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S-glutathionyl-(chloro)hydroquinone reductases: a new class of glutathione transferases functioning as oxidoreductases

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Abstract

Glutathione transferases (GSTs) are best known for transferring glutathione (GSH) to hydrophobic organic compounds, making the conjugates more soluble. However, the omega-class GSTs of animals and the lambda-class GSTs and dehydroascorbate reductases (DHARs) of plants have little or no activity for GSH transfer. Instead, they catalyze GSH-dependent oxidoreductions. The lambda-class GSTs reduce disulfide bonds, the DHARs reduce the disulfide bonds and dehydroascorbate, and the omega-class GSTs can reduce more substrates, including disulfide bonds, dehydroascorbate, and dimethylarsinate. Glutathionyl-(chloro)hydroquinone reductases (GS-HQRs) are the newest class of GSTs that mainly catalyze oxidoreductions. Besides the activities of the other three classes, GS-HQRs also reduce GS-hydroquinones, including GS-trichloro-*p*-hydroquinone, GS-dichloro-*p*-hydroquinone, GS-2-hydroxy-*p*-hydroquinone, and GS-*p*-hydroquinone. They are conserved and widely distributed in bacteria, fungi, protozoa, and plants, but not in animals. The four classes are phylogenetically more related to each other than to other GSTs, and they share a Cys-Pro motif at the GSH-binding site. Hydroquinones are metabolic intermediates of certain aromatic compounds. They can be auto-oxidized by O₂ to benzoquinones, which spontaneously react with GSH to form GS-hydroquinones via Michael's addition. GS-HQRs are expected to channel GS-hydroquinones, formed spontaneously or enzymatically, back to hydroquinones. When the released hydroquinones are intermediates of metabolic pathways, GS-HQRs play a maintenance role for the pathways. Further, the common presence of GS-HQRs in plants, green algae, cyanobacteria, and halobacteria suggest a beneficial role in the light-using organisms.

Keywords

Glutathione transferases; glutathione-dependent reductases; glutathione conjugates; S-glutathionyl-hydroquinones; pentachlorophenol

Introduction

Glutathione transferases (GSTs) usually transfer glutathione (GSH) to the electrophilic centers of toxic, hydrophobic compounds, with the conjugates being more soluble and less toxic (Sheehan et al., 2001). In animals, the conjugates are pumped out of the cells and transported to the kidney, where they are transformed into mercapturic acids for release in the urine (Commandeur et al., 1995). Plants can detoxify herbicides and endogenous compounds with GSTs, and the conjugates are transported into the plant vacuole for storage

(Rea et al., 1998). However, plants may transport the conjugates to the root for release in root exudates to avoid the accumulation of toxic conjugates (Schröder et al., 2007). Microorganisms may pump the conjugates out of the cells, but they remain in the vicinity (Kaluzna and Bartosz, 1997). When the conjugates are still toxic, the conjugation is not an effective means of detoxification. Consequently, it is unclear whether microorganisms use GSTs for detoxification in the same way as animals or plants. Despite the rich presence of GSTs in sequenced microbial genomes, only a limited number of bacterial GSTs have been characterized. The most characterized members have physiological roles that are not for detoxification, but are often involved in the metabolism of specific compounds (Allocati et al., 2009).

GSTs are divided into three major families, depending on the location inside a mammalian cell: cytosolic, mitochondrial (kappa-class GSTs), and microsomal GSTs (Hayes et al., 2005). The cytosolic GSTs are the most abundant and are further grouped into several divergent classes on the basis of origin, sequence similarity, catalytic amino-acid residue, and substrate specificity. The established classes include the alpha, Mu, and Pi classes found in mammals (Sheehan et al., 2001); the Phi, Tau, lambda, and dehydroascorbate reductase (DHAR) classes of plants (Dixon et al., 2002); the delta class of insects (Board et al., 1997); and the beta class of bacterial origin (Rossjohn et al., 1998). Theta, zeta, and omega are present in a variety of life forms (Board et al., 1997, 2000). The catalytic amino-acid residue is often conserved among closely related classes (Sheehan et al., 2001). An N-terminal region Tyr residue stabilizes the thiolate of the bound GSH (GS^-) in the GSTs of the alpha, Mu, and Pi classes. A Ser residue fulfills the same function in the theta, zeta, kappa, Phi, and Tau classes (Li et al., 2005). An N-terminal region Cys residue interacts with GSH in beta, omega, lambda, DHAR, and glutathionyl-(chloro) hydroquinone reductase (GS-HQR) classes (Board et al., 2000; Dixon et al., 2002; Xun et al., 2010). For the GSTs with either a Tyr or Ser catalytic residue, the residue stabilizes GS^- , which attacks the second substrate to generate GS-conjugates. For the GSTs with a Cys catalytic residue, there is no evidence that the residue stabilizes GS^- . In fact, most of the GSTs have little to no activities for transferring GSH to the “universal” GST substrate, 1-chloro-2,4-dinitrobenzene (CDNB).

The beta-class GSTs are the first reported GSTs with a Cys catalytic residue at the N-terminus because of the Cys residue (Cys 10) being covalently bound to GSH in the structure of GSTB1-1 of *Proteus mirabilis* (Rossjohn et al., 1998). GSTB1-1 of *P. mirabilis* can use the standard GST substrate CDBN as well as catalyzing thiol transfer, a typical reaction of glutaredoxin. The Cys residue is required for thiol transfer, but not for CDBN conjugation (Caccuri et al., 2002). The Cys residue of the beta-class GST of *Escherichia coli* is not needed for CDBN conjugation, either (Inoue et al., 2000). The mutation of the Cys residue (Cys 10) to Ala in the beta-class GST of *Ochrobactrum anthropi* results in increases in both k_{cat} and K_m values, with a net decrease in catalytic efficiency (k_{cat}/K_m) (Allocati et al., 2008). It appears that the Cys residue in the GST increases the affinity to GSH. Thus, the beta-class GSTs have both thiol transfer and conjugation activities.

The omega-class GSTs were discovered by BLAST searches of the human EST (expressed sequence tag) database with the N-terminal domain of human GSTZ1 (Board et al., 2000). Further bioinformatic analysis revealed that the orthologs of human GSTO1-1 are common in animals. The omega-class GSTs are different from other mammalian GSTs because they have a Cys residue, instead of Tyr or Ser, at the active site. The Cys residue forms a disulfide bond with a bound GSH in the crystal structure of human GSTO1-1. The omega-class GSTs have very weak activities toward CDBN, but actively reduce a mixed disulfide bond of GS-SX (thiol transfer), dehydroascorbate (DHA), and dimethylarsinate, with GSH as the reducing power (Whitbread et al., 2005).

The lambda-class GSTs and DHARs were identified from plants by BLAST research with the human GSTO1 sequence and are common in plants. The lambda-class GSTs are known to catalyze only thiol transfer, and the DHARs perform both thiol transfer and DHA reduction (Dixon et al., 2002). The limited substrate range may be attributed to the monomeric nature of these two classes of GSTs, because the dimer interface is involved in substrate binding in other GSTs (Sheehan et al., 2001).

The yeast GS-HQRs were originally identified as members in the omega class (Garcera et al., 2006). A BLAST search of the *Saccharomyces cerevisiae* genome with the human GSTO1 sequence identified three distantly homologous genes, and the recombinant proteins had the activities of the omega-class GSTs. One of them is *ecm4* (extracellular mutant 4), a protein possibly involved in cell-surface biosynthesis and architecture. The *ecm4* gene is identified as a transposon mutant hypersensitive to calcofluor white that binds to chitin, a component of the yeast cell wall. *S. cerevisiae ecm4* mutants show increased amounts of chitin (Lussier et al., 1997). However, the three yeast proteins share less than 20% sequence identity with human GSTO1, and the conserved domain database has an *ecm4* group COG0435, which is not assigned to the omega-class GSTs (Tatusov et al., 2003). Recently, the three yeast proteins have been regrouped as members in the new GST class, GS-HQRs, on the basis of sequence similarity and their ability to catalyze GSH-dependent reduction of GS-(chloro)hydro-quinones (Xun et al., 2010).

Discovery of GS-HQRs

PcpF, the first characterized GS-HQR, plays a maintenance role in the metabolic pathway of pentachloro-phenol (PCP) in *Sphingobium chlorophenolicum* ATCC 39723 (Huang et al., 2008). PCP is anthropogenic and was introduced into the environment as a wood preservative in the 1930s (Crosby, 1981). Although PCP is toxic to all cells, as it disrupts the integrity and function of cell membranes, some bacteria have evolved to degrade it in a short time (Saber and Crawford, 1985). The enzymes responsible for PCP degradation have been identified and characterized from *S. chlorophenolicum* ATCC 39723 (Figure 1) (Cai and Xun, 2002). PCP 4-monooxygenase (PcpB) oxidizes PCP to tetrachloro-*p*-benzoquinone (Xun and Orser, 1991), which is reduced by PcpD to tetrachloro-*p*-hydroquinone (TeCH) (Dai et al., 2003). TeCH reductive dehalogenase (PcpC) uses GSH to dechlorinate TeCH to trichloro-*p*-hydroquinone (TriCH) and then to 2,6-dichloro-*p*-hydroquinone (DiCH) (Xun et al., 1992; Orser et al., 1993). DiCH 1,2-dioxygenase (PcpA) opens the aromatic ring to produce 2-chloromaleylacetate (Xun et al., 1999), which is reduced by (chloro) maleylacetate reductase (PcpE) to maleylacetate and then to 3-oxoadipate, a common metabolic intermediate in the degradation of aromatic compounds (Cai and Xun, 2002). PcpC is a GST, and the Cys14 residue at the N-terminal region participates in the dechlorination of TeCH and TriCH. When the Cys residue is oxidatively damaged, the damaged PcpC produces S-glutathionyl-TriCH (GS-TriCH) and GS-DiCH conjugates (McCarthy et al., 1996). PcpC cannot transform the conjugates, which had been considered as dead-end products until the discovery of PcpF (Huang et al., 2008).

PcpF was discovered due to the proximity of *pcpF* and *pcpC* genes on the chromosome. The four essential genes (*pcpA*, *pcpB*, *pcpC*, and *pcpE*) of PCP degradation are located at four discrete sites on the chromosome of *S. chlorophenolicum* ATCC 39723 (Cai and Xun, 2002). Although most of the genes are induced by PCP, the *pcpC* gene is constitutively expressed. The *pcpF* gene is physically linked to *pcpC*. When the *pcpF* gene is disrupted in *S. chlorophenolicum* ATCC 39723, the mutant degrades PCP more slowly and becomes more sensitive to PCP (Huang et al., 2008). Further investigation of recombinant PcpF overproduced in *E. coli* demonstrated its ability to convert GS-TriCH and GS-DiCH to TriCH and DiCH at the expense of GSH. Only a small fraction of PcpC is oxidatively

damaged in *S. chlorophenolicum* ATCC 39723. Even the small fraction of the damaged PcpC produces some GS-TriCH and GS-DiCH conjugates. PcpF channels the conjugates back to the metabolic pathway (Figure 1). When *pcpF* is inactivated, the conjugates accumulate. The quinones in the conjugates are expected to undergo redox cycling (Lau et al., 1988; Buffinton et al., 1989), generating reactive oxygen species (ROS) that slow down cell growth and PCP metabolism. Thus, PcpF plays two roles: channeling the conjugates back to the metabolic pathway and preventing the accumulation of the harmful conjugates.

The wide distribution of GS-HQRs in all domains of life

Although PcpF and PcpC are both GSTs, they are very different. PcpC has been assigned to the zeta class on the basis of sequence similarity of amino-acid residues at the active site and shared enzymatic activities, including dichloroacetate dehalogenase and maleylacetone isomerase activities (Anandarajah et al., 2000). Despite of classification, PcpC does not have high homology with any GSTs. Its closest relative is a hypothetical GST (GenBank: AAZ25344), with only 27.3% sequence identity by a BLAST search of bacterial genomes. Unlike PcpC, PcpF has highly homologous proteins in all domains of life (with more than 30% sequence identity). The PcpF homologs have been characterized as GS-HQRs because of their sequence similarity and the ability of all tested PcpF homologs (three from bacteria and one from yeast) to catalyze the GSH-dependent reduction of GS-TriCH to TriCH (Xun et al., 2010). The four GS-HQRs can also reduce several other GS-hydroquinones, with GSH as the reducing power (Lam and Xun, unpublished data).

In Archaea, GS-HQRs are present in every sequenced genome in the order of Halobacteriales. In Bacteria, GS-HQRs are common in the phyla of Actinobacteria, Cyanobacteria, Firmicutes (containing orders of Bacillales, Clostridia, and Lactobacillales), and Proteobacteria. In Eucarya, GS-HQRs are well conserved in fungi, protozoa, and plants. Some fungi, protozoa, and plants have several PcpF homologs (e.g., three in *S. cerevisiae*) (Garcera et al., 2006), two (EFA80513 and EFA79998) in *Polysphondylium pallidum* PN500 and four in *Arabidopsis thaliana* (Xun et al., 2010). Further, GS-HQR homologs are found in the genomes of two green algae [*Chlamydomonas reinhardtii* and *Ostreococcus lucimarinus*] and one moss [*Physcomitrella patens* subsp. *Patens*], but not in two red algae (*Hemiselmis andersenii* and *Guillardia theta*) by BLAST search. GS-HQR genes are not found in sequenced animal genomes.

Phylogenetic analysis of GSTs with a catalytic Cys residue

The beta, omega, lambda, DHAR, and GS-HQR classes have a catalytic Cys residue at the beginning of the first α -helix (α_1 -helix) that interacts with GSH (Board et al., 2000; Dixon et al., 2002; Xun et al., 2010). Representative GSTs from each of the classes were analyzed by multisequence alignment, and the relationship is presented in a phylogenetic tree (Figure 2). In addition, representatives from the zeta class were included because many members of this class have a Cys residue at the beginning of the α_1 -helix. The Cys residue (Cys16) may contribute to GSH binding, but the adjacent Ser residue (Ser14) stabilizes GS^- for catalysis in human GSTZ1-1 (Board et al., 2003). The GS-HQRs selected for alignment were PcpF, ReYqjG and EcYqjG of bacteria, Atecm4a of plant, and *ecm4* of yeast. They form a distinct group, more related to the omega, lambda, and DHAR classes than to the beta class. Another bacterial GST, LigG, catalyzes a similar reaction as the GS-TriCH reduction, removing the GS moiety from α -GS- β -hydroxypropiovanillone, an intermediate of lignin metabolism (Masai et al., 2003). LigG is closely related to the omega, lambda, DHAR, and GS-HQR classes, but it does not belong to any class (Figure 2). PcpC and LinD are also included in the analysis because they are GSTs catalyzing GSH-dependent dechlorination of chloro-*p*-hydro-quinones (Orser et al., 1993; Miyauchi et al., 1998). An N-terminal-region Cys

residue is involved in the catalysis in PcpC (McCarthy et al., 1996). PcpC has been assigned to the zeta class in spite of low sequence similarities (Anandarajah et al., 2000); however, phylogenetic analysis groups PcpC with LinD, instead of the zeta class. The two chlorohydroquinone reductive dehalogenases share 22.9% sequence identity. It is not clear whether PcpC and LinD belong to the same class, as GSTs with less than 30% sequence identity are usually assigned to separate classes (Sheehan et al., 2001). Nonetheless, the grouping of PcpC and LinD together is in agreement with an extensive sequence analysis of known GSTs (Marco et al., 2004). Their close relatives are ganglioside-induced, differentiation-associated protein-1 (GDAP1) and orthologs in mammals, birds, amphibians, and fishes. GDAP1 orthologs have the cytosolic GST folds, but do not have detectable GST activities (Shield et al., 2006). They have a tail-anchor domain at the C-terminus, anchoring them in the outer membrane of mitochondria. They have been characterized as mitochondrial fission factors because over-expression of the GDAP1 gene induces fragmentation of mitochondria (Niemann et al., 2005).

GST structures

The cytosolic GSTs may share low sequence similarities, but usually have the basic structure of a thioredoxin-like N-terminal domain of four β -sheets and three α -helices arranged as $\beta_1\alpha_1\beta_2\alpha_2\beta_3\beta_4\alpha_3$ and all-helical C-terminal domain. The N-terminal domain is mainly responsible for GSH binding (the G-site), and the C-terminal domain is involved in the binding of the second (hydrophobic) substrate (the H-site) (Sheehan et al., 2001). Glutaredoxins are structurally related to GSTs, consisting of only the thioredoxin-like domain (Noguera et al., 2005). Glutaredoxins are usually monomeric and best known for their thiol transferase activity. The cytosolic GSTs are usually dimeric, with the exception of the monomeric DHARs and lambda-class GSTs (Dixon et al., 2002).

The catalytic Cys-Pro motif

The catalytic Cys residue is conserved in the classical glutaredoxins and the GSTs in the omega, lambda, DHAR, and GS-HQR classes, and it is immediately followed by a Pro residue that ensures the correct orientation of the Cys residue at the N-terminus of the β_1 -helix (Figure 3A) (Board et al., 2000; Dixon et al., 2002; Xun et al., 2010). The proximity of Cys32 to the positive end of the alpha-helix dipole promotes the formation of Cys32 thiolate in human GSTO1-1 (Board et al., 2000). The Cys thiolate is believed to be essential for the GSH-dependent reductions catalyzed by these GSTs. LigG also has the Cys-Pro motif at the active site (Figure 3A). However, PcpC, LinD, and the GSTs in the beta and zeta classes do not share this Cys-Pro motif (Figure 3B). Instead, the adjacent Ser residues are expected to stabilize GS^- for conjugations (Sheehan et al., 2001). The GSTs with the Cys-Pro motif at the active site and those without are divided into two major groups by phylogenetic analysis (Figure 2).

The reaction mechanism of GS-HQRs

The Cys-Pro residues are Cys53 and Pro54 in PcpF (Figure 3A). The Cys53 should be primarily present as a thiolate, stabilized by the alpha-helix dipole as in hGSTO1-1 (Board et al., 2000), and the sulfur atom of GS-TriCH should be slightly electron deficient due to the strong electron-withdrawing effect of the aromatic ring of TriCH (Figure 4A). Thus, the Cys53 thiolate attacks the sulfur atom of GS-TriCH to form GS-PcpF (PcpF-Cys53-S-SG) and release of TriCH (Figure 4A). A GSH then comes in the active site to exchange a disulfide bond with the formation of GS-SG and PcpF-Cys53⁻ (Figure 4B). Although this is a hypothesis, our kinetic analysis has shown that PcpF catalyzes the GSH-dependent reduction of GS-TriCH via a ping-pong mechanism (Figure 5), which supports the proposed reaction mechanism (Figure 4) (Xun et al., 2010). Because PcpF has high affinity to GS-

TriCH with the K_m value of 4.4 μ M and low affinity to GSH with the K_m value of 1.3 mM, we believe that the GS-moiety from GS-TriCH binds to the G-site and the GSH comes in at the H-site after TriCH is released. The hypothesis is also supported by the fact that other small thiols (e.g. L-cysteine, 2-mercaptoethanol, and dithiothreitol) can replace GSH for the reaction (Figure 5). Again, PcpF has low affinities for the small thiols (Xun et al., 2010), and they should also bind to the H-site. GSH binding at the H-site has been directly observed in some GSTs (Caccuri et al., 2002; Tocheva et al., 2006; Allocati et al., 2008).

The natural substrates of GS-HQRs

The physiological roles of GS-HQRs are likely involved in the reduction of GS-hydroquinones. Although they also have thiol-transferase and DHA-reductase activities, the activities are much weaker than those catalyzed by classical glutaredoxins (Xun et al., 2010). The physiological role of PcpF in PCP degradation has been demonstrated. It plays a maintenance role. A small amount of GS-TriCH and GS-DiCH conjugates produced by oxidatively damaged PcpC are channeled back to the metabolic pathway by PcpF during PCP degradation (Figure 1) (Huang et al., 2008). Interestingly, two other bacterial GS-HQRs and one yeast GS-HQR (*ecm4*) all catalyze the GSH-dependent reduction of GS-TriCH to TriCH (Xun et al., 2010). To date, only GS-HQRs are known to reduce GS-TriCH. It is clear that GS-TriCH is not a natural substrate of GS-HQRs, because PCP has only been recently introduced into the environment (Crosby, 1981), and GS-TriCH is a by-product of PCP metabolism in *S. chlorophenolicum* ATCC 39723 (Huang et al., 2008). GS-HQRs likely can use some natural GS-hydroquinones as the substrates. Bacteria, fungi, and plants are known to produce a variety of hydroquinones in their metabolisms (Romanova et al., 1977; Rieble et al., 1994; Pérez-Pantoja et al., 2008). The hydroquinones are the potential source for the formation of GS-hydroquinones.

Nonenzymatic formation of natural GS-hydroquinone conjugates

Besides enzymatic generation of GS-TriCH and GS-DiCH by oxidatively damaged PcpC (Figure 1), GS-hydroquinones can be generated by spontaneous reactions. GSH does not react with hydroquinones, but rapidly reacts with benzoquinones via Michael's addition (Figure 6A) (Hill et al., 1993) with direct formation of GS-hydroquinones (reduced form). Both *o*- and *p*-substituted benzoquinones can react with GSH to form substituted GS-hydroquinones (Ross et al., 1985). The reaction is not limited to single-ring quinones, as GS-menadione (also known as thiodione) can be readily formed by mixing GSH and menadione (Nickerson et al., 1963). Besides Michael's addition, GSH can also replace a halide (or other electron-withdrawing group) on a benzoquinone via substitution (Song et al., 2009) (Figure 6B). GS-hydroquinones remain toxic, as they are still subject to redox cycling, generating ROS (Lau et al., 1988; Buffinton et al., 1989); therefore, the conjugation is not the final detoxification. When laboratory animals are exposed to benzene, phenol, and *p*-hydroquinone, GS-*p*-hydroquinone is formed and transformed into the expected mercapturic acid, N-acetyl-L-Cys-*p*-hydroquinone, which is released in the urine (Nerland and Pierce, 1990). For bacteria, fungi, and plants, the fate of GS-hydroquinones should be different. When the corresponding hydroquinones can be further metabolized, the ideal solution is to break the conjugates and release the hydroquinones. The maintenance function of PcpF in PCP metabolism implies similar roles of GS-HQRs in the metabolism of other aromatic compounds.

Cellular localization

S. cerevisiae has three GS-HQRs (GTO1, EMC4, and GTO3). EMC4 and GTO3 are in the cytoplasm, whereas GTO1 is peroxisomal (Barreto et al., 2006). The C-terminus of GTO1 has a PTS1 (peroxisome-targeting sequence 1) that directs the protein to peroxisome. The

peroxisomal location of GTO1 has been demonstrated with a GFP fusion. Sequence analysis of GTO1 in 10 fungal genomes identified only one homolog in related yeast, *Saccharomyces paradoxus*, with a similar PTS1 (Barreto et al., 2006). Thus, most GS-HQRs are located in the cytoplasm, but a few are in the peroxisome in fungi. In plants, GS-HQRs are located in the chloroplast and cytoplasm. ChloroP is used to determine the signal-peptide targeting of GS-HQRs into the chloroplast (Emanuelsson et al., 1999). *A. thaliana* has four GS-HQRs, and two (NP_001031671 and NP_568632) of them have the signal peptides targeting to the chloroplast. Maize has at least two GS-HQRs by BLAST search of Maize Genetics and Genome Database (MaizeGDB): one in the cytoplasm (GRMZM2G105005_T01) and the other in the chloroplast (GRMZM2G102216_T01). Rice has at least one GS-HQR (NP_001048496) localized in the chloroplast. In Archaea and bacteria, GS-HQRs are expected to be in the cytoplasm, as no signal peptides have been found.

Gene organization and expression

The GS-HQR genes are usually found on the chromosome in bacteria, fungi, and plants. Although genes are often organized in operons in bacteria, GS-HQR genes are generally transcribed as monocistronic mRNA on the basis of their location on the chromosomes. Even when a GS-HQR gene is physically linked with another gene, the linkage is random from one organism to another. The three GS-HQR genes in yeast *S. cerevisiae* are located on three different chromosomes. In plant *A. thaliana*, three GS-HQR genes are on chromosome V and one is on chromosome IV.

Information regarding gene expression is limited for GS-HQR genes. The three GS-HQR genes of *S. cerevisiae* are inducible by oxidative agents that deplete cellular thiols (Barreto et al., 2006). The *ecm4* gene is strongly induced by diamide, CDNB, or tert-butyl hydroperoxide. GTO1 gene is induced by CDNB and diamide. GTO3 gene is marginally induced by tert-butyl hydroperoxide. In bacteria, two GS-HQR genes are constitutively expressed. The *pcpF* gene is constitutively expressed in *S. chlorophenolicum* ATCC 39723 (Huang et al., 2008). The *EcyqjG* gene is constitutively expressed under both aerobic and anaerobic conditions in *E. coli* K-12 MG1655 wild type and several deletion mutants of gene regulators that sense and respond to oxygen and oxidative stresses (Covert et al., 2004).

Evolution

Because the N-terminal domains of the cytosolic GSTs and glutaredoxins have the same structural fold, the GSTs are believed to be evolved from glutaredoxins that are oxidoreductases, with GSH as the reductant (Sheehan et al., 2001). GS-HQR, DHAR, and omega- and lambda-class GSTs have weak or no conjugating activities, and all have the thiol-transferase activity of glutaredoxin (Holmgren and Aslund, 1995). They may represent a branch of GSTs catalyzing mainly GSH-dependent oxidoreduction, rather than conjugation. The beta-class GSTs have both the thiol-transferring and GSH-conjugating activities. Thus, it may represent the class of GSTs in the evolution pathway from reductases to conjugating enzymes (Caccuri et al., 2002). The conjugating GSTs are more common in animals and plants (Frova, 2003). The specific classes and multicopies of each class in mammals, insects, and plants suggest that the pace of duplication and evolution has accelerated for the GSTs after phyla separation in eukaryotes. Given the wide distribution in all domains of life and the high sequence similarities among them (more than 30% identity), GS-HQRs are likely to share a monophyletic origin preceding the domain split or the eukaryotes had acquired them via horizontal gene transfer very early in the course of evolution. In the first scenario, animals might have lost GS-HQRs due to the development of phase III detoxifying enzymes, (e.g., GS-conjugate pumps) and the ability to remove GS-conjugates as mercapturic acids in the urine. In the second scenario, animals have never

acquired GS-HQRs via horizontal gene transfer. Another possibility is that the omega-class GSTs are evolved from GS-HQRs. The two classes are closely related by phylogenetic analysis (Figure 2) and share many enzymatic activities. Although the omega-class GSTs cannot reduce GS-TriCH (Xun et al., 2010), human GSTO1-1 reduces S-(phenacyl)glutathiones (Board and Anders, 2007). If the omega-class GSTs are evolved from GS-HQRs, the evolution started after the animal-plant separation, because they are present only in animals. The omega-class GSTs are phylogenetically more related to GS-HQRs than to other mammalian GSTs.

Perspectives of GS-HQRs in biodegradation, biosynthesis, and photosynthesis

The maintenance role of PcpF in PCP degradation by *S. chlorophenolicum* ATCC 39723 has been demonstrated (Figure 1) (Huang et al., 2008). Because the genome of *S. chlorophenolicum* was not available prior to 2010, *Cupriavidus necator* (ex *Ralstonia eutropha*) JMP134 was selected to examine the potential role of GS-HQRs in the biodegradation of aromatic compounds. *C. necator* JMP 134 has a single GS-HQR (CnYqjG) that shares 64% sequence identity with PcpF, and CnYqjG (ex ReYqjG) actively reduces GS-TriCH to TriCH at the expense of GSH (Xun et al., 2010). The genome of *C. necator* JMP134 has been determined, and detailed metabolic pathways of many aromatic compounds have been mapped onto the genome (Pérez-Pantoja et al., 2008). *C. necator* JMP134 degrades several aromatic compounds, with hydroquinones as metabolic intermediates. Some hydroquinone metabolic intermediates include 6-chloro-2-hydroxy-*p*-hydroquinone from 2,4,6-trichlorophenol degradation (Louie et al., 2002), 2-amino-*p*-hydroquinone from 3-nitrophenol degradation (Schenzle et al., 1999), gentisate (2,5-dihydroxybenzoate) from salicylate degradation (Pérez-Pantoja et al., 2008), and homogentisate [2-(2,5-dihydroxyphenyl)acetate] from phenylalanine (and tyrosine) degradation (Pérez-Pantoja et al., 2008). In addition, the bacterium metabolizes *p*-hydroquinone and hydroxy-*p*-hydroquinone (Pérez-Pantoja et al., 2008). These hydroquinones can be readily oxidized to the corresponding benzoquinones in air at neutral pH (Roof and Turner, 1955; Lustberg et al., 1971), which can react with GSH via Michael's addition to form GS-hydroquinones (Figure 6A). CnYqjG, PcpF, and *ecm4* have been demonstrated to convert GS-*p*-hydroquinone and GS-hydroxy-*p*-hydroquinone to *p*-hydroquinone and hydroxy-*p*-hydroquinone at the expense of GSH (Lam and Xun, unpublished data). When a GS-hydroquinone is a substrate of CnYqjG, the enzyme is likely to play a maintenance role in the pathway, with the hydroquinone as an intermediate. Although the deduced functions of CnYqjG in the degradation of aromatic compounds in *C. necator* JMP 134 need confirmation, the deduction also applies to fungi that can degrade lignin via hydroxy-*p*-hydroquinone as a metabolic intermediate (Rieble et al., 1994). Plant hydroquinones are likely intermediates or final products of biosynthesis (Romanova et al., 1977). When oxidized, the benzoquinones can react with GSH to form GS-hydroquinone conjugates that can be the substrates for plant GS-HQRs. So far, we have demonstrated four GS-hydroquinones (Figure 7) that can be used by GS-HQRs. They are *p*-hydroquinones with or without substitution. It remains to be tested whether other GS-hydroquinones would be substrates for GS-HQRs.

Bioinformatic analysis has pointed to a potential role of GS-HQRs in photosynthesis or light protection. The plant GS-HQRs are located in the cytoplasm as well as in the chloroplast. The cytosolic GS-HQRs should play a physiological role in the maintenance of metabolic pathways, whereas the chloroplastic GS-HQRs may have functions in photosynthesis or light protection. GS-HQRs are conserved and present in every one of the available genomes of 50 cyanobacteria, two green algae, and one moss at the NCBI (National Center for Biotechnology Information) website. GS-HQRs are present in each of the 12 available

genomes of halobacteria, the Archaea that can use bacteriorhodopsin to absorb light for alkaline triphosphate production. The common presence of GS-HQRs in light-using organisms suggests a beneficial, if not an essential, role for the organisms.

Acknowledgments

Declaration of interest

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References

- Allocati N, Federici L, Masulli M, Di Ilio C. Glutathione transferases in bacteria. *FEBS J.* 2009; 276:58–75. [PubMed: 19016852]
- Allocati N, Federici L, Masulli M, Favalaro B, Di Ilio C. Cysteine 10 is critical for the activity of *Ochrobactrum anthropi* glutathione transferase and its mutation to alanine causes the preferential binding of glutathione to the H-site. *Proteins.* 2008; 71:16–23. [PubMed: 18076047]
- Anandarajah K, Kiefer PMJ, Donohoe BS, Copley SD. Recruitment of a double bond isomerase to serve as a reductive dehalogenase during biodegradation of pentachlorophenol. *Biochemistry.* 2000; 39:5303–5311. [PubMed: 10820000]
- Barreto L, Garcerá A, Jansson K, Sunnerhagen P, Herrero E. A peroxisomal glutathione transferase of *Saccharomyces cerevisiae* is functionally related to sulfur amino acid metabolism. *Eukaryot Cell.* 2006; 5:1748–1759. [PubMed: 16936141]
- Board PG, Anders MW. Glutathione transferase omega-1 catalyzes the reduction of S-(phenacyl)glutathiones to acetophenones. *Chem Res Toxicol.* 2007; 20:149–154. [PubMed: 17226937]
- Board PG, Baker RT, Chelvanayagam G, Jermini LS. Zeta, a novel class of glutathione transferases in a range of species from plants to humans. *Biochem J.* 1997; 328:929–935. [PubMed: 9396740]
- Board PG, Taylor MC, Coggan M, Parker MW, Lantum HB, Anders MW. Clarification of the role of key active site residues of glutathione transferase zeta/maleylacetoacetate isomerase by a new spectrophotometric technique. *Biochem J.* 2003; 374:731–737. [PubMed: 12852784]
- Board PG, Coggan M, Chelvanayagam G, Eastale S, Jermini LS, Schulte GK, et al. Identification, characterization, and crystal structure of the omega class glutathione transferases. *J Biol Chem.* 2000; 275:24798–24806. [PubMed: 10783391]
- Buffinton GD, Ollinger K, Brunmark A, Cadenas E. DT-diaphorase-catalysed reduction of 1,4-naphthoquinone derivatives and glutathionyl-quinone conjugates. Effect of substituents on autoxidation rates. *Biochem J.* 1989; 257:561–571. [PubMed: 2494985]
- Caccuri AM, Antonini G, Allocati N, Di Ilio C, De Maria F, Innocenti F, et al. GSTB1-1 from *Proteus mimbilis*: a snapshot of an enzyme in the evolutionary pathway from a redox enzyme to a conjugating enzyme. *J Biol Chem.* 2002; 277:18777–18784. [PubMed: 11889135]
- Cai M, Xun L. Organization and regulation of pentachlorophenol-degrading genes in *Sphingobium chlorophenolicum* ATCC 39723. *J Bacteriol.* 2002; 184:4672–4680. [PubMed: 12169590]
- Commandeur JN, Stijntjes GJ, Vermeulen NP. Enzymes and transport systems involved in the formation and disposition of glutathione S-conjugates. Role in bioactivation and detoxication mechanisms of xenobiotics. *Pharmacol Rev.* 1995; 47:271–330. [PubMed: 7568330]
- Covert MW, Knight EM, Reed JL, Herrgard MJ, Palsson BO. Integrating high-throughput and computational data elucidates bacterial networks. *Nature.* 2004; 429:92–96. [PubMed: 15129285]
- Crosby DG. Environmental chemistry of pentachlorophenol. *Pure Appl Chem.* 1981; 53:1052–1080.
- Dai M, Rogers JB, Warner IR, Copley SD. A previously unrecognized step in pentachlorophenol degradation in *Sphingobium chlorophenolicum* is catalyzed by tetrachlorobenzoquinone reductase (PcpD). *J Bacteriol.* 2003; 185:302–310. [PubMed: 12486067]

- Dixon DP, Davis BG, Edwards R. Functional divergence in the glutathione transferase superfamily in plants. Identification of two classes with putative functions in redox homeostasis in *Arabidopsis thaliana*. *J Biol Chem*. 2002; 277:30859–30869. [PubMed: 12077129]
- Emanuelsson O, Nielsen H, von Heijne G. ChloroP, a neural network-based method for predicting chloroplast transit peptides and their cleavage sites. *Prot Sci*. 1999; 8:978–984.
- Frova C. The plant glutathione transferase gene family: genomic structure, functions, expression, and evolution. *Physiol Plant*. 2003; 119:469–479.
- Garcera A, Barreto L, Piedrafita L, Tamarit J, Herrero E. *Saccharomyces cerevisiae* cells have three omega class glutathione S-transferases acting as 1-Cys thiol transferases. *Biochem J*. 2006; 398:187–196. [PubMed: 16709151]
- Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. *Annu Rev Pharmacol Toxicol*. 2005; 45:51–88. [PubMed: 15822171]
- Hill BA, Kleiner HE, Ryan EA, Dulik DM, Monks TJ, Lau SS. Identification of multi-S-substituted conjugates of hydroquinone by HPLC-coulometric electrode array analysis and mass spectroscopy. *Chem Res Toxicol*. 1993; 6:459–469. [PubMed: 8374043]
- Holmgren A, Aslund F. Glutaredoxin. *Meth Enzymol*. 1995; 252:283–292. [PubMed: 7476363]
- Huang Y, Xun R, Chen G, Xun L. Maintenance role of a glutathionyl-hydroquinone lyase (PcpF) in pentachlorophenol degradation by *Sphingobium chlorophenolicum* ATCC 39723. *J Bacteriol*. 2008; 190:7595–7600. [PubMed: 18820023]
- Inoue H, Nishida M, Takahashi K. Effects of Cys10 mutation to Ala in glutathione transferase from *Escherichia coli*. *J Organomet Chem*. 2000; 611:593–595.
- Kaluzna A, Bartosz G. Transport of glutathione S-conjugates in *Escherichia coli*. *Biochem Mol Biol Int*. 1997; 43:161–171. [PubMed: 9315294]
- Lau SS, Hill BA, Highet RJ, Monks TJ. Sequential oxidation and glutathione addition to 1,4-benzoquinone: correlation of toxicity with increased glutathione substitution. *Mol Pharmacol*. 1988; 34:829–836. [PubMed: 3200250]
- Li J, Xia Z, Ding J. Thioredoxin-like domain of human kappa class glutathione transferase reveals sequence homology and structure similarity to the theta class enzyme. *Prot Sci*. 2005; 14:2361–2369.
- Louie TM, Webster CM, Xun L. Genetic and biochemical characterization of a novel 2,4,6-trichlorophenol degradation pathway in *Ralstonia eutropha* JMP134. *J Bacteriol*. 2002; 184:3492–3500. [PubMed: 12057943]
- Lussier M, White AM, Sheraton J, di Paolo T, Treadwell J, Southard SB, et al. Large scale identification of genes involved in cell surface biosynthesis and architecture in *Saccharomyces cerevisiae*. *Genetics*. 1997; 147:435–450. [PubMed: 9335584]
- Lustberg TJ, Schulman JD, Seegmiller JE. The preparation and identification of various adducts of oxidized homogentisic acid and the development of a new sensitive colorimetric assay for homogentisic acid. *Clin Chim Acta*. 1971; 35:325–333. [PubMed: 5125319]
- Marco A, Cuesta A, Pedrola L, Palau F, Marín I. Evolutionary and structural analyses of GDAP1, involved in Charcot-Marie-Tooth disease, characterize a novel class of glutathione transferase-related genes. *Mol Biol Evol*. 2004; 21:176–187. [PubMed: 14595091]
- Masai E, Ichimura A, Sato Y, Miyauchi K, Katayama Y, Fukuda M. Roles of the enantioselective glutathione S-transferases in cleavage of β -aryl ether. *J Bacteriol*. 2003; 185:1768–1775. [PubMed: 12618439]
- McCarthy DL, Navarrete S, Willett WS, Babbitt PC, Copley SD. Exploration of the relationship between tetrachlorohydroquinone dehalogenase and the glutathione S-transferase superfamily. *Biochem*. 1996; 35:14634–14642. [PubMed: 8931562]
- Miyauchi K, Suh SK, Nagata Y, Takagi M. Cloning and sequencing of a 2,5-dichlorohydroquinone reductive dehalogenase gene whose product is involved in degradation of gamma-hexachlorocyclohexane in *Sphingomonas paucimobilis*. *J Bacteriol*. 1998; 180:1354–1359. [PubMed: 9515900]
- Nerland DE, Pierce WM Jr. Identification of N-acetyl-S-(2,5-dihydroxyphenyl)-L-cysteine as a urinary metabolite of benzene, phenol, and hydroquinone. *Drug Metab Dispos*. 1990; 18:958–961. [PubMed: 1981544]

- Nickerson WJ, Falcone G, Strauss G. Studies on quinone-thioethers. I. Mechanism of formation and properties of thiodione. *Biochemistry*. 1963; 2:537–543. [PubMed: 14069543]
- Niemann A, Ruegg M, La Padula V, Schenone A, Suter U. Ganglioside-induced differentiation associated protein 1 is a regulator of the mitochondrial network: new implications for Charcot-Marie-Tooth disease. *J Cell Biol*. 2005; 170:1067–1078. [PubMed: 16172208]
- Noguera V, Walker O, Rouhier N, Jacquot JP, Krimm I, Lancelin JM. NMR reveals a novel glutaredoxin-glutaredoxin interaction interface. *J Mol Biol*. 2005; 353:629–641. [PubMed: 16181638]
- Orser CS, Dutton J, Lange C, Jablonski P, Xun L, Hargis M. Characterization of a *Flavobacterium* glutathione S-transferase gene involved in reductive dechlorination. *J. Bacteriol*. 1993; 175:2640–2644. [PubMed: 8478329]
- Pérez-Pantoja D, De la Iglesia R, Pieper DH, González B. Metabolic reconstruction of aromatic compounds degradation from the genome of the amazing pollutant-degrading bacterium *Cupriavidus necator* JMP134. *FEMS Microbiol Rev*. 2008; 32:736–794. [PubMed: 18691224]
- Rea PA, Li ZS, Lu YP, Drozdowicz YM, Martinoia E. From vacuolar GS-X pumps to multispecific ABC transporters. *Annu Rev Plant Physiol Plant Mol Biol*. 1998; 49:727–760. [PubMed: 15012252]
- Rieble S, Joshi DK, Gold MH. Purification and characterization of a 1,2,4-trihydroxybenzene 1,2-dioxygenase from the basidiomycete *Phanerochaete chrysosporium*. *J Bacteriol*. 1994; 176:4838–4844. [PubMed: 8050996]
- Romanova AS, Patudin AV, Ban'kovskii AI. Quinones of higher plants as possible therapeutic agents. *Pharm Chem J*. 1977; 11:927–937.
- Roof BS, Turner JC. Protein interactions of gentisic acid and certain of its oxidation products. *J Clin Invest*. 1955; 34:1647–1652. [PubMed: 13271548]
- Ross D, Thor H, Orrenius S, Moldeus P. Interaction of menadione (2-methyl-1,4-naphthoquinone) with glutathione. *Chem Biol Interact*. 1985; 55:177–184. [PubMed: 2998631]
- Rossjohn J, Polekhina G, Feil SC, Allocati N, Masulli M, Di Ilio C, et al. A mixed disulfide bond in bacterial glutathione transferase: functional and evolutionary implications. *Structure*. 1998; 6:721–734. [PubMed: 9655824]
- Saber DL, Crawford RL. Isolation and characterization of *Flavobacterium* strains that degrade pentachlorophenol. *Appl Environ Microbiol*. 1985; 50:1512–1518. [PubMed: 4091568]
- Schenzle A, Lenke H, Spain JC, Knackmuss HJ. 3-hydroxylaminophenol mutase from *Ralstonia eutropha* JMP134 catalyzes a Bamberger rearrangement. *J Bacteriol*. 1999; 181:1444–1450. [PubMed: 10049374]
- Schröder P, Scheer CE, Diekmann E, Stampfl A. How plants cope with foreign compounds. Translocation of xenobiotic glutathione conjugates in roots of barley (*Hordeum vulgare*). *Environ Sci Pollut Res Int*. 2007; 14:114–122. [PubMed: 17455821]
- Sheehan D, Meade G, Foley VM, Dowd CA. Structure, function, and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. *Biochem J*. 2001; 360:1–16. [PubMed: 11695986]
- Shield AJ, Murray TP, Board PG. Functional characterisation of ganglioside-induced differentiation-associated protein 1 as a glutathione transferase. *Biochem Biophys Res Commun*. 2006; 347:859–866. [PubMed: 16857173]
- Song Y, Wagner BA, Witmerl JR, Lehmler HJ, Buettner GR. Nonenzymatic displacement of chlorine and formation of free radicals upon the reaction of glutathione with PCB quinones. *Proc Natl Acad Sci U S A*. 2009; 106:9725–9730. [PubMed: 19497881]
- Tatusov RL, Fedorova ND, Jackson JD, Jacobs AR, Kiryutin B, Koonin EV, et al. The COG database: an updated version includes eukaryotes. *BMC Bioinformatics*. 2003; 4:41. [PubMed: 12969510]
- Tocheva EI, Fortin PD, Eltis LD, Murphy MEP. Ternary complexes of BphK, a bacterial GST that reductively dechlorinates PCB metabolites. *J Biol Chem*. 2006; 281:30933–30940. [PubMed: 16920719]
- Whitbread AK, Masoumi A, Tetlow N, Schmuck E, Coggan M, Board PG. Characterization of the omega class of glutathione transferases. *Meth Enzymol*. 2005; 401:78–99. [PubMed: 16399380]

- Xun L, Orser CS. Purification and properties of pentachlorophenol hydroxylase, a flavoprotein from *Flavobacterium* sp strain ATCC 39723. *J Bacteriol.* 1991; 173:4447–4453. [PubMed: 2066340]
- Xun L, Topp E, Orser CS. Purification and characterization of a tetrachloro-*p*-hydroquinone reductive dehalogenase from a *Flavobacterium* sp. *J Bacteriol.* 1992; 174:8003–8007. [PubMed: 1459949]
- Xun L, Bohuslavek J, Cai M. Characterization of 2,6-dichloro-*p*-hydroquinone 1,2-dioxygenase (PcpA) of *Sphingomonas chlorophenolica* ATCC 39723. *Biochem Biophys Res Comm.* 1999; 266:322–325.
- Xun L, Belchik S, Xun R, Huang Y, Zhou H, Sanchez E, et al. S-glutathionyl-(chloro)hydroquinone reductases: a novel class of glutathione transferases. *Biochem J.* 2010; 428:419–427. [PubMed: 20388120]

Figure 1.

PCP metabolic pathway in *S. chlorophenolicum* ATCC 39723. PCP is converted to II (tetrachloro-*p*-benzoquinone), TeCH (tetrachloro-*p*-hydroquinone), TriCH (trichloro-*p*-hydroquinone), DiCH (2,6-dichloro-*p*-hydroquinone), VI (2-chloromaleylacetate), maleylacetate, and 3-oxoadipate. PcpB, PCP 4-monooxygenase; PcpD, quinone reductase; PcpC, TeCH reductive dehalogenase; PcpC-ox, oxidatively damaged PcpC; PcpF, GS-HQR; PcpA, DiCH 1,2-dioxygenase; PcpE, (chloro)maleylacetate reductase.

Figure 2.

Inferred phylogenetic relationship among GSTs with a Cys residue at the N-terminus of α_1 -helix. The tree was constructed using the neighbor-joining method with pairwise deletion. The numbers on the branches are bootstrap values (percentage of 1,000 runs), indicating the frequency of grouping in the cluster. The distance correlates to the number of amino acids substituted per site. The GSTs and corresponding NCBI protein accession numbers are as follows: EcYqjG, NP_417573; ReYqjG, YP_295669; ECM4, NP_013002; PcpF, AAM96671; AtECM4a, NP_199315; HsGSTOI, NP_004823; HsGSTO2, NP_899062; ZmGSTL, CAA41447; OzGSTL, AAF70831; AtGSTL1, AL162973; TaGSTL, CAA76758; AtDHAR1, AAF98403; SoDHAR, AAG24945; LigG, BAA77216.1; RaGSTZ (of *Ralstonia*

sp. U2), 2JL4_A; HsGSTZI, AAB96392.1; AtGSTZI, AAO60039; PcpC, AAM96673; LinD, BAA14011; BphK, YP_556402; SpGSTB, 1F2ED; PmGSTB, 2PMTA; EcGSTB, 1A0F_A.

(A)

PcpF	YHLYAGFAC CP WAHRVLIMRALKGLEEMISVSMVNAYMG
EcYqjG	YHLYVSLAC CP WAHRTLIMRKLKGLEPFISVSVVNPLML
CnYqjG	YHLYVSLAC CP WAHRTLIMRRLKGLDDMIGVSVVHWLMR
ECM4	YWLYVSLAC CP WAHRTLITRALKGLTSVIGCSVVHWHLD
AtECM4a	YHLYISYAC CP WACRCLSYLKIKGLDEAITFSSVHAIWG
OzGSTL	TRLYICYF CP FAQRAWIIRNFKGLQDKIELVGIDLQDK
ZmGSTL	TRLYICYF CP FAQRAWVTRNLKGLQDKMELVAIDLQDK
ATGSTL1	TRLYISYT CP FAQRVWITRNLKGLQDEIKLVPIDLPNR
TaGSTL	TRLYICYI CP FAQRAWVTRNCKGLQEEIKLVAINLED
HsGSTO1	IRIYSMRF CP FAERTRLVLKAKGIRHEVININLNK--
HsGSTO2	IRIYSMRF CP YSHRTRLVLKAKDIRHEVVNINLRNK--
LigG	LTIYHIPG CP FSEVEIMLELKGLRMKDVEIDISKPRP
SoDHAR	-----D CP FCQRVLLTLEEKHLPYDMKLV--DLSNK
ATDHAR1	-----D CP FSQRALLTLEEKSLTYKIHLI--NLSDK

< β_1 -S > < α_1 -H > (hGSTO1-1)

(B)

EcGSTB	---MKLF-YKPGAC CS LASHITLRESGKDFTLVSVDLMK-KRLENG
PMGSTB	---MKLY-YTPGS CS LSPHIVLRETGLDFSIERIDLRT-KKTESG
SpGSTB	---MKLF-ISPGAC CS LAPHIALRETGADFEAVKVDLAV-RKTEAG
BphK	---MKLY-YSPGAC CS LSPHIALREAGLNFELVQVDLAS-KKTASG
PcpC	MPEVSLYNYTMSI CS MKTRLAMEEFGVDYDDKQVDIGFALENFEP
Lind	EPRFELFHFVFSV CS QKVRGTLMEKGVTFGSNELTILPPQSENYC
HsGSTZ1	--KPILYSYFRSS CS WRVRIALALKGIDYKTPINLIKDRGQQFS
RaGSTZ	--KMKLYNFWRS CS HTRLRIALNLKGVPEYLAHLGK--EEHLK
AtGSTZ1	EEKLKLYSYWRSS CA HVRRIALALKGLDYEYIPVNLLK--GDQFD

< β_1 -S> < α_1 -H > (hGSTZ1-1)

Figure 3.

Alignment of peptide sequences around α_1 -helix of the GSTs from Figure 2. The whole peptide sequences are aligned by ClustalW, and the alignment around α_1 -helix is presented here to show the location of the Cys residue at the beginning of the helix and GSTs with the Cys-Pro motif (A) and those without (B). Accession numbers are given in the legend for Figure 2.

Figure 4.

A proposed reaction between PcpF-Cys53 thiolate and GS-TriCH. **(A)** The Cys53 thiolate attacks the sulfur atom of GS-TriCH to generate GS-PcpF (PcpF-Cys53-S-SG) and release TriCH. **(B)** GS-PcpF is reduced by GSH or other small thiols to regenerate PcpF-Cys-S⁻.

Figure 5.

PcpF catalyzes GS-TriCH reduction via a ping-pong mechanism. The reaction mechanism is deduced from kinetic analysis (Xun et al., 2010). Small thiols can replace GSH in the second half of the reaction.

Figure 6.

Spontaneous formation of GS-hydroquinones. (A) Michael's addition results in GS-hydroquinones. (B) Substitution produces GS-benzoquinones, which can be reduced to GS-hydroquinones.

Figure 7.

GS-hydroquinone substrates of GS-HQRs. GS-TriCH and GS-DiCH have been reported as substrates (Huang et al., 2008; Xun et al., 2010), and the other two have not been reported (Lam and Xun, unpublished data).