Native and Inhibited Structure of a Mu class-related Glutathione S-transferase from Plasmodium falciparum*

Received for publication, September 2, 2003
Published, JBC Papers in Press, September 12, 2003, DOI 10.1074/jbc.M309663200

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The parasite Plasmodium falciparum causes malaria tropica, the most prevailing parasitic disease worldwide, with 300–500 million infections and 1.5–2.7 million deaths/year. The emergence of strains resistant to drugs used for prophylaxis and treatment and no vaccine available makes the structural analysis of potential drug targets essential. For that reason, we analyzed the three-dimensional structure of the glutathione S-transferase from P. falciparum (Pf-GST1) in the apoform and in complex with its inhibitor S-hexyl-glutathione. The structures have been analyzed to 2.6 and 2.2 Å, respectively. Pf-GST1 shares several structural features with the Mu-type GSTs and is therefore closely related to this class, even though alignments with its members display low sequence identities in the range of 20–33%. Upon S-hexyl-glutathione binding, the overall structure and the glutathione-binding site (G-site) remain almost unchanged with the exception of the flexible C terminus. The detailed comparison of the parasitic enzyme with the human host Mu-class enzyme reveals that, although the overall structure is homologous, the shape of the hydrophobic binding pocket (H-site) differs substantially. In the human enzyme, it is shielded from one side by the large Mu-loop, whereas in Pf-GST1 the Mu-loop is truncated and the space to recognize and bind voluminous substrates is extended. This structural feature can be exploited to support the design of specific and parasite-selective inhibitors.

Sophisticated defense strategies have evolved in organisms that enable them to deal with a broad range of foreign and endogenously derived toxic compounds. These structurally diverse usually highly non-polar molecules are by definition not required for normal metabolic functions, and enzyme systems that catalyze the rapid conversion of these compounds have been developed. However, instead of converting these compounds to useful metabolites as described for a vast array of highly specific enzymes among a large number of bacteria, higher life forms have opted for elimination of toxic compounds rather than their utilization (1). Generally, the pathways of detoxification can be divided into three phases. Phase I reactions consist of the so-called “functionalization” reactions, where a reactive group is demasked or introduced into the molecule. These reactive groups then can be conjugated with natural constituents of the organism (phase II) prior to excretion (phase III) (2).

Glutathione S-transferases (GSTs, EC 2.5.1.18) are a family of phase II detoxification enzymes found in most organisms. Eucaryotes usually contain multiple GSTs with different catalytic activities to accommodate a wide range of functions within the cells. Cytosolic GSTs of mammals are divided into seven classes named Alpha, Mu, Pi, Theta, Zeta, Sigma, and Omega on the basis of different amino sequences and substrate specificities. Additionally, the Kappa GST is a soluble mitochondrial enzyme. Studies of GSTs from non-mammalian species have revealed the existence of several new classes such as the Beta-class found in bacteria, the Phi- and Tau-classes of plants, and the Delta-class described in insects (3). Three-dimensional structures of several members of the different GST classes were determined by x-ray crystallography and have provided insights into their unique catalytic mechanism that are not designed to accommodate any particular substrate, exhibiting a broad specificity toward substrates and non-substrate ligands (4). The primary function of the GSTs lies in the protection of cellular macromolecules by catalyzing the nucleophilic addition of the thiol of reduced glutathione (GSH) to electrophilic centers of carcinogenic, mutagenic, toxic, and pharmacologically active compounds. Furthermore, they are also involved in metabolic pathways not associated with detoxification. Other functions include selenium-independent reduction of hydroperoxides and isomerization of specific metabolites (e.g. prostaglandin H2, 13-cis-retinoic acid). A recently discovered GST class also possesses dehydroascorbate reductase and thioltransferase activities (5). Besides their enzymatic functions, GSTs can also modulate activities of some key signaling proteins and transcription factors by direct interaction with the proteins, thereby preventing stress response pathways (6). They are involved in the quenching and with varying affinities in the transport of exogenous compounds such as pesticides, herbicides, anthelmintics, antibiotics, and endogenous metabolites as steroids and porphyrin metabolites. GSTs were identified as “ligandins” while searching for auxin- or cytokinin-binding proteins in plants, pointing to their role as binding and transport proteins for plant hormones (3, 7, 8). The participation of GSTs in regulatory pathways of stress response, in the neutralization of lipid peroxidation products, in the sequestration of potentially toxic compounds, and also in

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The atomic coordinates and structure factors (code 1PA3 and 1Q4J) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org).

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‡ The abbreviations used are: GST, glutathione transferase; Pf, P. falciparum; r.m.s., root mean square; G-site, glutathione-binding site; H-site, hydrophobic binding pocket.
Drug resistance (9) is especially important within a parasitic context.

The disease malaria caused by the intracellular parasite *P. falciparum* remains a major and growing threat to countries in the tropical and subtropical regions of the world with 300–500 million cases and >1 million deaths each year. The emergence of strains resistant to drugs used for prophylaxis and treatment presently no vaccine available makes it essential to identify novel targets and lead compounds active against them (10, 11).

GST activity has been detected in simian (*Plasmodium knowlesi*), rodent (*Plasmodium yoelii, Plasmodium berghei*), and human (*P. falciparum*) malarial parasites, and the initial conjugation of GSH to antimalarial drugs has been described previously (12–14). For the investigations we describe here, the conjugation of GSH to antimalarial drugs has been described (10, 11). The enzyme exhibits moderate non-selenium-dependent glutathione peroxidase activity and potentially contributes to the protection of the parasite during oxidative stress situations in the parasitized red blood cells. Interestingly, it was also shown that Pfs-GST1 interacts with ferriprotoporphyrin IX, the major toxic degradation product released during the digestion of hemoglobin. Therefore, it was postulated that, in addition to detoxification, Pfs-GST1 is involved in the sequestration of non-polymerized heme in the cytosol. The relative high concentration of Pfs-GST1 in the parasite makes it plausible that the protein serves as an *in vivo* buffer for the parasitotoxic ferriprotoporphyrin IX in the cytosol (17) and that an inhibition of the ligandin would effectively kill the parasite. Interfering with heme detoxification and hemozoin formation is also the mode of action of the known antimalarial drug chloroquine. Therefore, potential Pfs-GST1 inhibitors would have a synergistic effect by preventing parasite resistance against chloroquine (15).

Although the utility of this enzyme as a drug target against *P. falciparum* is yet to be established, the critical role played by Pfs-GST1 in detoxification makes it a viable drug target against malaria. The data reported here form the basis of structure-based design of selective inhibitors, which may serve as antimalarial drug leads.

**EXPERIMENTAL PROCEDURES**

Construction of the expression vector, expression, purification, and crystallization of Pfs-GST1 were carried out as described previously (15, 18). X-ray suitable crystals were obtained after 3 days at room temperature. The space group was assigned to be P212121 with cell dimensions of *a* = 61.2, *b* = 86.9, and *c* = 74.8 Å. The asymmetric unit contains two molecules resulting in a Matthews’s coefficient of 2.25 Å³/Da (19) and a corresponding solvent content of 44.4%. Heavy atom derivative search was performed at room temperature by dissolving heavy atom reagents in the reservoir solution. For the analysis of the GST-S-hexyl-GSH complex, native crystals were soaked in a reservoir solution containing additionally 5 mM S-hexyl-GSH. Soaking time and concentrations are summarized in Table I.

Native and heavy atom derivative data were collected at room temperature on a MARresearch 300-mm image plate detector using CuKα radiation from a Rigaku RU-200 rotating anode x-ray generator operating at 50 kV and 100 mA. Data of the GST-S-hexyl-GSH complex were collected with synchrotron radiation at the consortium beamline X13 (HASYLAB, DESY). The programs DENZO and SCALEPACK (20) were applied for data analysis and reduction. Further calculations were performed with the CCP4 program suite (21). Statistics for all of the datasets are provided in Table I.

The phase problem was successfully solved by multiple isomorphous replacement methods using copper (copper mercury phosphonate) and platinum (platinum tetracyanoplatinate) as heavy atoms. The heavy atom positions, occupancies, B-factors, and initial phases were calculated using the program SOLVE (22) including the anomalous signals of both derivatives. Further refinement of heavy atom parameters and phases was performed with PHENIX (21) and resulted in an overall figure of merit of 0.6. The initial electron density was calculated at 3.1 Å and improved by solvent flattening applying the program DM (21), allowing the identification of most sections of the structure and secondary structure elements. The following model building was carried out with TURBO-FRODO (23). The first model was constructed as polyalanine. The information from this model was used to combine phases and to calculate an electron density, which allowed the identification of amino acid side chains according to the known sequence. Both molecules in the asymmetric unit were built simultaneously. A successive cyclic procedure of manual model building and phase combining of multiple isomorphous replacement and model phases combined with phase expansion to 2.6 Å led to the first model of ~80% completeness.

After the initial model building, cyclic steps of refinement using the program Refmac5 (24) and model building were performed applying all of the data up to 2.6 Å. Non-crystallographic symmetry restraints were applied during the complete process of refinement. During this process, missing residues and 47 water molecules were added. The *R*-factor converged for the apoenzyme at 20.5% (*R*~merge~ = 25.3%). The monomers in the asymmetric unit show a r.m.s. deviation of 0.5 Å for all of the Co-atoms.

For the GST-S-hexyl-GSH complex, the refined apoenzyme was used as starting model. For the refinement, all of the data up to a 2.2-Å resolution were used. The inhibitor S-hexyl-glutathione was identified by difference Fourier techniques in both glutathione-binding sites (G-sites) of the two molecules in the asymmetric unit. Subsequently, 138

### Table I

| Concentration (mm) | Nativea | S-Hexyl-GSHb | Ethylmercury phosphate (EMP)c | Potassium tetra-cyanoplatinatea |
|--------------------|---------|-------------|-------------------------------|-------------------------------|
| Soaking time (h)   | 15      | 16          | 40                            | 40                            |
| Asymmetric monomers| 2       | 2           | 8                             | 8                             |
| Wavelength (Å)     | 2.6     | 2.15        | 2.9                           | 3.2                           |
| Limiting resolution (Å) | 13027  | 22273       | 9699                          | 7054                          |
| Last shell (Å)     | 2.6–2.69| 2.15–2.19   | 2.9–3.0                       | 3.2–3.3                       |
| Completeness (%)   | 97.6 (94.7) | 99.4 (99.8)| 99.5 (97.1)                  | 97.1 (96.5)                  |
| Mean redundancy    | 6.0 (5.6) | 6.4 (5.9)  | 7.6 (6.4)                     | 5.9 (5.5)                     |
| Mean I/Io         | 9.9 (2.3) | 12.3 (4.1) | 10.7 (3.7)                    | 6.8 (2.6)                     |
| R <sub>free</sub>/% | 13.0 (51.9) | 5.5 (31.0)| 12.8 (32.2)                  | 17.1 (46.7)                  |

| Phasing powerd | Centric | Acentric | Centric | Acentric |
|----------------|---------|----------|---------|----------|
| R<sub>value</sub>/% | 1.57 | 1.88 | 0.98 | 1.12 |
| Sites           | 109     | 65       | 75      | 82       |

| Symmetry        | Centric | Acentric |
|-----------------|---------|----------|
| Non-crystallographic symmetry restraints were applied during the complete process of refinement. During this process, missing residues and 47 water molecules were added. The *R*-factor converged for the apoenzyme at 20.5% (*R*~merge~ = 25.3%). The monomers in the asymmetric unit show a r.m.s. deviation of 0.5 Å for all of the Co-atoms. For the GST-S-hexyl-GSH complex, the refined apoenzyme was used as starting model. For the refinement, all of the data up to a 2.2-Å resolution were used. The inhibitor S-hexyl-glutathione was identified by difference Fourier techniques in both glutathione-binding sites (G-sites) of the two molecules in the asymmetric unit. Subsequently, 138
The Overall Structure of Pf-GST1—The regions of secondary structural elements are given in Fig. 1. The first domain (domain 1, residues 1–80) consists of four β-sheets with three flanking α-helices. Furthermore, domain 1 consists of distinct N-terminal and C-terminal motifs, which have a βαβ and ββα arrangement, respectively, and which are linked by an α-helix (α-2). This fold is similar to that of thioredoxin and T4 glutaredoxin, small proteins that are involved in a variety of redox reactions in cells (26–28). The first domain (Fig. 2a) begins with an N-terminal β-strand (β-1) followed by a short 310-helix at the top of an α-helix (α-1) and then a second β-strand (β-2), which is parallel to β-1. A loop region leads to a second α-helix (α-2), which connects to the C-terminal tail. This motif consists of two sequential β-strands (β-3 and β-4), which are antiparallel and are followed by a third α-helix (α-3) at the C terminus of the fold. The four β-sheets are essentially in the same plane with two helices (α-1 and α-3) below this plane and the α-2 helix above it facing the solvent. The loop that connects α-2 to β-3 features a characteristic proline residue, which is the in less favored cis-conformation found to be highly conserved in all of the GSTs. The entire domain 1 is highly conserved and provides most of the glutathione-binding site (G-site). It is connected to the second domain (domain 2) by a linker sequence.

Domain 2 (approximately residues 87–211) is build up entirely from five (α-4 to α-8) α-helical segments connected by a variety of loops (Fig. 2a). The first two helices are relatively long and have a similar conformation to the typical Alpha-class of enzymes (26). As described for Alpha-class enzymes, helix α-4 (residues 90–114) is smoothly distorted into a banana shape, whereas α-5 (residues 119–142) is distorted by a proline residue (Pro-129). Helices α-5 and α-6 are connected by an extended flexible loop. The biological significance of this loop is not clear. Helix α-6 (residues 159–175) is shorter than α-4 and α-5, forming a three-helix bundle and the core of the domain. The loop connecting helices α-6 and α-7 (residues 183–197) is much larger than described for the Alpha-class enzymes; however, α-7 is also almost right-angled to α-6. Pro-197 terminates α-7, and helix α-8 starts with another sharp changing of direction to domain 1. The typical Alpha-class extra helix α-9 is not found in this structure, which strongly supports the Pi- or Mu-class nature of this enzyme.

A comparison of the three-dimensional structure of S-hexyl-GSH complex with that of ligand-free enzyme revealed no significant changes. The G-site seems to be relatively rigid, and the overall structure is not appreciably affected by inhibitor binding (r.m.s. deviation of 0.4 Å for all of the Ca-atoms of the superimposed structures) with the exception of the rather flexible C-terminal tail and the loop connecting the helices α-4 and α-5. The result of the superposition is in good agreement with the structural analysis of the human GST M2-2 (29, 30).

The Biological Dimer—Glutathione S-transferases are functional dimers. The dimers are stable, and the molecular interactions at the subunit interface is class-specific. The two molecules in the asymmetric unit are slightly different (r.m.s. deviation of 0.5 Å), and they are not forming the functional biological homodimer. The biological homodimer (Fig. 2b) is formed by the crystallographic 2-fold axis by two identical molecules and has a central typical V-shaped crevice of ~40 Å long also found in other GSTs (26, 31–33). The intermolecular interactions at the subunit interface of the dimer are summarized in Table III. There are several characteristic hydrophobic interactions close to the 2-fold axis. Particularly, the side chain of residue Arg-77 (helix α-3) serves as a hydrophilic anchor and forms two hydrogen bridges to the side chain of residue Asp-97 (helix α-4) from the other subunit of the homodimer. This arginine-mediated intersubunit contact is very specific for Mu-class enzymes, whereas Arg-68 mediates this special contact in the Alpha- and the Pi-class (32). Although the arginines are not conserved in the Alpha- and Pi-classes on one hand and Mu-class on the other, they obviously occupy similar spatial positions.

One of the most striking similarities found in the Alpha-, Mu-, and Pi-classes is the type of interaction occurring at the intersubunit interface of the homodimers. These are dominated by hydrophobic interactions between residues from domain 1 of one subunit and domain 2 of the other. This “lock and key” motif is common for the Alpha-, Mu-, and Pi-classes (26). How-
ever, a detailed comparison of these structural similarities offers a good possibility to distinguish among the three classes (3). For \( \text{Pf\textsuperscript{-}} \)GST1, Phe-56 acts as the “key” extending from the loop preceding \( \beta \)-3, which fits into the hydrophobic “lock” provided by residues Trp-131, Phe-135, and Tyr-134 of the other subunit. This lock is located between helices \( \alpha \)-4 and \( \alpha \)-5. This structural feature is also unique for Mu-class GSTs, because in Alpha- and Pi-class GSTs, residue Phe-52 or Tyr-49 acts as the key residue, respectively.

The G-site—All of the GSTs are highly specific toward the natural substrate GSH. The G-site for binding of the tripeptide GSH appears as a pronounced cleft along the domain interface of domains 1 and 2 (Fig. 2a) and is located in equivalent positions in the structures of Alpha-, Mu-, and Pi-class enzymes. The molecular recognition of GSH or the specific inhibitor S-hexyl-GSH, respectively, is facilitated by a specific network of polar interactions between protein residues and the tripeptide shown in Fig. 3. Residues contributing to and involved in binding GSH are conserved within a gene class. These residues are typical for Mu-class GSTs. The three invariant G-site ligands, Tyr-9, Glu-71, and Asp-105 (from domain 2 of the adjacent subunit of the dimer), prove the Mu-class character of \( \text{Pf\textsuperscript{-}} \)GST1. The catalytic tyrosine is located at the end of the C terminus of strand \( \beta \)-1 and is followed by a conserved loop connecting \( \beta \)-1 and \( \alpha \)-1. Moreover, the four conservatively replaced G-site residues within the Mu-class Ser-72, Val-59, Lys-15, and Glu-58 are involved in GSH binding. Lys-15 is in close proximity to the S-hexyl-GSH sulfur and would therefore attribute to the further stabilization of GSH or the electrophilic co-substrate. In other Mu-class enzymes, Lys-15 is replaced by Leu, which is a remarkable difference (32, 34). In Alpha-class GSTs, Lys-15 is replaced by Arg and its guanidinium group forms a salt bridge to Glu-104 (26). This residue is also conserved in the Pi-class, but here it is involved in a salt bridge to Glu-96 (35). In \( \text{Pf\textsuperscript{-}} \)GST1, only Arg-13 is present but interestingly Arg 13 forms a salt bridge to Asp-170 and stabilizes the protein conformation at the G-site, comparable to the function of Arg-15 in Alpha- and Pi-class enzymes.

The \( \text{Mu-loop} \) and the Hydrophobic Binding Pocket (H-site)—Pi- and Mu-class enzymes adopt a very similar folding topology. However, one obvious difference is the characteristic “Mu-loop” of the Mu-class enzymes, which is located between \( \beta \)-2 and \( \alpha \)-2 (34) and is a consequence of the amino acid sequence found in domain 1. In the typical Mu-class enzyme, \( \beta \)-2 is extended and forms a reverse turn. In particular, the Mu-loop together with the C-terminal tail and the loop formed by Arg and its guanidinium group forms a deep active site cleft than the one found in the Pi-class. This deep cleft accommodates the putative H-site. The functional significance of this loop is that these side chains form part of the electrophilic substrate-binding H-site. In the structure of \( \text{Pf\textsuperscript{-}} \)GST1, the loop is formed by residues 35–42 and is significantly smaller than that typical for Mu-class proteins (Fig. 4). Consequently, there are no interactions between the residues of the Mu-loop and the C-terminal tail of the molecule, which increases the solvation exposure of G- and H-sites of \( \text{Pf\textsuperscript{-}} \)GST1 (Fig. 5a). In the typical Mu-class enzymes, one side of the H-site is shielded by the large Mu-loop (Fig. 5b).

Apart from the Mu-loop, the H-site is mainly formed by the top section of helix \( \alpha \)-4 (residues 100–112), the loop connecting \( \alpha \)-4 and \( \alpha \)-5 (residues 113–116), the loop connecting \( \alpha \)-1 and \( \beta \)-1.
Fig. 3. Stereoview showing the three-dimensional structure superposition using Pf-GST1 (in light blue) and the human GST M2-2 (in red). High deviations in the region of the Mu-loop include α-2, the C-terminal region, and the loop connecting α-4 and α-5 (residues 114–120). All of the three structural elements participate in binding the hydrophobic co-substrate. The loop connecting α-5 and α-6 (residues 143–150) has a different conformation. The program MOLSCRIPT (41) was used to produce the figures.

Fig. 4. Stereoview of a ball and stick presentation showing the binding of S-hexyl-GSH at the G-site of Pf-GST1. Carbon atoms are colored in gray, nitrogen atoms are in blue, oxygen atoms are in red, and the sulfur of the inhibitor is shown in yellow. Hydrogen-bonding interactions are indicated in dotted green lines. S-hexyl-GSH is highlighted in yellow. The figure was produced by MOLSCRIPT (41).

DISCUSSION

Comparison of the structural features of the different GST classes reveals several major similarities as well as crucial differences (3, 32). Based only on biochemical results, it is difficult to classify the Pf-GST1 (15, 16). It is generally accepted that GSTs belonging to one class should share at least 60% identity, whereas those with <30% identity are not members of the same class (3). The highest sequence similarity of Pf-GST1 to a Mu-class enzyme analyzed by x-ray crystallography can be observed with the isoenzyme 3-3 of rat liver (34) with only 28% identity. However, the comparison with the structure of rat liver Mu-class GST revealed that the overall fold is similar (r.m.s. deviation for Ca atoms of 3.4 Å). The r.m.s. deviation is mainly caused by a relatively poor fit in regions of the Mu-loop, the C-terminal tail, and the loop formed by α-4 and α-5 with r.m.s. deviations up to 11. The comparison of Pf-GST1 with the human Mu-class enzyme (isoenzyme 2-2; GST M2-2) (36) revealed that the sequence identity of ~24% is lower than the Mu-class from the rat but that the overall fold is slightly closer related with an r.m.s. deviation of 2.6 Å. Again, the highest differences are in the regions of the Mu-loop, the C-terminal tail, and the loop formed by α-4 and α-5 (Fig. 4). Interestingly, in typical Mu-class enzymes, the Mu-loop and C terminus are connected by interchain hydrogen bonds (Fig. 5b) and form in a characteristic deep cleft shielding the G- and H-sites from one side. However, the Pf-GST1 G- and H-sites are not shielded by the Mu-loop and the C terminus and therefore are much more exposed to the solvent (Fig. 5a). The additional space between the Mu-loop and the C terminus found in the plasmodial enzyme may support the design of parasite-specific synthetic inhibitors. The mainly hydrophobic nature of this groove near the G-site is created by the residues Phe-10, Asp-11, Ala-41, Tyr-211, and Val-210 (Fig. 5a). In the human enzyme, Tyr-211 is replaced by Met, perhaps one of the major amino acid substitutions in this region. Furthermore, for Pf-GST, Lys-15 is found to be in the position of Leu-12 of the human GST M2-2 enzyme (Fig. 5a and b), one of the essential and conserved residues that characterizes the H-site of Mu-class isoenzymes (37). As described before, Lys-15 might be a promising target residue for structure-based drug design experiments.

Although the G-site of Pf-GST1 and mammalian Mu-class GSTs is structurally similar, the H-site of the GSTs differs significantly. The most significant difference is the replacement of the typical Mu-loop by a short turn in Pf-GST1. This structural feature is also described for the GST from Schistosoma japonicum, a trematode that causes schistosomiasis (38). A further comparison of the H-site from Pf-GST1 with that of other GST classes indicates a higher similarity to that of a typical Pi-class enzyme. In principle, the G- and the H-sites of Pf-GST1 combine structural features of the Pi- and the Mu-classes. Therefore, we can consider the Pf-GST-1 as a “chimeric enzyme.” This general conclusion is supported by the biochemical characterization of the enzyme (15, 16).

It is widely accepted that the differences in domain 2 are responsible for different substrate affinities. In mammalian Mu-class enzymes, the C terminus folds as a coil and seems to act as a flexible cap for the H-site. In comparison with the human enzyme, the C terminus of the malarial enzyme is eight residues shorter (see Figs. 1 and 4) and therefore the C terminus is more similar to a Pi-class enzyme. Nevertheless, our comparison between the apoenzyme and the S-hexyl-GSH-inhibited enzyme revealed that the C-terminal part is rather flexible and that the conformation of this region is less well defined. This finding is in agreement with results obtained for the structure of the human enzyme (36). It was mentioned that the conformational heterogeneity of the C terminus (36, 39)
may have functional significance in the catalytic cycle of these enzymes. The hypothesis was that different conformational states will result in significant changes in the nature of the hydrophobic binding site and therefore the different conformers of this region impart different substrate binding and catalytic properties to the enzyme, also explaining how different hydrophobic substrates can be accommodated by a single enzyme (36). With the present structure, we cannot prove or
refuse this hypothesis. Therefore, it will be necessary to analyze different inhibitor/substrate complexes.

The structure of Pf-GST1 in the apoform and in complex with S-hexyl-GSH revealed key features of the G- and H-sites and gave first insights into ligand binding. Future crystallographic and docking experiments will reveal the non-substrate binding site of ferrisprotoporphyrin IX, the major toxic degradation product released during the digestion of hemoglobin by the malarial parasite. In summary, the vital role played by Pf-GST1 in detoxification coupled with substantial differences observed between the parasite and the host enzymes in the H-site make Pf-GST1 a promising target for drug design investigations of adjuvants to chloroquine, the dominant antimalarial drug. In conclusion, the presented structures will open new ways for the development of new and efficient antimalarial drugs.

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