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Glutathione: A multifunctional tripeptide

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Abbreviations:

AscH	Ascorbate
BCNU	1,3-*bis*-Chloroethyl-1-Nitrosourea
BSO	Buthionine sulfoximine
DTNB	5,5' -Dithio- *bis*-(2-nitrobenzoic acid)
GPx	Glutathione peroxidase
GR	Glutathione disulfide reductase
Grx	Glutaredoxin
GSH	Glutathione
GSSG	Glutathione disulfide
HPLC	High performance liquid chromatography
LOOH	Lipid hydroperoxides
PhGPx	Phospholipid hydroperoxide glutathione peroxidase
ROS	Reactive oxygen species
TNB	5-thio-2-nitrobezoic acid
SOD	Superoxide dismutase
Trx	Thioredoxin
Vit	Vitamin

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1. Abstract

Glutathione is a tripeptide found in almost all mammalian cells. One of the residues of the tripeptide, cysteine, contains a sulfhydryl (-SH) group. The –SH group is key to nearly all reactions of glutathione (GSH). GSH provides reducing equivalents to the cell under conditions of oxidative stress and in the process, is oxidized to glutathione disulfide (GSSG). In this way, glutathione maintains a reducing intracellular environment and protects cells from oxidative damage. Thus, GSH is regarded as a small antioxidant molecule. GSH also contributes in cell signaling and acts as a coenzyme. Methods have been developed to quantify both GSH and GSSG and estimate the capacity of GSH as an antioxidant, by using the Nernst equation. This report summarizes the synthesis, quantification, antioxidant properties and biological significance of glutathione.

2. Introduction

A reducing environment is essential for cell survival. Nature has therefore devised various ways of overcoming oxidative stress to the cell. Three primary ways of maintaining the intracellular reducing environment are [1]:

- Antioxidant enzymes (*e.g.* SOD, Catalase, GPx)
- Antioxidant molecules (*e.g.* Vit C, GSH, Trx.)
- Metal Chelators (*e.g.* Transferrin for Iron, Ceruloplasmin for Copper)

Out of all the small antioxidant molecules present in the cell, the glutathione system is one of the most important redox buffers [1]. GSH is a tripeptide of glutamate, cysteine and glycine residues (molecular weight 307.4 g mol^{-1}) (**Figure 1**).

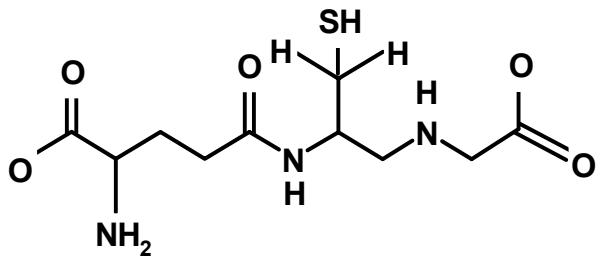

Figure 1: Chemical structure of GSH. The systematic name of the molecule is glutamyl-cysteinylglycine. The name comes from the three constituent amino acids in the tripeptide.

The important reaction center of GSH is the –SH group present on the Cys residue. This allows GSH to act as a sulfhydryl buffer in the cell, which maintains the Cys residues of hemoglobin *etc*. in the reduced state. In this process, GSH is oxidized to GSSG [2].

$$2GSH \longrightarrow GSSG + 2e^- + 2H^+ \quad (1)$$

However, as soon as GSSG is formed in the cell, it is rapidly reduced back to GSH by the enzyme glutathione disulfide reductase (GR), which uses NADPH as a cofactor. Thus, the oxidation of GSH and subsequent reduction of GSSG form a cyclic process. GSH is also used by

the peroxidase family of enzymes like GPx to scavenge H_2O_2 and form water (**Figure 2**). Since the conversion of GSSG to GSH is very fast, at a given instant, GSSG concentration in the cell is much lower than GSH. Typically, GSH concentration in cells range from 0.1-10 mM while GSSG is 5-15 μM, depending on the redox environment of the cell [2].

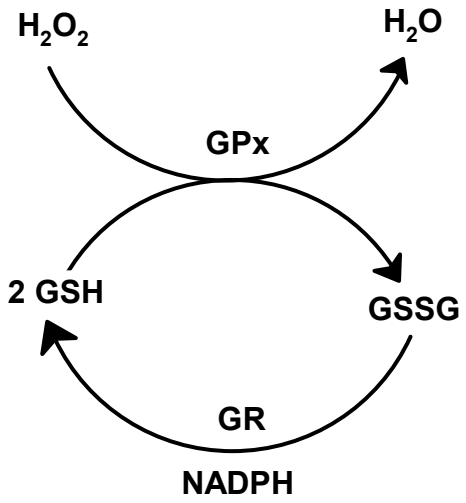

Figure 2: Glutathione system needs a continuous supply of NADPH to maintain the reducing environment *via* GSH and GR. The enzyme GPx consumes GSH to reduce hydroperoxides.

3. Synthesis of GSH

GSH is not a constituent of the human diet. Thus, the only source of GSH in the body is the intracellular synthesis by the enzymes γ–glutamylcysteine synthetase (γ–GCS, EC 6.3.2.2) and

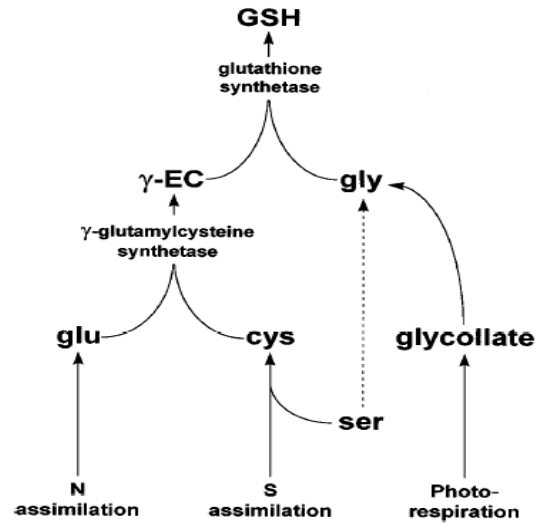

Figure 3: Synthesis of GSH from glu, cys and gly. The two important enzymes involved are γ-glutamylcysteine synthetase and GSH synthetase. Both these enzymes are ATP dependent. Thus GSH synthesis requires energy.

GSH synthetase (GS,EC 6.3.2.3) [3]. Synthesis of GSH from metabolites of the body is shown in **Figure 3** [4]. The enzymes γ-glutamyl transpeptidase,

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γ-glutamyl cyclotransferase and 5-oxoprolinase, constitute the γ-glutamyl cycle for GSH synthesis. GSH acts as a feedback inhibitor of the cycle because it can competitively inhibit γ–GCS [3].

4. Measurement of glutathione

Various biochemical assays have been used to measure the intracellular and extracellular glutathione concentrations. Some of these methods directly measure both GSH and GSSG, while others measure only [GSH] and obtain the concentration of GSSG from the stoichiometric equation. These concentrations can then be plugged into the well-known Nernst equation (see below) to obtain the redox potential for the GSSG/2GSH couple [5].

$\mathbf{E_{cell} = \{-252-(RT/nF)\ ln\ ([GSH]^2/[GSSG])\}\ mV}$, where $E^{\circ\prime}_{cell}$ (pH 7.2) = -252 mV*

Some of the most common techniques used to measure glutathione concentrations are summarized below.

4.1 The recycling assay

The GSH-GSSG recycling assay was first described by Tietze back in 1969 [6], and later adapted to a 96-well microtiter plate by Baker *et. al.* [2] . Since none of the reactants or products of the glutathione system (see Figure 2) have a high extinction coefficient, a dye 5,5' -Dithio- *bis*-(2-nitrobenzoic acid) (DTNB) is used. DTNB oxidizes GSH, forming a colored compound called 5-thio-2-nitrobezoic acid (TNB) (ε_{412} = 13,000 $M^{-1}cm^{-1}$) [6]. GSSG concentration is measured indirectly by first blocking all the intracellular GSH. Next, GSSG is enzymatically reduced to

GSH and this GSH reacts with DTNB. Rate of TNB accumulation is equated to the GSH or GSSG concentrations (**Figure 4**).

* If the intracellular pH is assumed 7.2, the E^{o}_{cell} at 37ºC turns out to be –252 mV using the equation: $E^{o'}_{pH} = E^{o'}_{(7.0)} + [(pH - 7) \times (\Delta E / \Delta pH)]$ mV [5].

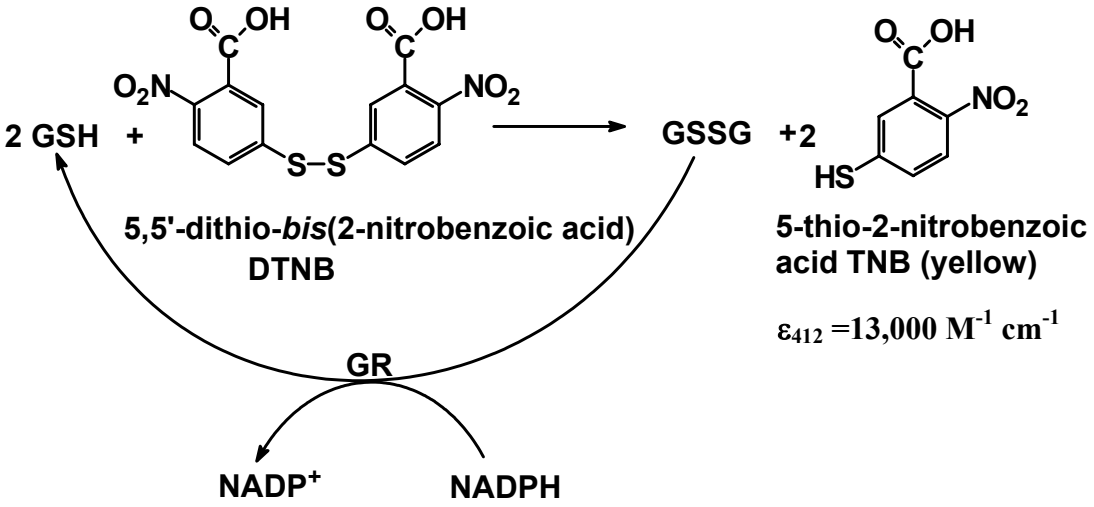

Figure 4: The GSH-GSSG recycling assay in the presence of DTNB. Two molecules of GSH oxidize to form one molecule of GSSG and in the process reduce the indicator molecule DTNB to the yellow colored TNB. Since the enzyme and NADPH are present in excess the reaction continues to recycle and TNB accumulates. The rate of TNB production can be related to the total GSH present in the system. (Figure by Freya Schafer).

4.2 HPLC detection of Glutathione

High performance liquid chromatography (HPLC) has been used to detect GSH, GSSG, cysteine, homocysteine and other redox buffers in cells. HPLC method has a lower detection limit than the recycling assay [7]. Usually a methanol/water solvent system and a UV detector are used in the HPLC method. The samples are derivatized with the fluorescent S-Dansyl chloride before analysis [7]. However, by this method only GSH concentration can be measured. Amount of GSSG present has to be deduced from [GSH]. The HPLC method can also use an electrochemical detector. The advantage of an electrochemical detector is that both oxidized and

reduced forms of glutathione can be measured simultaneously. However, the disadvantage of this method is that the electrode response can drift with time and so the standards need to be calibrated more often [8].

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5. Glutathione as an antioxidant

GSH is a strong reducing agent owing to the presence of –SH group. It can donate electrons to species and in the process becomes oxidized (redox reaction). The antioxidant properties of GSH are due to this electron-donating nature of GSH. Some examples of reactions of GSH with radicals and hydroperoxides are shown below [9]:

$GSH + HO^{\bullet} \longrightarrow GS^{\bullet} + H_2O$	(Hydroxyl radical quenching)	**(2)**
$GSH + R^{\bullet} \longrightarrow GS^{\bullet} + RH$	(Secondary radical quenching)	**(3)**
$GSH + DNA^{\bullet} \longrightarrow GS^{\bullet} + DNA$	(DNA radical quenching)	**(4)**
$2GSH + LOOH \xrightarrow{GPx} GSSG + LOH + H_2O$	(Lipid hydroperoxide reduction)	**(5)**
$2GSH + PSSX \xrightarrow{Grx} GSSG + P(SH)_2X$	(Maintaining protein sulfhydryls)	**(6)**
$2GSH + DHA \longrightarrow GSSG + AscH^-$	(Recycling of Vit C)	**(7)**

Thus, glutathione in the reduced form can act as a radical scavenger. Two $GS^{\bullet}$ species, **(Reaction 2-4**) can form the GSSG molecule, which can be recycled back to GSH by GR. These reactions form the basis of the antioxidant properties of GSH. Most of these reactions are catalyzed by the glutathione peroxidase family of enzymes (*e.g.* GPx, PhGPx *etc*.). The rate of catalysis by these enzymes is independent of the nature of the hydroperoxide present [10].

Glutathione status in the cell can be used as an indicator of the redox status of the cell. Under conditions of oxidative stress, GSH is consumed in scavenging free radicals and removing ROS. Thus, GSH levels decrease and GSSG levels increase. The ratio of [GSH]/[GSSG] and the reduction potential (in millivolts) are two ways to express the intracellular redox environment of the cell. However, it is important to recognize that the GSH system is only one of the redox

buffers of the cell. The complete representation of the intracellular redox environment should take into account other buffers such as Trx, Cys *etc.* The antioxidant reactions of glutathione are summarized in **Figure 5** [11].

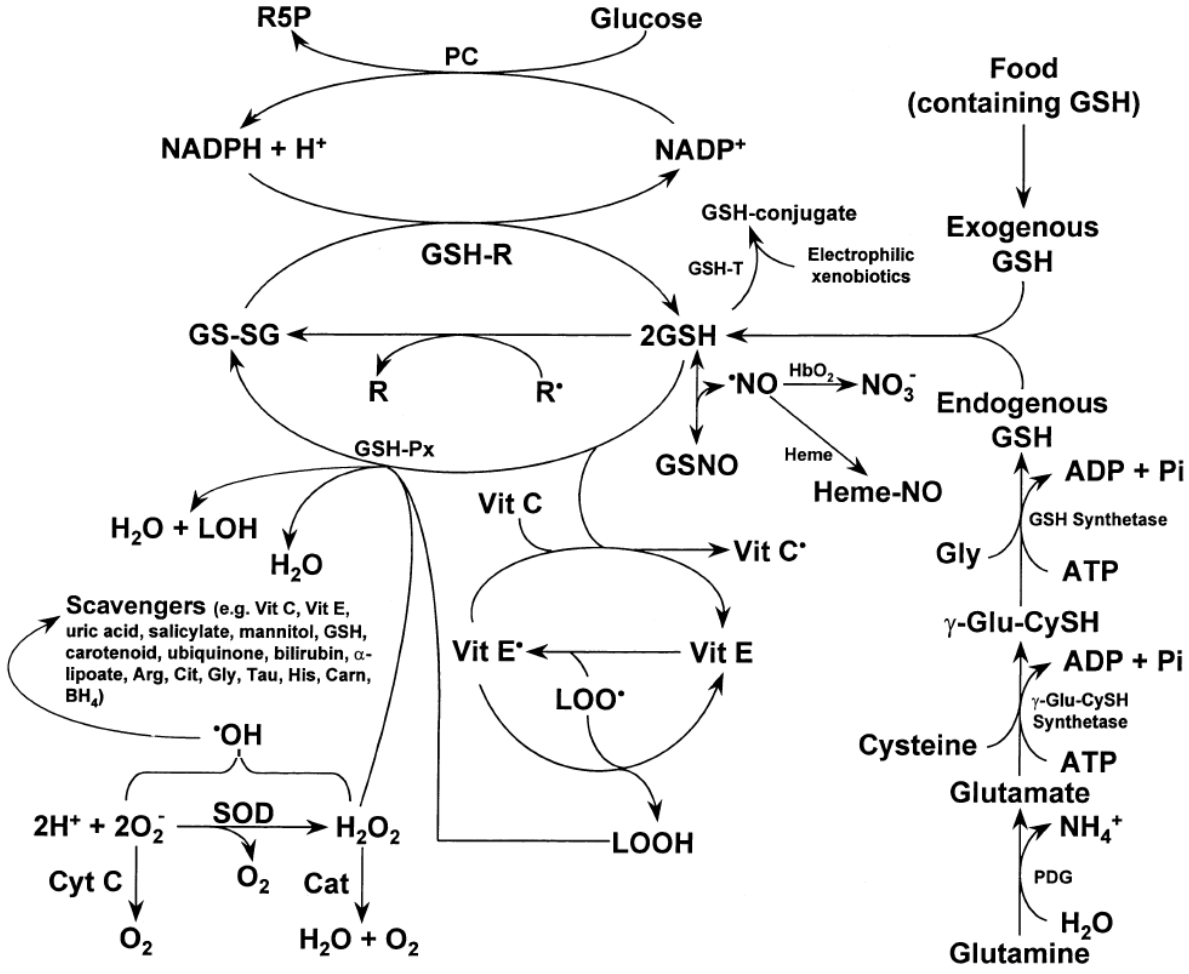


Figure 5: Glutathione plays a central role in scavenging various ROS. It is consumed by the peroxidase family of enzymes to remove H_2O_2 produced by SOD [11].

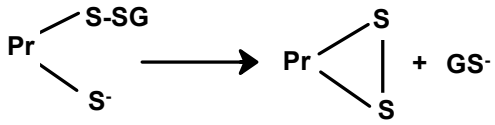
7. Some biologically significant reactions of glutathione

7.1 Glutathionylation of proteins

Proteins with –SH group can also respond to oxidative stress. This response is sensitive to GSH/GSSG ratio [12]. When GSH/GSSG ratio is decreased, protein thiols form mixed disulfides with glutathione (**Reaction 8**).

(8)

These mixed disulfide can be maintained as the glutathionylated protein, or it can react with an adjacent thiol (if available), to form an intra-protein disulfide (**Reaction 9**)



(9)

When the oxidative stress subsides, GR returns the GSH/GSSG ratio to normal, enabling the reversal of protein thiol redox changes. Protein disulfides can also be reduced by enzymes thioredoxin-2 and glutatredoxin-2 [13].

7.2 Autooxidation of GSH

Autooxidation of glutathione leads to a decrease in molecular oxygen concentration. Metal ions, especially copper, appear to play an important role in the process. It has been shown that autooxidation of thiols in general, is associated with superoxide production [13]. Thus, these reactions can cause ROS production in the cell and lead to oxidative stress. However, most of these studies have been done in chemical settings and their real biological consequences are not well understood. One of the mechanisms proposed for autooxidation of GSH leading to peroxide production is shown in **Figure 7.**

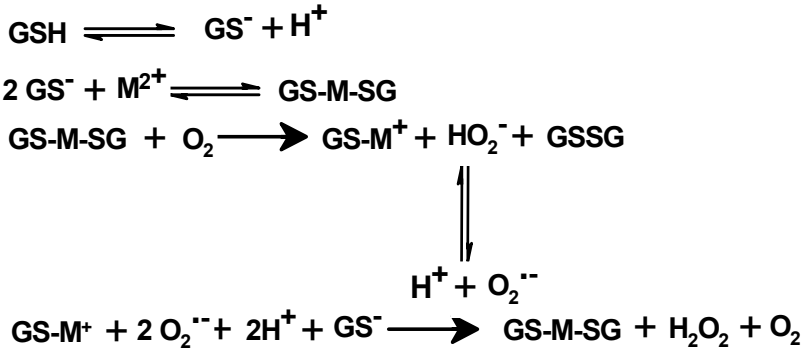


Figure 7: A proposed mechanism for GSH autooxidation. Production of H_2O_2 and superoxide radical, combined with the depletion of GSH levels can lead to oxidative stress [13].

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8. Significance of glutathione in human life [14]

Glutathione appears to be of utmost importance to the intracellular reducing environment. GSH deficiency has been associated with several disease states *e.g.* myocardial infarction, Parkinsons's disease, uncelrative colitis, ischemia-reperfusion injury, *etc*. Efforts have been made to design GSH therapy to overcome genetic defects in GSH synthesis. Reagents like BSO and BCNU have been used to block GSH synthesis in drug-resistant tumors that overproduce GSH. Due to its importance in various cellular functions, a constant effort is being made to understand this mutli-functional tripeptide.

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