Characterization of a Phanerochaete chrysosporium Glutathione Transferase Reveals a Novel Structural and Functional Class with Ligandin Properties*[S]

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Background: GSTs are detoxification enzymes poorly characterized in fungi.

Results: GSTFuA1 possesses a unique three-dimensional structure and binds wood degradation compounds at or near the glutathione binding pocket.

Conclusion: This GST is a new fungal isomorph that we name GSTFuA1.

Significance: GSTs with binding properties could be of great interest in various biotechnological applications.

Glutathione S-transferases (GSTs) form a superfamily of multifunctional proteins with essential roles in cellular detoxification processes. A new fungal specific class of GST has been highlighted by genomic approaches. The biochemical and structural characterization of one isoform of this class in Phanerochaete chrysosporium revealed original properties. The three-dimensional structure showed a new dimerization mode and specific features by comparison with the canonical GST structure. An additional β-hairpin motif in the N-terminal domain prevents the formation of the regular GST dimer and acts as a lid, which closes upon glutathione binding. Moreover, this isoform is the first described GST that contains all secondary structural elements, including helix α4 in the C-terminal domain, of the presumed common ancestor of cytosolic GSTs (i.e. glutaredoxin 2). A sulfate binding site has been identified close to the glutathione binding site and allows the binding of 8-anilino-1-naphthalene sulfonic acid. Competition experiments between 8-anilino-1-naphthalene sulfonic acid, which has fluorescent properties, and various molecules showed that this GST binds glutathionylated and sulfated compounds but also wood extractive molecules, such as vanillin, chloronitrobenzoic acid, hydroxycacetophenone, catechins, and aldehydes, in the glutathione pocket. This enzyme could thus function as a classical GST through the addition of glutathione mainly to phenethyl isothiocyanate, but alternatively and in a competitive way, it could also act as a ligandin of wood extractive compounds. These new structural and functional properties lead us to propose that this GST belongs to a new class that we name GSTFuA, for fungal specific GST class A.

Glutathione S-transferases (GSTs) are a superfamily of proteins widespread in animals, plants, fungi, and bacteria. From a functional point of view, GSTs usually catalyze glutathione (GSH) transfer onto hydrophobic molecules (glutathionylation activity) or GSH removal from specific substrates (deglutathionylation) (1). These enzymes have broad substrate acceptance, although each GST possesses its own specific catalytic profile. Most GSTs are dimeric proteins. Each monomer is composed of a conserved thioredoxin domain containing the GSH binding pocket (G site) and a more variable α-helical domain (H site) containing the binding site for the GSH acceptor substrate (2). Most studies have focused on the GSH-dependent catalytic activities involved both in detoxification processes and endogenous metabolism. However, some GSTs have also been identified as proteins that selectively bind organic anions such as tetrapyrroles in mammals and plants (3–5). This “ligandin” property has been defined as the capacity of the protein to bind nonsubstrate ligands (3). In plants, it could be involved in the intracellular transport of hydrophobic compounds, such as pigments, and in temporary storage of phytohormones (6, 7). Fun-
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gal GSTs have been less studied than their plant or animal counterparts. Nevertheless, the recent burst of fungal genome sequencing programs has highlighted evolutionary specificities of GSTs in these organisms (8–10). Currently, at least seven different classes of cytosolic GSTs have been defined in fungi: MAK16, EFBγ, Ure2p, Omega, S-glutathionyl-(chloro)hydroquinone reductase, GTT, and etherase-like. Although some isoforms from the Ure2p, Omega, S-glutathionyl-(chloro)hydroquinone reductase, and GTT have been characterized in yeast (11–13), Aspergillus sp. (14, 15), and Phanerochaete chrysosporium (9, 16), the etherase-like class has never been studied to date. Etherase-like GSTs are fungal specific, although they show weak homology with LigE, a bacterial protein responsible for the cleavage of β-aryl ether linkages of lignin in Sphingobium sp. SYK-6 (8, 17). The etherase-like class is expanded in wood decaying fungi, because, for instance, 11 etherase-like encoding genes are present in the genome of Postia placenta. Although less expanded, they are, however, present in pathogenic fungi as Aspergillus sp. or Fusarium sp. for example (8).

P. chrysosporium is a white rot fungus able to completely mineralize lignocellulose by degrading recalcitrant compounds. During the wood decaying process, phenolic acids and phenolic aldehydes are released. These compounds are problematic in biofuel production, but at the same time, they can have beneficial properties for human health. As an example, vanillin possesses antimicrobial activity, antioxidant properties, antimutagenic effects, and chemoprotective activity in inflammation and cancer (for a review, see Ref. 18). Syringaldehyde is also a naturally occurring aromatic compound, which exhibits both a detrimental action on cellulose hydrolysis and fermentation processes and a good antioxidant activity, being 2- and 10-fold more effective than quercetin and trolox, respectively (19).

In this study, we describe for the first time the biochemical and structural characterization of a GST belonging to the previously named “etherase-like” class in P. chrysosporium and demonstrate the ligandin property of this enzyme toward compounds derived from lignin degradation.

EXPERIMENTAL PROCEDURES

Materials—Hydroxyethyl disulfide was from Pierce. α-O-Methylumbelliferyl-β-hydroxypropiovanillone was a gift from Dr. Masai (Nagako University of Technology, Japan). S-(phenylacetophenone)-glutathione and 2-methyl-S-glutathionyl-naphtoquinone were synthesized as described previously (16). para-Nitrophenyl sulfate potassium salt was from Acros Organics. 5-Chloromethylfluorescein diacetate (CMFDA) was from Invitrogen. All other reagents were from Sigma-Aldrich.

Cloning of GSTS118, GSTS118-S22A, and GSTS118-S22C—The enzyme studied here is referred to as GSTS118 based on its protein identification number in the Joint Genome Institute database. The open reading frame sequence encoding P. chrysosporium GSTS118 was amplified from a cDNA library using GSTS118 forward and reverse primers (5’-CCCCCATGGGCTCAGCCCATCGTGT-3’ and 5’-CCCGGATCCCTATACATCAACCTGCT-3’, respectively) and cloned into the NcoI and BamHI restriction sites (underlined in the primers) of pET-3d (Novagen). The amplified sequence encodes a protein in which an alanine has been inserted after the initiator methionine to improve protein production. Two mutants of the putative catalytic Ser-22 into Ala or Cys residues were also generated using two complementary mutagenic primers (supplemental Table 1).

Expression and Purification of the Recombinant Proteins—For protein production, the Escherichia coli BL21(DE3) strain, containing the pSBET plasmid, was co-transformed with the different recombinant plasmids (20). Cultures were progressively amplified up to 2 liters in LB medium supplemented with ampicillin and kanamycin at 37 °C. Protein expression was induced at exponential phase by adding 100 μM isopropyl β-D-thiogalactopyranoside for 4 h at 37 °C. The cultures were then centrifuged for 15 min at 4400 × g. The pellets were resuspended in 30 ml of TE NaCl (30 mM Tris-HCl, pH 8.0, 1 mM EDTA, 200 mM NaCl) buffer. Cell lysis was performed on ice by sonication (3 × 1 min with intervals of 1 min), and the soluble and insoluble fractions were separated by centrifugation for 30 min at 27,000 × g at 4 °C. All subsequent steps were performed in the cold. The soluble part was then fractionated with ammonium sulfate in two steps, and the protein fraction precipitating between 40 and 80% of saturation contained the recombinant protein, as estimated by 15% SDS-PAGE. The protein was purified by size exclusion chromatography after loading on an ACA44 (5 × 75-cm) column equilibrated in TE NaCl buffer. The fractions containing the protein were pooled, dialyzed by ultrafiltration to remove NaCl, and loaded onto a DEAE-cellulose column (Sigma) in TE (30 mM Tris-HCl, pH 8.0, 1 mM EDTA) buffer. The proteins were eluted using a 0–0.4 M NaCl gradient. Finally, the fractions of interest were pooled, dialyzed, concentrated by ultrafiltration under nitrogen pressure (YM10 membrane; Amicon), and stored in TE buffer at −20 °C. Purity was checked by SDS-PAGE. Protein concentrations were determined spectrophotometrically using a molar extinction coefficient at 280 nm of 68,870 M⁻¹ cm⁻¹.

For the production of the selenomethionine-substituted GSTS118, a methionine auxotroph strain, BL21(DE3)Met⁻, was cotransformed with pET-GSTS118 and pSBET. Cultures were done as described previously (21), and the labeled protein was purified following a procedure identical to that described for unlabeled GSTS118.

Activity Measurements—The activity measurements of GSTS118, GSTS118-S22C, and GSTS118-S22A proteins in thiol transferase activity with a hydroxyethyl disulfide assay or for reduction of dihydroascorbate were performed as described by Couturier et al. (22). The GSH transferase activity was assessed with phenethyl isothiocyanate (phenethyl-ITC) prepared in 2% (v/v) acetonitrile, 1-chloro-2,4-dinitrobenzene (CDNB), and 4-nitrophenyl butyrate (PNP-butyrate) prepared in DMSO. For these three substrates, the reactions were monitored at 274, 340, and 412 nm, respectively, following the increase in absorbance arising from the formation of the S-glutathionylated adduct. The reactions with CDNB and PNP-butyrate were performed in 100 mM phosphate buffer, pH 7.5, in
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the presence of GSH (5 mM), whereas the reaction with phenethyl-ITC was performed at pH 6.5 with an identical GSH concentration. Esterase activity was measured in microplates using CMFDA as substrate, which releases fluorescence upon activation by esterases (23). Experiments were performed in 50 mM phosphate buffer, pH 8.0, in a total volume of 200 μl. The reactions were started by the addition of the purified enzyme, and fluorescence was measured every minute for 1 h with excitation at 485 nm and emission at 535 nm on a VICTORM™ X5 plate reader (PerkinElmer Life Sciences). Catalytic parameters were calculated using the GraphPad® software from steady state experiments performed using 0–25 μM CMFDA and 5 mM GSH.

Peroxidase activities were monitored as follows. 1 mM peroxide (hydrogen peroxide, tert-butylic hydroperoxide, and cumene hydroperoxide) in 30 mM Tris-HCI, pH 8.0, was incubated in the presence of 2 mM GSH, 200 μM NADPH, 0.5 IU of glutathione reductase. The activity was followed by monitoring the decrease in absorbance arising from NADPH oxidation in this coupled enzyme assay system, showing the formation of oxidized glutathione (GSSG). The reactions were started by the addition of the purified enzyme and monitored with a Cary Eclipse (VARIAN) spectrophotometer. The excitation wavelength was set at 290 nm, emission spectra were recorded from 305 to 560 nm. The fluorescence was measured every minute for 1 h with excitation at 485 nm and emission at 535 nm on a VICTOR™ X5 plate reader (PerkinElmer Life Sciences). IC50 values were obtained by fitting data to Equation 2,

\[ Y = \frac{\text{Bottom} + (\text{Top} - \text{Bottom})}{(1 + 10^{\text{Log}[\text{Ligand}] - \text{Log ICS50})} \]  

where \( Y \) represents the fluorescence signal observed after background subtraction, Top is the fluorescence signal of ANS bound onto GST5118 without any ligand, and Bottom is the fluorescence signal of ANS at the highest ligand concentration.

Inhibition Kinetics—Competition tests between ANS and phenethyl-ITC or CMFDA were performed by measuring GSH transferase and esterase activities. Activity with phenethyl-ITC was monitored in a total volume of 500 μl containing 50 μM GSH, 50 μM ANS, and 10 μM to 10 mM phenethyl-ITC. Inhibition tests of esterase activity with CMFDA by ANS or vanillin were performed in a total volume of 200 μl with or without 50 μM GSH, 50 μM ANS, or 300 μM vanillin and CMFDA (0 μM to 25 μM). Similar tests were performed with 25 μM CMFDA, 300 μM vanillin, and 0–5 mM GSH. The reactions were started by the addition of the purified enzyme, and the catalytic parameters were calculated using the GraphPad® software.

Crystallization—Two different samples were crystallized, the selenomethionine derivative protein (apoGST5118) and the native protein with bound GSH (holoGST5118). The crystallization experiments were set up using the microbatch under oil (paraffin) method at 277 K. Selenomethionine-apoGST5118 crystals appeared after 4–5 days from droplets containing 2 μl of protein solution (15–20 mg/ml protein in TE buffer) and 2 μl of precipitating solution (30% PEG 8000, 200 mM sodium acetate, and 100 mM sodium cacodylate, pH 6.5). Crystals of GST5118 in complex with GSH (holoGST5118) were obtained in co-crystallization experiments. The best crystals grew after 2 months in droplets containing a mixture of 485 μM protein solution (14 mg/ml) and 6 mM GSH in TE buffer and precipitating solution (30% PEG 8000, 200 mM sodium acetate, and 100 mM HEPES, pH 7.0). Prior to data collection, the crystals were transferred to a cryosolution containing 20% glycerol with mother liquor and flash-cooled to 100 K.

Data Collection and Processing, Structure Solutions, and Refinements—ApoGST5118 diffraction data were collected on the synchrotron beamline PX1 at SOLEIL (France) at 0.9791 Å, and holoGST5118 x-ray data were measured on the beam-
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| TABLE 1 |
|---------|
| Statistics of x-ray diffraction data collection and model refinement |
| ApoGST5118 | HoloGST5118 |
| Data collection | | |
| Beam line | PX1, SOLEIL | BM30A/FIP, ESRF |
| Space group | P2₁₁₁,2₂₂₂,2₄₄ | P2₁₁₁,2₂₂₂,2₄₄ |
| No. of dimers in the asymmetric unit | 2 | 2 |
| Cell dimensions a, b, c (Å) | 86.05 88.46 157.16 | 86.57 88.15 156.49 |
| Resolution (Å) | 44.23–1.80 (1.89–1.80)* | 48.48–2.00 (2.11–2.00) |
| Rmerge (%) | 0.08 (0.619) | 0.069 (0.375) |
| Mean I/σ(I) | 22.2 (3.6) | 20.0 (4.7) |
| Completeness (%) | 99.7 (98.1) | 100.0 (99.9) |
| n observations | 1,443,573 (112,670) | 555,508 (71,179) |
| Redundancy | 12.8 (7.2) | 6.8 (6.0) |
| Wilson B factor (Å²) | 20.1 | 21.8 |
| Phasing method | SAD* |
| Refinement | | |
| Resolution (Å) | 42.54–1.80 (1.84–1.80) | 44.90–2.00 (2.02–2.00) |
| Rfree (%) | 19.3 | 17.8 |
| Rmerge (%) | 22.7 (30.1) | 22.1 (32.5) |
| Average B-factor (Å²) | 25.1 | 24.6 |
| Protein atoms | 28.1 | 30.6 |
| Ligand atoms | 29.7 | 31.2 |
| Ramachandran statistics (%) | 98.5 | 98.6 |
| Residues in preferred regions | 1.1 | 1.0 |
| Residues in allowed regions | 0.4 | 0.4 |
| Outlier residues | 0.007 | 0.007 |
| Root mean square deviations | 1.025 | 1.034 |
| Bond length (Å) | | |
| Root mean square deviations | | |
| Bond angle (degrees) | | |
| Outlier residues | | |
| | | |

* Values in parentheses are for highest resolution shell.
* Single-wavelength anomalous dispersion.
* Rₚ was determined from all of the reflections (working set + test set), whereas Rmerge corresponds to a subset of reflections (test set).

RESULTS

GST5118 X-ray Structures—Two structures of GST5118 (apo and holo with bound GSH) were solved by using the single-wavelength anomalous diffraction data collected from crystals of the selenomethionylated protein. Each corresponded to the same local packing with two homodimers arranged similarly in the asymmetric units. In each structure, the four monomers adopted quite similar structures. Average root mean square deviations of the six possible superpositions are 0.34 and 0.27 Å for apo and holo structures, respectively. Root mean square deviations increased by at least 0.2 Å when apo and holo monomers were compared. This increase is mainly contributed by the displacement of β-hairpin β2'–β2 during the apo-holo transition (see below).

The GST5118 monomer adopts the GST canonical fold, which consists of an N-terminal thioredoxin-like motif (B₁a₁B₂a₂B₃B₄a₃) and a C-terminal domain of at least four helices (a₄a₅a₆a₇). The two most conserved residues in GST
are present in GST5118: cis-Pro-74 in the N-terminal domain, which is assumed to maintain the enzyme in a catalytically competent structure (38), and Asp-205, which is important for the C-terminal domain cohesion through its participation in the N-capping box (39). Nevertheless, the GST5118 structure contains unique features in both N- and C-terminal domains (Fig. 1). The first one is an elongation of about 8 residues of the loop β1-α1 (11–22) when compared with almost all GSTs. Only GST-like chloride intracellular channel proteins and the recently characterized GST from Agrobacterium tumefaciens (40) exhibit this property. In both GST5118 and A. tumefaciens GST structures, this long β1-α1 loop closes one part of the electrophilic substrate pocket (H site). In addition, a β-hairpin motif (β2' β2") between α2 and β3 is observed for the first time in a GST fold. This motif hinders the formation of the regular GST dimer and partially covers the GSH binding site (G site). The C-terminal domain includes two extra helices: α4', which follows α4 in an antiparallel way, and α6' located between α6 and α7. This second supplementary helix is a characteristic of plant Phi GSTs (41). As for helix α4', to the best of our knowledge, it has been observed only in GRX2 (glutaredoxin 2) (Protein Data Bank entries 3ir4 and 1g7o (42)). This protein is assumed to be the common ancestor of GSTs (43). Furthermore, α4' lies in a position similar to the C-terminal helix of the human Alpha and Theta GSTs (supplemental Fig. S3). It has been proposed that a secondary structure in this position covers the H site and reduces its accessibility (44, 45).

GST5118 exhibits a new dimerization mode with an accentuated open V-shape where both subunits are related by a C2 symmetry. In the canonical GST assembly, the α4 and α5 helices of one monomer pack against the N-terminal domain of its partner. In GST5118, the additional β-hairpin prevents this proximity. Helix α4 of one subunit packs in the groove between helices α4 and α5 of the other protomer (Fig. 1 and supplemental Fig. S4). Interestingly, this new oligomeric structure conserves a large cleft in the center of the dimer facing both of the active sites. The size of the buried interface (1850 Å²) is significant and comparable with that observed in the “open” classical GST dimers like in human Omega GST (46) or in Drosophila Sigma GST (47). There are five hydrogen bonds and a hydrophobic lock-and-key motif that hold the two protomers together. The key residue of the lock-and-key motif is Phe-113 from helix α4. It fits in the pocket of the other subunit formed by Pro-107 from loop α3-α4; Gln-114 and Phe-117 from helix α4; Leu-195, Met-196, and Thr-203 from the α5-α6 loop; and Ser-205 from helix α6 (supplemental Fig. S5). Interestingly, this motif is present in Alpha, Mu, and Pi GSTs (48), where the lock is also located between helices α4 and α5. However, in the latter cases, the key is located in loop α2-β3, which is replaced by the protruding β-hairpin in GST5118.

In the catalytic center of the holo structure, the GSH molecule fits in the canonical G site, where the anchoring motifs for the γ-glutamyl and cysteyln moieties are conserved in GSTs from this class. The imidazolone group of His-70, from the β-hairpin motif, interacts via a water molecule with the carbonyl group of the γ-glutamyl moiety and with the amide group of the glycylic moiety (Fig. 2). Its carboxylate group is stabilized by the side chains of Tyr-148 and Arg-153 from α4'. The thiol sulfur of GSH forms a hydrogen bond with the hydroxyl group of the putative catalytic Ser-22, which is located at the N-terminal end of α1 in the thioredoxin domain. This Ser-22 occupies the same position as the catalytic residue of the Cys/Ser-GSTs (supplemental Fig. S3). The two residues Trp-21 and Pro-23, which surround the above-mentioned catalytic serine and take part in the H site, are invariant in GSTs from this class (supplemental Fig. S6). Trp-21 is a very well conserved residue in thioredoxins that is involved in enzyme substrate recognition (49), and Pro-23 is a well conserved residue in the active site motif in the class I glutaredoxins (50).

Upon GSH binding, significant conformational changes occur in the GST5118 structure. In the G site, the β-hairpin motif comes closer to the glycylic moiety, allowing its interaction with the side chain of His-70, which adopts a new rotamer (from m-70 to t60). The side chain conformation of Asp-87 also changes to stabilize the N-terminal end of GSH (from σ70 to t0). In the H site, Trp-122 and Phe-127 from α4 undergo confor-
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Table 2: Kinetic parameters of GST5118 and GST5118-S22A in enzymatic assays

| Substrate          | GST5118 | GST5118-S22A | GST5118 | GST5118-S22A | GST5118 | GST5118-S22A |
|--------------------|---------|-------------|---------|-------------|---------|-------------|
|                     | $K_m$  | $k_m$  | $k_m$  | $K_m$  | $k_m$  | $K_m$  | $k_m$  |$rac{k_m}{K_m}$ |$K_m$  | $k_m$  |$rac{k_m}{K_m}$ |
| CDNB               | ND     | 653.8 ± 49.4 | ND     | 1765.0 ± 115.6 | ND     | 2.69 ± 0.02 |
| HED                | ND     | 178.6 ± 7.8  | ND     | 57.3 ± 6.8  | ND     | 0.32 ± 0.02 |
| DHA                | ND     | ND          | ND     | ND          | ND     | ND          |
| Phenethyl-ITC      | ND     | 119.0 ± 14.4 | ND     | 510.9 ± 16.0 | ND     | 4.3 ± 0.3   |
| H$_2$O$_2$         | ND     | ND          | ND     | ND          | ND     | ND          |
| Terbutyl-OOH       | ND     | ND          | ND     | ND          | ND     | ND          |
| Cu-OOH             | ND     | 3767.0 ± 437.0 | ND     | 62.6 ± 1.5  | ND     | 0.011 ± 0.002 |
| GOuaroO            | ND     | ND          | ND     | ND          | ND     | ND          |
| PNP-butyrate       | ND     | 774.3 ± 88.3 | ND     | 165.0 ± 5.6 | ND     | 0.021 ± 0.001 |
| CMFDA without GSH  | 3.6 ± 0.6 | 1.1 ± 0.1 | 1.0060 ± 0.0002 | 0.021 ± 0.002 | 0.0017 ± 0.0002 | 0.24 ± 0.01 |
| CMFDA with GSH     | 8.0 ± 0.8 | 1.5 ± 0.1 | 0.017 ± 0.04 | 1.021 ± 0.002 | 0.022 ± 0.001 |
| GSH                | 216.4 ± 34.9 | 615.4 ± 44.2 | 662.0 ± 14.2 | 13.8 ± 0.6 | 3.1 ± 0.4   |

By esterases (23). GST5118 is active at transferring GSH onto phenethyl-ITC and PNP-butyrate but not CDNB. Surprisingly, GST5118 also exhibits two types of esterase activity on CMFDA, one with and another one without GSH (Table 2). Still, in the absence of GSH, the activity remains weak, with a catalytic efficiency 12-fold lower than in the presence of GSH. By contrast, no thiol transferase activity could be detected with hydroxethyl disulfide, and the enzyme was also inactive in the reduction of dehydroascorbate. In addition, no peroxidase activity could be detected with either hydrogen peroxide, tert-butyldihydroperoxide, or cumene hydroperoxide. Etherase activity was also tested using the synthetic substrate $\alpha$-O-methylumbelliferyl-$\beta$-hydroxypropiovanillone. This compound is an analog of lignin fragments containing an ether linkage that can be cleaved by Lig proteins (17). Using LigF from Sphingobium sp. SYK-6 as control (53), we showed that GST5118 does not possess etherase activity with $\alpha$-O-methylumbelliferyl-$\beta$-hydroxypropiovanillone. GST5118 does not display sulfatase activity using para-nitrophenyl sulfate potassium, although a sulfate binding site has been identified in the structure.

Based on sequence alignments and structural data, we postulated that Ser-22 (GST5118 numbering) could be the catalytic residue responsible for GST5118 activity. Two directed mutations have been performed replacing Ser-22 by an alanine (GST5118-S22A) or a cysteine (GST5118-S22C). Both proteins lost GSH transferase activity using phenethyl-ITC. Surprisingly, GST5118-S22A gained activity with CDNB, hydroxethyl disulfide, and cumene hydroperoxide (Table 2). On the other hand, GST5118-S22C was inactive with all substrates used in this study (data not shown).

ANS Binding Site—ANS is an environment-sensitive fluorescent dye, the fluorescence quantum yield of which increases upon binding to hydrophobic sites of proteins (54). ANS binding onto GST5118 was accompanied by the appearance of a characteristic fluorescence emission spectrum with a maximum at 475 nm, the excitation wavelength being fixed at 385 nm (Fig. 3A). The fluorescence signal, resulting from the interaction between the protein and ANS, was established rapidly in less than 30 s and increased with the molar ratio of ANS to GST5118, revealing a saturation curve. For concentrations...
higher than 50 μM ANS, a loss of signal was observed as previously shown for BSA or MurA interaction with ANS (54). A dissociation constant of 10.65 ± 0.5 μM was obtained by plotting the concentration of ANS bound onto GST5118 against the concentration of free ANS. Data were fitted to Equation 1. The number of ANS binding sites, determined as described under “Experimental Procedures,” was 1.008 ± 0.015, suggesting the binding of only one ANS molecule per monomer (Fig. 3B).

In additional experiments, tryptophan-based fluorescence of GST5118 was measured using 290 and 340 nm as excitation and emission wavelengths, respectively, in the presence or absence of ANS (Fig. 3C). Fluorescence resonance energy transfer (FRET), revealed by the appearance of a signal at 475 nm, was observed between Trp and ANS, confirming the binding of ANS onto the protein, the energy transfer being highly dependent on the distance between the donor and acceptor molecules (Fig. 3C). This result is in accordance with the presence of four Trp residues in the vicinity of the Ser-22 (Trp-21, Trp-26, Trp-122, and Trp-215).

Similar experiments have been performed using the mutated protein GST5118-S22C. Interestingly, the GST5118-S22C mutant is able to bind ANS only after reduction with DTT (Fig. 3A). Besides giving information about the ANS binding site in GST5118, these data demonstrate the robustness of the method. Mass spectrometry experiments performed on GST5118-S22C showed that it covalently binds a GSH adduct. Indeed, purified GST5118-S22C exhibited a molecular mass of 29,033 Da, whereas the previously DTT-treated protein exhibited the expected molecular mass calculated from the primary sequence of 28,728 Da. Additionally, after treatment with GSSG, S-(phenylacetophenone)-glutathione, and 2-methyl-S-glutathionyl-naphthoquinone, the mass of the reduced protein increased by 305 Da (29,033 Da), which corresponds to a GSH adduct. By contrast, no GSH adduct was detected on the wild type protein, suggesting that the mutated protein is able to bind GSH covalently at the newly added cysteinyl residue, this addition preventing ANS binding onto the protein. ANS binding onto GST5118 was investigated in the presence of various GSH concentrations (up to 10 mM), demonstrating that GSH inhibits ANS binding. The obtained data were fitted to Equation 2, leading to an IC_{50} value of 261.5 ± 39.8 μM (supplemental Fig. S7). This measured value is in accordance with the K_m for GSH (216.4 ± 34.89 μM) found in the enzymatic assays. Additionally,
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### TABLE 3

**Competition experiments between ANS and various compounds**

Values given are IC50 fluorescence inhibition obtained by fitting data to Equation 2.

| GST5118 | K \text{ ANS} | No. of fixation sites at 50 μM ANS | Glutathione |
|---------|---------------|------------------------------------|-------------|
|         | 10.6 ± 0.5 μM | 1.008 ± 0.015                      | 261.5 ± 39.8 μM |

**Beech compounds**

|                      | K \text{ ANS} | Glutathione |
|----------------------|---------------|-------------|
| Coniferaldehyde      | 61.3 ± 1.2 μM |             |
| Vanillin             | 216.6 ± 8.5 μM|             |
| 2-Hydroxy-4-methoxybenzophenone | 15.6 ± 0.1 mU |             |
| 4-Chloro-3-nitrobenzoic acid | 2.3 ± 0.4 mU |             |
| 4’-Hydroxycetophenone | 17.1 ± 0.1 mU |             |
| Gallic acid          | 79.4 ± 1.6 μM |             |
| Vanillic acid        | 584.3 ± 98.7 μM|             |
| 3-Hydroxy-4-methoxycinnamic acid, predominantly trans | — | |

**Glutathionylated compounds**

|                      |              |              |
|----------------------|--------------|--------------|
| 2-Bromo-4’-phenylacetophenone | 99.7 ± 46.5 μM |             |
| Phenylacetophenone-SG | 83.7 ± 33.4 μM |             |

**Sulfated compounds**

|                      |              |              |
|----------------------|--------------|--------------|
| 1,2-Naphthoquinone-4-sulfonic acid | 334.5 ± 56.8 μM |             |
| p-Xylene-2-sulfonic acid hydrate | 928.7 ± 258.8 μM |             |
| p-Xylene             | 1.6 ± 0.0 μM |             |
| 4-Aminotoluene-3-sulfonic acid | 223.6 ± 53.2 μM |             |
| 4-Methylumbelliferyl-sulfate | — |             |
| 4-Methylumbelliferone | — |             |

In the vicinity of catalytic Ser-22, a sulfate/acetate binding site has been detected in the GST5118 structure. Because ANS possesses a sulfonic acid motif, various sulfated compounds were tested for their ability to interact with the ANS binding site in GST5118. ANS binding is inhibited in the presence of 4-aminotoluene-3-sulfonic acid, p-xylene-2-sulfonic acid hydrate, 1,2-naphthoquinone-4-sulfonic acid sodium, and also 4-methylumbelliferyl sulfate potassium (Table 3). Moreover, this inhibition seems to be sulfate/sulfonate-dependent because 4-methylumbelliferol and p-xylene do not compete with ANS, suggesting that the ANS binding site also overlaps with the sulfate-binding site in GST5118.

Competition experiments between ANS and various substrates have been performed in order to investigate possible overlapping between the substrate binding site and the ANS binding site. As shown above, GST5118 exhibits a relatively high affinity for CMFDA, phenethyl-ITC, and PNP-butyrate with and without GSH (Table 3). ANS binding onto GST5118 remained mainly unaltered in the presence of either phenethyl-ITC or CMFDA (supplemental Fig. S9). Moreover, GST5118 activity against phenethyl-ITC is inhibited in the presence of ANS (supplemental Fig. S10). Concerning CMFDA, only esterase activity with GSH is inhibited by ANS (Fig. 4A). Due to quenching phenomena, PNP-butyrate was not used in this test.

excitation transfer between Trp and bound ANS was fully abolished in the presence of 1 mM GSH (supplemental Fig. S8). S-(phenylacetophenone)-Glutathione and 2-methyl-S-glutathionyl-naphtoquinone were also able to inhibit ANS binding, whereas the same compounds deprived of the GSH adduct could not (Table 3). Taking together, these data suggest strongly that in GST5118, the ANS binding site overlaps at least in part the glutathione binding site (G site).

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### FIGURE 4

**Inhibition of GSH-dependent esterase activity by ANS or vanillin.** Data are represented as mean ± S.D. (n = 3). A, noncompetitive inhibition of esterase activity against CMFDA without inhibitor (○) or in the presence of 50 μM ANS (●) or 300 μm vanillin (▲). K\text{i} values were calculated resolving to the non-linear noncompetitive inhibition equation \((r^2 > 0.99); V_{\text{max}} = V_{\text{max}} / (1 + /K_i); Y = V_{\text{max}} X / K_{\text{inhib}} + X); V_{\text{max}} and K_{\text{inhib}} represent the maximum enzyme velocity and the Michaelis-Menten constant without inhibitor, whereas \(V_{\text{max}}\) represents the maximum enzyme velocity for one concentration of inhibitor, and \(K_{\text{i}}\) is the inhibition constant. B, competitive inhibition of esterase activity GSH-dependent against GST without inhibitor (○) or in the presence of 300 μm vanillin (▲). The \(K_{\text{i}}\) value was calculated resolving to the non-linear competitive inhibition equation \((r^2 > 0.99); K_{\text{inhib}} = K_{\text{inhib}} \times (1 + [I]/K_i); Y = V_{\text{max}} X / K_{\text{inhib}} + X); V_{\text{max}} and K_{\text{inhib}} represent the maximum enzyme velocity and the Michaelis-Menten constant without inhibitor, whereas \(K_{\text{inhib}}\) represents the Michaelis-Menten constant in the presence of inhibitor, and \(K_{\text{i}}\) is the inhibition constant.

Taken together, these data suggest that the ANS binding site does not overlap with the substrate binding site because the probe does not compete with hydrophobic substrates as phenethyl-ITC or CMFDA for binding. By contrast, the competition between ANS and GSH (Fig. 4B) and the inhibition of GSH-requiring activities show an overlap between ANS and the G site of GST5118.

**Ligand Screening**—Using ANS as a screening tool, competition experiments have been performed with 11 structurally variable beech compounds (supplemental Fig. S11). Seven of them were recognized by GST5118 (Table 3). Among these products, those exhibiting the better affinity for GST5118 are either aldehydes or compounds harboring electrophilic motifs or atoms such as chlorine or nitrogen. Steric hindrance is not a factor influencing ligand binding because epicatechin and catechin hydrate, two of the largest compounds used, are both bound by GST5118.
To confirm the interaction between these compounds and GST5118, additional experiments were conducted using vanillin and testing the ability of GST5118 to react with CFMDA in the presence and absence of glutathione. Vanillin strongly inhibited the activity in the presence of GSH (Fig. 4) but had no effect in the absence of GSH (data not shown). Kinetic experiments revealed, as expected, a non-competitive inhibition against CFMDA (i.e. different binding sites for vanillin and CFMDA) and a competitive inhibition against GSH (i.e. overlapping binding sites for vanillin and GSH). In both cases, the $K_i$ value ($K_i$ around 200 μM) is in accordance with the $IC_{50}$ value calculated from the experiments performed with ANS (216.6 ± 8.5 μM).

As summarized in Fig. 5, all of these data suggest that GSH, ANS, sulfate/acetate, and ligands such as vanillin bind to GST5118 through overlapping binding sites, which are different from the substrate-binding site.

**DISCUSSION**

GST5118 is a newly identified fungal GST with unique properties both in terms of structural organization and biochemistry. GST5118 monomer exhibits two extra secondary structures in addition to the canonical GST framework: 1) the β-hairpin β2'β2" in the N-terminal domain, which hinders the formation of the regular GST dimer and which may be described as a shield covering the G site once GSH is bound, and 2) the helix α4', which closes the presumed electrophilic substrate binding site and involves a basic side chain in the anion binding site of the enzyme. Based on structural alignments, all five *P. chrysosporium* GSTs from this class should share the same original properties, allowing us to define a new structural GST class with a novel dimerization mode. This class holds the unique property of having conserved all the secondary structures of monomeric glutaredoxin 2, which is the presumed common ancestor of the cytosolic GSTs (43). All other structures solved so far lost at least helix α4'. The catalytic Ser-22 is in hydrogen-bonding contact with the thiol sulfur atom of the cysteinyl moiety of GSH and thus could participate in the stabilization of the thiolate anion. The GST5118-S22A mutant retains the ability to conjugate GSH onto electrophilic substrates and exhibits weak peroxidase and thiol transferase activities. This suggests that another residue could stabilize the thiolate form of GSH and thus lower its pKₐ. In the vicinity of GSH, Asn-24 which is conserved in isoforms of this class (supplemental Fig. S6) could fulfill this role with few conformational changes. In accordance, this Asn residue is conserved in Ure2p class and has been shown to be crucial for peroxidase activity of *Saccharomyces cerevisiae* Ure2p (55). This observation could explain why GST5118-S22A shows such an activity pattern.

A sulfate binding pocket was identified in the N-terminal domain of GST5118 structure, close to the GSH binding site. This could explain the binding of ANS, which possesses a sulfonic acid moiety, at the G site and the competition between ANS and GSH. The unique binding mode of ANS onto GST5118 allowed us to use it as a ligand screening tool. Glutathionylated and sulfur-containing compounds as well as products resulting from wood degradation compete with ANS, suggesting that all of them bind at the G site. This is in accordance with the structural data concerning glutathionylated and sulfated compounds; however, it was unexpected for wood compounds because the recognition of hydrophobic substrates has never been described to occur at the G site.

The consequence of binding wood compounds at the G site is the inability of GST5118 to accept and therefore transfer GSH anymore. This enzyme could thus have two functions, possibly depending on the intracellular concentration of GSH and wood compounds: a classical GSH transferase activity, especially with phenethyl-ITC as substrate, and a ligandin property toward wood extractive compounds. The affinity for ligand binding is likely to be driven by very fine interactions. An example of this fine ligand specificity is the pair vanillin/vanillic acid, in which the first compound is well recognized by GST5118, whereas the latter cannot displace ANS. The only difference between these two molecules is the addition of a single atom of oxygen, transforming the aldehyde function into an acidic one. Vanillin is
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known to be one of the key intermediates found during wood decay, and it was shown to be taken up by fungal cells (56). However, vanillin is not a nutrient substrate but rather acts as a chemical stress on fungal cells (57). It has also been suggested that it can be responsible for activation of fungal metabolic pathways for phenolic compounds (58). This ligandin property of GST5118 could thus be helpful to protect the cell against highly reactive xenobiotics or secondary metabolites. Similar functions were assigned to plant Phi and Tau GSTs that participate in the transport of highly reactive products to the vacuole without catalyzing their conjugation and thus sequestering them away from critical intracellular targets (59, 60).

In conclusion, the structural and functional properties of GST5118 allow us to define a new GST class that we name GSTFUa (for fungal specific class A). Accordingly, GST5118 from *P. chrysosporium* can thus be renamed GSTFUa1.

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REFERENCES

1. Hayes, J. D., Flanagan, J. U., and Jowsey, I. R. (2005) Glutathione trans-ferases. *Annu. Rev. Pharmacol. Toxicol.* 45, 51–88

2. Mannervik, B. (2012) Five decades with glutathione and the GSTome. *Annu. Rev. Nutr.* 32, 235–242

3. Oakley, A. J., Lo Bello, M., Nuccetelli, M., Mazzetti, A. P., and Parker, M. W. (1999) The ligandin (non-substrate) binding site of human Pi class glutathione transferase is located in the electrophile binding site (H-site). *J. Mol. Biol.* 291, 913–926

4. Lederer, B., and Böger, P. (2003) Binding and protection of porphyrins by glutathione S-transferases of Zea mays L. *Biochim. Biophys. Acta 1621, 226–233

5. Dixon, D. P., Lapthorn, A., Madesis, P., Mudd, E. A., Day, A., and Edwards, R. (2008) Binding and glutathione conjugation of porphyrinogens by plant glutathione transferases. *J. Biol. Chem.* 283, 20268–20276

6. Bilang, J., Macdonald, H., King, P. J., and Sturm, A. (1993) A soluble auxin-cation, molecular cloning, and characterization of glutathione *S*-transferase acting as 1-Cys thiol transferases of *Phanerochaete chrysosporium*. *Annu. Rev. Pharmacol. Toxicol.* 33, 51–88

7. Morel, M., Ngadin, A. A., Droux, M., Jacquot, J. P., and Gelhaye, E. (2011) Functional diversification of fungal glutathione *S*-transferases acting as 1-Cys thiol transferases. *BMB Rep.* 45, 265–274

8. Morel, M., Ngadin, A. A., Droux, M., Jacquot, J. P., and Gelhaye, E. (2003) Roles of the enantioselective glutathione S-transferases in cleavage of *β*-aryl ether. *J. Bacteriol.* 185, 1768–1775

9. Lee, S., Monnappa, A. K., and Mitchell, R. J. (2012) Biological activities of lignin hydroxylate-related compounds. *BMB Rep.* 45, 265–274

10. Lee, C. Y., Sharma, A., Cheong, J. E., and Nelson, J. L. (2009) Synthesis and antioxidant properties of dendrphilic polyacrylamids. *Biores. Eng. Chem. Lett.* 19, 6326–6330

11. Schenk, P. M., Baumann, S., Mattes, R., and Steinbiss, H. H. (1995) Improved high level expression system for eukaryotic genes in *Escherichia coli* using T7 RNA polymerase and rare ArgtRNAAs. *BioTechniques* 19, 196–198, 200

12. D’Ambrosio, K., Kauffmann, B., Rouhier, N., Benedetti, E., Jacquot, J. P., Aubry, A., and Corbier, C. (2003) Crystallization and preliminary x-ray studies of the glutaredoxin from poplar in complex with glutathione. *Acta Crystallogr. D Biol. Crystallogr.* 59, 1043–1045

13. Couturier, J., Koh, C. S., Zaffagnini, M., Winger, A. M., Gualberto, J. M., Corbier, C., Decottignies, P., Jacquot, J. P., Lemaire, S. D., Didierjean, C., and Rouhier, N. (2009) Structure-function relationship of the chloroplastic glutaredoxin S12 with an atypical WCSYS active site. *J. Biol. Chem.* 284, 9299–9310

14. Zhang, J., Shibata, A., Ito, M., Shuto, S., Ito, Y., Mannervik, B., Abe, H., and Morgenstern, R. (2011) Synthesis and characterization of a series of highly fluorogenic substrates for glutathione transferases, a general strategy. *J. Am. Chem. Soc.* 133, 14109–14119

15. Kim, E. D., Kim, K. H., Bae, Y. I., Lee, J. H., Jang, Y. H., and Nam, S. W. (2005) Purification and characterization of the recombinant arylsulfatase cloned from *Pseudomonas aeruginosa*. *Protein Expur. Purif.* 39, 107–115

16. Koh, C. S., Navrot, N., Didierjean, C., Rouhier, N., Hirasawa, M., Knaff, D. B., Wingle, G., Samian, R., Jacquot, J. P., Corbier, C., and Gelhaye, E. (2008) An atypical catalytic mechanism involving three cysteines of thioredoxin. *J. Biol. Chem.* 283, 23062–23072

17. Kabsch, W. (2010) *XDS*. *Acta Crystallogr. D Biol. Crystallogr.* 66, 125–132

18. Evans, P. (2006) Scaling and assessment of data quality. *Acta Crystallogr. D Biol. Crystallogr.* 62, 72–82

19. Wann, M. D., Ballard, C. C., Cowtan, K. D., Dodson, E. J., Emsley, P., Evans, P. R., Keegan, R. M., Krissinel, E. B., Leslie, A. G., McCoy, A., McNicholas, S. J., Murshudov, G. N., Pannu, N. S., Potterson, E. A., Powell, H. R., Read, R. J., Vagin, A., and Wilson, K. S. (2011) Overview of the CCP4 suite and current developments. *Acta Crystallogr. D Biol. Crystallogr.* 67, 235–242

20. Adams, P. D., Afonine, P. V., Bunkóczi, G., Chen, V. B., Davis, E. N., Echols, N., Headd, J. J., Hung, L. W., Kapral, G. J., Grosse-Kunstleve, R. W., McCoy, A. J., Moriarty, N. W., Oeffner, R., Read, R. J. I., Richardson, D. C., Richardson, J. S., Terwilliger, T. C., and Zwart, P. H. (2010) PHENIX: A comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr. D Biol. Crystallogr.* 66, 213–221

21. Terwilliger, T. (2004) SOLVE and RESOLVE. Automated structure solution, density modification, and model building. *J. Synchrotron Radiat.* 11, 49–52

22. Emsley, P., Lohkamp, B., Scott, W. G., and Cowtan, K. (2010) Features and development of Coot. *Acta Crystallogr. D Biol. Crystallogr.* 66, 486–501

23. Chen, V. B., Arendall, W. B., 3rd, Headd, J. J., Keedy, D. A., Immormino, R. M., Kapral, G. J., Murray, L. W., Richardson, J. S., and Richardson, D. C.
Ligandin Properties of a New Fungal GST

(2010) MolProbit. All-atom structure validation for macromolecular crystallography. Acta Crystallogr. D Biol. Crystallogr. 66, 12–21

33. Kriissinel, E., and Henrick, K. (2007) Inference of macromolecular assemblies from crystalline state. J. Mol. Biol. 372, 774–797

34. Holm, L., and Rosenström, P. (2010) Dali server. Conservation mapping in 3D. Nucleic Acids Res. 38, W545–W549

35. Gille, C. (2006) Structural interpretation of mutations and SNPs using STRAP-NT. Protein Sci. 15, 208–210

36. Cole, C., Barber, J. D., and Barton, G. J. (2008) The Jpred 3 secondary structure prediction server. Nucleic Acids Res. 36, W197–W201

37. Gouet, P., Robert, X., and Courcelle, E. (2003) ESPript/ENDscript. Extracting and rendering sequence and 3D information from atomic structures of proteins. Nucleic Acids Res. 31, 3320–3323

38. Allocati, N., Casalone, E., Masulli, M., Ceccarelli, I., Carletti, E., Parker, M. W., and Di Ilio, C. (1999) Functional analysis of the evolutionarily conserved proline S3 residue in Proteus mirabilis glutathione transferase B1-1. FEBS Lett. 445, 347–350

39. Cocco, R., Stenberg, G., Dragani, B., Rossi Principe, D., Paludi, D., Manervik, B., and Aceto, A. (2001) The folding and stability of human Alpha class glutathione transferase A1-1 depend on distinct roles of a conserved N-capping box and hydrophobic staple motif. J. Biol. Chem. 276, 32177–32183

40. Skopelitou, K., Dhavala, P., Papageorgiou, A. C., and Labrou, N. E. (2012) Structural interpretation of mutations and SNPs using STRAP-NT. Protein Sci. 21, 1237–1242

41. Board, P. G., Coggan, M., Chelvanayagam, G., Easteal, S., Jenner, L. S., Schulte, G. K., Danley, D. E., Hoth, L. R., Griffor, M. C., Kamath, A. V., Rosner, M. H., Chrunyk, B. A., Perregaux, D. E., Gabel, C. A., Geoghegan, K. F., and Pandit, J. (2000) Identification, characterization, and crystal structure of the Omega class glutathione transferases. J. Biol. Chem. 275, 24798–24806

42. Frova, C. (2006) Glutathione transferases in the genomics era. New in-...