ROS

RESEARCH HIGHLIGHTS

GSH: A Double-Edged Sword

Robert Z. Hopkins

AIMSCI Research Institute, P.O. Box 37504, Raleigh, NC 27626, USA

Correspondence: rzh@aimsci.com (R.Z.H.)

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ABSTRACT | As the most abundant thiol compound, the reduced form of glutathione (GSH) has won a great title of being a major intracellular antioxidant. Indeed, GSH has been well known to play a major role in the detoxification of reactive oxygen species (ROS) and electrophilic compounds either via direct reaction with the ROS or electrophiles or serving as an electron donor for enzymatic antioxidants, including glutathione peroxidase, glutathione S-transferase, and glutaredoxin. On the other hand, under certain conditions, GSH may become a detrimental factor for the host cells. In this context, several recent studies suggest that GSH may augment bacterial virulence, promotes tumorigenesis, and even contributes to the development of anxiety. This Research Highlights article briefly surveys the classical and atypical biological activities of GSH to better define this famous intracellular tripeptide as a double-edged sword.

KEYWORDS | Antioxidant; Anxiety; Electrophile; GSH; Reactive oxygen species; Signaling; Tumorigenesis; Virulence

ABBREVIATIONS | Grx, glutaredoxin; γGCL, γ-glutamylcysteine ligase; GSH, reduced form of glutathione; GSSG, glutathione disulfide; ROS, reactive oxygen species

CONTENTS

1. OVERVIEW

The term glutathione, if not specified, may refer to both the reduced form (GSH) and the oxidized form (GSSG) of glutathione. GSH is a tripeptide (γ-glutamylcysteinylglycine) with a molecular mass of 307 (structure in **Figure 1**). In mammalian cells, the cytosolic concentrations of GSH are in the range of 1–10 mM. GSH is also present in high concentrations in the mitochondria and nuclei. In contrast, the extracellular levels of GSH are much lower, and GSH levels in the plasma are typically in the range of lower micromolar concentrations. In mammalian cells, GSH synthesis involves two cytosolic enzymes, namely, γ-glutamylcysteine ligase (γGCL) and glutathione synthetase (GSS). γGCL consists of two subunits: the heavy catalytic subunit designated as GCLC with a molecular mass of ~73 kDa and the light modifier subunit designated as GCLM with a molecular mass of ~31 kDa. GCLC and GCLM are encoded by separate genes that are localized on chromosomes 6p12 and 1p22.1, respectively, in humans. The human GSS gene is localized on chromosome 20q11.2.

2. BIOCHEMISTRY

GSH is synthesized from three amino acids via two successive enzymatic reactions in the cytoplasm (**Figure 2**). The first step involves combination of cysteine and glutamate to produce γ-glutamylcysteine. This reaction is catalyzed by γGCL, also formerly known as γ-glutamylcysteine synthetase (γGCS). This reaction requires coupled ATP hydrolysis. The next step involves the enzyme GSS, which catalyzes the addition of glycine to the dipeptide to form γ-glutamylcysteinylglycine (GSH). This enzyme also requires coupled hydrolysis of adenosine triphosphate (ATP).

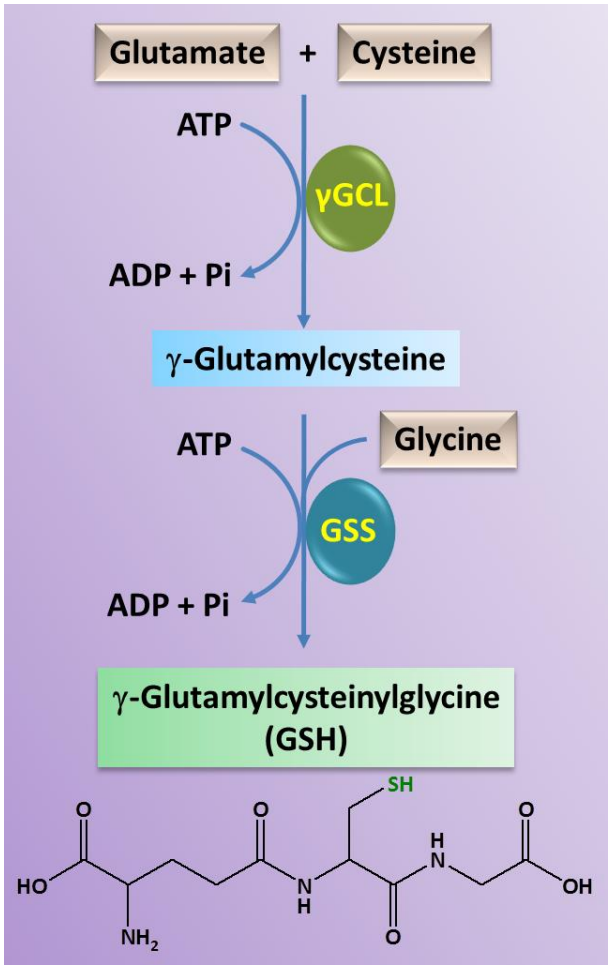

FIGURE 1. Biosynthesis of the reduced form of glutathione (GSH) in mammalian cells. As illustrated, GSH is synthesized from three amino acids through two successive reactions catalyzed by γ-glutamylcysteine ligase (γGCL) and glutathione synthetase (GSS), respectively. Two molecules of adenosine triphosphate (ATP) are consumed for synthesizing each molecule of GSH. It is noteworthy that γGCL is the key enzyme of GSH biosynthesis. This enzyme is subject to Nrf2/ARE-mediated transcriptional regulation.

3. BIOLOGICAL ACTIVITIES

3.1. Classical Biological Activities

In mammalian systems, GSH is a major antioxidant involved in at least four types of biochemical reactions. They are: (1) reaction with reactive oxygen species (ROS) leading to their detoxification; (2) reaction with electrophilic compounds; (3) regeneration of α-tocopherol (a form of vitamin E) and vitamin C; and (4) protein deglutathionylation catalyzed by glutaredoxin, an enzyme that uses GSH as the electron donor.

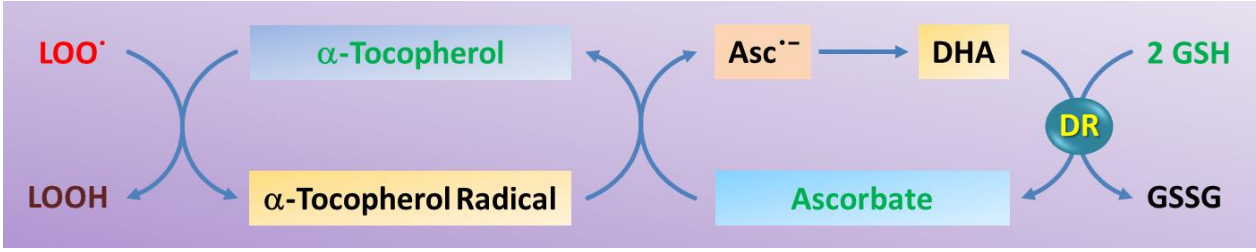

FIGURE 2. Role of the reduced form of glutathione (GSH) in the regeneration of α-topopherol and ascorbate. As illustrated, α-tocopherol reduces lipid peroxyl radical (LOO·) to lipid hydroperoxide (LOOH), and in the reaction, α-tocopherol is oxidized to α-tocopherol radical. The α-tocopherol radical can be reduced back to α-tocopherol by ascorbate, which is oxidized to ascorbate radical ($Asc^{\cdot-}$) and then to dehydroascorbate (DHA). DHA can be reduced back to ascorbate by a GSH-dependent reaction catalyzed by DHA reductase (DR). During this reaction, GSH is oxidized to glutathione disulfide (GSSG).

3.1.1. Reaction with ROS

GSH is a major defense against ROS. GSH can directly react with ROS, leading to the detoxification of these reactive species. GSH is also used as a cofactor by glutathione peroxidase in the detoxification of H_2O_2 and other peroxides, as well as peroxynitrite. In addition, the reaction between GSH and nitric oxide (or nitric oxide-derived species) leads to the formation of S-nitrosoglutathione. In biological systems, S-nitrosoglutathione may act as a second messenger to transduce nitric oxide bioactivity [1, 2].

3.1.2. Reaction with Electrophilic Compounds

Electrophiles (electron-deficient species), such as reactive aldehydes, are derived from xenobiotic biotransformation as well as lipid peroxidation. GSH reacts with electrophiles, forming less reactive conjugates. Thus, conjugation with GSH represents an important mechanism for the detoxification of electrophilic species. The conjugation reactions with GSH may occur spontaneously, but are markedly accelerated by glutathione S-transferase. It is noteworthy that for certain xenobiotics, GSH conjugation may result in their bioactivation, thereby leading to increased toxicity [3].

3.1.3. Regeneration of Vitamin E and Vitamin C

While GSH is the most abundant non-protein thiol antioxidant in mammalian cells, there are also many other cellular non-protein antioxidants, such as α-tocopherol and vitamin C (also known as ascorbate). α-Tocopherol reduces lipid peroxyl radical to form lipid hydroperoxide, and in the reaction α-tocopherol is oxidized to α-tocopherol radical. The α-tocopherol radical can be reduced back to α-tocopherol by ascorbate, which is oxidized to ascorbate radical and then to dehydroascorbate (DHA). DHA is reduced to ascorbate by a GSH-dependent reaction catalyzed by dehydroascorbate reductase (**Figure 3**). Because of this role GSH deficiency results in decreased tissue levels of vitamin C in animal models [4, 5].

3.1.4. Protein Deglutathionylation

Reversible protein S-glutathionylation (protein–SSG) is an important post-translational modification involved in redox signaling. Analogous to protein dephosphorylation catalyzed by phosphatases, glutaredoxin using GSH as a cofactor/electron donor catalyzes deglutathionylation of proteins. This participates in regulating diverse intracellular signaling pathways [6, 7].

3.2. Atypical Biological Activities

3.2.1. Anxiety

In addition to the above well-established functions, GSH also possesses atypical biological activities, including both beneficial and detrimental effects. For example, GSH is the cofactor for glyoxalase 1, an

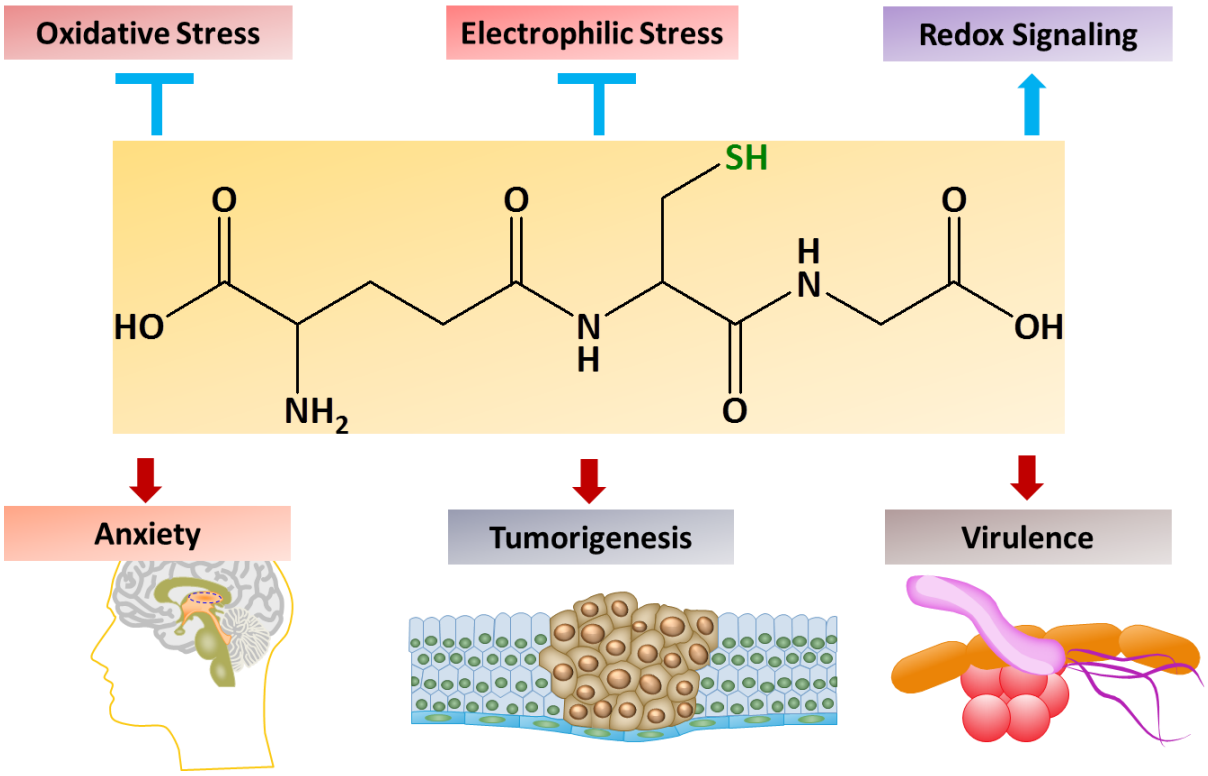

FIGURE 3. The reduced form of glutathione (GSH) acting as a double-edged sword. As illustrated, GSH is a major cellular defense against oxidative and electrophilic stress. It also plays an important role in redox signaling. On contrary, this tri-peptide, under certain conditions, make the host cell or organism more susceptible to such conditions as anxiety, pathogen infections, and tumorigenesis.

enzyme involved in the detoxification of glycolysis-derived α-oxoaldehyde, methylglyoxal, and protection against hyperglycemia-induced oxidative stress [8–10]. On the contrary, as a GSH-dependent enzyme, glyoxalase 1 may be also involved in the development of anxiety (**Figure 3**). Local overexpression of glyoxalase 1 in the mouse brain results in increased anxiety-like behavior, while local inhibition of glyoxalase 1 expression by RNA interference decreases the anxiety-like behavior [11]. This is possibly due to the ability of glyoxalase 1 to decrease brain levels of methylglyoxal, a γ-aminobutyric acid (GABA) type A receptor agonist [12].

3.2.2. Pathogen Virulence

Intracellular GSH may play a role in promoting the virulence of pathogens in host cells (**Figure 3**). Infection by the human bacterial pathogen Listeria monocytogenes is mainly controlled by the positive regulatory factor A (PrfA), a member of the Crp/Fnr family of transcriptional activators. Activation of PrfA is dependent on host cell GSH, and the GSH-dependent PrfA activation is mediated by allosteric binding of GSH to PrfA [13]. By binding to PrfA in the cytosol of the host cell, GSH induces the correct fold of the HTH motifs, thus priming the PrfA protein for DNA interaction and promoting the virulence [14].

3.2.3. Tumorigenesis

Increased GSH content in cancer cells is a major mechanism underlying cancer cell resistance to chemotherapy [15, 16]. In this context, many classical anticancer drugs are electrophilic compounds that

are detoxified by GSH conjugation in the cancer cells. In experimental models, GSH and thioredoxin synergize to drive cancer initiation, proliferation, and progression [17, 18] (**Figure 3**). Likewise, combined inhibition of GSH and thioredoxin antioxidant pathways leads to a synergistic cancer cell death in vitro and in vivo, suggesting both GSH and thioredoxin as potential targets for cancer therapeutic intervention.

4. CONCLUSION AND PERSPECTIVES

As one of the most extensively characterized cellular antioxidant, GSH is not a molecule of always doing good things to the host cells. It actually acts like a double-edged sword. On the one side, GSH protects the host cells against oxidative and electrophilic stress as well as assists in cell signaling. On the other side, this abundant intracellular thiol compound may represent a potential danger to the host cells. This notion of GSH acing as a double-edged sword also applies to other cellular antioxidants as well as ROS. Hence, a thorough appraisal of a particular antioxidant molecule or ROS requires examination of its both sides. This practice would be instrumental in designing effective modalities for the intervention of redox-related disease pathophysiology and for the promotion of health span.

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