GLUTATHIONE TRANSFERASES

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GSTs

• How many enzymes
• Structures
THREE SUPERFAMILIES

• SOLUBLE GLUTATHIONE-TRANSFERASES (25 kDa, dimers) aerobic organisms

• MEMBRANE BOUND GLUTATHIONE-TRANSFERASES (17 kDa, trimer) aerobic organisms

• FOSFOMYCIN RESISTANCE PROTEIN (Fos A) (16 kDa, dimer) bacterial
FOSFOMYCIN RESISTANCE
(Fos A)

- Bacterial (plasmid or chromosomal)
- Specific
- Fosfomycin is a stable! epoxide that inhibits cell wall-synthesis in bacteria
CYTOSOLIC GLUTATHIONE TRANSFERASES

- SEVERAL FAMILIES: alfa, mu, pi, theta, sigma, zeta, omega, beta, phi (incl. ≥1)

Monomers:

Form dimers:
Within a family homo- and heterodimers
Human soluble GSTs

<table>
<thead>
<tr>
<th>Gene family</th>
<th>alpha</th>
<th>mu</th>
<th>theta</th>
<th>pi</th>
<th>zeta</th>
<th>sigma</th>
<th>kappa</th>
<th>omega</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genes</td>
<td>A1-A5</td>
<td>M1-M5</td>
<td>T1,T2</td>
<td>P1</td>
<td>Z1</td>
<td>S1</td>
<td>K1</td>
<td>O1</td>
</tr>
<tr>
<td>Chromosome</td>
<td>6p</td>
<td>1p</td>
<td>22q</td>
<td>11q</td>
<td>14q</td>
<td>4q</td>
<td>7q</td>
<td>10q</td>
</tr>
</tbody>
</table>

Enzyme Nomenclature: GSTP1-1 or GSTA1-2.
Tissue-distribution (human)

1, Standard
2, brain
3, heart
4, kidney
5, liver
6, lung
7, pancreas
8, prostate
9, muscle
10, intestine
11, spleen
12, testis

Sherratt et al., Biochem. J. (1997) 326, 837
Evolutionary aspects

Thioredoxin fold

Domain addition

GST Theta ➔ Cytosolic GSTs

Alpha, Mu, Pi, Sigma, Beta Zeta, Omega, Phi, Tau, Delta, etc

Mitochondrial GST Kappa

Domain insertion
DIMER-STRUCTURE

H-site

G-site
MGST1 TRIMER 3-D model (3.2 Å)
Tissue distribution:

**NARROW**

MPGES1

**WIDE**

MGST1 

MGST2 

MGST3

5-Lipoxygenase activating protein

Leukotriene C4 synthase

**NARROW**

GSH-dep. oxido-reductase

Glutathione peroxidases

GST:s
GSTs

Mechanism and Substrate specificity
GSH binding
Making GSH more reactive

\[ \text{GSH} \rightarrow \text{GS}^- + \text{H}^+ \]

\( pK_a^{\text{GSH}} \) lowered from 9 to ≈ 6 in the enzyme

GS\(^-\) thiolate is 10\(^9\) times more reactive than the protonated thiol
(Thiolate/CDNB ≈ 5 M\(^{-1}\) s\(^{-1}\); Selenolate/CDNB ≈ 23 M\(^{-1}\) s\(^{-1}\))
GSH is bound in an Extended Conformation where all possible interactions are used.

- $\text{GS}^{-}$ thiolate
- Tyrosoyl-OH
An model second substrate and convenient assay

\[
\text{GSH} + \text{Cl} \text{NO}_2 \text{NO}_2 \xrightarrow{\text{GS}} \text{GS} \text{NO}_2 \text{NO}_2 + \text{HCl}
\]
New fluorogenic substrates
The H-site

(IN)

Cys

gama-Glu

Gly

(OUT)
Multiple Functions

BCNU (cytostatic)

Aflatoxin (carcinogen)

Atrazine (herbicide)
Reactive compounds are common in biology

- **Cyanobacteria:** microcystine

- **Mustard oil:** allylisothiocyanate
Reactive compounds are formed continuously in the cell

Lipid peroxidation gives rise to:

Hydroxyalkenals:

Hydroperoxides:
Enzymes that can reduce lipid hydroperoxide in the membrane

cGST/GPX1

PHGPX4

MGST1
Conjugate export and processing

- GSH conjugates are exported out of the cell by membrane transporters called MDR (multidrug resistance proteins)
- Conjugates are often processed to mercapturic acids before excretion in urine or bile
GSTs

• In REDOX regulation
GST protection

$\text{H}_2\text{O}_2$ is not a substrate for GTs.
GSTP catalyses protein S-glutathionylation (H$_2$O$_2$ challenge) Tyr 7, and Cys 47/101.
GSTP knockout leads to increased c-Jun signalling = increased proliferation
Therapeutic use

Telintra - binds to GSTP at the G-site

Multimeric JNK complex inhibited by GSTP

GSTP oligomer

Retains peroxidase activity

Cascade to MYELOPROLIFERATION

Tew KD, Biochem. Pharm. 73, 1257
GSTP & Prdx6 = GSH Peroxidase

Tew et al, FRBM 51, 299.
Table 2. GST knockout mice.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genetic background</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gsta3</td>
<td>C57BL/6</td>
<td>↑ AFB1-DNA adducts</td>
<td>Ilic et al. (2010)</td>
</tr>
<tr>
<td>Gsta4</td>
<td>129</td>
<td>↑ Sensitivity to paraquat</td>
<td>Engle et al. (2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ Sensitivity to CCl₄</td>
<td>Dwivedi et al. (2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ Protein carboxylation, mitochondrial dysfunction, and ROS</td>
<td>Curtis et al. (2010)</td>
</tr>
<tr>
<td>Gstm1</td>
<td>129xC57BL/6</td>
<td>↓ Activity toward DCNB</td>
<td>Arakawa et al. (2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>development of methaemoglobinemia</td>
<td>Fujimoto et al. (2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ Deficits in social behaviours</td>
<td>Yochum et al. (2010)</td>
</tr>
<tr>
<td>Gsto1</td>
<td>DBA/1lacI</td>
<td>Marginal change in arsenic sensitivity</td>
<td>Chowdhury et al. (2006)</td>
</tr>
<tr>
<td>Gstp1/p2</td>
<td>129xF1</td>
<td>↑ Skin tumorigenesis</td>
<td>Henderson et al. (1998b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ Lung tumorigenesis</td>
<td>Ritchie et al. (2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ Colon tumorigenesis</td>
<td>Ritchie et al. (2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ Acetaminophen hepatotoxicity; altered JNK regulation</td>
<td>Henderson et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>129xC57BL/6</td>
<td>↑ Myeloproliferation</td>
<td>Gate et al. (2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ Spontaneous tumours</td>
<td>Gate et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>C57BL/6</td>
<td>↑ Cisplatin nephrotoxicity</td>
<td>Townsend et al. (2009b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ Protein S-glutathionylation</td>
<td>Townsend et al. (2009a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ Cyclophosphamide-induced bladder toxicity</td>
<td>Conklin et al. (2009a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ Acetyl-choline-induced arterial relaxation</td>
<td>Conklin et al. (2009b)</td>
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<tr>
<td></td>
<td>SW</td>
<td>↑ MPTP sensitivity of dopaminergic neurons</td>
<td>Smeyne et al. (2007)</td>
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<tr>
<td>Gsts1</td>
<td>Unknown</td>
<td>↓ Allergic reactivity</td>
<td>Urade et al. (2004)</td>
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<tr>
<td></td>
<td>C57BL/6</td>
<td>↑ Severity and duration of delayed-type hypersensitivity reaction</td>
<td>Trivedi et al. (2006)</td>
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<tr>
<td>Gstt1</td>
<td>C57BL/6</td>
<td>↓ Activity toward GSTT substrates</td>
<td>Fujimoto et al. (2007)</td>
</tr>
<tr>
<td>Gstz1</td>
<td>129 or C57BL/6</td>
<td>↑ Accumulation of tyrosine metabolites; dietary phenylalanine lethal</td>
<td>Fernandez-Canon et al. (2002)</td>
</tr>
<tr>
<td></td>
<td>Balb/c</td>
<td>↑ Oxidative stress; enlarged liver, kidneys and spenic atrophy; dietary</td>
<td>Lim et al. (2004)</td>
</tr>
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<td></td>
<td></td>
<td>phenylalanine lethal</td>
<td></td>
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</table>
GSTs are induced via Nrf2 regulation (and several other pathways)
MGST1 activation by reactive compounds or redox

One of the intruders bites the microsomal glutathione transferase in the heel...

Which is seized by rage and multiplies its efficiency.
MGST1 is activated by sulfhydryl reagents

SH - SH - SH + NEM → SNEM - SNEM - SNEM

2 µmol/min mg 30 µmol/min mg

At the single cysteine-49 of the homo-trimer (subunits Mr ≈ 17 kDa)
Activation does occur under toxic and oxidative stress in vivo!
Activation of MGST1 by S-thiolation

In vitro by GSSG

GSSG/GSH ratio = 50 at half maximal activation

In vivo by hydroperoxide

Sies et al, ABB 322, 288
P-SH + DTNB = P-S-S-NB

P-S-S-NB + GSH = PS-SG
PS-SG + GSH = P-SH + GSSG

P-S-H + DTT = P-SH + DTT (ox)

Fig. 1. Glutathione S-transferase activity in microsomes activated with 0.1 mM DTNB (●) subsequently treated with 5 mM GSH (□) or 0.3 mM (▲) and 5 mM dithioerythritol (△). DTNB-dithioerythritol treated microsomes could be activated again with 3 mM DTNB (+). Control microsomes incubated without DTNB or with 5 mM dithioerythritol only (○).
Activation increases the rate of Thiolate Anion formation

\[ \text{GSH} + E \rightarrow E\cdot\text{GS}^- + H^+ \]

Activation increases the rate of thiolate anion formation (not the chemical step)
Capacity and throughput

**CAPACITY:**
0.2 mM Glutathione transferase in liver + 5 mM GSH = 25 turnovers empties the liver of GSH (e.g. paracetamol overdose) Theoretically this can happen in less than a second!!!!!
Humans excrete 0.1 mmol glutathione conjugates per day = Equal to one turnover per enzyme every second day
CONCLUSION

Glutathione dependent protection has to be highly abundant and efficient to serve as an interception system.

In all cellular compartments.