Glutathione – the very basics 1

- full name: \( \gamma \)-L-glutamyl-L-cysteinyl-glycine

- GSH (glutathione)
  - M.W. = 370.3 g mol\(^{-1}\)
  - often improperly called reduced glutathione

- GSSG (glutathione disulfide)
  - M.W. = 612.6 g mol\(^{-1}\)
  - often improperly called oxidized glutathione
Glutathione – the very basics 2

- Glutathione is the most abundant non-protein thiol in the cell, often found in the millimolar range (1 to 10 mM, depending on cell type).

- Glutathione is a tri-peptide that has a gamma linkage between the first two amino acids (instead of the typical alpha linkage), which resists degradation by intracellular peptidases.
Glutathione – the very basics 3

Projection Drawing

\[
\begin{align*}
&\text{O} = \text{C} = \text{O} \\
&\text{H} \quad \text{H}_2 \quad \text{C} \quad \text{C} \quad \text{C} \quad \text{N} \quad \text{C} \quad \text{C} \\
&\text{NH}_3^+ \quad \text{H} \quad \text{H}_2 \quad \text{C} \quad \text{O} \\
&\text{O} \quad \text{O} \\
&\text{S} \quad \text{H} \quad \text{C} \quad \text{C} \quad \text{O} = \text{O} \quad \text{O} \\
\end{align*}
\]
Glutathione – uses and recycling

- GSH is consumed in many enzymatic and non-enzymatic reactions, where it serves as a source of reducing equivalents.

- GSH is used by glutathione peroxidases, and can exchange with mixed disulfides to yield GSSG.

- GSSG, via the action of glutathione reductase, regenerates GSH at the expense of NADPH. This is a redox-cycling mechanism to prevent GSH loss.
Glutathione - redox cycling

NADPH

GSH

H₂O₂

LOOH

GSSG

PSSG

PSSP

H₂O

LOH

PSH

Glutathione Reductase

Glutathione Peroxidase

NADP⁺
Glutathione – uses and losses

- The glutathione S-transferases (GST) serve a protective role by adding GSH to a molecule, targeting it for export from the cell. In this reaction, GSH is ‘lost’ from the cell and must be replaced.

- Replacement is by either the salvage pathway, or more prominently, by *de novo* synthesis.
de novo synthesis - 1

- Enzymatic synthesis occurs from the component amino acids (glutamate, cysteine, and glycine) via the sequential action of two ATP-dependent, cytosolic enzymes.

- The rate of de novo synthesis is responsive to environmental factors; it is regulated at many levels, and is the topic of another lesson.
The first enzyme of the two enzymes, according to IUBMB nomenclature, is properly called glutamate-cysteine ligase (GCL, E.C. 6.3.2.2).

Formerly referred to as $\gamma$-glutamylcysteine synthetase (GCS).
**de novo synthesis - 3**

- The GCL holoenzyme is a heterodimer of ~104 kDa. It can be separated under non-denaturing conditions to yield two subunits.

- Increased GCL activity usually results from increased content of the GCL subunits, usually due to increased gene expression for the subunits.

- The GCL holoenzyme can also be regulated by S-nitrosation, phosphorylation, and oxidation.
The ‘heavy’ subunit (~73 kDa) has the catalytic activity, and is the site of GSH feedback inhibition.

The ‘light’ (~28 kDa), or modulatory subunit alters, or regulates, the activity of the holoenzyme by reducing the $K_m$ for glutamate and elevating the $K_i$ for GSH, thereby making the enzyme more efficient and less sensitive to feedback inhibition.
de novo synthesis - 5

γ-glutamylcysteine → Cysteine

Glutathione (GSH) synthesis involves the following reactions:

1. Formation of γ-glutamylcysteine from glutamate and cysteine catalyzed by GCL (γ-glutamylcysteine ligase).
2. Formation of glutathione by the addition of glutamate to γ-glutamylcysteine.

Reagents used: ADP and ATP.
The second enzyme in *de novo* synthesis is named, according to IUBMB, glutathione synthase (GS, E.C. 6.3.2.3), formerly called glutathione synthetase.

This enzyme is a homodimer of ~118 kDa.

In an ATP-dependent manner, GS adds glycine to \(\gamma\)-glutamylcysteine to form GSH.
de novo synthesis - 7

\[
\gamma\text{-glutamylcysteinylglycine} \rightarrow \gamma\text{-glutamylcysteine} \rightarrow \text{GSH synthase} \rightarrow \text{GSH (GSH)}
\]

\[
\text{Glycine} \quad \text{ADP} \quad \text{ATP}
\]
Glutathione – breakdown 1

- The linkage of glutamate to cysteine via the gamma carbon makes GSH refractory to standard proteases. Only one enzyme is known to breakdown GSH.

- IUBMB officially named this enzyme \( \gamma \)-glutamyltransferase (GGT, E.C. 2.3.2.2). Sometimes called \( \gamma \)-glutamyltranspeptidase.
Glutathione – breakdown 2

- GGT is an ectoenzyme (it exists functionally on the outside of cells). It functions in an ATP-dependent manner to cleave the gamma linkage between glutamate and cysteine to transfer the glutamyl residue to another amino acid, often cystine (cysteine disulfide). This reaction also generates cysteinylglycine.

- Cysteinylglycine is cleaved by an external dipeptidase to yield free cysteine and glycine.
The dipeptidase products cysteine and glycine re-enter the cell by specific amino acids transporters. This is critical, as cysteine is often a limiting amino acid in \textit{de novo} GSH biosynthesis.

The \( \gamma \)-glutamyl-amino acid couple also re-enters the cell by an amino acid transporter. Once in the cell the amino acid and the \( \gamma \)-glutamyl moiety are separated. The carrier amino acid is often cystine, and this process has been hypothesized to be important in the re-cycling of cysteine (via subsequent reduction of cystine).

The \( \gamma \)-glutamyl residue forms 5-oxoproline, which by the action of 5-oxoprolinase, yields glutamate.
The Glutathione Cycle - 1

- The processes of *de novo* GSH biosynthesis by GCL and GS;
- its use in protective reactions and subsequent export from the cell;
- its breakdown by GGT; and
- the re-entry of the amino acids into the cell form a cycle, as originally proposed by Meister’s group a quarter of a century ago.

The Glutathione Cycle – 2 – lesson summary

Glutathione - Synthesis

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Dickinson et al. 19