Glutathione Metabolism and Its Implications for Health

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ABSTRACT Glutathione (γ-glutamyl-cysteinyl-glycine; GSH) is the most abundant low-molecular-weight thiol, and GSH/glutathione disulfide is the major redox couple in animal cells. The synthesis of GSH from glutamate, cysteine, and glycine is catalyzed sequentially by two cytosolic enzymes, γ-glutamylcysteine synthetase and GSH synthetase. Compelling evidence shows that GSH synthesis is regulated primarily by γ-glutamylcysteine synthetase activity, cysteine availability, and GSH feedback inhibition. Animal and human studies demonstrate that adequate protein nutrition is crucial for the maintenance of GSH homeostasis. In addition, enteral or parenteral cystine, methionine, and N-acetylcysteine, and L-2-oxothiazolidine-4-carboxylate are effective precursors of cysteine for tissue GSH synthesis. Glutathione plays important roles in antioxidant defense, nutrient metabolism, and regulation of cellular events (including gene expression, DNA and protein synthesis, cell proliferation and apoptosis, signal transduction, cytokine production and immune response, and protein glutathionylation). Glutathione deficiency contributes to oxidative stress, which plays a key role in aging and the pathogenesis of many diseases (including drug resistance, virus replication, and some chronic diseases). New knowledge of the nutritional regulation of GSH metabolism is critical for the development of effective strategies to improve health and to treat these diseases.

KEY WORDS: amino acids, oxidative stress, cysteine, disease

The work with glutathione (γ-glutamyl-cysteinyl-glycine; GSH) has greatly advanced biochemical and nutritional sciences over the past 125 years. Specifically, these studies have led to the free radical theory of human diseases and to the advancement of nutritional therapies to improve GSH status under various pathological conditions. Remarkably, the past decade witnessed the discovery of novel roles for GSH in signal transduction, gene expression, apoptosis, protein glutathionylation, and nitric oxide (NO) metabolism. Most recently, studies of in vivo GSH turnover in humans were initiated to provide much-needed information about quantitative aspects of GSH synthesis and catabolism in the whole body and specific cell types. This article reviews the recent developments in GSH metabolism and its implications for health and disease.

Abundance of GSH in Cells and Plasma. Glutathione is the predominant low-molecular-weight thiol (0.5–10 mmol/L) in animal cells. Most of the cellular GSH (85–90%) is present in the cytosol, with the remainder in many organelles (including the mitochondria, nuclear matrix, and peroxisomes) (8). With the exception of bile acid, which may contain up to 10 mmol/L GSH, extracellular concentrations of GSH are relatively low (e.g., 2–20 μmol/L in plasma) (4,9). Because of the cysteine residue, GSH is readily oxidized nonenzymatically to glutathione disulfide (GSSG) by electrophilic substances (e.g., free radicals and reactive oxygen/nitrogen species). The GSSG efflux from cells contributes to a net loss of intracellular GSH. Cellular GSH concentrations are reduced markedly in response to protein malnutrition, oxidative stress, and many pathological conditions (8,9). The GSH + 2GSSG concentration is usually denoted as total glutathione in cells, a significant amount of which (up to 15%) may be bound to protein (1). The [GSH]:[GSSG] ratio, which is often used as an indicator of the cellular redox state, is >10 under normal physiological conditions (9). GSH/GSSG is the major redox couple that determines the antioxidative capacity of cells, but its value can be affected by other redox couples, including NADPH/NADP + and thioredoxin/thioredoxin ox (4).

GSH Synthesis. The synthesis of GSH from glutamate, cysteine, and glycine is catalyzed sequentially by two cytosolic enzymes, γ-glutamylcysteine synthetase (GCS) and GSH synthetase (Fig. 1). This pathway occurs in virtually all cell types, with the liver being the major producer and exporter of GSH. In the GCS reaction, the γ-carboxyl group of glutamate reacts with the amino group of cysteine to form a peptidic γ-linkage, which protects GSH from hydrolysis by intracellular peptidases. Although γ-glutamyl-cysteine can be a substrate for γ-glutamylcyclotransferase, GSH synthesis is favored in animal cells because of the much higher affinity and activity of GSH synthetase.

Mammalian GCS is a heterodimer consisting of a catalytically active heavy subunit (73 kDa) and a light (regulatory) subunit (31 kDa) (8). The heavy subunit contains all substrate binding sites, whereas the light subunit modulates the affinity of the heavy subunit for substrates and inhibitors. The Km values of mammalian GCS for glutamate and cysteine are 1.7 and 0.15 mmol/L, respectively, which are similar to the intracellular concentrations of glutamate (2–4 mmol/L) and cysteine (0.15–0.25 mmol/L) in rat liver (9). Mammalian GSH
Glutathione synthesis and utilization in animals. Enzymes that catalyze the indicated reactions are: 1) γ-glutamyl transpeptidase, 2) γ-glutamyl cyclotransferase, 3) 5-oxoprolinease, 4) γ-glutamylcysteine synthetase, 5) glutathione synthetase, 6) dipeptidase, 7) glutathione peroxidase, 8) glutathione reductase, 9) superoxide dismutase, 10) BCAA transaminase (cytosolic and mitochondrial), 11) glutaminase, 12) glutamate dehydrogenase, 13) glutamine-fructose-6-phosphate transaminase (cytosolic), 14) nitric oxide synthase, 15) glutathione S-transferase, 16) NAPDH oxidase and mitochondrial respiratory complexes, 17) glycolysis, 18) glutathione-dependent thioldisulfide or thioltransferase or nonenzymatic reaction, 19) transsulfuration pathway, 20) deacetylase, and 21) serine hydroxymethyltransferase. Abbreviations: AA, amino acids; BCKA, branched-chain α-ketoacids; GlcN-6-P, glucosamine-6-phosphate; GS-NO, glutathione–nitric oxide adduct; KG, α-ketoglutarate; LOO, lipid peroxyl radical; LOOH, lipid hydroperoxide; NAC, N-acetylcysteine; OTC, L-2-oxothiazolidine-4-carboxylate; R, radicals; R, nonradicals; R-S-P, ribose-5-phosphate; X, electrophilic xenobiotics.

synthetase is a homodimer (52 kDa/subunit) and is an allosteric enzyme with cooperative binding for γ-glutamyl substrate (10). The K_m values of mammalian GSH synthetase for ATP and glycine are ∼0.04 and 0.9 mmol/L, respectively, which are lower than intracellular concentrations of ATP (2–4 mmol/L) and glycine (1.5–2 mmol/L) in rat liver. Both subunits of rat GCS and GSH synthetase have been cloned and sequenced (9), which facilitates the study of molecular regulation of GSH synthesis. γ-Glutamylcysteine synthetase is the rate-controlling enzyme in de novo synthesis of GSH (8).

Knowledge regarding in vivo GSH synthesis is limited, due in part to the complex compartmentalization of substrates and their metabolism at both the organ and subcellular levels. For example, the source of glutamate for GCS differs between the small intestine and kidney (e.g., diet vs. arterial blood). In addition, liver GSH synthesis occurs predominantly in perivenous hepatocytes and, to a lesser extent, in perportal cells (11). Thus, changes in plasma GSH levels may not necessarily reflect changes in GSH synthesis in specific cell types. However, recent studies involving stable isotopes (5–7) have expanded our understanding of GSH metabolism. In healthy adult humans, the endogenous disappearance rate (utilization rate) of GSH is 25 μmol/(kg · h) (6), which accounts for 65% of whole body cysteine flux [38.3 μmol/(kg · h)]. This finding supports the view that GSH acts as a major transport form of cysteine in the body. On the basis of dietary cysteine intake [9 μmol/(kg · h)] in healthy adult humans (6), it is estimated that most of the cysteine used for endogenous GSH synthesis is derived from intracellular protein degradation and/or endogenous synthesis. Interestingly, among extrahepatic cells, the erythrocyte has a relatively high turnover rate for GSH. For example, the whole-blood fractional synthesis rate of GSH in healthy adult subjects is 65%/d (6), which means that all the GSH is completely replaced in 1.5 d; this value is equivalent to 3 μmol/(kg · h). Thus, whole blood (mainly erythrocytes) may contribute up to 10% of whole-body GSH synthesis in humans (5,6).

Regulation of GSH Synthesis by Amino Acids. Cysteine is an essential amino acid in premature and newborn infants and in subjects stressed by disease (14). As noted above, the intracellular pool of cysteine is relatively small, compared with the much larger and often metabolically active pool of GSH in cells (15). Recent studies provide convincing data to support the view that cysteine is generally the limiting amino acid for GSH synthesis in humans, as in rats, pigs, and chickens (6,14,15). Thus, factors (e.g., insulin and growth factors) that stimulate cysteine (cystine) uptake by cells generally increase intracellular GSH concentrations (8). In addition, increasing the supply of cysteine or its precursors (e.g., cysteine, N-acetylcysteine, and L-2-oxothiazolidine-4-carboxylate) via oral or intravenous administration enhances GSH synthesis and prevents GSH deficiency in humans and animals under various nutritional and pathological conditions (including protein malnutrition, adult respiratory distress syndrome, HIV, and AIDS) (2). Because cysteine generated from methionine catalyzes via oral or intravenous administration enhances GSH synthesis and prevents GSH deficiency in humans and animals under various nutritional and pathological conditions (including protein malnutrition, adult respiratory distress syndrome, HIV, and AIDS) (2). 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Despite the efflux of cysteine from the liver, the plasma concentration of cysteine is maintained by the hepatic secretion of cysteine and reduction of cystine in the portal vasculature (6). Glutathione, a thiol-containing tripeptide [γ-glutamyl-cysteinyl-γ-glutathione (GSH)], is the most abundant reduced tripeptide in the body (7). It is stored in all cells of the body, including the liver (8). It is synthesized in the liver in response to cysteine and glutamate (Fig. 1). The liver is the major source of GSH, which is transported to other tissues with the help of carrier-mediated transport systems (9). GSH is synthesized from cysteine and glutamate, and is involved in various cellular functions, including protection against free radicals (10). In this section, we will discuss the role of GSH in health and disease, and the factors that influence its synthesis and transport.

Roles of GSH. Glutathione participates in many cellular reactions. First, GSH effectively scavenges free radicals and other reactive oxygen species (e.g., hydroxyl radical, lipid peroxyl radical, peroxynitrite, and H2O2) directly, and indirectly through enzymatic reactions (24). In such reactions, GSH is oxidized to form GSSG, which is then reduced to GSH by the NADPH-dependent glutathione reductase (Fig. 1). In addition, glutathione peroxidase (a selenium-containing enzyme) catalyzes the GSH-dependent reduction of H2O2 and other peroxides (25).

Second, GSH reacts with various electrophiles, physiological metabolites (e.g., estrogen, melanins, prostaglandins, and leukotrienes), and xenobiotics (e.g., bromobenzene and acetaldehyde) to form mercapturates (24). These reactions are initiated by glutathione-S-transferase (a family of Phase II detoxification enzymes).

Third, GSH conjugates with NO to form an S-nitroso-glutathione adduct, which is cleaved by the thioredoxin system to release GSH and NO (24). Recent evidence suggests that the targeting of endogenous NO is mediated by intracellular GSH (26). In addition, both NO and GSH are necessary for the hepatic sensitizing agents (27), indicating their critical role in regulating lipid, glucose, and amino acid utilization.

Fourth, GSH serves as a substrate for formaldehyde dehydrogenase, which converts formaldehyde and GSH to formaldehyde-glutathione (2). The removal of formaldehyde (a carcinogen) is of physiological importance, because it is produced from the metabolism of methionine, choline, methanol (alcohol dehydrogenase), sarcosine (sarcosine oxidase), and xenobiotics (via the cytochrome P450–dependent monooxygenase system of the endoplasmic reticulum).

Fifth, GSH is required for the conversion of prostaglandin H2 (a metabolite of arachidonic acid) into prostaglandins D2 and E2 by endoperoxide isomerase (8).

Sixth, GSH is involved in the glyoxalase system, which converts methylglyoxal to d-lactate, a pathway active in microorganisms. Finally, glutathionylation of proteins (e.g., thioredoxin, ubiquitin-conjugating enzyme, and cytochrome c oxidase) plays an important role in cell physiology (2).

Thus, GSH serves vital functions in animals (Table 1). Adequate GSH concentrations are necessary for the proliferation of cells, including lymphocytes and intestinal epithelial cells (28). Glutathione also plays an important role in spermatogenesis and sperm maturation (1). In addition, GSH is essential for the activation of T-lymphocytes and polymorphonuclear leukocytes as well as for cytokine production, and therefore for mounting successful immune responses when the host is immunologically challenged (2). Further, both in vitro and in vivo evidence show that GSH inhibits infection by the...
influenza virus (29). It is important to note that shifting the
GSH/GSSG redox toward the oxidizing state activates several
signaling pathways (including protein kinase B, protein phos-
tathases 1 and 2A, calcineurin, nuclear factor kB, c-Jun
terminal kinase, apoptosis signal-regulated kinase 1, and
mitogen-activated protein kinase), thereby reducing cell
proliferation and increasing apoptosis (30). Thus, oxidative
stress (a deleterious imbalance between the production and
removal of reactive oxygen/nitrogen species) plays a key role
in the pathogenesis of many diseases, including cancer, inflam-
mation, kwashiorkor (predominantly protein deficiency), sei-
zure, Alzheimer's disease, Parkinson's disease, sickle cell ane-
emia, liver disease, cystic fibrosis, HIV, AIDS, infection, heart
attack, stroke, and diabetes (2,31).

Concluding Remarks and Perspectives. GSH displays
remarkable metabolic and regulatory versatility. GSH/GSSG
is the most important redox couple and plays crucial roles in
antioxidant defense, nutrient metabolism, and the regulation
of pathways essential for whole body homeostasis. Glutathione
deficiency contributes to oxidative stress, and, therefore, may
play a key role in aging and the pathogenesis of many diseases.
This presents an emerging challenge to nutritional research.
Protein (or amino acid) deficiency remains a significant nutri-
tional problem in the world, owing to inadequate nutritional
supply, nausea and vomiting, premature birth, HIV, AIDS,
cancer, cancer chemotherapy, alcoholism, burns, and chronic
digestive diseases. Thus, new knowledge regarding the effective
utilization of dietary protein or the precursors for GSH syn-
thesis and its nutritional status is critical for the develop-
ment of effective therapeutic strategies to prevent and treat a wide
array of human diseases, including cardiovascular complica-
tions, cancer, and severe acute respiratory syndrome.

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