Glutathione Conjugation

I. Glutathione Metabolism, Homeostasis
II. GSH Conjugation Reactions
III. Glutathione S-Transferases
IV. Reactions of GSH Conjugates

Bill Atkins
H172Q, winky@uw.edu

Suggested Reading

Bernard Testa, Stefanie D. Krämer. The Biochemistry of Drug Metabolism - An Introduction : Part 4. Reactions of Conjugation and Their Enzymes. Chemistry and Biodiversity, vol. 5(11): 2171-2336 (2008)

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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 Nov 15;360(Pt 1):1-16

Townsend DM, Tew KD. The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene. 2003 Oct 20;22(47):7369-75

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van Bladeren PJ. Glutathione conjugation as a bioactivation reaction.
Chem Biol Interact. 2000 Dec 1;129(1-2):61-76

Anders MW. Glutathione-dependent bioactivation of haloalkanes and haloalkenes.
Drug Metab Rev. 2004 Oct;36(3-4):583-9.

I. Glutathione Metabolism and Homeostasis

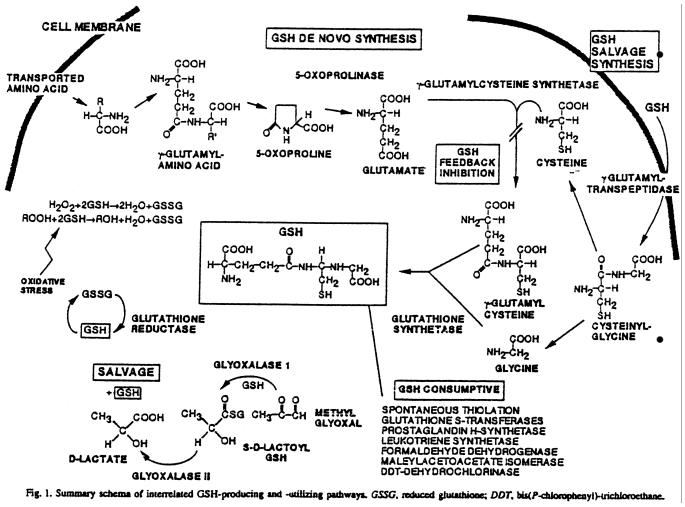


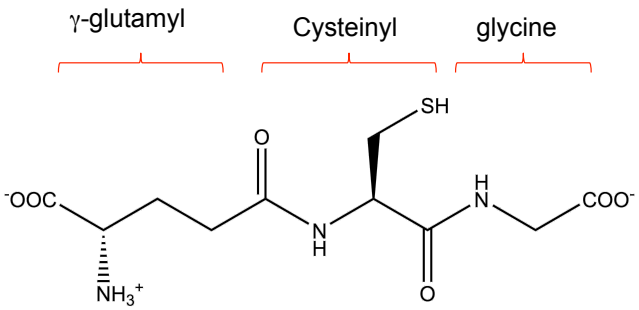
Fig. 1. Summary schema of interrelated GSH-producing and -utilizing pathways. *GSSG*, reduced glutathione; *DDT*, bis(*P*-chlorophenyl)-trichloroethane.

important in:
- xenobiotic deactivation,
- regulating 'redox' state of cells, maintenance of reduced cysteines,
- general oxidative stress responses

tissue distribution is variable, but generally high (1-15 mM). In hepatocytes there are two pools of GSH:

mitochondrial pool ~ 20%
cytosolic pool ~ 80%

I. Glutathione: Chemical Properties



- Nucleophilic cysteine- chemical strategy is to have a good nucleophile on a polar, water-soluble, co-factor. In contrast to UDPGA and PAPS, electrophilic drugs react.
- Redox active cysteine
- Unusual peptide linkage; γ-glutamyl-cys-gly

I. Glutathione Metabolism & Homeostasis

- Enzymatic Reactions of GSH

GSH Peroxidase: $2GSH + ROOH \rightarrow GSSG + ROH + H_2O$

Detoxification of peroxides, control of oxidative stress

GSSG Reductase: $GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$

Maintenance of GSH levels

Thiol transferases, Glutaredoxins, Thioredoxins others:

$2GSH + RSSR \rightarrow GSSG + 2RSH$

Protein folding, protein regulation

Glutathione S-transferases: detoxification

Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. Deponte M. Biochim Biophys Acta. 2013. 1830(5):3217-66

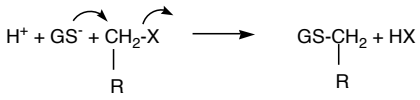
II. GSH Reactions

A ranking of nucleophilic and electrophilic sites in bio(macro)molecules

Nucleophilic sites	Increasing hardness	Electrophilic sites
Thiol groups (R–SH) in Cys residues in proteins and peptides (*e.g.*, GSH)		Aldehydes, polarized double bonds
Thioether groups (R–S–R') in Met residues		Epoxides, strained lactones, alkyl sulfates, alkyl halides
Amino groups in Arg, Lys, and His residues		Arylcarbonium ions
Amino groups of bases in RNA and DNA		Benzylic carbonium ions, nitrenium ions
Oxygen atoms (=O) of purines and pyrimidines		Alkylcarbonium ions
Phosphate oxygen (P=O) of RNA and DNA		

GSH is a ‘soft’ nucleophile due to the polarizability of Sulfur. GSH ‘prefers’ soft electrophiles.

II. GSH Reactions

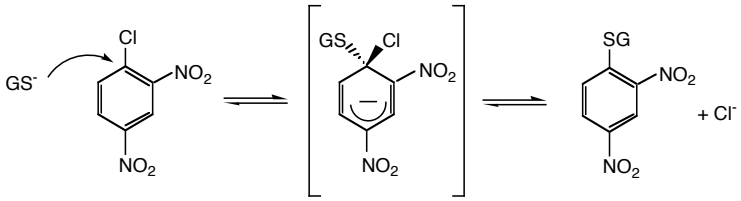


R = alkyl, aryl, benzylic, allylic
X = Br, Cl, I, OSO_3^-, OSO_2R, $OPO(OR)_2$

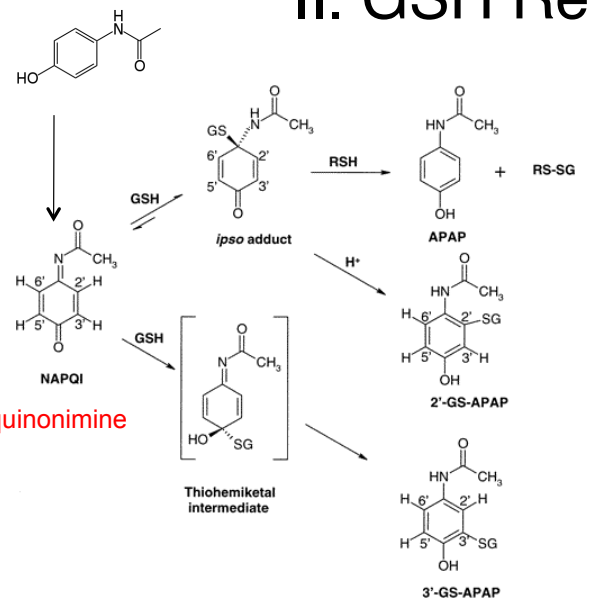
A. nucleophilic substitutions and nucleophilic aromatic substitutions

the 'universal' GST substrate

1-chloro-2,4-dintrobenzene (CDNB)



II. GSH Reactions



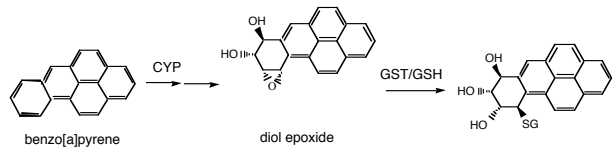
quinonimine

B. nucleophilic additions
ketones, aldehydes, esters, nitriles, α,β unsaturated Michael acceptors.

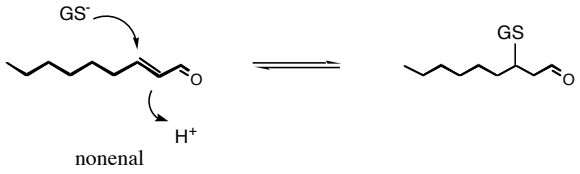
e.g. NAPQI

II. GSH Reactions

B. Nucleophilic Additions (cont'd)

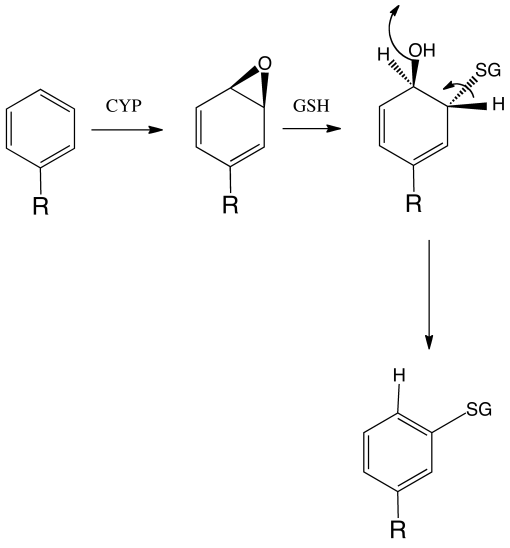


e.g. epoxides



e.g. α,β-unsaturated aldehydes

II GSH Addition to Aryl Epoxides: Stereoselectivity

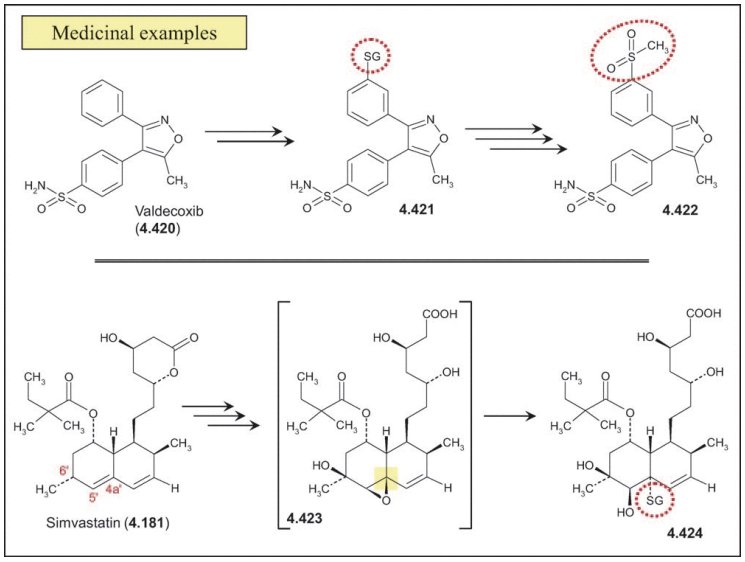


- Addition to aryl epoxides is 'anti' and yields a hydroxy glutathione conjugate.
- Dehydration is driven by energetically favorable aromatization.

II. Reactions: Examples of GSH addition to Drug Epoxide Metabolites

Epoxidation, followed by GSH attack and dehydration

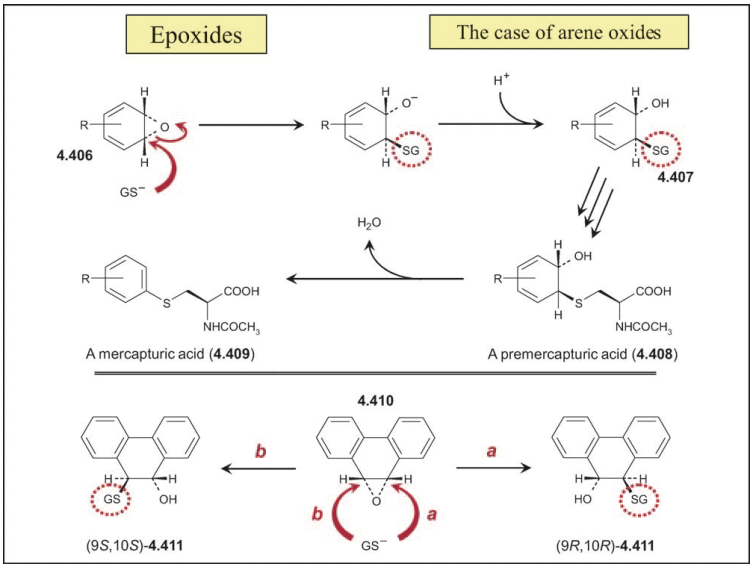
Epoxidation, followed by GSH attack but no dehydration



II. GSH Reactions Addition to Epoxides: Mechanistic Aspects

SN_2-type, 'inversion' of stereochemical configuration. And here, aromatization drives dehydration, after mercapturic acid pathway. Aromatization after addition to aryl epoxides is common and important.

Regio-and stereoselectivity of addition depends on the GST isoform.

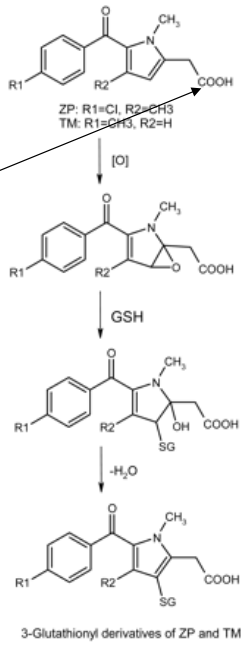


II. GSH Reactions: In vitro trapping 'bioactivated drugs' as a mechanistic tool to detect electrophilic intermediates

Contrast to glucuronidation:

Zomepirac, Tolmetin are 'toxic' NSAIDS.

Dogma has been: acyl glucuronide adducts proteins.

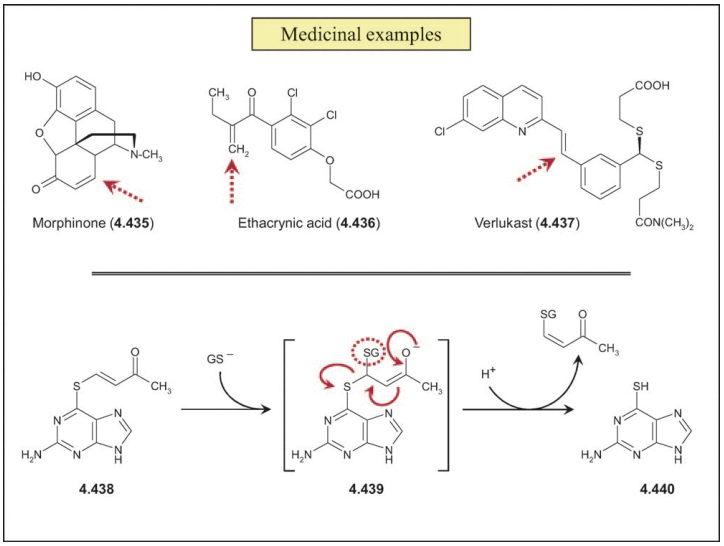


- Epoxidation - metabolic activation of zomepirac.
- CYP-dependent epoxidation, followed by GSH addition.

  epoxidation: alternative possible mechanism for toxicity of zomepirac, vs.acyl glucuronide formation.

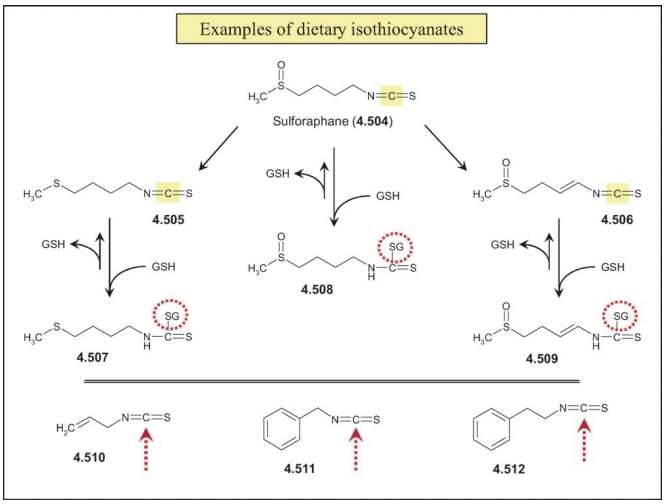
Chen et al., 2005 DMD 34:151-155.

II. GSH Reactions: Polarized Alkenes, Alkenals



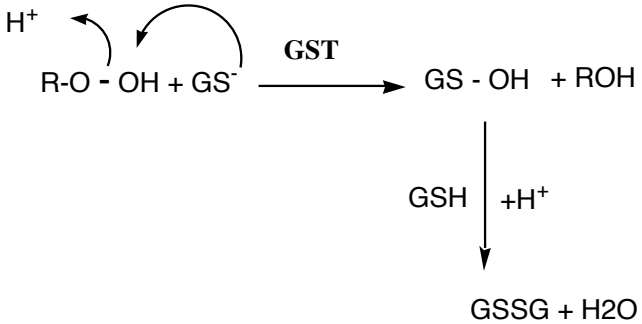
Addition-elimination has been used as a Glutathione-dependent strategy for pro-drug activation.

II. GSH Reactions: Addition to Isocyanates and Isothiocyanates



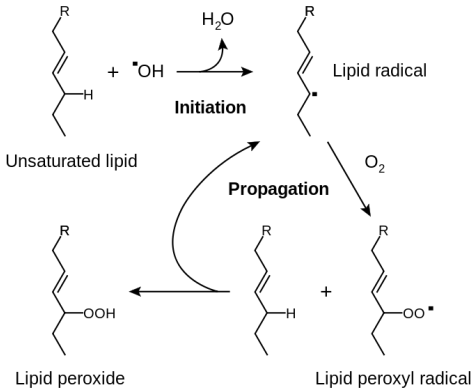
- Found in cruciferous vegetables.
- Isocyanates used in industrial processes. Bhopal disaster, 1984 release of methylisocyanate. $H_3C-N=C=O$
- Induce GSTs.
- Reversible: can serve to transport drug/toxin as the GSH conjugate.

II. GSH Reactions

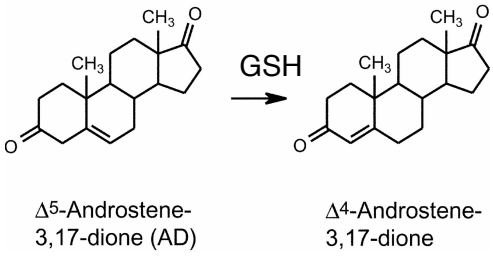


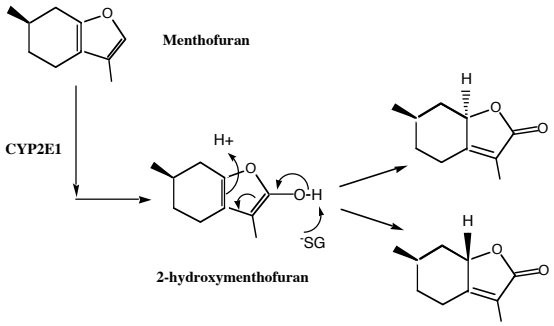
C. Reduction of peroxides

e.g. lipid peroxides



II. GSH Reactions





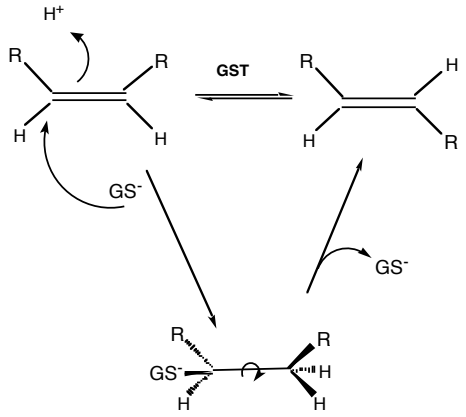
D. Double Bond Isomerization

GS^- is base catalyst (C-4 protons), no net consumption of GSH

e.g. positional isomerization of androstenedione

e.g. hydroxymenthofuran/ a sort of keto-enol isomerization

II. GSH Reactions



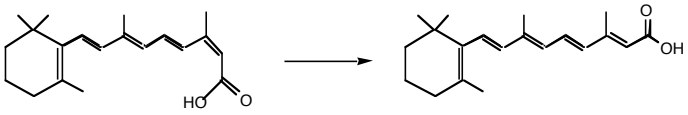
D. Double Bond Isomerization

cis-trans isomerization

e.g. maleate ⇔ fumerate prostaglandins

atypical example:

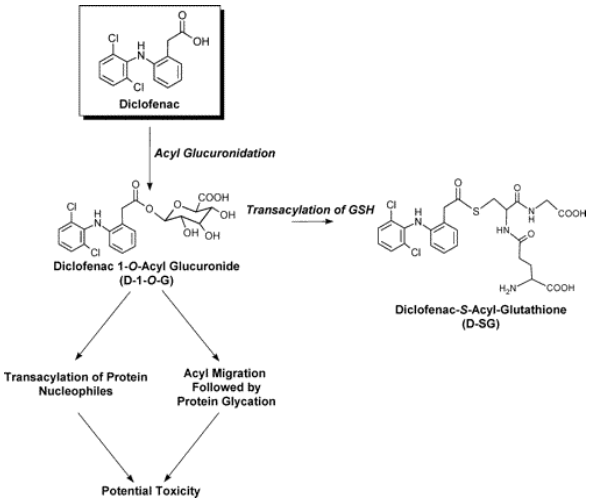
Retinoic acid isomerization catalyzed by GSTP1-1 does not require GSH.



13-cis retinoic acid

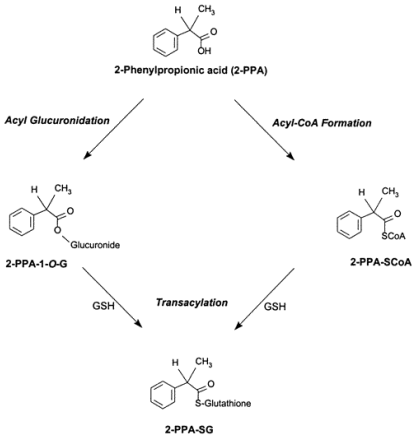
all-trans retinoic acid

II. GSH Reactions



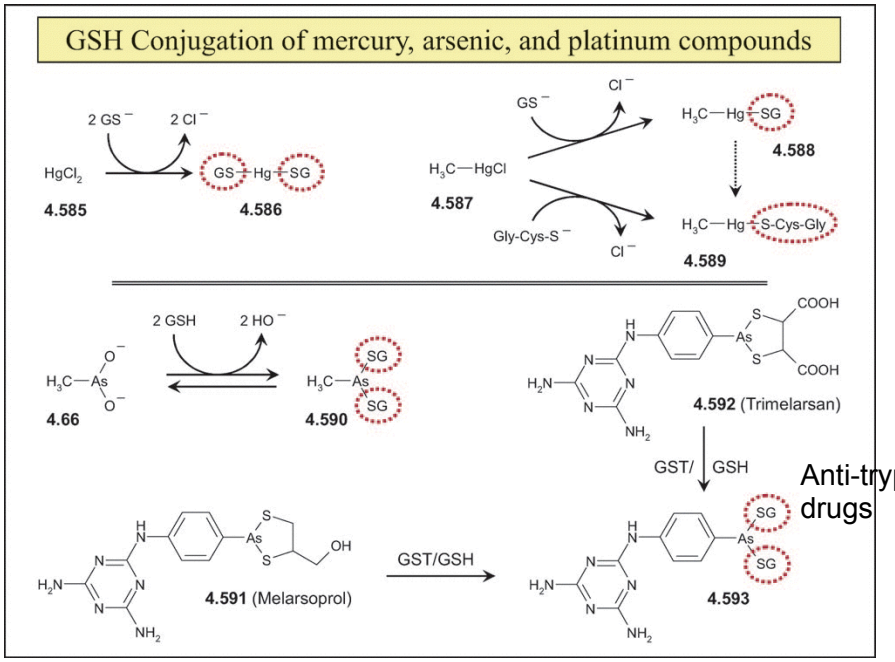
E. Transacylation. May contribute to the formation of glutathione conjugates from other primary metabolites.

e.g.s Glucuronides, CoA thioesters



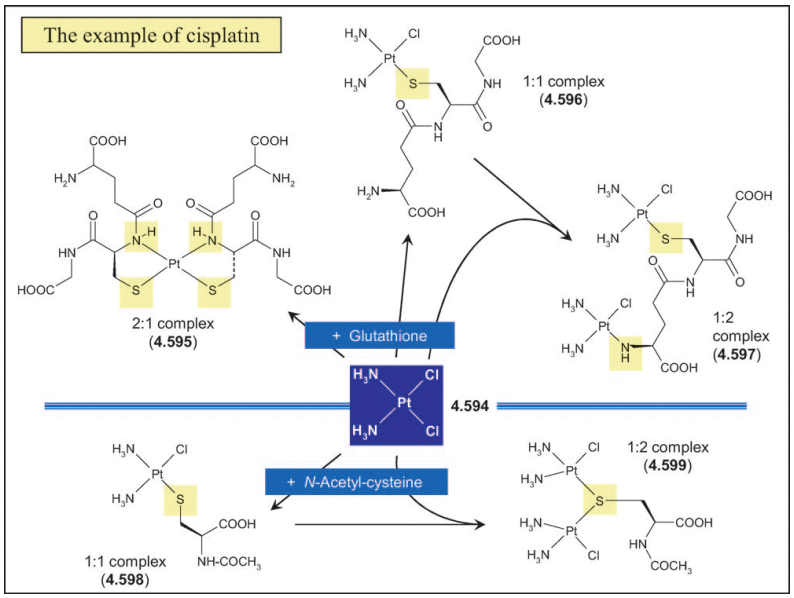
e.g.s zomepirac, diclofenac, clofibrate

II. GSH Reactions: Substitution on Organometallics



Anti-trypanosomal drugs

II. GSH Reactions: Cisplatin



III. Glutathione S-Transferases

Two structurally unrelated families of GST:

MAPEGs: membrane-associated proteins involved in eicosanoid and glutathione metabolism.

- Family of 13 proteins is based on sequence and structural properties and functions. Six human members: 5-lipoxygenase-activating protein (FLAP), leukotriene C4 (LTC4) synthetase, Microsomal MGST1, MGST2, MGST3, and MGST-like 1 (mGSTL1).
- MAPEG GSTs are <u>not</u> closely related to the cytosolic GSTs. Relatively small proteins, ~17 kD.
- MGSTI appears to contribute to drug metabolism. MGST2 and 3 may contribute to drug metabolism, but also likely contribute to lipid peroxide metabolism, and hence protection from oxidative stress.

Cytosolic GSTs have multiple functions, including detoxification, drug metabolism, antioxidative stress.

Human GSTs involved in xenobiotic metabolism

The GST gene superfamilies	Human enzymes
Microsomal GST superfamily (homotrimers)	
MGST1, MGST2, MGST3	MGST1, MGST2, MGST3
Cytoplasmic GST superfamily (μ homodimers, and a few heterodimers)	
GSTA1, GSTA2, GSTA3, GSTA4, GSTA5	**Alpha class**: GST A1-1, A1-2, A2-2, A3-3, A4-4, A5-5
GSTK1	**Kappa class**: GST K1-1 (mitochondrial and peroxisomal)
GSTM1, GSTM2, GSTM3, GSTM4, GSTM5	**Mu class**: GST M1a-1a, M1a-1b, M1b-1b, M1b-2, M2-2, M2-3, M3-3, M4-4, M5-5
GSTO1, GSTO2	**Omega class**: GST O1-1, O2
GSTP1	**Pi class**: GST P1-1
GSTS1	**Sigma class**: GST S1
GSTT1	**Theta class**: GST T1-1, T2
GSTZ1	**Zeta class**: GST Z1-1

III. MAPEG Structure and Function

MAPEGs are functional trimers

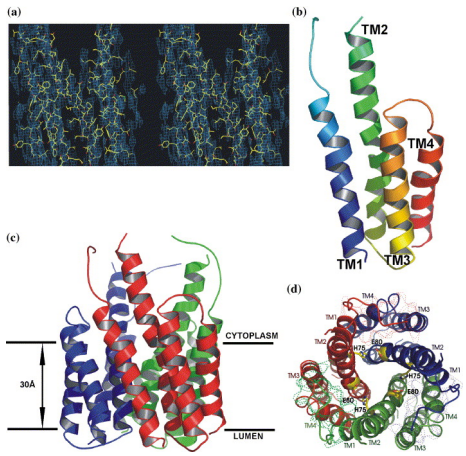
ER membrane proteins

4-transmembrane helices/monomer

III. MAPEG Structure and Function

Structure available for MGST1 indicates a four helix bundle, with 4 transmembrane helices/subunit.

Trimers form along helical axes.

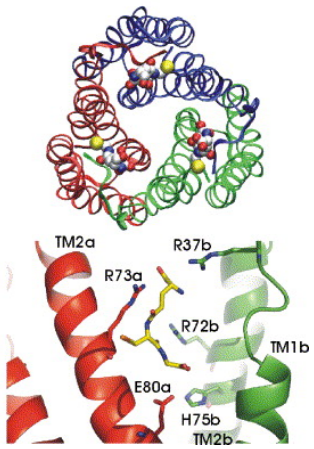


III. MAPEGs: GSH Binding Site

GSH binding site includes intersubunit interactions.

Contacts to each GSH include two subunits within the trimer.

Binding sites near the top of the trimer at the cytosolic surface - no 'latency' as with UGTs.



III. Cytosolic Glutathione S-transferases:

Nomenclature

Isoforms designated on the basis of sequence homology, but unlike CYPs, SULTs, and UGTs the abbreviations include letter (family) and two numbers designating subunit composition.

In humans, A- (alpha-), P- (pi-), M- (mu-), O- (omega) and T- (theta), sigma (S)-classes are cytosolic, xenobiotic metabolizing enzymes.

The cytosolic GSTs are dimeric enzymes, usually homodimers, although intra-class heterodimers are documented. No inter-class heterodimers have been reported. The nomenclature attempts to define gene class and subunit composition: A1-1, M3-3 etc, where A1-2 represents a heterodimer of alpha-class subunits 1 and 2.

Formally - not 'S-transferases'

III. Glutathione S-transferases: Tissue distribution

Human Isoform	Tissues
A1-1	Liver > kidney, adrenal > pancreas
A2-2	Liver, pancreas>kidney
A3-3	Ovary, testis, adrenal, placenta
A4-4	low but everywhere
M1-1	Liver>testis>brain
M2-2	Brain>testis>heart
M3-3	Testis>brain
M4-4	
M5-5	
P1-1	Brain>lung, placenta>kidney, pancreas
T1-1	Kidney, liver>small intestine>brain
T2-2	liver ?
O1-1	liver, heart
O2-2	testis
K1-1	many - mitochondrial

III. Glutathione S-transferases

Polymorphisms

GSTM1-1, high frequency of Caucasians are homozygous for a GSTM1-1 deletion. Possible increased risk of bladder and lung cancer.

M3-3, lower frequency in Caucasians may be associated with increased risk of skin cell carcinomas.

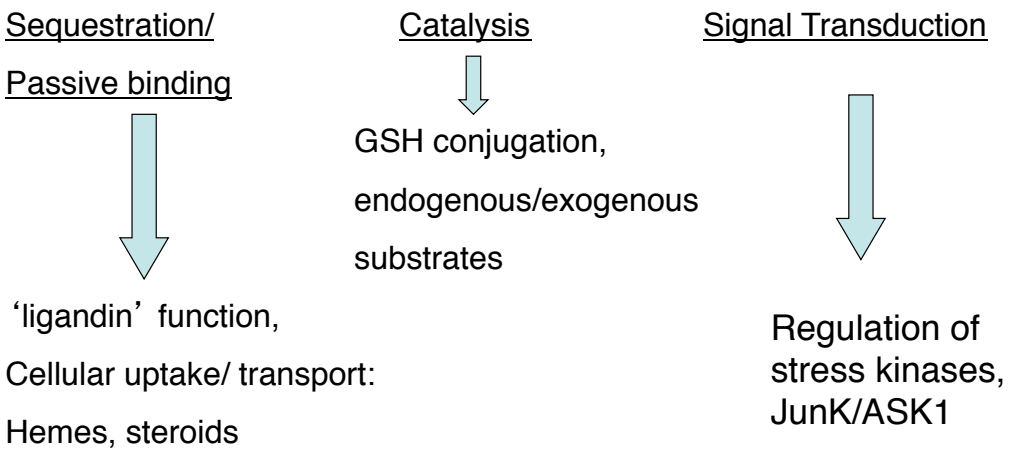
A1-1, small percentage (?) have deletion in the promoter region that leads to null phenotype. Possible increased risk of colon cancer.

P1-1, several allelic 'SNP' variants produce GSTP's with altered function: Ile104→Val, Ala113→Val, double mutant. Have altered catalytic function/ substrate selectivity.

T1-1, gene deletion in 12-60% depending on ethnic origin.
May be associated with increased risk of tumors in several tissues, but this is not well studied.

III. Glutathione S-transferases

Functional Overview



III. Glutathione S-transferases

Over-expression of GSTs in Cancer Cells

- A-, P-, M-, and T-class cytosolic enzymes have each been shown to be induced 3 -50-fold in various transformed model cell lines. The increased levels of GSTs may contribute to multidrug resistance of tumor cells, but the causal relationship between GST levels and drug resistance is still debated.

- GSTP1-1 may have a 'unique' function related to cancer cell response – GST inhibits c-Jun kinase. That is, GSTP1-1 also regulates signal transduction pathways involved in apoptotic/proliferative responses.

III. Glutathione S-transferases

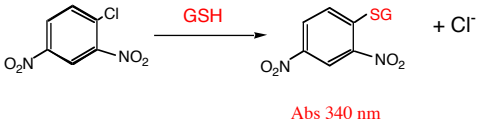
Substrate Selectivity:

Class and isoform-dependent substrate selectivity is broad and overlapping, as with the other detoxification enzymes. A few generalizations are:

M-class GSTs have high activity toward planar aromatic hydrocarbon epoxides.

T-class have high affinity for aryl sulfates, catalyze desulfation.

A1-1,A2-2 have relatively high activity toward organic peroxides. GSTA4-4 selective for lipid hydroxy-enals (4-Hydroxy-Nonenal, HNE). A3-3 great with androstenedione.



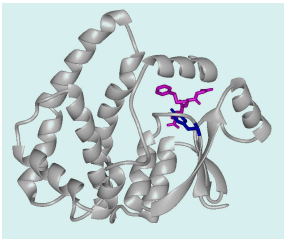
Abs 340 nm

Universal substrate:

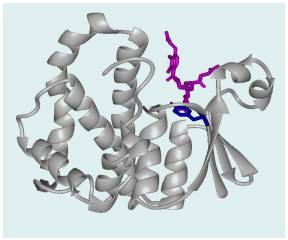
CDNB

III. Glutathione S-transferases

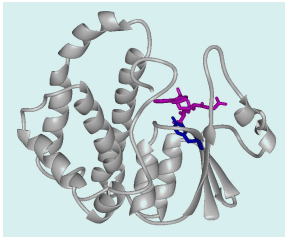
Structures: Overall fold



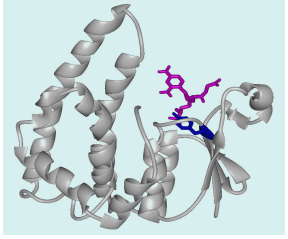
GST A1-1



GST P1-1



GST M3-3



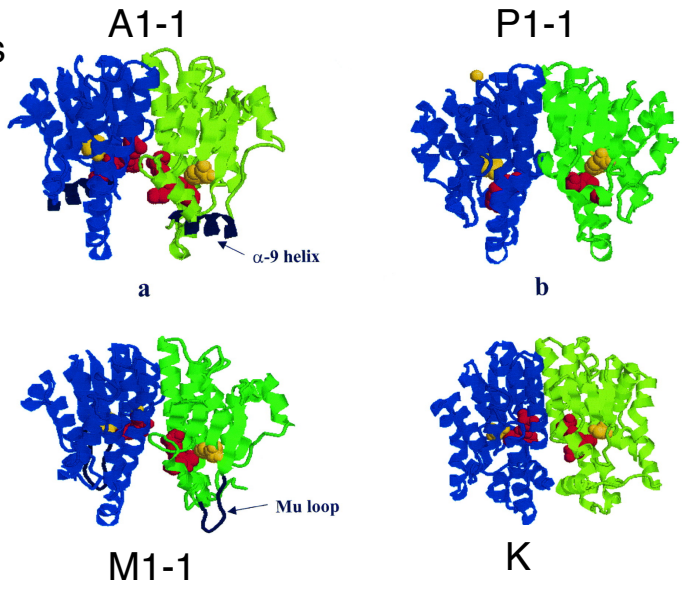
Sigma GST

Cytosolic GSTs are dimers. Overall subunit structure is very similar, with two distinct domains. The N-terminal Domain binds glutathione (G-site) and consists of 3 or 4 helices and a 4-stranded β-sheet. The much larger C-terminal domain is loops and helices, and contributes to the xenobiotic binding site (H-site) which lies between the domains.

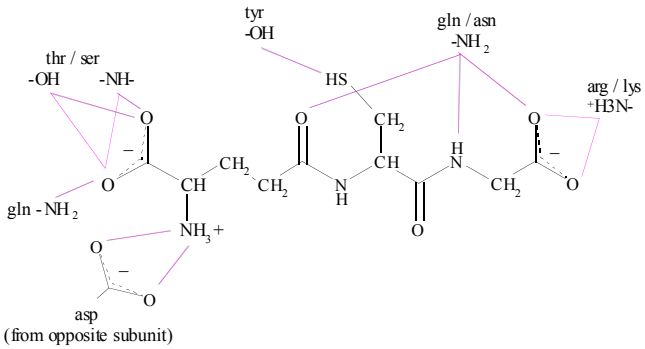
III. GSTs

GSTs are dimers, interclass subunit-subunit interactions are incompatible.

Large intersubunit cleft of varying size and character.



III. GSTs: G-site



Spectroscopic experiments indicate that GSH bound at the active site of GST has a pKa of ~ 6.5 (M)- (P) -7.4 (A). Thus, the nucleophilic GS^- is bound, rather than GSH. This is due to a hydrogen bond to a conserved tyrosine (M, P, A), Cys (O) or ser (T). For some A class isoforms, the tyrosine has 'unusual' properties as well.

structures from each class indicate that salt bridges and hydrogen bonds between active site and GSH peptide are functionally conserved, but structurally distinct.

III. GSTs: H-site, M-class

For each of the GST isoforms, the H-site is lined with hydrophobic residues. In some cases the H-site includes an appropriately placed residue that aids in catalysis of specific substrates i.e. substrate specificity.

e.g. In GSTM an H-site Tyr provides a general base to the 'leaving' oxygen of epoxide substrates.

M-class

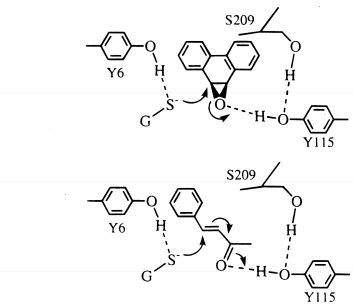
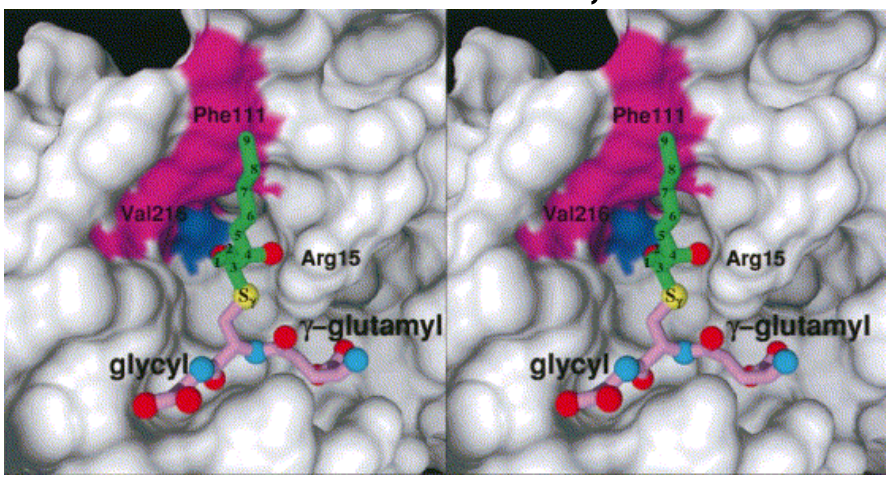
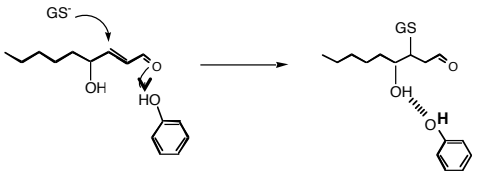


Figure 9. Proposed role of the hydroxyl group of Y115 as an electrophilic participant in catalysis of Michael additions and oxirane ring openings. Taken from ref 26.

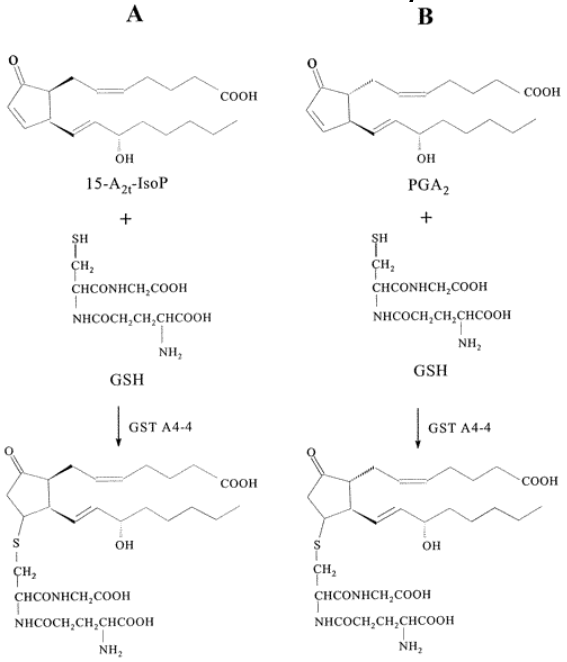
III. GSTs: H-sites, A4-4





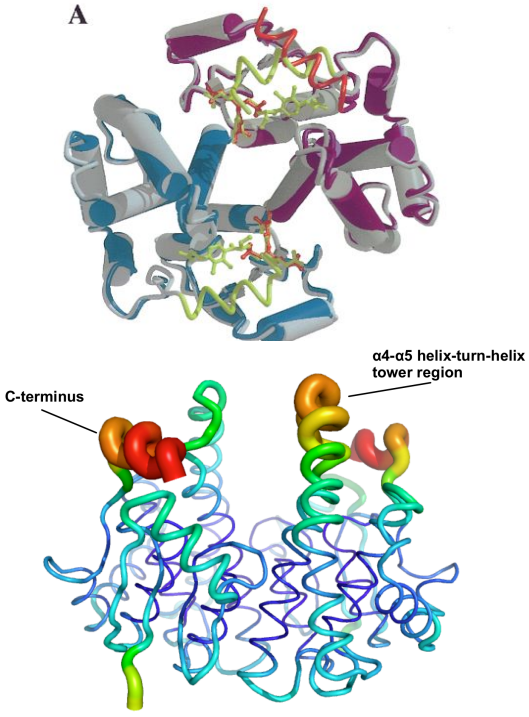
A4-4 has Tyr-212 which acts as general acid for 4-HNE conjugation

III. GSTs, H-sites A4-4



A4-4 also likes isoprostane oxidation products of prostaglandins A(2) and J(2), possible toxic metabolites of oxidative stress.

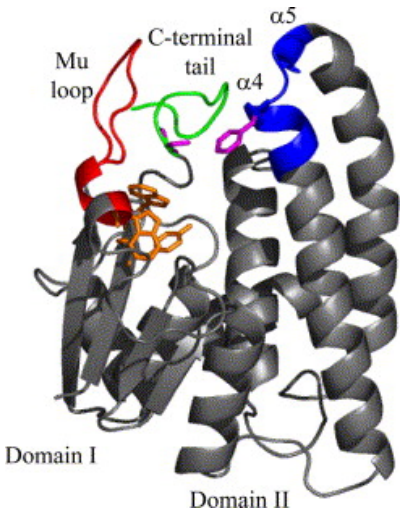
III. GSTs: Dynamics in GSTA1-1



C-terminal helix of A1-1 (208-222) is dynamic.

Location depends on which ligands are bound: helix closes over the active site with GS-conjugates

III. Dynamics in GSTM3-3



'Mu-loop' (red) and C-terminus (green) are mobile and restrict egress of products from the active site.

Blue segment is also dynamic and includes the catalytic Tyr-115

IV. Reactions of Glutathione Conjugates

Degradation by peptidases to Cysteine Conjugates occurs in the kidneys. Normally, the cysteine conjugate is eliminated in urine. Conversion of GSH conjugate to Cys conjugate is the 'Mercapturic Acid Pathway.

Mercapturic acid pathway

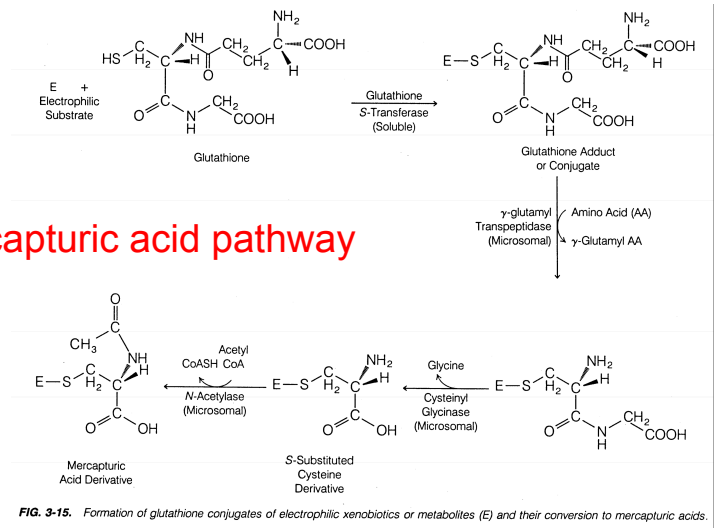
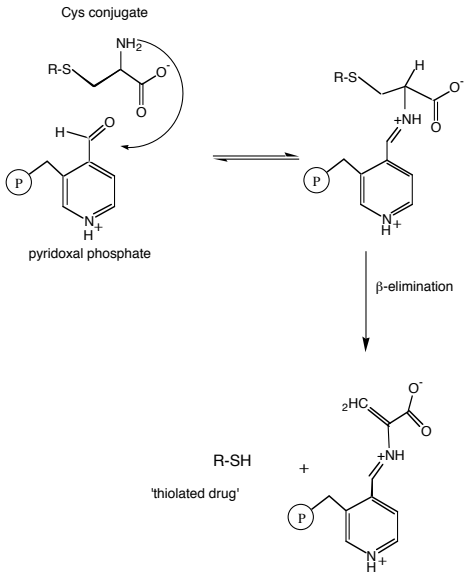


FIG. 3-15. Formation of glutathione conjugates of electrophilic xenobiotics or metabolites (E) and their conversion to mercapturic acids.

IV. Reactions of Glutathione Conjugates: Cysteine Conjugate Metabolism

The enzyme Cysteine S-Conjugate β-Lyase further degrades Cys conjugates. The product may be toxic.

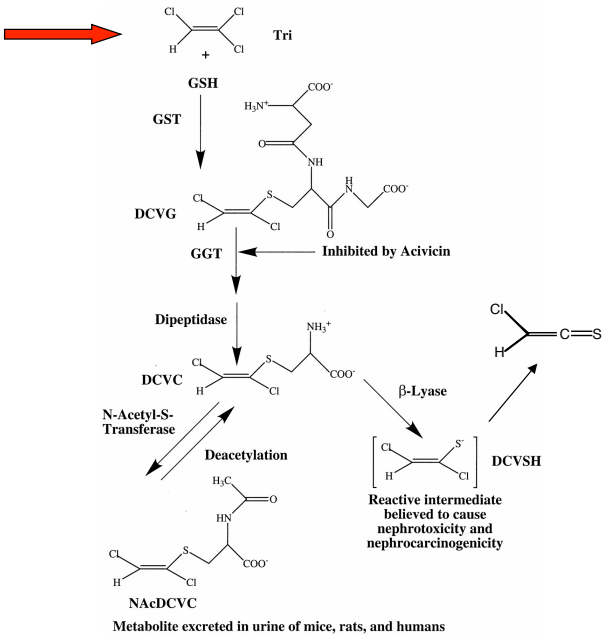
e.g.' s
Cisplatin
Halogenated anesthetics
TCE and halogenated toxins
Catechol neurotransmitters



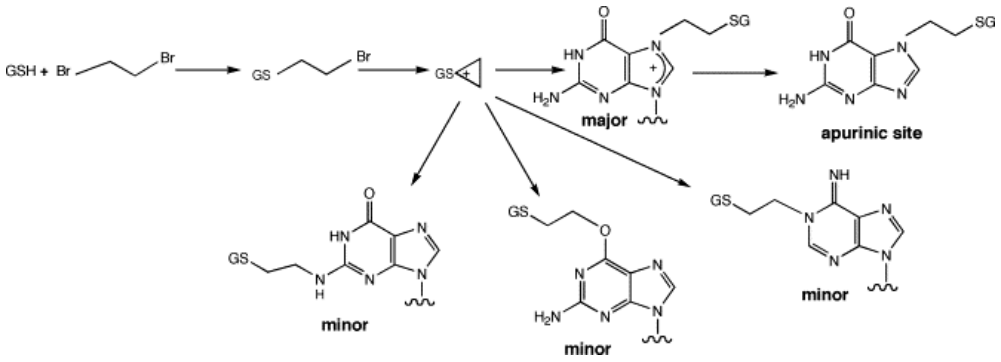
Cysteine β-lyase pathway

IV. Reactions of Glutathione Conjugates

TCE, industrial solvent



IV. Reactions of Glutathione Conjugates: Ethylene Dibromide



IV. Reactions of GSH Conjugates: Busulfan

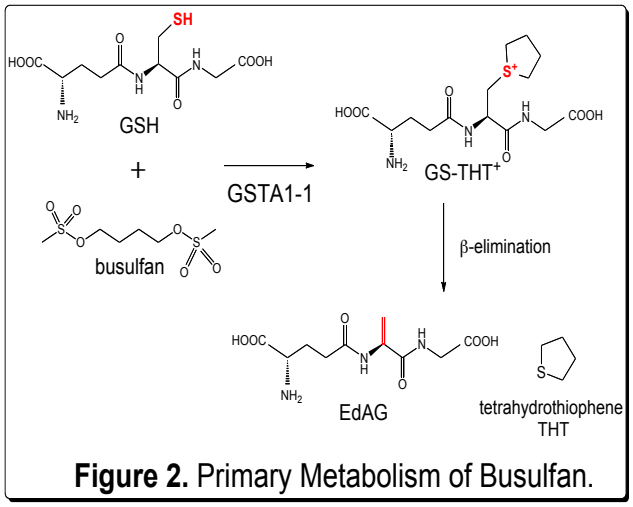


Figure 2. Primary Metabolism of Busulfan.

Busulfan is a DNA crosslinker used in leukemia, lymphomas other bone marrow diseases, to clear cancer cells prior to transplant of healthy cells. Busulfan metabolism starts with GSH conjugation followed by interesting rearrangements.

IV. Reactions of GSH Conjugates: Transport

GSH conjugates are effluxed by several ATP-dependent transporters, MRP1, MRP2. RLIP.

In addition, GSH and GSH conjugates stimulate the transport by MRP's of other drugs and conjugates including glucuronides. Mechanism?

IV. Reduction of GS-Conjugates