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Glutathione *S*-transferases: Family of enzymes

by

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Abbreviations:

- GST: Glutathione S-transferase
- GSH: Glutathione
- ATPase: Adenosine tri-phosphatase

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Abstract

The glutathione S-transferases (GSTs) are major phase II detoxification enzymes found mainly in the cytosol. They catalyze the initial step in the formation of *N*-acetylcysteine (mercapturic acid) derivatives of a diverse group of foreign compounds. In addition to their role in catalyzing the conjugation of electrophilic substrates to glutathione (GSH), these enzymes also carry out a range of other functions like nucleophilic aromatic substitution reactions, reversible michael additions to α,β-unsaturated aldehydes and ketones, isomerizations, epoxide ring openings, and for few GSTs, peroxidase reactions. GSTs share greater than 60% identity within a class. Much emphasis tends to be placed on the primary structure at the N-terminus because, within the classes, this region tends to be better conserved than others, as it includes an important part of the active site. This paper primarily focuses on the classification and crystal structure of GSTs, and their catalytic reactions.

Introduction

The glutathione S-transferases (GSTs) are a family of enzymes that catalyze the initial step in the formation of *N*-acetylcysteine (mercapturic acid) derivatives of a diverse group of foreign compounds [1]. These enzymes are involved in the detoxification of enzymes and in few instances activation of a wide variety of chemicals and also have extensive ligand binding properties in addition to their catalytic activities [4]. The mammalian GSTs are localized in both the cytoplasm and the endoplasmic reticulum however, the cytosolic glutathione activities are usually 5 to 40 times greater than the microsomal activity. They have also been implicated in a variety of resistance phenomena involving cancer chemotherapy agents, insecticides, herbicides and microbial antibiotics [2]. They are ubiquitous, with the greatest activity found in the testis, liver, intestine, kidney, and adrenal gland. Since their discovery in 1970s, thousands of articles have been written on the structure, function, and toxicologic significance of GSTs. This paper primarily focuses on the classification and crystal structure of GSTs, and their catalytic reactions.

Crystal structure of Glutathione S-transferases

All the classes of GSTs follow a similar canonical fold, with each subunit consisting of two distinct domains (Figure 1a) [2]. Domain 1 is highly conserved and provides most of the GSH binding site, and is connected to domain 2 by a short linker sequence (Figure 1b). Domain 2 begins at the C-terminus of the linker sequence. N-terminal domain 1 adopts a similar topology as in thioredoxin fold, consisting of four β-sheets with three flanking α-helices (Figure 1c). The fold consists of distinct N- and C-terminal motifs and have $\beta\alpha\beta$ and $\beta\beta\alpha$ arrangement respectively, and which are linked by a α-helix.

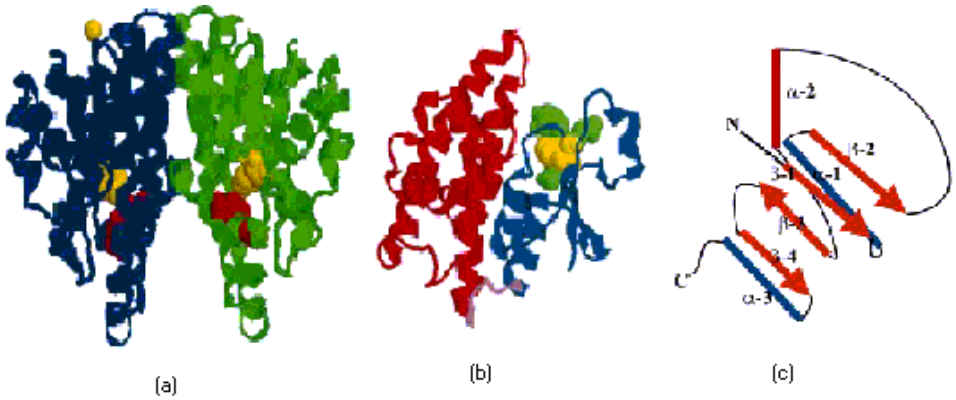

Figure 1: (a) GST structure showing two subunit- blue (left-hand) and green (right hand), and the inter-subunit cleft; (b) 3-D structure of an individual subunit, N-terminal domain 1 is blue (right) and C-terminal domain 2 is red (left) and linker strands shown in violet and; (c) representation of thioredoxin fold, α-helices are shown as cylinders, β-sheets are shown as orange arrows [2].

Classification of glutathione S-transferases

The GSTs enzyme family has been divided into a number of classes based on criteria, including amino acid/nucleotide sequence, immunological, kinetic and tertiary/quaternary structural properties [2,3]. These classes follow similar folds, with structural differences concentrated especially around the active site and at the inter-subunit interface in the crystal structures.

A. Mammalian GSTs

Mammalian GSTs share more than 60% identity within a class, and those with less than 30% identity are assigned to separate classes. N-terminus region in the primary structure tends to be conserved better within the mammalian classes and contains a catalytically essential tyrosine, serine or cysteine residue that interacts with thiol group of GSH, lowering the pK_a to 6-7 from its normal value of 9. Based on the criteria described above, mammalian GSTs are divided into four main classes, alpha, mu, pi and theta. C-terminal domain is less similar in alpha, mu and pi classes than the N-terminal domain. Theta class, on the other hand, shows only 7% of overall identity with the other classes, and has a catalytically essential serine rather than a tyrosine in the region of N-terminus as in other classes. Theta class is also present in plant GSTs. C-terminal consists of 5 α-helices in pi and mu classes, while 6 α-helices in alpha class and this extra helix

packs on to the hydrophobic binding site resulting in smaller and stronger hydrophobic site than that in pi and mu classes. Aromatic residue in alpha, pi and mu classes respectively, acts a 'key' extending from domain 1 loop preceding β-3, which fits into a hydrophobic 'lock' provided by helices α-4 and α-5 of the other subunit. Theta class lacks this 'lock and key' motif, and thus the cleft between the two subunits is significantly less pronounced. The mu class enzymes have a characteristic 'mu loop' located between β-2 and α-2 which results in deeper active site cleft than pi class. Some other classes other than alpha, pi, mu and theta are the kappa and omega classes. Kappa class also lacks the 'lock and key' motif like in alpha, pi or mu classes. Omega class has 7 α-helices in domain 2, and there is an interaction between cysteine-32 and mixed disulfide group of GSH. Also the inter-subunit interface is dominated by non-polar interactions and is more open in this class than in other GST classes.

B. Non-mammalian GSTs [2]

- **Sigma class:** The GSTs purified from squid, grouped in this class, shows 42-44% similarity with cephalopod S-crystallin, the major protein of the eye lens. There is no 'lock and key' motif in sigma class and electrostatic interactions near the active site appear to be especially important in this regard. The active site of sigma class binds with GSH in the same fashion as the alpha or pi classes in mammalian GSTs.
- **Zeta class:** It's a phylogenetaically highly conserved class of GSTs and is present in spectrum of species ranging from plants to humans. Zeta class GST is identical with maleylacetate isomerase, an enzyme of catabolic pathway for tyrosine as shown in Table 1 (see below). This class has the poor catalytic activity with most of the GST substrates, because of the presence of very small polar active site due to the diverse protein folding

around α-2 helix. A methionine residue acts as 'lock and key' motif linking the dimer together. Also, the V-shaped dimer is lacking in this class same as that in theta class.

- **Beta class:** Three-dimensional structure reveals a mixed disulfide between a conserved cysteine residue and the thiol group of GSH, similar to that observed in mammalian omega class. The dimer interface is close-packed and dominated by polar interactions in the beta class as compared to theta class. Also, none of the cysteine, tyrosine and serine residues at the N-terminus are catalytically essential for beta class but C-terminal histidine-106 and lysine-107 may contribute to the interaction with GSH.

C. Fungal GSTs [2]

Fungal GSTs show limited similarity within N-terminal region of theta class but are quite distinct from alpha, pi and mu classes. The two major enzymes sequenced in fungal GSTs are GST Y-1 and Y-2. Y-1 shows conservation of N-terminal catalytically essential serine of the theta class whereas in Y-2 serine is replaced by threonine lowering the catalytic activity than Y-1.

D. Plant GSTs [2]

Plant GSTs are mainly classified in four classes namely theta, zeta, phi and tau. Theta and zeta classes are already been discussed above, as they are present in a range of species while phi and tau classes are plant specific. Phi includes GSTs with herbicide-detoxifying activities, and their genes contain 3 exons, whereas tau includes auxin-inducible GSTs containing 2 exons. N-terminal domain and hydrophobic interface subunit are quite similar in plant GSTs, and differences are concentrated in C-terminal domain where a hydrophobic substrate is likely to bind, hence C-domain is considerably broader in plant than in mammalian GSTs.

E. Insect GSTs [2]

There are two immunologically distinct classes for insect GSTs, class I and II. Class I, also known as delta class, is closely related to theta class in mammalian GSTs having serine as the residue in N-terminal, whereas class II is closely related to sigma class. Class I is generally intronless in the coding region, but may be interrupted by introns in a 5' untranslated part of gene. Not much is known about class II of plant GSTs, but recently it was found out that several members of omega, zeta, sigma and theta classes are common with class II GSTs.

F. Helminth GSTs [2]

Helminth (parasitic) organisms have low levels of phase I (see below) and other detoxification enzymes, but express GSTs in response to drug treatment. The enzymes purified from parasitic nematode *Ascaridia galli* shows relationship with GSH-dependent prostaglandin-H E-isomerase. Helminth GSTs resembles the mu, pi and sigma classes. *A. galli* GSTs resembles sigma class, *Ascaris suum* GSTs resembles the pi class lacking both the mu loop and the extra α-helix, and S*chistoma japonicum* (causing schistosomiasis) GSTs resembles the mu class.

Role of glutathione S-transferases in detoxification of enzymes

The enzymatic detoxification of xenobiotics has been classified into three distinct phases. Phases I and II involve the conversion of a lipophilic, non-polar xenobiotic into a more water-soluble less toxic metabolite, which is then eliminated more easily from the cell [2]. Phase I is catalyzed mainly by the cytochrome P450 system [6]. Phase II enzymes catalyze the conjugation of activated xenobiotics to an endogenous water-soluble substrate, such as reduced glutathione (GSH), glucuronic acid or glycine. Conjugation to GSH catalyzed by the GSTs is the major phase II reaction in many species [2, 3]. The GSH–xenobiotic conjugate is too hydrophilic to diffuse freely from the cell, and has to be pumped out actively by a transmembrane ATPase such

as the GS-X pump, which is phase III [5]. This results in the unidirectional excretion of the xenobiotic from the cell, since the hydrophilic GSH moiety prevents re-diffusion across the plasma membrane. Ultimately, this conjugate is excreted from mammals as mercapturic acids. A schematic is shown in Figure 2.

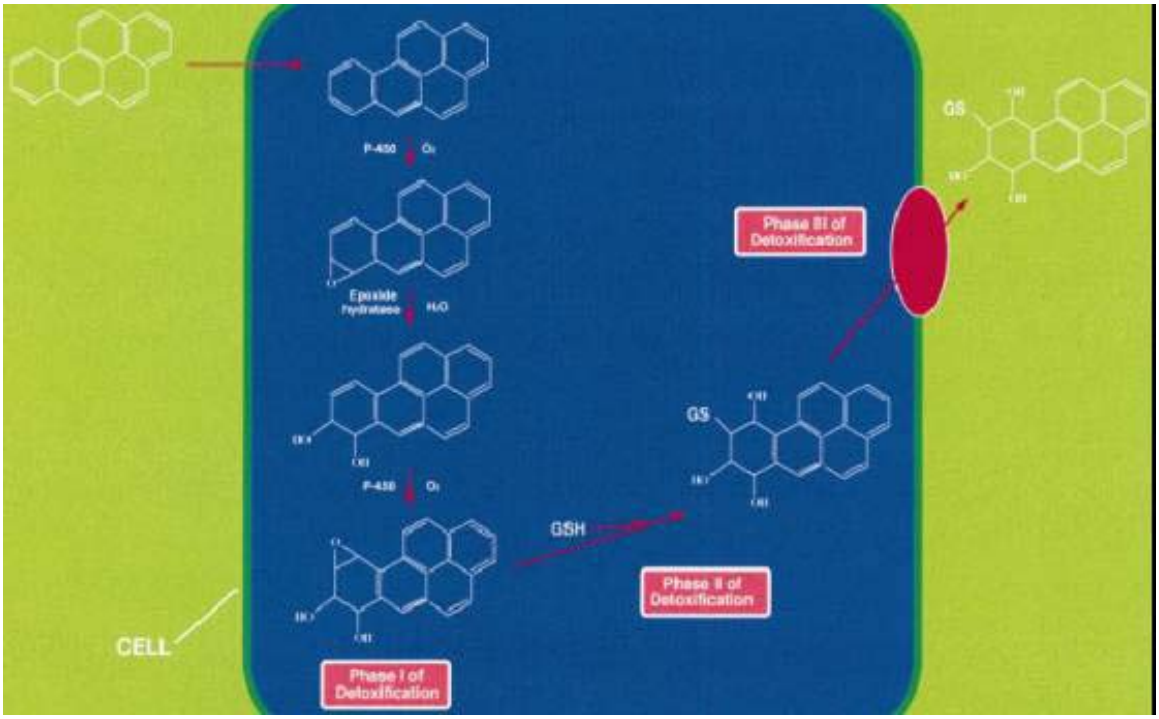

Figure 2: Overview of enzyme detoxification [2]

Glutathione S-transferases catalytic reactions

GSTs catalyze the general reaction:

$$GSH + R\text{-}X \rightarrow GSR + HX \quad (1)$$

The main function of these enzymes is to bring the substrate into close proximity with GSH by binding both GSH and electrophilic substrate to the active site of the protein, and activate the sulfhydryl group on GSH, thereby alowing for nucleophilic attack of GSH in the electrophilic substrate (R-X) [7]. GSTs catalyze various reactions like nucleophilic aromatic substitution reactions, reversible michael additions to α,β-unsaturated aldehydes and ketones, isomerizations, epoxide ring openings, and for few GSTs, peroxidase reactions [3,4]. The electrophilic functional

center of the substrates can be carbon, nitrogen or sulfur. Few reactions catalyzed by GSTs in addition to their detoxification function are given in Table 1.

Table 1: Reactions catalyzed by GSTs in addition to detoxification [2]

Reaction 2	Steroid isomerization (catalyzed by alpha class)	Δ^5 – androstene-3,17-dione → Δ^4 – androstene-3,17-dione
Reaction 3	Isomerization of retinoic acid (catalyzed by Pi class)	13-*cis*- retinoic acid → All-*trans*-retinoic acid
Reaction 4	Prostaglandin synthase (catalyzed by sigma class)	PGH_2 → PGD_2
Reaction 5	Maleylacetoacetate isomerase (catalyzed by Zeta class)	Maleylacatoacetic acid → Fumarylacetoacetic acid
Reaction 6	Anthocyanin synthesis (catalyzed by plant classes Phi and tau)	Cyanidin-3-glucoside + GSH → Glutathione conjugate → anthocyanins

Summary

Glutathione S-transferases play a fundamental role in protection against toxic chemicals. Three-dimensional structures confirm a similar overall fold in GST subunits, with class specific feature found in the active site, inter-subunit interface and domain 2. GSTs bring the substrate into close proximity with GSH by binding both GSH and electrophilic substrate to the active site of the protein, and activate the sulfhydryl group on GSH. Different classes of GSTs and their subclasses are summarized below in Table 2.

Table 2: GSTs and their classes [2,3]

GST	**Subclasses**	**Catalytic essential in N-terminus**	**No. of α-helices in domain 2**
Mammalian	Alpha	Tyrosine	6
	Mu	Tyrosine	5
	Pi	Tyrosine	5
	Theta	Serine, but no 'key and lock' motif	-
	Kappa	No 'lock and key' motif	-
	Omega	Cysteine-32 and –SH	7
Non-mammalian	Sigma	No 'lock and key' motif	-
	Zeta	Methionine	-
	Beta	Not required, but histidine-106 and lysine-107 required in C-terminal	-
Fungal	Y-1	Serine	-
	Y-2	Threonine	-
Plant	Phi	Not required, interaction occurs in C-terminal domain	-
	Tau		
Insect	I / Delta	Serine, resembles sigma class	-
	II	Resembles omega, sigma, zeta and theta classes	
Helminth		Resembles sigma, pi and mu classes	

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