Plant glutathione S-transferases: enzymes with multiple functions in sickness and in health

Robert Edwards, David P. Dixon and Virginia Walbot

Glutathione S-transferases (GSTs) are abundant proteins encoded by a highly divergent, ancient gene family. Soluble GSTs form dimers, each subunit of which contains active sites that bind glutathione and hydrophobic ligands. Plant GSTs attach glutathione to electrophilic xenobiotics, which tags them for vacuolar sequestration. The role of GSTs in metabolism is unclear, although their complex regulation by environmental stimuli implies that they have important protective functions. Recent studies show that GSTs catalyse glutathione-dependent isomerizations and the reduction of toxic organic hydroperoxides. GSTs might also have non-catalytic roles as carriers for phytochemicals.

Cellular survival requires the management of reactive oxygen species, endogenous phytochemicals and exogenous toxins, including diverse xenobiotics added to the environment by humans. Furthermore, many cellular processes function optimally only within a narrow redox range. Glutathione (GSH), the tripeptide γ-Glu-Cys-Gly, plays a central role in the crucial processes of detoxification and redox buffering. GSH is abundant in plants, typically exceeding 1 mM in the cytoplasm. Plants also contain several GSH-dependent detoxifying enzymes, most notably glutathione S-transferases (GSTs), which collectively constitute >1% of the soluble protein in maize leaves. Individual gene analysis and genomics projects indicate that plants have 25 or more genes coding for GSTs, and that the proteins share as little as 10% amino acid identity. In both crops and weeds, GSTs detoxify electrophilic herbicides by catalysing their conjugation with GSH (Ref. 4; Fig. 1). The S-glutathionylated metabolites are tagged for vacuolar import by ATP binding cassette (ABC) transporters, which selectively transport GSH conjugates. GSTs are composed of two subunits with molecular masses in the range of 25–27 kDa and are either homodimers of a single gene product or heterodimers of subunits encoded by different genes. Individual GST isoenzymes can selectively detoxify specific xenobiotics, with species differences in GST specificity and capacity determining herbicide selectivity.

Paradoxically, in spite of the specific and crucial roles of individual GST enzymes in xenobiotic metabolism, their roles in endogenous plant metabolism have remained an enigma. There are only a few reports of natural products (such as the phytotoxic medicarpin (Fig. 1) being conjugated with GSH (Ref. 6) in spite of a burgeoning literature showing that the expression of specific GSTs vary markedly during plant development (cell division, senescence) and after exposure to pathogens, changes in environmental conditions and chemical treatments. GSTs with high affinity for auxins and cytokinins have been suggested to contribute to hormone homeostasis. Indeed, the enhancement of GST expression has become a marker for plant response to stress, although the functional significance of selective GST expression is only just emerging.

One role of this article is to introduce the diversity of GSTs, highlighting novel features of the plant GST family compared with other eukaryotes. The complexity and antiquity of GST genes and their functionality as homo- and heterodimeric proteins present challenges in both classification and nomenclature. However, the core of the article is a consideration of the functions of GSTs in endogenous plant metabolism. In particular that GSTs:

1. Catalyse conjugation reactions with natural products similar to those observed with xenobiotics.
2. Function as binding and carrier proteins for phytochemicals between cellular compartments.
3. Catalyse alternative GSH-dependent biotransformation reactions.

GST-gene diversity; who counts as a family member?

GSTs are an ancient and diverse protein family, existing as multi-gene families in bacteria, fungi, animals and plants (Fig. 2). The ‘classic’ definition of a GST requires the catalytic formation of GSH conjugates with xenobiotic substrates. The compound 1-chloro-2,4-dinitrobenzene (CDNB) is used as a model substrate (Fig. 1) but some GSTs show little activity with this compound. Furthermore, highly electrophilic substrates spontaneously conjugate with GSH under physiological conditions, with GSTs accelerating the reaction only modestly. Whatever their substrate, GSTs should selectively bind GSH or natural homologues, such as homoglutathione (γ-Glu-Cys-Ala) and hydroxymethyl glutathione (γ-Glu-Cys-Ser), which are commonly found in legumes and grasses, respectively. Affinity chromatography using GSH as a ligand is widely used to extract GSTs, although resins with GSH conjugates can be useful in the recovery of specific GSTs from cellular extracts.

Members of the GST family show the greatest sequence similarity in their GSH-binding domain; four highly conserved amino acids in this domain are a ‘signature motif’ for GSTs, but this motif is not sufficient to identify a GST. By applying the dual definition of GSH binding and catalysis of GSH conjugation with electrophiles, we can distinguish GSTs from proteins with related sequences that have evolved alternative functions. Proteins related to the GST superfamily, such as the crystallin protein in the squid lens and elongation factor 1-γ proteins, have lost their capacity either to bind GSH or to use the thiol in conjugation reactions. Some offshoots of the GST family currently have no assignable functions. For example, several stress-induced proteins in bacteria resemble GSTs (Ref. 13), as does the herbicide safener inducible 27-kDa In2-1 protein from maize.

Classification of plant GSTs based on sequence

An accurate GST classification scheme would require knowledge of the proteins’ in vivo role(s) to name individual GST enzymes.
Three distinct types of plant GSTs were recognized initially. Type I included GSTs with herbicide-detoxifying activity; these genes have three exons. The other large group, type III, consisted mainly of auxin-induced GSTs, with the genes containing two exons. Types I and III GSTs show >50% sequence divergence and have now been placed in separate classes\(^5\,6\). Type II GSTs have ten exons and are much closer to the mammalian zeta GSTs (Ref. 2). Recently, a Type-IV grouping was proposed for several Arabidopsis genes that are similar to classical mammalian theta enzymes\(^5\).

During 1999, dozens of plant GST sequences were reported from diverse angiosperm sequencing projects (e.g. maize, tomato, soybean and cotton), with >25 genes for GSTs identified to date in the Arabidopsis genome alone. This diversity makes the catch-all theta classification inappropriate. Some plant GSTs clearly group with specific mammalian types but there are two distinct plant-specific types\(^7\,8\).

Because the principle of Greek-letter designations is widely used for non-plant GSTs (Ref. 15), we suggest that a new nomenclature system be adopted for plant GST genes (Box 1; Table 1). Therefore, the classification of plant GSTs should be amended to include the following new classes:

- **Phi (F)** - a plant-specific class replacing Type I.
- **Zeta (Z)** - replacing Type II.
- **Tau (U)** - a plant-specific class replacing Type III.
- **Theta (T)** - replacing Type IV.

This nomenclature is applicable to *Synechocystis* spp., whose genome contains genes for four GST-like proteins without the intron-exon gene structure of higher plants. At the predicted-amino-acid level, the gene designated *gst1* (SwissProt P74665) is a zeta GST; ORF *Q55139* (P73835) shows the greatest similarity to the tau-class gene, maize In2-1 (P92548). The gzt gene (Q55139) is a phi-class member, and ORF sbt0216 (P72650) has similarity to the theta GSTs. We can conclude that the two plant-specific classes, tau and phi, are characteristic of both simple and complex photosynthetic organisms and might be required to accommodate the metabolic consequence of active oxygen generated by photosynthesis.

Another interesting feature of the GST family is the genes’ arrangement in repeating units on plant chromosomes, which strongly suggests that there have been numerous gene duplications. Chromosome II of Arabidopsis contains seven adjacent tau-class genes, and all three of the known theta-class genes in the Arabidopsis genome occur as a cluster of theta enzymes\(^5\).

**Structure of plant GSTs**

X-ray crystallography has shown a similar three-dimensional topology for three phi-class GSTs from Arabidopsis\(^5\,9\) and maize\(^10\,11\), which share only 20% sequence identity. Each subunit has an active

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**Fig. 1. Glutathione conjugation reactions.** Glutathione (GSH) forms conjugates with the chalcone isoliquiritigenin (a) and the prenylflavone medicarpin (b). These addition reactions occur spontaneously under basic conditions and can be readily reversed by reducing the pH (c). The phenylpropanoids cinnamic acid and coumaric acid undergo addition reactions with GSH to form the respective conjugates. This reaction is radical driven and is catalysed by an ascorbate peroxidase. (d and e) The classic detoxification reactions catalysed by GSTs with the model substrate 1-chloro-2,4-dinitrobenzene and the herbicide alachlor, respectively. In both cases, the glutathione substitution products are stable and the reaction irreversible.
site that includes a conserved GSH-binding site (G-site) located in the N-terminal domain and a C-terminal co-exit-binding domain (H-site), which will accommodate a diverse range of hydrophobic compounds. The G-site is specific for GSH and facilitates the formation of the catalytically active thiolate anion of GSH. In all three phi GSTs, the active sites are situated on either side of a large, open cleft formed between the subunits, allowing access to large planar and spherical molecules (Fig. 3). However, each active site interacts minimally with the adjacent subunit. Crystalllography has also shown that certain GSTs can bind an additional GSH molecule adjacent to the active site, although the functional significance of this secondary binding site has received little attention.

In addition to the diversity of GST genes in each species, heterodimer formation could give rise to up to \((1 + 2 + 3 + \ldots + n)\) distinct heterodimers, where \(n\) is the number of GST genes in a particular class. The importance of GST dimerization in xenobiotic metabolism was shown by determining the cause of sensitivity to the herbicide alachlor in an inbred maize line (Fig. 1). ZmGST II (Table 1) subunits were unable to dimerize with one another to form the ZmGST II homodimer, which is highly active in conjugating and detoxifying alachlor (Fig. 1).

Studies with overexpressed GST subunits in recombinant bacteria suggest that dimerization is spontaneous but restricted to subunits of the same class. In the case of the maize tau GSTs ZmGST V and ZmGST VI, heterodimerization was as likely as homodimerization, whereas heterodimer formation was favoured with the maize phi GSTs ZmGST I and ZmGST II (Ref. 22).

Do heterodimers perform unique roles? The available kinetic measurements, performed with xenobiotic substrates in vivo, have detected only ‘additive’ properties in heterodimers. We propose that plant genes be named using a species designation, a gene-class identifier, and a number within that class. For example, XyGSTZ1 indicates that the gene was isolated from species Xy (such as Xy for *Arabidopsis*, *Zm* for *Zea mays*). Zm denotes zeta class and the numeral 1 indicates that it is the first gene of the zeta class from that species.

At the protein level, XyGSTZ1-1 would indicate a homodimer encoded by XyGSTZ1, XyGSTZ1-2 would indicate a heterodimer encoded by the XyGSTZ1 and XyGSTZ2 genes. For example, the maize enzyme GST V VI (Ref. 21) would become ZmGSTU1-2. This system is compatible with the current practice for animal GSTs as well as with the plant-genetics conventions of using italics for genes and standard type for proteins. Table 1 shows the application of this nomenclature to maize and *Arabidopsis* GSTs.

We propose a new gene and enzyme nomenclature for glutathione S-transferases (GSTs) to simplify future assignments and to align designations more closely to evolutionary history. The current naming of GSTs is confusing because enzymes are named in their order of purification and genes by their order of sequencing. This produces situations in which enzyme I is encoded by gene 3. Heterodimeric proteins are given a specific name, which further obscures which genes encode the subunits.

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### Table 1. Suggested new nomenclature applied to maize and *Arabidopsis* GSTs

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<tr>
<th>Species</th>
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<th>Proposed name</th>
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GSTs of herbivorous animals have long been accorded the role of metabolizing dietary toxins, most of which are plant natural products. Interestingly, GSTs are frequently highly abundant in crops, a characteristic exploited in selective herbicides, where the GSTs which detoxify them can be 20-fold more abundant in the crop than in the competing weeds. In an evolutionary context, the evidence available in wheat and its relatives suggests that enhanced GST activity towards xenobiotics, the dominant GST-dependent activities that have little to do with conventional conjugating natural products, has been conserved in wheat 10 have a surprisingly similar spectrum of activities. If the phi and tau GSTs have distinct functions, these are likely to be evident only with endogenous substrates.

GSTs conjugating natural products

After glutathionation, xenobiotic conjugates are rapidly imported into the vacuole before being further metabolized into a range of known sulphur-containing metabolites. By contrast, the GST conjugate of cufic acid identified in wine must and a sulphur-containing metabolite of gibberellic acid, termed gibberthione, are among the few candidates for GST conjugation of endogenous plant products. This suggests that GST functions are important for detoxification of endogenous metabolites or that the conjugates are unstable and have escaped detection.

Stress-inducible GSTs might act to conjugate metabolites arising from oxidative damage. For example, cytotoxic alkenals derived from the peroxidation of natural products, such as fatty acids, are well-defined substrates of GSTs in oxidatively stressed animal cells. In plants, 4-hydroxyxenon, a toxic alkene released following oxidative damage of membranes, is actively detoxified by phi GSTs from sorghum, and tau GSTs from wheat have similar activities. Pathogen-inducible GSTs probably also detoxify exogenous natural products, though of exogenous origin, such as phytoxins produced by competing plants or by invading microbial pathogens. A good example of this is the detoxification of isothiocyanates, reactive compounds released from glucosinolate precursors formed in Brassica species. These allelochemicals can suppress the growth of competing plants but are excellent substrates for the phi and phi GSTs in wheat and maize.

Although inducible GSTs might use stress metabolites as substrates, their role in GST conjugation of natural products in healthy plants is less clear. Until recently, the best candidates for endogenous phytochemicals detoxified by GSTs were the phenylpropanoids sinapic acid and coumaric acid (Fig. 1). Intriguingly, these results suggest that GSH conjugation of electrophilic natural plant products could be more widespread than previously thought; a role in vacuolar targeting might have been missed because the conjugates are degraded in the acidic conditions of most vacuoles or in the course of phytochemical analysis.

GSTs functioning as binding proteins

A hypothesis to explain why a specific GST is needed for anthocyanin sequestration is that these proteins act as carriers. The concept of GSTs as carrier proteins or ‘ligandin’ was first proposed in the early 1970s, based on the fact that several GSTs were identified as the cellular binding factor for diverse steroids, drugs and mammalian metabolites. Later, cloning and identification of ‘ligandin’ as a GST resulted in the same quandary currently faced
bolites fit the definition of ligandins. 

Evidence that specific plant GSTs that bind defined plant metabolites function in the binding and transport of plant hormones. Similarly, both tetrapyrroles and plant hormones, binding inhibits GST activity toward xenobiotics, but the inhibitory ligands do not undergo conjugation. Similarly, the CDNB conjugating activity of petunia AN9, a GST, is inhibited by flavonols, flavones and anthocyanins. There is ample evidence that specific plant GSTs that bind defined plant metabolites fit the definition of ligandins.

**GSTs catalysing GSH-dependent peroxidase and isomerase reactions**

By catalysing the nucleophilic attack of GSH on hydroperoxides, GSTs can catalyse the reduction of organic hydroperoxides to the less-toxic monohydroxy alcohols, the resulting sulfenic acid derivatives of GSH then spontaneously forming a disulfide with another GSH molecule (Fig. 4). Tobacco seedlings overexpressing a tobacco tau GST with a high glutathione peroxidase activity are more tolerant of chilling and osmotic dehydration than wild-type plants.

A further link between GSTs functioning as glutathione peroxidases and oxidative-stress tolerance was discovered in black grass (Alopecurus myosuroides). Herbicide-resistant weeds that are cross-resistant to multiple classes of herbicides express a phi GST (AmpGSTF1) that is a highly active glutathione peroxidase; this GST is barely detectable in herbicide-sensitive black grass. AmpGSTF1 appears to contribute to herbicide resistance by preventing the accumulation of cytotoxic hydroperoxides of natural products, which can be formed either directly or indirectly as a result of injury by herbicides with diverse modes of action. Significantly, the phi GSTs that are induced in crops following treatment with herbicide safeners, providing a connection between safener-mediated protection of crops and oxidative stress tolerance.

GSTs are also essential for the isomerization of specific metabolites (Fig. 4). The proposed mechanism involves the transient formation of a GSH adduct, spontaneous isomerization of the compound and finally the release of the isomer and GSH. An excellent example is the human zeta GST, which functions as a malonylacetacetate isomerase (MAAI), a key enzyme in phenylalanine catabolism. MAAI catalyses the cis-trans isomerization of maleylacetacetate to fumarylacetacetate (Fig. 4). This reaction has been known for some time to be GSH dependent and associated with a GST; the recent complementation of an MAAI-deficient strain of Aspergillus nidulans with the human zeta GST confirming its identity as the MAAI enzyme. This GST-mediated cis-trans isomerase reaction involves the reversible addition of GSH to the cis double bond; after rotation, GSH is eliminated and the trans isomer is formed. In view of their sequence similarity, plant zeta GSTs probably have a similar activity. The carnation (Dianthus caryophyllus) zeta genes are induced during senescence, which is consistent with a role in the degradation of aromatic amino acids.

GST-mediated isomerase reactions recently identified in animals include prostaglandin-H E isomerase activity and the isomerization of 13-cis retinoic acid to all-trans retinoic acid. In the latter case, the enzyme catalysing the reaction is clearly a GST, yet the reaction does not require GSH and these enzymes appear to have recruited other proteaceous thiods to catalyse the reaction. In plants, the isomerase activity of GSTs has been demonstrated using thiadiazolidine herbicides; these are bioactivated by GST-mediated isomerization to triazolidines, which are potent inhibitors of protoporphyrinogen oxidase. A likely mechanism for isomerization involves nucleophilic attack at the carbonyl group by GSH, forming a connection between safener-mediated protection of crops and the formation of the GSH-conjugated intermediate.

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**Fig. 4.** Examples of glutathione-S-transferase (GST)-mediated and glutathione (GSH)-dependent reactions that do not result in the formation of GSH conjugates as end products. (a) GST functioning as a glutathione peroxidase, using GSH to reduce an organic hydroperoxide (ROOH) to the monohydroxy alcohol (ROH). (b) A zeta GST catalysing a cis-trans isomerization of malonylacetacetate to fumarylacetacetate. (c) GST catalysing the isomerization of a thiadiazolidine proherbicide to the phytotoxic triazolidine, showing the proposed reaction mechanism and the formation of the GSH-conjugated intermediate.

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**Conclusions**

Recent advances in molecular genetics and biochemistry have revealed a surprising complexity in the variety of GST genes and enzymes in plants. We have tried to highlight some tantalizing reasons for this diversity: members of an ancient gene family have been recruited to perform diverse physiological functions based around their ability to activate a readily available cellular thiol while coordinately binding a diverse range of hydrophobic ligands. GST functions have direct cytoprotective activities and they might...
be essential to preserve plants during environmental stress and disease, as well as supporting normal development. We consider the frontier of research to involve matching individual GSTs with their in vivo ligands and showing which interactions result in catalysis.

Because of the extreme divergence among GSTs, sequence analysis alone cannot uncover function. Perhaps even more intriguing are the hints that some GSTs might be carrier proteins for reactive plant metabolites, controlling their activity and cellular distribution. In the coming years, our ability to manipulate the expression of GSTs in plants through gene disruption and transgenic approaches will allow us to test these models of GST function in vivo.

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