione S-transferases (GSTs) of Plasmodium parasites are potential targets for antimalarial drug and vaccine development. We investigated the equilibrium unfolding, functional activity regulation and stability characteristics of the unique GST of Plasmodium vivax (PvGST). Despite high sequence, structural, functional, and evolutionary similarity, the unfolding behavior of PvGST was significantly different from Plasmodium falciparum GST (PfGST). The unfolding pathway of PfGST was non-cooperative with stabilization of an inactive dimeric intermediate. The absence of any compact, folded monomeric intermediate during the unfolding transition suggests that inter-subunit interactions play an important role in stabilizing the protein. Presence of salts effectively inhibited PvGST enzymatic activity by quenching the nucleophilicity of the thiolate anion of GSH. Based on the present findings, together with our previous studies on PfGST, we propose that the regulation of GST enzymatic activity through a dimer-tetramer transition via GSH binding is an exclusive feature of Plasmodium.

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Introduction
Glutathione S-transferases (GSTs) are dimeric cytosolic proteins with a subunit molecular mass of about 25 kDa. They are multifunctional enzymes which mediate the conjugation of toxic electrophilic compounds to GSH, thereby playing an important role in cellular detoxification [1,2]. On the basis of amino acid sequence similarity, immunological cross reactivity and substrate specificity, the GSTs have been grouped into at least 13 classes [2,3]. Interestingly, the sequence similarity between the classes is low, but the global architecture of the proteins is highly similar. The three-dimensional structure of GST isoenzymes from various classes reveals that they have a conserved overall topology [4-6]. Each subunit of the dimeric GST consists of two domains; the N-terminal thioredoxin-like domain linked via a loop to the C-terminal domain which is mostly α-helical. The N-terminal domain contains the key structural determinants for the recognition of GSH, whereas the C-terminal domain provides the structural elements associated with the second substrate specificity. The active site is located along the interface between the two domains with each domain contributing essential residues for functional activity and consisting of 2 sites—a conserved GSH binding G-site and an H-site where a variety of substrates can bind. The catalytic mechanism proceeds through GSH binding to the enzyme, activation of GSH by promoting and stabilizing the thiolate anion group (GS-), nucleophilic attack by the GS- to the hydrophobic substrate possessing an electrophilic centre, product formation and finally product release [1,7].

The presence of a single isoform of GST in Plasmodium spp. underlines its functional importance and drug target characteristics [8-10]. The GST from Plasmodium vivax shares almost 85% sequence identity with Plasmodium falciparum GST and thus both the proteins show high structural and functional similarity [9,11]. The crystal structure of PfGST shows distinct uniqueness among other GSTs as it possesses prominent structural differences in the active site region with a more solvent-accessible H-site where a large spectrum of molecules including inhibitors can bind [8,12-15]. Thus, structurally, Plasmodium GSTs have not been classified in any of the previously known GST classes [9,12]. Our recent work showed that changes in the oligomeric status via GSH binding regulates the functional activity of PfGST [3]. This observation is unique for PfGST as no other GST has been reported to show similar characteristics to date.

Understanding the folding/unfolding and assembly of parasite enzymes is of importance in understanding the oligomerization process, the influence of quaternary structure and subunit