Biochimica et Biophysica Acta 1830 (2013) 3143–3153

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Glutathione synthesis ☆,☆☆

Shelly C. Lu *

Division of Gastroenterology and Liver Diseases, USC Research Center for Liver Diseases, Southern California Research Center for ALPD and Cirrhosis, Keck School of Medicine USC, Los Angeles, CA 90033, USA

ARTICLE INFO

Article history:
Received 16 July 2012
Received in revised form 5 September 2012
Accepted 10 September 2012
Available online 17 September 2012

Keywords:
GSH
Glutamate-cysteine ligase
GSH synthase
Nrf2
MafG
Antioxidant response element

ABSTRACT

Background: Glutathione (GSH) is present in all mammalian tissues as the most abundant non-protein thiol that defends against oxidative stress. GSH is also a key determinant of redox signaling, vital in detoxification of xenobiotics, and regulates cell proliferation, apoptosis, immune function, and fibrogenesis. Biosynthesis of GSH occurs in the cytosol in a tightly regulated manner. Key determinants of GSH synthesis are the availability of the sulfur amino acid precursor, cysteine, and the activity of the rate-limiting enzyme, glutamate cysteine ligase (GCL), which is composed of a catalytic (GCLC) and a modifier (GCLM) subunit. The second enzyme of GSH synthesis is GSH synthetase (GS).

Scope of review: This review summarizes key functions of GSH and focuses on factors that regulate the biosynthesis of GSH, including pathological conditions where GSH synthesis is dysregulated.

Major conclusions: GCL subunits and GS are regulated at multiple levels and often in a coordinated manner. Key transcription factors that regulate the expression of these genes include NF-E2 related factor 2 (Nrf2) via the antioxidant response element (ARE), AP-1, and nuclear factor kappa B (NFκB). There is increasing evidence that dysregulation of GSH synthesis contributes to the pathogenesis of many pathological conditions. These include diabetes mellitus, pulmonary and liver fibrosis, alcoholic liver disease, cholestatic liver injury, endotoxemia and drug-resistant tumor cells.

General significance: GSH is a key antioxidant that also modulates diverse cellular processes. A better understanding of how its synthesis is regulated and dysregulated in disease states may lead to improvement in the treatment of these disorders. This article is part of a Special Issue entitled Cellular functions of glutathione.

1. Introduction

Glutathione (GSH) is a tripeptide, γ-L-glutamyl-L-cysteinylglycine, present in all mammalian tissues at 1–10 mM concentrations (highest concentration in liver) as the most abundant non-protein thiol that defends against oxidative stress. GSH is also a key determinant of redox signaling, vital in detoxification of xenobiotics, and modulates cell proliferation, apoptosis, immune function, and fibrogenesis. This review is focused on factors that determine GSH synthesis and pathologies where dysregulation in GSH synthesis may play an important role with emphasis on the liver. This is because the liver plays a central role in the interorgan GSH homeostasis [1].

2. Structure and functions of GSH

GSH exists in the thiol-reduced and disulfide-oxidized (GSSG) forms [2]. GSH is the predominant form and accounts for >98% of total GSH [3–5]. Eukaryotic cells have three major reservoirs of GSH. Most (80–85%) of the cellular GSH are in the cytosol; 10–15% is in the mitochondria and a small percentage is in the endoplasmic reticulum [6–8]. Rat liver cytosolic GSH turns over rapidly with a half-life of 2–3 h. The structure of GSH is unique in that the peptide bond linking glutamate and cysteine of GSH is through the γ-carboxyl

Abbreviations: 4-HNE, 4-hydroxynonenal; 15d-PGJ$_2$, 15-deoxy-Δ^{12,14}-prostaglandin J$_2$; AP-1, activator protein-1; As^{3+}, trivalent arsenite; α-SMA, a-smooth muscle actin; ARE, antioxidant response element; ATRA, all-trans retinoic acid; BDL, bile duct ligation; BHMT, betaine homocysteine methyltransferase; β-NF, β-naphthoflavone; BSO, buthionine sulfoximine; CBS, cystathionine β synthase; CMK, Ca^{2+}-calmodulin kinase II; CNC-bZIP, cap 'n' collar-basic leucine zipper proteins; CREB, c-AMP-response element binding protein; DEM, diethyl maleate; ECM, extracellular matrix; EpRE, electrophile response element; GCL, glutamate-cysteine ligase; GCLC, GCL-catalytic subunit; GCLM, GCL-modifier subunit; GGT, γ-glutamyltranspeptidase; GPx, GSH peroxidase; GS, GSH synthase; GSH, glutathione; GSSG, oxidized GSH; HCC, hepatocellular carcinoma; Hcy, homocysteine; HGF, hepatocyte growth factor; HSC, hepatic stellate cell; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MAT, methionine adenosyltransferase; MRE, metal response element; MS, methionine synthase; MT, methyltransferase; NFE2, nuclear factor erythroid 2; NO, nitric oxide; Nrf2, nuclear factor-erythroid 2 related factor 2; PKA, protein kinase A; PKC, protein kinase C; RNS, reactive nitrogen species; RXRα, retinoid X receptor α; ROS, reactive oxygen species; SAH, S-adenosylhomocysteine; SAMe, S-adenosylmethionine; TAA, thioacetamide; TBH, tert-butyl hydroquinone; TGF-β1, transforming growth factor-β1; TLR4, toll like receptor 4; TNFα, tumor necrosis factor α; UDCA, ursodeoxycholic acid

☆ This article is part of a Special Issue entitled Cellular functions of glutathione.
☆☆ This work was supported by NIH grant R01DK092407.

* HMR Rm 415, Department of Medicine, Keck School of Medicine USC, 2011 Zonal Ave., Los Angeles, CA 90033, USA. Tel.: +1 323 442 2441; fax: +1 323 442 3234.
E-mail address: shellylu@usc.edu.

0304-4165/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbagen.2012.09.008

group of glutamate rather than the conventional α-carboxyl group. The only enzyme that can hydrolyze this unusual bond is γ-glutamyltranspeptidase (GGT), which is only present on the external surfaces of certain cell types [9]. As a consequence, GSH is resistant to intracelluar degradation and is only metabolized extracellularly by cells that express GGT. This allows for released GSH to be broken down and its constituent amino acids taken up by cells and reincorporated into GSH (so called γ-glutamyl cycle, see below). The bulk of plasma GSH originates from the liver, which plays a central role in the interorgan homeostasis of GSH by exporting nearly all of the GSH it synthesizes into plasma and bile [1,10,11]. Thus, dysregulation of hepatic GSH synthesis has impact on GSH homeostasis systemically.

GSH serves several vital functions including antioxidant defense, detoxification of xenobiotics and/or their metabolites, regulation of cell cycle progression and apoptosis, storage of cysteine, maintenance of redox potential, modulation of immune function and fibrogenesis [4,5,9,12–15]. Some of these key functions, namely antioxidant defense, redox signaling, storage of cysteine via the γ-glutamyl cycle, regulation of growth and death are described in more detail below.

2.1. Antioxidant function of GSH

The antioxidant function of GSH is accomplished largely by GSH peroxidase (GPx)-catalyzed reactions, which reduce hydrogen peroxide and lipid peroxide as GSH is oxidized to GSSG. GSSG in turn is reduced back to GSH by GSSG reductase at the expense of NADPH, forming a redox cycle [13]. Organic peroxides can also be reduced by GPx and GSH S-transferase. Catalase can also reduce hydrogen peroxide but it is present only in peroxisome. This makes GSH particularly important in the mitochondria in defending against both physiologically and pathologically generated oxidative stress [16,17]. As GSH to GSSG ratio largely determines the intracellular redox potential (proportional to the log of $[GSH]^2/[GSSG]$) [5], to prevent a major shift in the redox equilibrium when oxidative stress overcomes the ability of the cell to reduce GSSG to GSH, GSSG can be actively exported out of the cell or react with a protein sulfhydryl group leading to the formation of a mixed disulfide. Thus, severe oxidative stress depletes cellular GSH [13] (Fig. 2).

2.2. GSH in redox signaling

GSH regulates redox-dependent cell signaling. This is largely accomplished by modifying the oxidation state of critical protein cysteine residues [5,18]. GSH can be reversibly bound to the -SH of protein cysteinyl residues (Prot-SH) by a process called glutathionylation, generating glutathionylated proteins (Prot-SSG), which can either activate or inactivate the protein [18]. This is a mechanism to protect sensitive protein thiols from irreversible oxidation and may also serve to prevent loss of GSH under oxidative conditions (Fig. 2). Deglutathionylation can then occur through glutaredoxin and sulfiredoxin-catalyzed reactions using GSH as a reductant [15]. Many transcription factors and signaling molecules have critical cysteine residues that can be oxidized and this is an important mechanism whereby reactive oxygen and nitrogen species (ROS and RNS) regulate protein function and cell signaling that can be modulated by GSH [13,15].

2.3. GSH and the γ-glutamyl cycle

Alton Meister first described the γ-glutamyl cycle in the early 1970's, which allows GSH to serve as a continuous source of cysteine [19] (Fig. 3). This is an important function as cysteine is extremely unstable and rapidly auto-oxidizes to cystine extracellularly, which can generate potentially toxic oxygen free radicals [19]. In the γ-glutamyl cycle, GSH is released from the cell and the ecto-enzyme GGT transfers the γ-glutamyl moiety of GSH to an amino acid (the best acceptor being cystine), forming γ-glutamyl amino acid and cysteinylglycine. The γ-glutamyl amino acid can be transported back into the cell and once inside, the γ-glutamyl amino acid can be further metabolized to release the amino acid and 5-oxoproline, which can be converted to glutamate and used for GSH synthesis. Cysteinylglycine is broken down by dipeptidase to generate cysteine and glycine. Most cells readily take up cysteine. Once taken up, the majority of cysteine is incorporated into GSH, some is incorporated into protein, and some is degraded into sulfate and taurine [19].

2.4. GSH regulates growth and death

In many normal and malignant cell types, increased GSH level is associated with a proliferative response and is essential for cell cycle progression [20–26]. In normal hepatocytes, GSH level increases when cells shift from G_0 to G_1 phase of the cell cycle in vitro [25], and after 2/3 partial hepatectomy prior to the onset of increased DNA synthesis [27]. If this increase in GSH was blocked, DNA synthesis following partial hepatectomy was reduced by 33% [26]. In liver cancer and metastatic melanoma cells, GSH status also correlated with growth [26,28]. Interestingly, hepatocyte growth factor (HGF) induces the expression of GSH synthetic enzymes and acts as a mitogen in liver cancer cells only under subconfluent cell density condition and the mitogenic effect required increased GSH level [29]. A key mechanism for GSH's role in DNA synthesis relates to maintenance of reduced glutaredoxin or thioredoxin, which is required for the activity of ribonucleotide reductase, the rate-limiting enzyme in DNA synthesis [30]. In addition, the GSH redox status can affect the expression and activity of many factors important for cell cycle progression. Of particular interest is the finding that GSH co-localizes to the nucleus at the onset of proliferation, which through redox changes can affect the activity of many nuclear proteins including histones [14,31]. These recent studies show that a reducing condition in the nucleus is necessary for cell cycle progression [14].

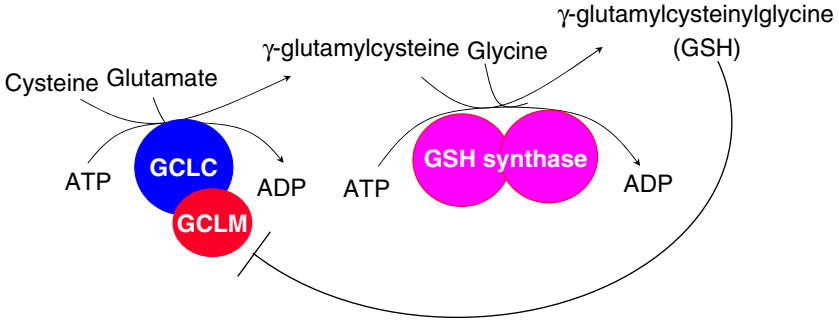

Fig 1. GSH synthesis. Synthesis of GSH occurs via a two-step ATP-requiring enzymatic process. The first step is catalyzed by glutamate-cysteine ligase (GCL), which is composed of catalytic and modifier subunits (GCLC and GCLM). This step conjugates cysteine with glutamate, generating γ-glutamylcysteine. The second step is catalyzed by GSH synthase, which adds glycine to γ-glutamylcysteine to form γ-glutamylcysteinylglycine or GSH. GSH exerts a negative feedback inhibition on GCL.