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Glutathione, a ubiquitous thiol

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Abbreviations:

ATP, Adenosine triphosphate; GPx, Glutathione peroxidase; $GS^{\bullet}$, Glutathiyl radical; GS^{-}, Glutathione anion; GSH, Glutathione; GSSG, Glutathione disulfide; NADPH, Nicotinamide adenine dinucleotide phosphate; NMR, Nuclear magnetic resonance; PhGPx, Phospholipid hydroxide glutathione peroxidase; ROH, Alcohol; ROOH, Hydroperoxides.

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Abstract

Glutathione, the most abundant nonprotein thiol compound in mammalian cells, is biosynthesized from amino acid precursors. The thiol group dominates its chemical reactivity, which includes one-electron and two-electron oxidation-reduction processes. Glutathione acts as a cosubstrate for glutathione peroxidase and dehydroascorbate reductase, and as a product of glutathione reductase-catalyzed reactions. Glutathione also behaves as a direct free radical scavenger. This paper will review the general chemical and biochemical properties of GSH, its biosynthesis and degradation and its biological functions.

Introduction

Glutathione (L-γ-glutamyl-L-cysteinylglycine; GSH), a widely distributed tripeptide (Figure 1), is the major nonprotein thiol/sulfhydryl compound in mammalian cells. It has been estimated that more than 90% of the nonprotein sulfur of cells is in the form of GSH [1]. GSH occurs in virtually all animal cells, often in relatively high concentrations (0.1-10 mM) [1]. Most of free glutathione is in the reduced thiol form (GSH) rather than its disulfide from (GSSG) [2]. GSSG is derived from GSH by oxidation of the thiol group on the cysteine residue. Nevertheless, up to one-third of the total cellular glutathione may be present as "mixed" disulfides with other compounds that contain thiol groups, such as cysteine, coenzyme A, and the cysteine residues of proteins [3].

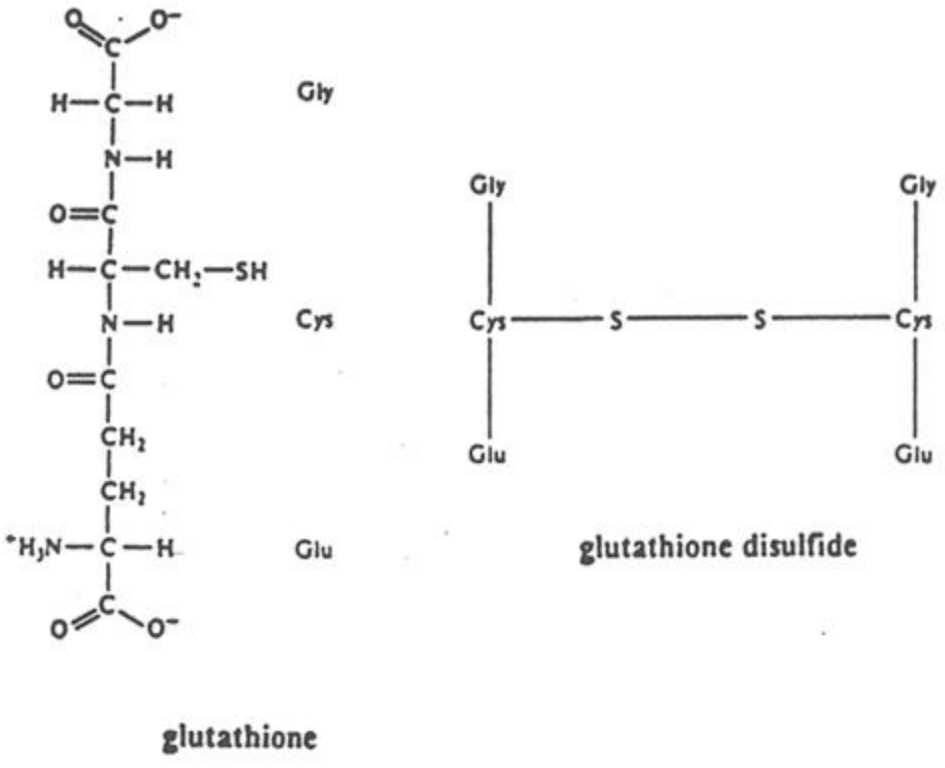

Figure 1. Structures of glutathione and glutathione disulfide [1].

GSH participates in a variety of detoxification, transport, and metabolic processes. The antioxidant functions of GSH are closely associated with its role in providing cells with its reducing milieu. The depletion of GSH has been speculated to be an important contributing factor to some serious human diseases, such as chronic renal failure, malignant disorders, diabetes, alcoholism, Parkinson's disease and cataract formation [4]. All these diseases are directly or indirectly associated with oxidative stress. More recently, GSH and thiol redox status has been demonstrated to regulate gene transcription involved in the pathogenesis of cancer, AIDS, diabetes, and atherosclerosis [5].

This paper will discuss the general chemical and biochemical properties of GSH, its biosynthesis and degradation and its biological functions.

Chemical Properties of Glutathione

The structures of GSH and its disulfide, GSSG, are shown in Figure 1. GSH has two peptide bonds, two carboxylic acid groups, one amino group, and one thiol group. The high number of hydrophilic functional groups combined with a low molecular weight allows GSH to dissolve easily in polar solvents, such as water, dilute ethanol, liquid ammonia, or dimethylformamide [1].

Of the chemically reactive groups present in GSH, the most important active group with respect to its biological and biochemical activity is the thiol group. When GSH is oxidized to form GSSG, its antioxidant functions disappear. Taking advantage of the substantial increase of ultraviolet absorption by RS^- in the region of 225 nm, Benesch and coworkers have determined the thiol pKa for GSH as 9.2 [6], which was later confirmed by NMR studies [7]. The acidity of the thiol group makes GSH react mostly

via its anion (RS^-) in an aqueous environment. Reaction rate constants increase with the increasing of pH value.

Biosynthesis and Degradation

GSH is synthesized from L-glutamate, L-cysteine, and L-glycine by the consecutive action of γ-glutamylcysteine synthetase and GSH synthetase in two ATP-dependent reactions [4], as shown in Figure 2. The first reaction is the rate-limiting step and is effectively inhibited by GSH feedback. However, when GSH is consumed, feedback inhibition is lost and the availability of L-cysteine as a precursor can become the rate-limiting factor [8]. The second reaction is not subject to negative feedback by GSH.

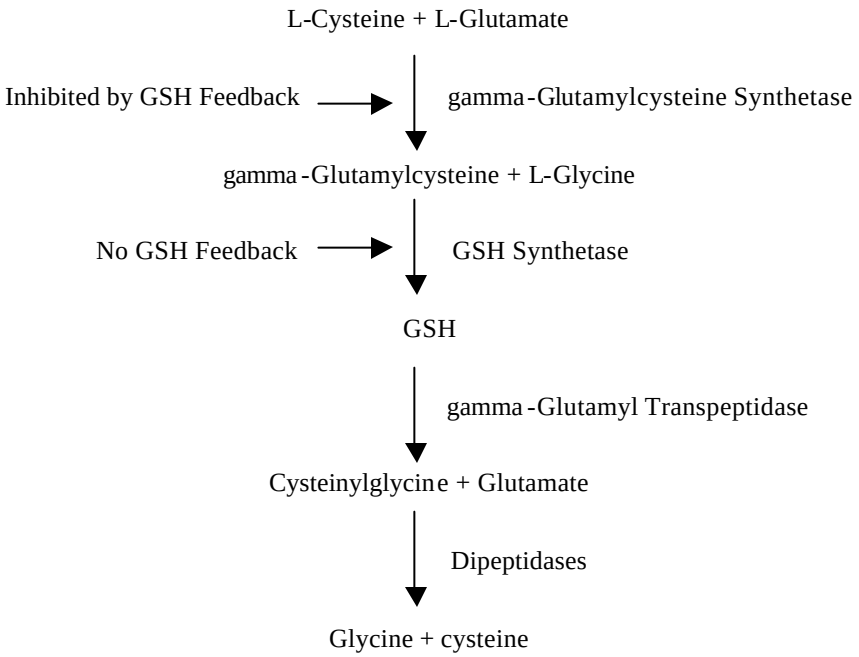

Figure 2. Biosynthesis and degradation of GSH [4].

Once GSH is produced, it will either function by itself or be degraded to participate in other metabolic pathways such as the γ-glutamyl cycle that can supply amino acid precursors for GSH synthesis [4]. The enzymes involved in GSH degradation are γ-glutamyl transpeptidase and dipeptidases (Figure 2).

Basic Oxidation-reduction Reactions

In general, thiols aare reducing agents as they can easily donate a hydrogen atom. Therefore, they are very reactive toward free oxidizzing radicals, including most carbon-, oxygen-, and nitrogen-centered radicals. The hydrogen atom abstraction is one of the most important aspects of GSH reactivity considering its function as an antioxidant.

1. One-electron processes

GSH can undergo oxidation reactions with free radicals ($R^{\bullet}$) through a one-electron process, in which GSH is oxidized to form a sulfur-centered glutathiyl radical ($GS^{\bullet}$) [3], as shown in Reaction 1.

$$GSH + R^{\bullet} \rightarrow GS^{\bullet} + RH \qquad \text{(Reaction 1)}$$

Sulfur-centered glutathiyl radicals can also be formed through photoionization of glutathione anion (Reaction 2) or through electron transfer to a metal ion (Reaction 3) [9].

$$GS^{-} \rightarrow GS^{\bullet} + e^{-} \qquad \text{(Reaction 2)}$$

$$GS^{-} + M^{n+} \rightarrow GS^{\bullet} + M^{(n-1)+} \qquad \text{(Reaction 3)}$$

2. Two-electron processes

Glutathione anion can be oxidized to produce glutathione cation (GS^+, which is usually written as GSOH), a sulfenic acid through two-electron oxidation (Reaction 4) [9]. On the other hand, glutathione anion and cation can be converted into glutathione disulfide via one-electron oxidation-reduction (Reaction 5) [9].

$$GS^- \rightarrow GSOH \qquad \text{(Reaction 4)}$$

$$GS^- + GSOH \rightarrow GSSG + OH^- \qquad \text{(Reaction 5)}$$

Biological Functions of Glutathione

1. Glutathione as a cosubstrate of glutathione peroxidase

Glutathione peroxidases (GPx) are known to decrease free radical production by reducing lipid hydroperoxides (ROOH) or hydrogen peroxide (H_2O_2) [3]. These enzymes catalyze the oxidation of GSH to GSSG and reduce ROOH (Reaction 6) or H_2O_2 (Reaction 7) to alcohol (ROH) or water, respectively [3]. Glutathione peroxidases are specific for GSH as a cosubstrate. However, they can accept a wide range of peroxides. There are two important types of glutathione peroxidases. One is selenium-dependent GSH peroxidase (GPx), which can catalyze the decomposition of both lipid hydroperoxides and H_2O_2. GPx is made up of four protein subunits, each of which contains one atom of selenium at its active site. GSH reduces the selenium atom and the reduced form of the enzyme then reacts with lipid hydroperoxides or hydrogen peroxide [3].

$$2GSH + ROOH \xrightarrow{GPx} GSSG + ROH + H_2O \qquad \text{(Reaction 6)}$$

$$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O \qquad \text{(Reaction 7)}$$

The other type of peroxidase is phospholipid hydroxide peroxidase (PhGPx), which is also selenium-dependent. PhGPx appears to mainly act on membrane-bound ROOH and cholesterol -OOH, but it also works on DNA hydroperoxides [10].

2. Glutathione as a cosubstrate of dehydroascorbate reductase

GSH also participates in the reduction of dehydroascorbate catalyzed by dehydroascorbate reductase [3]. This reaction regenerates ascorbate, which is a well-known antioxidant.

$$\text{dehydroascorbate} + 2\text{GSH} \rightarrow \text{GSSG} + \text{ascorbate} \qquad \text{(Reaction 8)}$$

3. Glutathione as a product of glutathione reductase-catalyzed reactions

To maintain the balance between GSH and GSSG, the oxidized form of glutathione (GSSG) is reduced to GSH by glutathione reductase, which uses NADPH as a cofactor (Rection 9). Glutathione reductase can also catalyze reduction of certain "mixed" disulfides, such as that between GSH and coenzyme A.

$$\text{GSSG} + \text{NADPH} + \text{H}^{+} \rightarrow 2\text{GSH} + \text{NADP}^{+} \qquad \text{(Reaction 9)}$$

4. Glutathione as a direct free radical scavenger

In 1993, Dr. Buettner published an important paper [11], in which he summarized the reduction potentials of a selected set available antioxidants and free radicals in a pecking order as shown on Table 1. The species listed at the top of the table possess a very positive reduction potential and display non-selective reactivity with different cell components. The species listed in the middle of the table can be antioxidants, which can scavenge reactive free radicals located on the top of the table, producing the corresponding antioxidant-derived radicals. Following the concept, GSH can act as a

direct scavenger of almost all of the noxious free radicals, such as $HO^{\bullet}$, $O_2^{\bullet-}$, and other oxygen-, carbon-, and nitrogen-centered free radicals, and itself is converted into the relatively stable $GS^{\bullet}$.

Table 1. The pecking order of free radicals and antioxidants [11].

Redox Couple	**$E^{0'}$/mV**
$HO^{\bullet}$, H^+/H_2O	+ 2310
$RO^{\bullet}$, H^+/ROH (aliphatic alkoxyl radical)	+ 1600
$ROO^{\bullet}$, H^+/ROOH (alkyl peroxyl radical)	+ 1000
$GS^{\bullet}/GS^-$ (glutathione)	+ 920
$PUFA^{\bullet}$, H+/PUFA-H (bis-allylic-H)	+ 600
$TO^{\bullet}$,H^+/TOH	+ 480
H_2O_2,H^+/H_2O, $HO^{\bullet}$	+ 320
Ascorbate$^{\bullet-}$, H^+/Ascorbate monoanion	+ 282
Fe(III) EDTA/ Fe(II) EDTA	+ 120
$O_2/ O_2^{\bullet-}$	- 330
Paraquat/ Paraquat$^{\bullet-}$	- 448
Fe(III) DFO/ Fe(II) DFO	- 450
RSSR/ RSSR$^{\bullet-}$ (GSH)	- 1500
H_2O/ e^-_{aq}	- 2870

Summary

Glutathione, with a reactive thiol group, is involved in many biologically important processes. It can serve as a specific cosubstrate of glutathione peroxidase, which is involved in the reduction of lipid hydroperoxide and hydrogen peroxide. Glutathione also participates in regeneration of other antioxidants, such as ascorbate. In addition, glutathione acts as a direct free radical scavenger. These biological functions make it one of the important endogenous small molecule antioxidants.

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