

Structure of the Major Cytosolic Glutathione S-Transferase from the Parasitic Nematode *Onchocerca volvulus**

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Onchocerciasis is a debilitating parasitic disease caused by the filarial worm *Onchocerca volvulus*. Similar to other helminth parasites, *O. volvulus* is capable of evading the host's immune responses by a variety of defense mechanisms, including the detoxification activities of the glutathione S-transferases (GSTs). Additionally, in response to drug treatment, helminth GSTs are highly up-regulated, making them tempting targets both for chemotherapy and for vaccine development. We analyzed the three-dimensional x-ray structure of the major cytosolic GST from *O. volvulus* (*Ov*-GST2) in complex with its natural substrate glutathione and its competitive inhibitor S-hexylglutathione at 1.5 and 1.8 Å resolution, respectively. From the perspective of the biochemical classification, the *Ov*-GST2 seems to be related to π -class GSTs. However, in comparison to other π -class GSTs, in particular to the host's counterpart, the *Ov*-GST2 reveals significant and unusual differences in the sequence and overall structure. Major differences can be found in helix α -2, an important region for substrate recognition. Moreover, the binding site for the electrophilic co-substrate is spatially increased and more solvent-accessible. These structural alterations are responsible for different substrate specificities and will form the basis of parasite-specific structure-based drug design investigations.

Onchocerciasis is a parasitic disease caused by the filarial worm *Onchocerca volvulus*. It is often called river blindness because of its most extreme manifestation and because the blackflies that transmit the disease breed in fast flowing waters. It is the world's second leading infectious cause of blindness. About 18 million people are infected with *O. volvulus* in Africa, the Arabian Peninsula, and Central and South America. Moreover, roughly 50 million individuals are at risk of acquiring the parasite. Onchocerciasis causes chronic suffering and severe disability. Furthermore, it significantly impedes the socio-economic development in affected communities (www.

who.int/tdr/index.html).

Chemotherapeutic approaches to control parasite transmission and to treat onchocerciasis rely on drugs that only kill microfilariae and infectious larvae but not the adult parasites. So far this necessitates the continuous use of these microfilaricides until the adult worms die. Therefore, the development of drugs that also effectively kill the adult worms would greatly support the control and treatment of *O. volvulus* infections (1). Furthermore, the potential development of drug-resistant strains of the nematode also demands the identification of alternative drug candidates to control the disease (2).

O. volvulus, similar to other parasitic organisms, is capable of surviving in an immunologically competent host by employing a variety of immune evasion strategies and defense mechanisms, including the detoxification and the repair mechanisms of glutathione S-transferases (GSTs¹; EC 2.5.1.18). GSTs are an ancient and diverse superfamily of multifunctional proteins represented by a number of species-independent gene classes. They play prominent roles in detoxification metabolism by catalyzing the nucleophilic addition of reduced glutathione (GSH) to numerous endobiotic and xenobiotic electrophilic substrates, usually promoting their inactivation, degradation, and excretion (3). GSTs participate in the complex network of catalytic functions that protect tissue against oxidative damage by detoxifying hydroperoxides of phospholipids, fatty acids, and DNA before they become engaged in free radical propagation reactions that can ultimately lead to the destruction of intracellular macromolecules (4). Furthermore, a potential modulating activity, mediated through protein interactions, on oxidative stress responsive cascades has been proposed (5). Several additional functions attributed to GSTs include the binding, transport, and storage of hydrophobic ligands (6), the isomerization of maleylacetoacetate, and the regulation of stress kinases and apoptosis (7–9). The importance of GSTs as a promising therapeutic target is underlined by their significant role in a variety of clinically relevant processes, such as susceptibility to disease and drug resistance (10). In a parasitic context, it is especially important to consider their function in the regulation of oxidative stress response, in drug resistance, and possibly in the modulation of the host immune defense mechanisms.

In previous studies, we have characterized three different GSTs from *O. volvulus* (*Ov*-GST1–3) that demonstrate different physiological functions within the parasite. The extracellular glycosylated *Ov*-GST1 is located directly at the parasite-host interface and has GSH-dependent prostaglandin D synthase activity. It therefore has the potential to participate in the modulation of immune cell functions by contributing to

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The atomic coordinates and structure factors (codes 1TU7 and 1TU8) 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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¹ The abbreviations used are: GST, glutathione S-transferase; GSH, reduced glutathione; r.m.s., root mean square.

the production of parasite-derived prostanoids. On the basis of gene structure, amino acid sequence, and immunological and kinetic properties, it was possible to place the *Ov*-GST1 in close vicinity to the σ -class (11, 12). The *Ov*-GST3 belongs to the ω -class and represents a highly stress-responsive GST that is up-regulated in response to oxidative stress, presumably providing a defense against immune-initiated peroxidation of parasite membranes (13). The *Ov*-GST2 is the major cytosolic GST in *O. volvulus*, in which the intracellular amount estimated constitutes about 0.1% of the total protein content in adult filarial worms. The general distribution of the highly abundant enzyme in all tissues and life stages of the parasite indicates its essential function. We propose that one of the biological functions of the *Ov*-GST2 is to neutralize, via GSH conjugation, glutathione peroxidase activity, or passive binding, the cytotoxic lipid peroxidation products arising from immune-initiated attack on parasitic membranes (14, 15). Representative crystal structures of π -class GSTs from different mammalian hosts, such as bovine (16), murine (17), porcine (18), and human (19–21) are available and can be used for structural comparisons. Homology modeling and sequence alignment predicted the *Ov*-GST2 to be topologically related to the mammalian π -class GSTs, and it was suspected that the hydrophobic substrate-binding site of the parasitic enzyme is more solvent-accessible than the typical π -class enzyme of the host (13). Only a few crystal structures of GSTs from endoparasites are known, namely those from *Schistosoma japonica* (22) and *Schistosoma hematobium* (23), the liver fluke *Fasciola hepatica* (24), and the malarial parasite *Plasmodium falciparum* (25, 26). The essential role of *Ov*-GST2 in detoxification emphasizes this protein as a viable drug target against filarial parasites.

EXPERIMENTAL PROCEDURES

Heterologous expression of the *Ov*-GST2 in *Escherichia coli*, purification, and crystallization were performed as described previously (27).

Diffraction data were collected with synchrotron radiation at the consortium beamline X13 (Hamburger Synchrotron Laboratory/Deutsches Elektronen-Synchrotron). The programs DENZO and SCALEPACK (28) were used for data analysis and reduction. Further calculations were performed applying the CCP4 program suite (29).

For crystals of the *Ov*-GST2-GSH complex, the space group was assigned to be $P2_1$ with cell dimensions of $a = 51.6$, $b = 82.3$, $c = 56.7$ Å, and $\beta = 95.9^\circ$. The asymmetric unit contains one dimer, resulting in a Matthews coefficient of 2.49 Å³/Da (30) and a corresponding solvent content of 50.3%. For crystals of the *Ov*-GST2-S-hexylglutathione complex, the space group was also assigned to be $P2_1$ but with cell dimensions of $a = 51.9$, $b = 82.0$, $c = 107.11$ Å, and $\beta = 91.2^\circ$, with a c-axis

almost twice as long as that for crystals of the *Ov*-GST2-GSH complex. Accordingly, the asymmetric unit contains two dimers, resulting in a Matthews coefficient of 2.36 Å³/Da (30) and a corresponding solvent content of 47.5%. Statistics for the datasets are summarized in Table I.

The structure of the *Ov*-GST2-GSH complex was determined by molecular replacement, using the program package AMoRe (31). A π -class GST dimer from human placenta (Protein Data Bank entry code 1AQW) was used as a search model. All data within the resolution range of 10–4 Å were used for the calculations, revealing the position of the dimer in the asymmetric unit. A rigid body refinement of the initial model reduced the R -factor from 45.2 to 39.7% for all data in the resolution range of 20–3 Å. The structure of the *Ov*-GST2-S-hexylglutathione complex was also solved by molecular replacement later on using the refined structure of the *Ov*-GST2 as a search model. Two dimers were positioned in the asymmetric unit. All non-conserved res-

TABLE I
Summary of the refinement statistics

Ligand	Glutathione	S-Hexylglutathione
Space group	$P2_1$	$P2_1$
a (Å)	51.6	51.9
b (Å)	82.3	82.0
c (Å)	56.7	107.11
β (°)	95.9	91.2
V_M (Å ³ /Da)	2.49	2.36
Solvent content (%)	50.3	47.5
Resolution (Å)	20–1.5	20–1.8
No. of reflections used in refinement	70,356	77,235
R_{crystal} (%) ^a	15.0 (17.6)	17.9 (22.3)
No. of reflections used in R_{free}	3,729	4,062
R_{free} (%) ^b	18.1 (20.0)	21.6 (27.3)
Protein atoms	6,840	13,680
Solvent molecules	807	763
Ligand atoms	72	144
Average B factor (Å ²):		
Main-chain atoms	15.5	16.2
Side-chain atoms	18.1	18.9
Root mean square deviation:		
Bonds (Å)	0.014	0.012
Bond angles (°)	1.51	1.35
Residues in regions of the Ramachandran plot (%):		
Most favored	91.9	91.8
Allowed	8.1	8.2
Disallowed		

^a $R_{\text{crystal}} = \sum |F_{\text{obs}} - F_{\text{calc}}| / \sum |F_{\text{obs}}|$, where F_{obs} and F_{calc} are observed and calculated structure factors, respectively.

^b R_{free} was calculated using randomly selected 5% of the reflections.

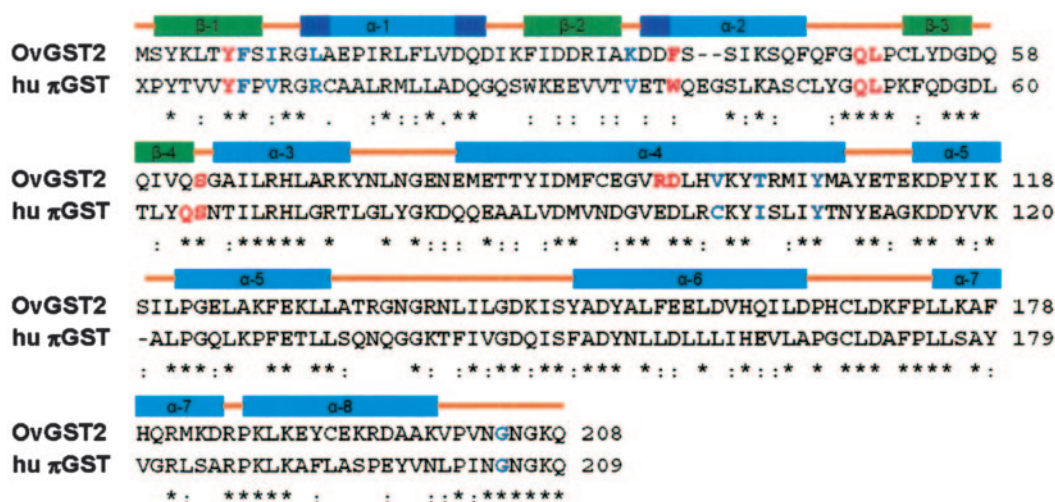


FIG. 1. Sequence alignment of *Ov*-GST2 from *O. volvulus* and the human (*hu*) placenta π -class GST. Residues of the G-site are colored in red, and residues of the H-site are in blue. The secondary structure elements are indicated accordingly. The * marks sequence identities, and : marks sequence homologies.

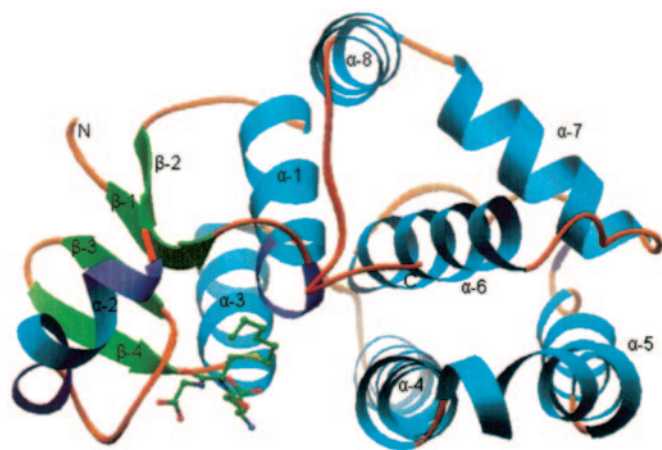


FIG. 2. **Ribbon diagram of *Ov*-GST2.** The *Ov*-GST2 monomer emphasizing the secondary structural elements. Helices are colored light blue, β -strands are green, and the 3_{10} helices are dark blue. The *S*-hexylglutathione molecule bound to the active site is shown in ball-and-stick representation. The program RIBBONS (67) was used to produce the figure. N, N terminus.

ides in the search model were replaced by alanines. Insertions and deletions were not included in the first refinement cycle. Both complex structures were refined using similar protocols. The refinement was performed by program REFMAC5 (32), and the progress was monitored by the continuous decrease of the free *R*-value (33). Because the asymmetric unit contained two GST monomers, use was made of non-crystallographic symmetry restraints for all non-hydrogen atoms throughout the course of refinement. The CCP4 program suite (29) and the program TURBO-FRODO (34) were used for calculations and model building, respectively. The ligands were identified by difference Fourier techniques in all G-sites of the molecules in the asymmetric unit. Solvent molecules were automatically added at the end of the refinement process using the program WARP (35) and checked for chemically reasonable positions where difference density exceeded 3σ . The quality of the final models was verified using the program PROCHECK (36). A summary of the refinement statistics is shown in Table I.

RESULTS

The structures of *Ov*-GST2 in complex with the physiological substrate GSH and the competitive inhibitor *S*-hexylglutathione were determined independently to characterize the substrate binding site (G-site) and the electrophilic binding site (H-site) of the enzyme. Both complexes have been crystallized under identical conditions, and the space group was assigned to be P2₁. Interestingly, although crystals of *Ov*-GST2 in complex with GSH reveal one dimer/asymmetric unit, crystals of *Ov*-GST2 in complex with *S*-hexylglutathione reveal two dimers/asymmetric unit. The structure of the *Ov*-GST2-GSH complex was refined to a resolution of 1.5 Å (*r* = 15.0%, *R*_{free} = 17.6%) and of the *Ov*-GST2-*S*-hexylglutathione complex to 1.8 Å (*r* = 19.9%, *R*_{free} = 22.3%). No significant changes in the tertiary structure of both complexes were observed. The root mean square (r.m.s.) deviation of the C α atoms is within the range of deviations calculated between each monomer of one structure. The superimposed monomers merely show a r.m.s. deviation in the range of 0.3–0.4 Å for all C α atoms. The G-site seems to be relatively rigid, and the overall structure is not notably affected by different ligands. The bound ligands do not affect the crystal packing, as they are not involved in any crystal contacts. Similar effects are also observed for the human π -class (19) and α -class (37) enzyme. The high resolution obtained facilitates the determination of solvent molecules with high accuracy. Both structures are well ordered, and the final models include all residues of the enzyme. The positions of the ligands are clearly defined by the electron density. The statistics of the model quality are summarized in Table I. The overall fold of *Ov*-GST2 shows that it is clearly related to π -class GSTs with

TABLE II
Intermolecular contacts between *Ov*-GST2 and *S*-hexylglutathione

Hydrogen bonds		
Donor	Acceptor	Distance
GTX X 1 N1	ASP B 96 OD1	2.93
ARG A 95 NH2	GTX X 1 O11	3.16
SER A 63 OG	GTX X 1 O11	2.59
SER A 63 N	GTX X 1 O12	2.89
GTX X 1 N2	LEU A 50 O	2.78
LEU A 50 N	GTX X 1 O2	2.87
GTX X 1 O32	GLN A 49 OE1	2.97
TYR A 7 OH	GTX X 1 SG2	3.11
Hydrophobic interactions		
Atom 1	Atom 2	Distance
GTX X 1 C4S	GLYT A 204 CA	3.78
GTX X 1 C3	PHE A 38 CE2	3.86
GTX X 1 SG2	LEU A 13 CD1	3.63
GTX X 1 C1	LEU A 13 CD1	3.75
GTX X 1 CB1	LEU A 13 CD1	3.67
GTX X 1 CG1	LEU A 13 CD1	3.73
GTX X 1 CD1	LEU A 13 CD1	3.87
GTX X 1 C4S	ILE A 10 CD1	3.79
GTX X 1 C2	PHE A 8 CE1	3.85

secondary elements given in Figs. 1 and 2. The monomers consist of two domains linked by a loop between the helices α -3 and α -4. Domain 1 (residues 1–74) is built from a four-stranded mixed β -sheet that is surrounded by 3 α -helices ($\beta\alpha\beta\alpha\beta\alpha$ motif). Domain 2 (residues 82–208) is largely α -helical. The typical α -class extra helix α -9, the most important structural feature to distinguish between α - and π -class enzymes, is not present in this structure.

There are two residues that adopt cis-conformation. One is a characteristic proline residue (Pro⁵¹), found to be highly conserved in all GSTs, which is located in the loop that connects α -2 to β -3. The other residue, Leu⁵⁰, is a G-site residue that has two hydrogen bonds to GSH and *S*-hexylglutathione and also adopts a cis-configuration in both complexes.

In their biologically active form, cytosolic GSTs are dimers, and *Ov*-GST2 conforms to that rule. The dimeric structure is stable, with molecular recognition at the subunit interface being class-specific. Intersubunit contacts are mediated by hydrophobic contacts as well as by hydrogen bonds. Intersubunit hydrogen bonds and salt links between the monomers within 3.5 Å are: Phe^{47A}–Lys^{127B}, Arg^{68A}–Asp^{88B}, Arg^{72A}–Glu^{81B}, and Arg^{72A}–Thr^{85B}. All contacts are related by local symmetry. Particularly, the side-chain of residue Arg⁶⁸ (helix α -3) serves as a hydrophilic anchor and forms two hydrogen bridges to the side-chain of residue Asp⁸⁸ (helix α -4) from the other subunit of the homodimer. This arginine-mediated intersubunit contact is very specific for α - and π -class enzymes (38).

In a classical π -class enzyme, Tyr⁴⁹ acts as a hydrophobic “key.” However, for *Ov*-GST2, Phe⁴⁷ (corresponds to Tyr⁴⁹ in the human π -GST) (Fig. 1) acts as the hydrophobic key extending from the loop preceding β -3, which fits into the hydrophobic “lock” provided by the residues Gly⁹³, Leu¹³¹, Lys¹²⁷, and Phe¹²⁸ of the other subunit. This lock is located between helices α -4 and α -5. Comparison of the *Ov*-GST2 structure with the human π -class enzyme yields an overall r.m.s. value on C α atoms of 1.5 Å (Fig. 3a), which is significantly higher than expected for members within the same class (<0.7 Å). With regard to sequence identity, this result is consistent with the low level of only 42% versus an expected sequence identity of >75%. In comparison with that, the human, pig, and mouse π -class GSTs can be superpositioned with r.m.s. values of 0.5–0.6 Å. The most significant differences observed between the human π -class enzyme and the *Ov*-GST2 occur in the regions of

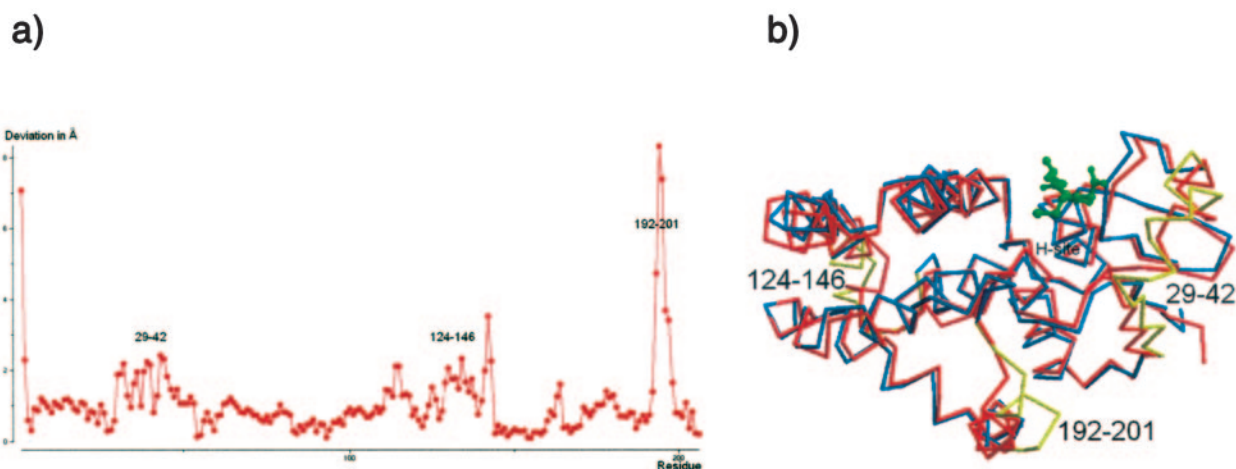


FIG. 3. **Superposition of *Ov*-GST2 and human π -class GST.** *a*, high deviations of corresponding α positions can be observed in regions 29–42, 124–146, and 192–201. The overall r.m.s. deviation on α carbon atoms is 1.5 Å. *b*, regions with largest deviations are colored in yellow. Other regions of human π -class GST are colored in light blue, and *Ov*-GST2 is in red. The program MOLSCRIPT (68) was used to produce the figure.

residues 29–42, 124–146, and 192–201 (Fig. 3). Region 29–42 includes strand β -2 and helix α -2, with a two-residue insertion (40 and 41) in the human enzyme (Fig. 1) leading to a bent helical structure. It is highly remarkable that this region also involves a G-site residue, Phe³⁸, which is replaced by Trp in the human, mouse, and pig enzyme. The largest deviation, however, is placed at the end of helix α -8 (residues 192–201). The sequence is not conserved, and the human enzyme reveals a distorted helix instead of a regular helix in *Ov*-GST2 in this region (Fig. 3).

The G-site part of the active site is formed by helix α -2, by residues connecting helix α -2 and strand β -3, and by a segment connecting strand β -4 to helix α -3. The overall geometry is similar but not identical to that of the human π -class GST. The H-site is formed by the loop connecting strand β -1 and helix α -1, by the C-terminal part of helix α -4, and by residues of the C terminus following helix α -8. Residues in contact with GSH and the GSH part of *S*-hexylglutathione are Tyr⁷, Phe⁸, Leu¹³, Phe³⁸, Gln⁴⁹, Leu⁵⁰, Ser⁶³, Arg⁹⁵, and Asp⁹⁶ (from the other monomer) as summarized in Table II.

In *Ov*-GST2, the α -carbonyl group of the γ -glutamyl moiety is aligned by two hydrogen bonds of Ser⁶³ and one hydrogen bond of Arg⁹⁵ (Fig. 4), which is a remarkable difference to the situation found in the human π -class GST. Here, Gln⁶⁴ takes over the task of Arg⁹⁵. The α -amino group interacts with Asp⁹⁶ from the other monomer, and the backbone of the γ -glutamyl moiety is stabilized by van der Waals contacts to Leu¹³. In the human counterpart, Leu¹³ is exchanged by Arg (Figs. 1 and 5). This exchange is the most drastic change, influencing the active site geometry and resulting in a reduction of the volume of the H-site binding pocket (Fig. 5). The backbone of the Cys moiety of GSH forms two hydrogen bonds with Leu⁵⁰, a residue in cis-conformation. The Gly moiety is bound by Gln⁴⁹, the backbone being further stabilized by van der Waals contacts to Phe⁸ and Phe³⁸. The third amino acid exchange in the G-site is Phe³⁸ against Trp in the human enzyme. In *Ov*-GST2, Phe³⁸ is only involved in van der Waals contacts, whereas in the human enzyme Trp forms a hydrogen bond to the Gly moiety. When comparing the *Ov*-GST2-GSH complex with the *Ov*-GST2-*S*-hexylglutathione complex, there are no differences in the positions of residues participating in the G-site (Fig. 4). A similar positioning of the sulfur atom has been observed in π -class enzymes from pig (38), mouse (17), and human (19–21). It is generally accepted that GSH predominantly reacts as the anionic thiolate and that, in the π -class, a network of hydrogen

bonds may be responsible for the activation of the GSH sulfur. Already, it has been shown for the human π -class enzyme that Tyr⁷ stays in the identical position in both the apo- and the GSH-complexed structures (21). This observation supports the proposal that Tyr⁷ will not be ionized in the reaction mechanism (39). This situation is also observed for the *Ov*-GST2 structure with Tyr⁷ in hydrogen bonding distance to the sulfur atom of GSH and to the amide nitrogen of Leu¹³.

The entrance of the H-site can be described as a pocket with side walls formed by Phe⁸ and Tyr¹⁰⁸. The bottom is formed by Lys³⁵, Ile¹⁰, and Gly²⁰⁴ and the deep end by Leu¹³, Thr¹⁰², and Val⁹⁹ (Fig. 5b). The *S*-hexyl-moiety of *S*-hexylglutathione is sandwiched between the aromatic rings of Phe⁸ and Tyr¹⁰⁸ and is kept in place by Ile¹⁰ and Gly²⁰⁴. Phe⁸, Ile¹⁰, and Gly²⁰⁴ are in van der Waals contacts within the range of 3.7–3.8 Å (Figs. 4 and 5). Tyr¹⁰⁸ has been shown to enhance Michael additions of GSH to electrophilic substrates (21, 40). This residue is strictly conserved in all π -class GSTs and hydrogen bonding interaction between the hydroxyl group of Tyr¹⁰⁸, and the amide nitrogen of Gly²⁰⁴ has also been observed in mouse, pig, and human π -class structures (17, 21, 38). Even though mammalian π -class GSTs share high levels of sequence identity, they can display large variations in their specific activities toward various substrates (41, 42). These variations can be attributed to rather small differences in the H-sites of each enzyme. Direct comparison of *Ov*-GST2 with the host's counterpart reveals that only Phe⁸, Tyr¹⁰⁶, and Gly²⁰⁴ are conserved; all other H-site residues are exchanged, resulting in a different binding environment for potential co-substrates (Figs. 1 and 5). As already mentioned, the most crucial difference is probably the exchange of Leu¹³ against Arg in the human enzyme. In the human enzyme, Arg¹³ and Glu⁹⁷ form two hydrogen bridges. Because of the Leu-Arg exchange, more space of ~40 Å³ is available for an electrophilic co-substrate in the region between Leu¹³, Thr¹⁰², Val⁹⁹, and Arg⁹⁵ (Fig. 5, *a* and *b*). Moreover, the exchange of Thr¹⁰² against Ile increases the H-site by 20 Å³ once again.

DISCUSSION

The *Ov*-GST2 is structurally related to the π -class GSTs. This GST class plays an important part in the detoxification of mutagens and toxins and is implicated in neoplastic development and drug resistance. High levels of π -class GST expression have been shown to be directly associated with tumor drug resistance; however, the precise role played by the enzyme

periments, a series of β -carbonyl-substituted GSH conjugates was evaluated as potential inhibitors of *Ov*-GST2. Their specificity for the parasite-derived protein was assessed through comparison with their inhibition of human π -class GST. Selective inhibition of *Ov*-GST2 has been demonstrated at low micromolar concentrations of β -carbonyl GSH conjugates. Selectivity for *Ov*-GST2 over human π -class of >25-fold was generated through appropriate substitutions of the conjugate moiety. Aryl and long chain or bulky alkyl groups enhance *Ov*-GST2 selectivity (13). This is in accordance with the expectations of a more open active site in parasitic enzymes (53). Our x-ray structures prove that the H-site of *Ov*-GST2 is spatially increased in comparison to the host enzyme, and this is also in good agreement with the results discussed above. Only three residues of the H-site are conserved in comparison with the human counterpart, and we can use these structural differences to design specific inhibitors.

The human π -class GST recognizes GSH by an induced-fit mechanism (54) and in the apoenzyme helix α -2 was especially identified as being rather flexible (55). Because we did not investigate the apo-form of the *Ov*-GST2, we cannot confirm this finding or refute it. However, it is noteworthy that the identified π -class key residues for this induced-fit mechanism, Tyr⁴⁹ and Cys⁴⁷, are not conserved in *Ov*-GST2 and are replaced by Phe (Fig. 1).

Oxidation of the human π -class GST leads to the formation of an intramonomer disulfide bond between Cys⁴⁷ and Cys¹⁰¹, with subsequent inactivation of the enzyme (56–58). Both sulfur atoms of these residues show a distance of >18 Å in the active enzyme, again indicating the high flexibility of helix α -2. In *Ov*-GST2, however, the second Cys (Cys¹⁰¹) residue is replaced by Val, making it debatable whether *Ov*-GST2 can be inactivated by oxidation at all. An exchange of Cys⁴⁷ to Ala made the human enzyme more resistant toward irreversible inactivation by 0.1 mM *N*-ethylmaleimide. Furthermore, the mutant Ala⁴⁷ was also more resistant to inactivation by the physiological disulfides, cystamine or cystine, which cause mixed disulfide and/or intra- or intersubunit disulfide bond formation (58).

The C-terminal part of helix α -8 (residues 192–201) is a region demonstrating large structural deviations between the human and parasitic GST. Helix α -8 is neighboring the loop that connects strand β -1 and helix α -1, and therefore it is in close distance to Val¹⁰ and Arg¹³ of the active site. Nevertheless, the distance to the H-site is >15 Å, and the biological meaning of this structural difference remains unclear.

Tyr⁷, one of the G-site residues, seems to be protonated, accepting a hydrogen bond from the main chain amide group of Leu¹³. Tyr⁷ does not appear to play a role as a general base in the enzymatic reaction. Similar conclusions have been reported for other mammalian π -class GSTs (19, 21, 59).

In the absence of a protective vaccine, control of onchocerciasis relies on measures directed against the vector and on chemotherapy. The only available drug for mass treatment of onchocerciasis is ivermectin (60).² Because control of the disease is at present largely dependent on ivermectin and will probably be so in the near future, it is a worrying possibility that resistance may be emerging in *O. volvulus*. Ivermectin resistance emerged relatively quickly in helminths of veterinary concern (62–64). Recent open case-controlled investigations, carried out in two onchocerciasis-endemic foci in Ghana, confirm the existence of populations of adult female *O. volvulus* that respond poorly to repeated doses of ivermectin (65, 66). As

there are no practical alternative strategies for the mass control of *O. volvulus*, there is an urgent need for the development of new drugs, notably drugs that kill or sterilize adult worms.

Our structural analysis of the GST2 from *O. volvulus* shows that it is a suitable drug target for intervention in filarial infections, with the potential advantage of not compromising the human host. Knowing the three-dimensional shape and chemical nature of the *Ov*-GST2 binding pocket will aid in the discovery and design of novel anti-filarial compounds. We suggest that neutralization of the *Ov*-GST2 may tip the molecular balance in favor of the host during this characteristically chronic nematode infection.

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