Glutathione – Synthesis

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Glutathione – the very basics 1

- full name: γ-L-glutamyl-L-cysteinyl-glycine
- GSH (glutathione)
  - M.W. = 307.3 g mol^{-1}
  - often improperly called reduced glutathione
- GSSG (glutathione disulfide)
  - M.W. = 612.6 g mol^{-1}
  - often improperly called oxidized glutathione

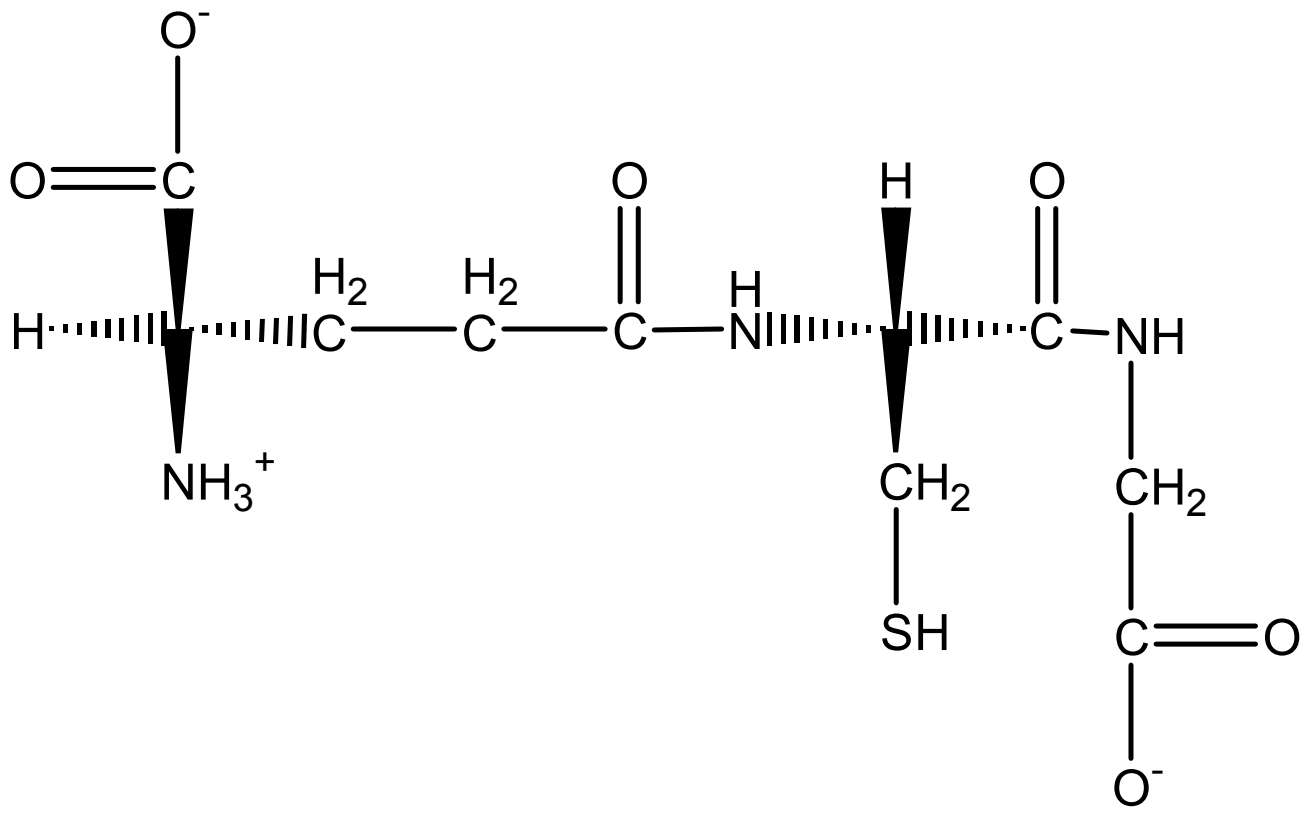
Glutathione – the very basics 2

- 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

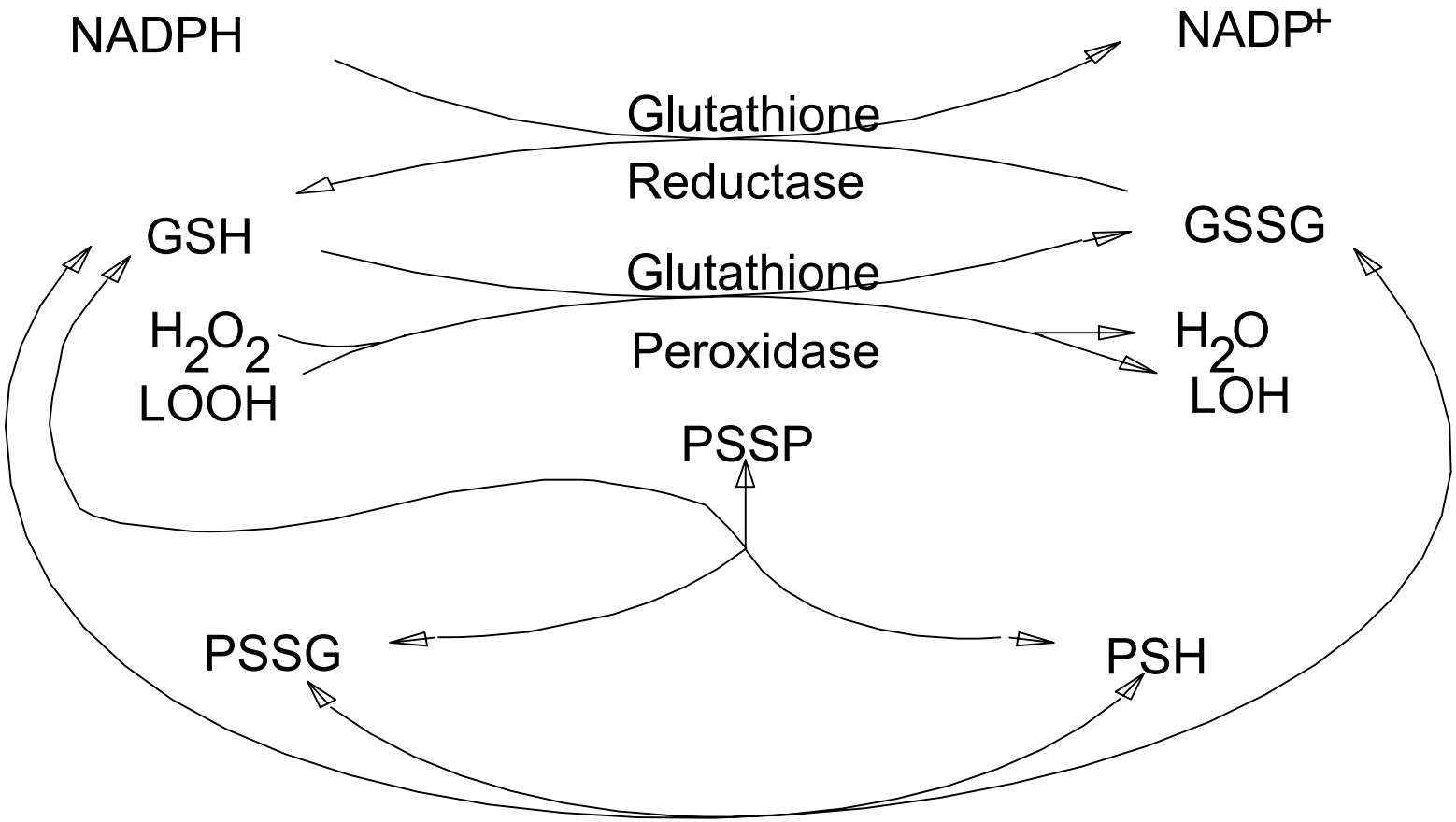
Projection Drawing



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



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.

de novo synthesis - 2

- 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 γ-glutamylcysteine synthetase (GCS).

de novo synthesis - 3

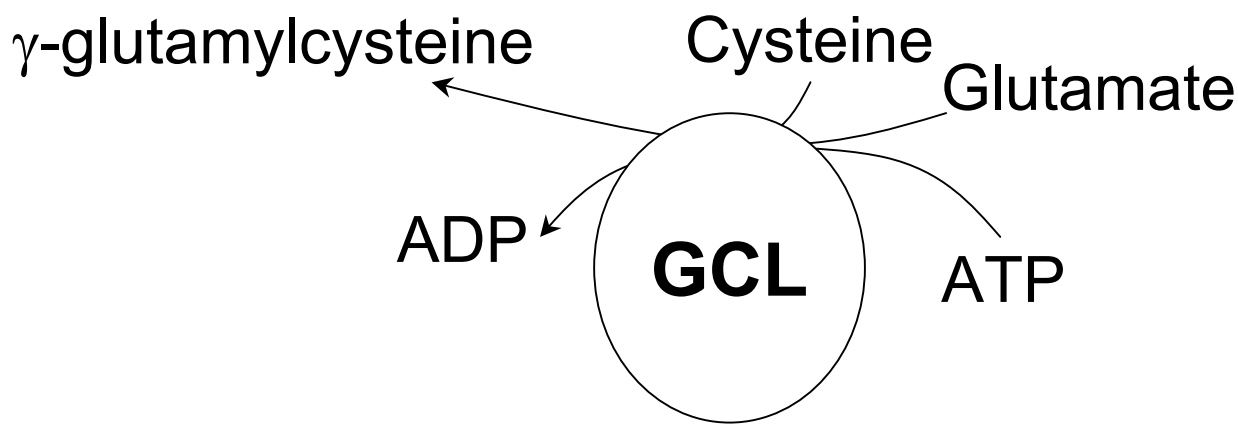
- The GCL holoenzyme is a heterodimer of ~104 kDa. It can be separated under non-denaturing conditions to yield two subunits.
  - Seeling *et al*., *J Biol Chem* **259:** 9345; 1984.
- Increased GCL activity usually results from increased content of the GCL subunits, usually due to increased gene expression for the subunits.
- The GCL holoenzyme can also be regulated by S-nitrosation, phosphorylation, and oxidation.
  - Griffith, *Free Radic Biol Med*, **27:** 922; 1999.
  - Sun *et al.*, *Biochem J*, **320:** 321; 1996.
  - Ochi, *Arch Toxicol*, **70:** 96; 1995.

de novo synthesis - 4

- The ‘heavy’ subunit (~73 kDa) has the catalytic activity, and is the site of GSH feedback inhibition.
  - Seeling et al., *J Biol Chem* **259:** 9345; 1984.

- 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.
  - Tu and Anders, *Arch Biochem Biophys* **354:** 247; 1998.
  - Choi *et al.*, *J Biol Chem* **275:** 3696; 2000.

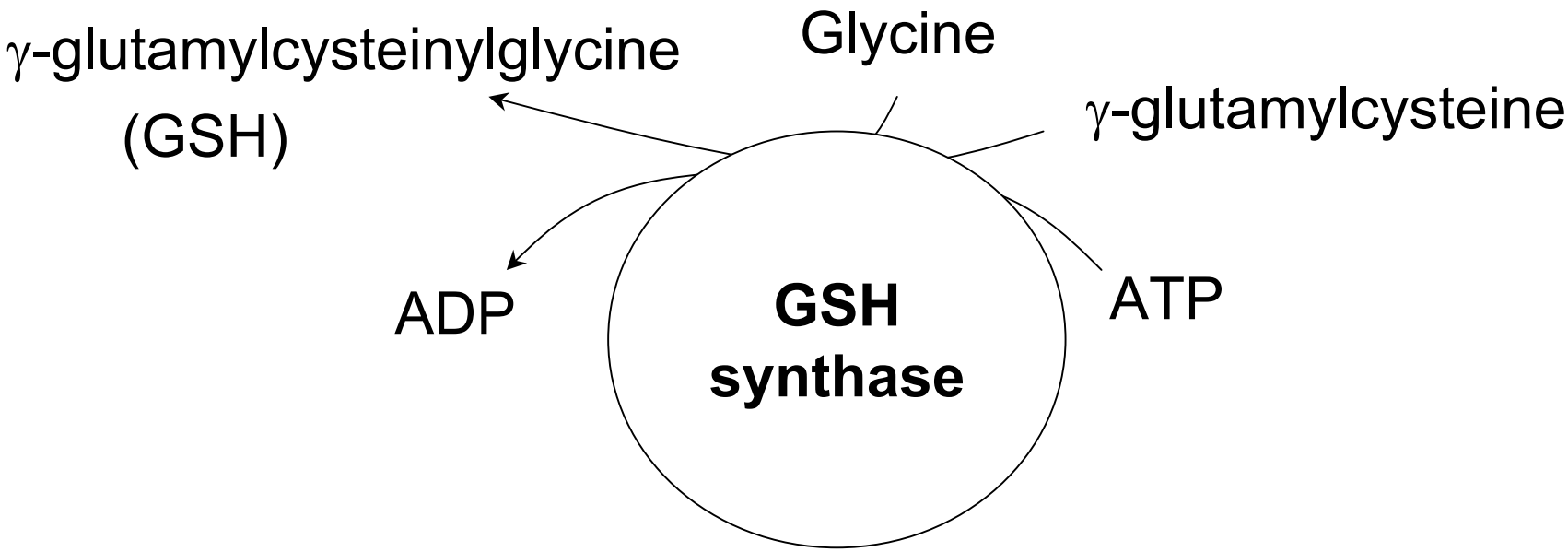
de novo synthesis - 5



de novo synthesis - 6

- 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 γ-glutamylcysteine to form GSH.

de novo synthesis - 7



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 γ-glutamyltransferase (GGT, E.C. 2.3.2.2). Sometimes called γ-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.

Glutathione – breakdown 3

- 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 *de novo* GSH biosynthesis.

- The γ-glutamyl-amino acid couple also re-enters the cell by an amino acid transporter. Once in the cell the amino acid and the γ-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 γ-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.

  – Griffith *et al.*, *PNAS,* **75:** 5405; 1978.

The Glutathione Cycle – 2 – lesson summary