Glutathione

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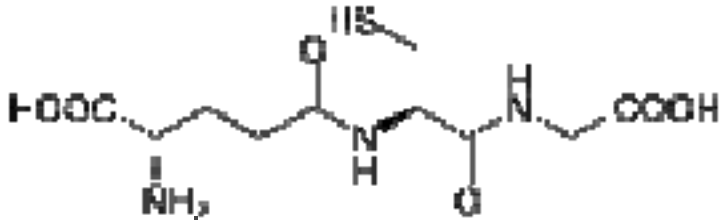

Glutathione (**GSH**) is an organic chemical that is found in plants and animals. It is a tripeptide with a gamma peptide linkage between the amine group of cysteine (which is attached by normal peptide linkage to a glycine) and the carboxyl group of the glutamate side-chain. It is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides.[2]

Thiol groups are reducing agents, existing at a concentration of approximately 5 mM in animal cells. Glutathione reduces disulfide bonds formed within cytoplasmic proteins to cysteines by serving as an electron donor. In the process, glutathione is converted to its oxidized form, glutathione disulfide (GSSG), also called L-(–)-glutathione.

Once oxidized, glutathione can be reduced back by glutathione reductase, using NADPH as an electron donor.[3] The ratio of reduced glutathione to oxidized glutathione within cells is often used as a measure of cellular toxicity.[4]

Biosynthesis

Glutathione is not an essential nutrient, since it can be synthesized in the body from the amino acids L-cysteine, L-glutamic acid, and glycine. The sulfhydryl (thiol) group (SH) of cysteine serves as a proton donor and is responsible for the biological activity of glutathione. Cysteine is the rate-limiting factor in cellular glutathione synthesis, since this amino acid is relatively rare in foodstuffs.

Cells make glutathione in two adenosine triphosphate (ATP)-dependent steps:

- First, *gamma*-glutamylcysteine is synthesized from L-glutamate and cysteine via the enzyme gamma-glutamylcysteine synthetase (glutamate cysteine ligase, GCL). This reaction is the rate-limiting step in glutathione synthesis.[5]
- Second, glycine is added to the C-terminal of *gamma*-glutamylcysteine via the enzyme glutathione synthetase.

Animal glutamate cysteine ligase (GCL) is a heterodimeric enzyme composed of a catalytic (GCLC) and modulatory (GCLM) subunit. GCLC constitutes all the enzymatic activity, whereas GCLM increases the catalytic efficiency of GCLC. Mice lacking GCLC (i.e., lacking all *de novo* GSH synthesis) die before birth.[6] Mice lacking GCLM demonstrate no outward phenotype, but exhibit marked decrease in GSH and increased sensitivity to toxic insults.[7][8][9]

While all cells in the human body are capable of synthesizing glutathione, liver glutathione synthesis has been shown to be essential. Mice with genetically-induced loss of GCLC (i.e., GSH synthesis) only in the liver die within 1 month of birth.[10]

The plant glutamate cysteine ligase (GCL) is a redox-sensitive homodimeric enzyme, conserved in the plant kingdom.[11] In an oxidizing environment, intermolecular disulfide bridges are formed and the enzyme switches to the dimeric active state. The midpoint potential of the critical cysteine pair is -318 mV. In addition to the redox-dependent control is the plant GCL enzyme feedback inhibited by GSH.[12] GCL is exclusively located in plastids, and glutathione synthetase is dual-targeted to plastids and cytosol, thus are GSH and gamma-glutamylcysteine exported from the plastids.[13] Both glutathione biosynthesis enzymes are essential in plants; knock-outs of GCL and GS are lethal to embryo and seedling.[14]

The biosynthesis pathway for glutathione is found in some bacteria, like cyanobacteria and proteobacteria, but is missing in many other bacteria. Most eukaryotes synthesize glutathione, including humans, but some do not, such as *Leguminosae*, *Entamoeba*, and *Giardia*. The only archaea that make glutathione are halobacteria.[15][16]

Function

Glutathione exists in both reduced (GSH) and oxidized (GSSG) states. In the reduced state, the thiol group of cysteine is able to donate a reducing equivalent ($H^+ + e^-$) to other unstable molecules, such as reactive oxygen species. In donating an electron, glutathione itself becomes reactive, but readily reacts with another reactive glutathione to form glutathione disulfide (GSSG). Such a reaction is probable due to the relatively high concentration of glutathione in cells (up to 5 mM in the liver).

GSH can be regenerated from GSSG by the enzyme glutathione reductase (GSR):[3] NADPH reduces FAD present in GSR to produce a transient FADH-anion. This anion then quickly breaks a disulfide bond (Cys58 - Cys63) and leads to Cys63's nucleophilically attacking the nearest sulfide unit in the GSSG molecule (promoted by His467), which creates a mixed disulfide bond (GS-Cys58) and a GS-anion. His467 of GSR then protonates the GS-anion to form the first GSH. Next, Cys63 nucleophilically attacks the sulfide of Cys58, releasing a GS-anion, which, in turn, picks up a solvent proton and is released from the enzyme, thereby creating the second GSH. So, for every GSSG and NADPH, two reduced GSH molecules are gained, which can again act as antioxidants scavenging reactive oxygen species in the cell.

In healthy cells and tissue, more than 90% of the total glutathione pool is in the reduced form (GSH) and less than 10% exists in the disulfide form (GSSG). An increased GSSG-to-GSH ratio is considered indicative of oxidative stress.

Glutathione has multiple functions:

- It is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds, as well as maintaining exogenous antioxidants such as vitamins C and E in their reduced (active) forms.[17]
- Regulation of the nitric oxide cycle, which is critical for life but can be problematic if unregulated[18]
- It is used in metabolic and biochemical reactions such as DNA synthesis and repair, protein synthesis, prostaglandin synthesis, amino acid transport, and enzyme activation. Thus, every system in the body can be affected by the state of the glutathione system, especially the immune system, the nervous system, the gastrointestinal system and the lungs.[*citation needed*]
- It has a vital function in iron metabolism. Yeast cells depleted of or containing toxic levels of GSH show an intense iron starvation-like response and impairment of the activity of extra-mitochondrial ISC enzymes, followed by death.[19]

Function in animals

GSH is known as a substrate in both conjugation reactions and reduction reactions, catalyzed by glutathione S-transferase enzymes in cytosol, microsomes, and mitochondria. However, it is also capable of participating in non-enzymatic conjugation with some chemicals.

In the case of *N*-acetyl-*p*-benzoquinone imine (NAPQI), the reactive cytochrome P450-reactive metabolite formed by paracetamol (or acetaminophen as it is known in the US), which becomes toxic when GSH is depleted by an overdose of acetaminophen, glutathione is an essential antidote to overdose. Glutathione conjugates to NAPQI and helps to detoxify it. In this capacity, it protects cellular protein thiol groups, which would otherwise become covalently modified; when all GSH has been spent, NAPQI begins to react with the cellular proteins, killing the cells in the process. The preferred treatment for an overdose of this painkiller is the administration (usually in atomized form) of *N*-acetyl-L-cysteine (often as a preparation called Mucomyst[20]), which is processed by cells to L-cysteine and used in the *de novo* synthesis of GSH.

Glutathione (GSH) participates in leukotriene synthesis and is a cofactor for the enzyme glutathione peroxidase. It is also important as a hydrophilic molecule that is added to lipophilic toxins and waste in the liver during biotransformation before they can become part of the bile. Glutathione is also needed for the detoxification of methylglyoxal, a toxin produced as a by-product of metabolism.

This detoxification reaction is carried out by the glyoxalase system. Glyoxalase I (EC 4.4.1.5) catalyzes the conversion of methylglyoxal and reduced glutathione to *S*-D-lactoyl-glutathione. Glyoxalase II (EC 3.1.2.6) catalyzes the hydrolysis of *S*-D-lactoyl-glutathione to glutathione and D-lactic acid.

Glutathione has recently been used as an inhibitor of melanin in the cosmetics industry. In countries like Japan and the Philippines, this product is sold as a skin whitening soap. Glutathione competitively inhibits melanin synthesis in the reaction of tyrosinase and L-DOPA by interrupting L-DOPA's ability to bind to tyrosinase during melanin synthesis. The inhibition of melanin synthesis was reversed by increasing the concentration of L-DOPA, but not by increasing tyrosinase. Although the synthesized melanin was aggregated within one hour, the aggregation was inhibited by the addition of glutathione. These results indicate that glutathione inhibits the synthesis and agglutination of melanin by interrupting the function of L-DOPA."[21]

Function in plants

In plants, glutathione is crucial for biotic and abiotic stress management. It is a pivotal component of the glutathione-ascorbate cycle, a system that reduces poisonous hydrogen peroxide.[22] It is the precursor of phytochelatins, glutathione oligomers that chelate heavy metals such as cadmium.[23] Glutathione is required for efficient defence against plant pathogens such as *Pseudomonas syringae* and *Phytophthora brassicae*.[24] APS reductase, an enzyme of the sulfur assimilation pathway uses glutathione as electron donor. Other enzymes using glutathione as substrate are glutaredoxin, these small oxidoreductases are involved in flower development, salicylic acid and plant defence signalling.[25]

Supplementation

Raising GSH levels through direct supplementation of glutathione is difficult. Research suggests that glutathione taken orally is not well absorbed across the gastrointestinal tract. In a study of acute oral administration of a very large dose (3 grams) of oral glutathione, Witschi and coworkers found "it is not possible to increase circulating glutathione to a clinically beneficial extent by the oral administration of a single dose of 3 g of glutathione."[26][27] However, it is possible to increase and maintain appropriate glutathione levels by increasing the daily consumption of cysteine-rich foods and/or supplements.[*non-primary source needed*][28]

Calcitriol (1,25-dihydroxyvitamin D_3), the active metabolite of vitamin D_3, after being synthesized from calcifediol in the kidney, increases glutathione levels in the brain and appears to be a catalyst for glutathione production.[29] Calcitriol was found to increase GSH levels in rat astrocyte primary cultures on average by 42%, increasing protein concentrations from 29 nmol/mg to 41 nmol/mg, 24 and 48 hours after administration; this effect was reduced to 11%, relative to the control, 96 hours after administration.[30] It takes about ten days for the body to process vitamin D_3 into calcitriol.[31]

In addition, plasma and liver GSH concentrations can be raised by administration of certain supplements that serve as GSH precursors.

Other supplements, including *S*-adenosylmethionine (SAMe)[32][33][34] and whey protein[35][36][37][38][39][40] have also been shown to increase cellular glutathione content.

N-acetyl cysteine (NAC) is available both as a drug and as a generic supplement, and has positive effects on glutathione production. Alpha lipoic acid restores intracellular glutathione.[41][42] Melatonin stimulates a related enzyme, glutathione peroxidase.[*non-primary source needed*][43]

Glutathione is a tightly regulated intracellular constituent, and is limited in its production by negative feedback inhibition of its own synthesis through the enzyme gamma-glutamylcysteine synthetase, thus greatly minimizing any possibility of overdosage. Glutathione augmentation using precursors of glutathione synthesis or intravenous glutathione is a strategy developed to address states of glutathione deficiency, high oxidative stress, immune deficiency, and xenobiotic overload in which glutathione plays a part in the detoxification of the xenobiotic in question (especially through the hepatic route).

Low glutathione is commonly observed in wasting and negative nitrogen balance,[44] as seen in cancer, HIV/AIDS, sepsis, trauma, burns and athletic overtraining. Glutathione supplementation can oppose this process, and in AIDS, for example, result in improved survival rates.[45] However, studies in many of these conditions have not been able to differentiate between low glutathione as a result of acutely (as in septic patients) or chronically (as in HIV) increased oxidative stress and increased pathology as a result of preexisting deficiencies.

A limited series of case reports and small clinical trials suggest that oxidative stress may be a factor underlying the pathophysiology of bipolar disorder, major depressive disorder and schizophrenia.[*undue weight? – discuss*] Replenishment of glutathione using *N*-acetyl cysteine reduces symptoms of both disorders.[*non-primary source needed*][46] Glutathione (GSH) is the major free radical scavenger in the brain.[47] Diminished GSH levels elevate cellular vulnerability towards oxidative stress; characterized by accumulating reactive oxygen species. GSH depletion has also been implicated in cellular predisposition to apoptosis.[*non-primary source needed*][48] Glutathione may also contain significant amounts of brain glutamate.[49]

Cancer

Preliminary results indicate glutathione changes the level of reactive oxygen species in isolated cells grown in a laboratory,[50][51] which may reduce cancer development.[52][53] None of these tests were performed on humans.

However, once a cancer has already developed, by conferring resistance to a number of chemotherapeutic drugs, elevated levels of glutathione in tumour cells protect cancerous cells in bone marrow, breast, colon, larynx and lung cancers.[54]

Pathology

Excess glutamate at synapses, which may be released in conditions such as traumatic brain injury, can prevent the uptake of cysteine, a necessary building-block of glutathione. Without the protection from oxidative injury afforded by glutathione, cells may be damaged or killed.[55]

Methods to determine glutathione

Reduced glutathione may be visualized using Ellman's reagent or bimane derivates such as monobromobimane. The monobromobimane method is more sensitive. In this procedure, cells are lysed and thiols extracted using a HCl buffer. The thiols are then reduced with dithiothreitol (DTT) and labelled by monobromobimane. Monobromobimane becomes fluorescent after binding to GSH. The thiols are then separated by HPLC and the fluorescence quantified with a fluorescence detector. Bimane may also be used to quantify glutathione in vivo. The quantification is done by confocal laser scanning microscopy after application of the dye to living cells.[56] Another approach, which allows to measure the glutathione redox potential at a high spatial and temporal resolution in living cells is based on redox imaging using the redox-sensitive green fluorescent protein (roGFP)[57] or redox sensitive yellow fluorescent protein (rxYFP)[58]

Importance in winemaking

The content of glutathione in must determines the browning effect during the production of white wine by trapping the caffeoyltartaric acid quinones generated by enzymic oxidation as grape reaction product (GRP).[59]